GASTROINTESTINAL BLOOD FLOW IN CHINOOK SALMON (*Oncorhynchus tshawytscha*).

by

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Gastrointestinal blood flow in chinook salmon (Oncorhychus tshawytscha)

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Abstract

Salmonids are known for their ability to swim continuously without compromising growth. However, it is not known how their cardiovascular system simultaneously meets the O_2 demands of the locomotory muscles, growth and digestion. This thesis is the first to examine these various cardiovascular demands in chinook salmon by: (1) measuring changes in intestinal blood flow ($\dot{q}_{IA}$) postprandially and during swimming; (2) examining the significance of changes in $\dot{q}_{IA}$ during exercise for O_2 delivery to muscles and swimming performance; (3) examining the ability of chinook salmon to swim and feed simultaneously; and (4) estimating the maximum swimming speed that chinook can likely sustain without compromising growth.

Intestinal blood flow and resting cardiac output ($\dot{Q}_{rest}$) in chinook salmon were 12-18 $mL \cdot min^{-1} \cdot kg^{-1}$ and 35 $mL \cdot min^{-1} \cdot kg^{-1}$, respectively. Therefore, $\dot{q}_{IA}$ accounted for 34% of $\dot{Q}_{rest}$. Postprandially, $\dot{q}_{IA}$ increased by 81%, peaking 23 h after feeding.

Unfed fish, swimming maximally, reduced $\dot{q}_{IA}$ by 60-70% because splanchnic vascular resistance (R_{SPLANC}) increased. Concurrently, $\dot{Q}$ increased by 86% from 35 to 65 $mL \cdot min^{-1} \cdot kg^{-1}$. A negative linear relationship between $\dot{q}_{IA}$ and oxygen consumption ($\dot{V}_{O_2}$) suggests that $\dot{q}_{IA}$ regulation is related to the O_2 demands of the locomotory muscles. Exercise-trained fish could better maintain $\dot{q}_{IA}$ and O_2 transport to the gut while swimming.

Model calculations indicated that blood flow redistributed from the gut during exercise was equal to 14% of the total O_2 transport to the locomotory muscles at the maximum prolonged swimming speed (U_{crit}). Moreover, blood pressure in the dorsal aorta (P_{DA}) would have been reduced by 65% at U_{crit} if R_{SPLANC} had not increased when muscle perfusion increased. Hence, the increase in R_{SPLANC} is of critical importance for regulation of blood pressure during swimming.

The postprandial increase in $\dot{V}_{O_2}$, otherwise known as heat increment (HI), was 128% of resting $\dot{V}_{O_2}$. The HI (32 μmol·min^{-1}·kg^{-1}) was unaffected by swimming speed and the maximum O_2 uptake ($\dot{V}_{O_{2max}}$) of starved and recently fed fish was the same. However, the postprandial U_{crit} was 9% lower than that of starved fish, probably because less blood flow was available for the locomotory muscles. These results are consistent with the hypothesis that $\dot{V}_{O_{2max}}$ is limited by cardiovascular O_2 delivery.

The high $\dot{V}_{O_{2max}}$ of chinook salmon is supported by an internal O_2 transport capacity that is higher than that in other fish species at the same temperature. Even though HI in chinook salmon is comparable to other fish species, chinook salmon likely support digestive function at a higher swimming velocity than other temperate fish species, because their scope for increasing $\dot{V}_{O_2}$ is greater.

The estimated maximum swimming velocity that chinook salmon could maintain with a positive energy budget was between 1.5 and 2 bl·s^{-1} or 56-74% of U_{crit}. At this swimming speed there is no evidence that $\dot{q}_{IA}$ limits intestinal function since the HI and $\dot{q}_{IA}$ were not reduced at this velocity.

(Said the Count) "There is reason that all things are as they are, and did you see with my eyes and know with my knowledge, you would perhaps better understand."

(Stoker, 1889)

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Table of contents

List of Tables

List of Figures.

Key to abbreviations and units of measurements used in the thesis

CMA: Coeliacomesenteric artery.

Ca_{O_2}: O_2 content of arterial blood ($mL \cdot dL^{-1}$).

Cv_{O_2}: O_2 content of venous blood ($mL \cdot dL^{-1}$).

DA: Dorsal aorta.

$\dot{D}_{O_2}$: Internal O_2 convection = $\dot{Q}$ (Ca_{O_2} - Cv_{O_2}) ($mL\ O_2 \cdot min^{-1} \cdot kg^{-1}$).

$\dot{D}_{O_2max}$: Maximum internal O_2 convection.

f_H: Heart rate (beats per minute).

E_{O_2}: Fraction of O_2 extracted from the blood in tissues.

E_{O_2max}: Maximum extraction of O_2 from blood in tissues.

[Hb]: Concentration of haemoglobin ($g \cdot dL^{-1}$).

HI: Heat increment.

Hct: Haematocrit (% red blood cell fraction of blood).

IA: Intestinal artery.

O_2: Oxygen.

Pa_{O_2}: Partial pressure of O_2 in arterial blood (kPa).

P_{DA}: Blood pressure in the dorsal aorta (kPa).

$\dot{Q}$: Cardiac output ($mL\ blood \cdot min^{-1} \cdot kg^{-1}$)

$\dot{Q}_{max}$: Maximum cardiac output.

$\dot{Q}_{rest}$: Resting cardiac output.

$\dot{q}_{IA}$: Intestinal blood flow (either as % change from resting levels or as absolute blood flow $mL \cdot min^{-1} \cdot kg^{-1}$).

R_{PERIF}: Periferal vascular resistance ($kPa \cdot mL^{-1} \cdot min \cdot kg$).

R_{SPLANC}: Splanchnic vascular resistance($kPa \cdot mL^{-1} \cdot min \cdot kg$).

R_{SYS}: Systemic vascular resistance($kPa \cdot mL^{-1} \cdot min \cdot kg$).

SV_H: Stroke volume of the heart ($mL \cdot kg^{-1}$).

$\dot{T}_{O_2}$: Cardiovascular O_2 transport = $\dot{Q} \cdot Ca_{O_2}$ ($mL\ O_2 \cdot min^{-1} \cdot kg^{-1}$).

$\dot{T}_{O_2max}$: Maximum cardiovascular O_2 transport.

U_{crit}: Critical swimming velocity ($cm \cdot s^{-1}$ or $bl \cdot s^{-1}$).

U_{opt}: Optimum swimming velocity. The swimming speed where the energetic cost of swimming a given distance is minimal.

$\dot{V}_{O_2}$: O_2 uptake (mL $O_2 \cdot min^{-1} \cdot kg^{-1}$).

$\dot{V}_{O_{2max}}$: Maximum O_2 uptake.

$\dot{V}_{O_{2rest}}$: Resting O_2 uptake.

Chapter 1

General Introduction

After hatching from eggs, anadromous salmonids remain for varying lengths of time in fresh water before migrating to the ocean (Pearcy, 1992). There they exploit the abundant food resources of the sea and accumulate most of their weight before returning again to fresh water to spawn. The growth of fry in fresh water is limited by the availability of food. However the growth of wild fish in seawater may surpass even the best growth attained by cultured fish that are fed to excess with specially formulated diets (Brett, 1983).

While foraging in the ocean, the fish may swim continuously (Brett, 1983), and salmonids are known to be able to maintain relatively high swimming velocities without compromising growth. Indeed, chinook salmon can swim continuously at velocities higher than 50% of their maximum prolonged swimming speed (U_{crit}: In this thesis, the maximum swimming speed that can be maintained for up to 20 minutes) without compromising growth (Kiessling & Higgs, in review), and rainbow trout swimming at 43% of U_{crit} grow faster than fish kept in minimal current (Farrell *et al.* 1990). Exercise training at moderate velocities may improve growth of salmonids (Leon, 1986; Houlihan & Laurent, 1987; Christiansen & Jobling, 1990) as compared with tank rested controls. However, at higher training velocities growth is reduced (Davison & Goldspink, 1977; Greer-Walker & Emerson, 1978; Christiansen & Jobling, 1990). It is not known what limits the growth of salmonids at higher swimming velocities, but it could be either a physiological limitation of digestion and assimilation at high swimming speeds, or that the energetic cost of locomotion equals or exceeds the maximum energy intake.

The digestive function of the intestine depends on adequate blood flow, both to supply O_2 for metabolic demands and to transport absorbed substances from the gut (Mailman,

1982). Blood flow to the intestines of fish increases during the postprandial stage (Axelsson *et al.* 1989; Axelsson & Fritsche, 1991). An additional role for the intestines in seawater fish is osmoregulation. Seawater fish must drink to regain water lost to the hypertonic environment (Eddy, 1982; Kirsch *et al.* 1984), a process that likely depends also on intestinal blood flow. When gas exchange increases in exercising fish, even more water may be lost across the gills (Gonalez & McDonald, 1992), requiring an increased drinking rate to maintain water balance. Intestinal blood flow is reduced in exercising fish (Randall & Daxboeck, 1982; Axelsson *et al.* 1989; Axelsson & Fritsche, 1991). Therefore, the ability of fish to maintain their normal growth rate and osmotic balance would appear to be compromised by inadequate gut blood flow.

When fish swim and feed, the cardiovascular system must supply O_2 to the locomotory muscles while simultaneously supplying the blood flow required for intestinal function and the metabolic cost of growth. Therefore, the capacity of the cardiovascular system to deliver O_2 may limit how fast fish can swim while supporting basic maintenance functions and growth.

Oxygen uptake.

Here is given a brief introduction to oxygen uptake in swimming and fed fish. A more detailed discussion of the subject is in the introduction to Chapter 3. In this thesis, O_2 consumption and O_2 uptake will be used interchangeably. Strictly speaking they are not the same and a mismatch will occur when the body stores of O_2 (in blood and tissues) are depleted or restored. However, here it is assumed that the fish are in equilibrium when O_2 uptake is measured and that O_2 uptake reflects O_2 consumption.

The energy required for swimming at sustainable velocities (swimming speed that can be maintained for more than 2 hours) is derived almost entirely from aerobic metabolism (Johnston & Moon, 1980a). As swimming speed increases, oxygen uptake ($\dot{V}_{O_2}$)

increases exponentially (Brett, 1964, 1965; Webb, 1975), and it is generally assumed that maximum O_2 uptake ($\dot{V}_{O2max}$) is achieved at U_{crit} (Jones & Randall, 1978).

The oxygen uptake ($\dot{V}_{O_2}$) of fish increases following feeding (see Jobling, 1981) and this increase in $\dot{V}_{O_2}$ depends both on the amount ingested and the composition of the food (Brett, 1976; Jobling & Davis, 1980; Legrow & Beamish, 1986). This increase in $\dot{V}_{O_2}$ following feeding is known by a variety of interchangeable terms such as: specific dynamic action, heat of nutrient metabolism, thermogenic action, and calorigenic effect of food (Beamish & Tripple, 1990). Following the recommendations of the NRC (1981), the term heat increment (HI) will be used here. The HI appears to reflect primarily the energetic cost of increased protein turnover and therefore represents the cost of growth (Coulson *et al.* 1978; Jobling, 1981; Brown & Cameron, 1991a; Lyndon *et al.* 1992). Most of the protein synthesis occurs in the white muscle mass (McMillan & Houlihan, 1988), which is approximately 66% of total body mass in salmonids (Stevens, 1968). However, the maximum rate of protein synthesis in the liver and the gut may account for as much as 40% of the total increase in $\dot{V}_{O_2}$ (Brown & Cameron, 1991; Lyndon *et al.* 1992).

When fed fish are swimming, their cardiovascular system must meet the challenge of supplying O_2 both for the locomotory muscles and the HI. If fish reach $\dot{V}_{O2max}$ while swimming at U_{crit}, then they should not be able to support the processes causing the HI while swimming maximally. Moreover, whatever limits $\dot{V}_{O2max}$ in fish may also limit their ability to simultaneously swim and grow.

Oxygen transport in fish.

The following sections give a brief overview of the steps in the O_2 cascade from the environment to fish tissues, and the pertinent literature.

The diffusion of O_2 from the environment to tissues is driven by the partial pressure gradient of oxygen (P_{O_2}). In fish, O_2 reaches tissues through two pathways, either by diffusing to the blood across the gills and then by internal convection through the cardiovascular system or by direct diffusion from the external environment. Although most of the oxygen consumed by salmonids is delivered by the cardiovascular system to tissues, a significant portion of $\dot{V}_{O_2\,rest}$ (10-15%) may be supported by direct diffusion (see below).

Ventilation.

In resting fish, water flows almost continuously across the gills of fish because of pressure differences created by alternate changes in the volume of the buccal and opercular cavities (Shelton, 1970). During exercise the ventilation volume increases in step with $\dot{V}_{O_2}$ (Stevens & Randall, 1967b; Kiceniuk & Jones, 1977; Piiper *et al.* 1977; Appendix II). As swimming speed increases above a critical level, salmonids shift from active to ram ventilation, where most of the power required for ventilation is generated by the locomotory muscles (Steffensen, 1985). In the ram mode of ventilation, fish adjust ventilation volume by changing mouth gape (Bushnell *et al.* 1984). Ram ventilation is more efficient in terms of ventilatory work (Farrell & Steffensen, 1987b) and therefore $\dot{V}_{O_2}$ is reduced after the transition to ram ventilation (Steffensen, 1985).

O_2 exchange across the gills.

The secondary lamellae are the main gas exchange surfaces of the gills. Water flows over the secondary lamellae in a direction opposite to that of the internal flow of blood (a counter current system). This allows the partial pressure of O_2 in the dorsal aorta (Pa_{O_2}) to be higher than the P_{O_2} of the expired water. The rate of O_2 diffusion between water and blood is proportional to the partial pressure gradient for oxygen at any location across the lamellae. The total conductance for O_2 between water and blood, termed either

transfer factor (Randall *et al.* 1967) or diffusing capacity (Piiper & Scheid, 1975), includes the combined conductance of the external water, a boundary layer of water over the gill epithelium, the gill epithelium, the plasma, the red blood cells, and the reaction of O_2 with haemoglobin. Furthermore, shunting of water and blood flow away from the respiratory surfaces may decrease the apparent conductance (Piiper & Scheid, 1982,1984; Scheid, 1985) by creating regional inhomogenities in perfusion, ventilation, and conductance of the gills (Malte & Weber, 1989). The exact contribution of each factor to the total conductance is not known (Randall & Daxboeck, 1984).

During exercise, the pattern of blood flow through the gills changes to increase the effective respiratory surface as well as to reduce the mean diffusion distances between water and blood. At rest, only two-thirds of the lamellae are perfused (Booth, 1978; Farrell *et al.* 1979), mostly in the basal regions of the filaments. The increase in blood pressure in the ventral aorta during exercise recruits the more distal lamellae on each filament (Farrell *et al.* 1980) and thus increases the effective respiratory surface area. Furthermore, the higher blood pressure causes proportionately more blood to flow through central than through basal region of each lamellae than at rest, which reduces the mean diffusion distance between blood and water (Farrell *et al.* 1980). Catecholamines may also change the blood flow pattern of the gills (Booth, 1978; Randall, 1982) and may increase the permeability of the gill epithelium to O_2 during exercise (Isaia *et al.* 1978).

Based on results from studies using perfused trout preparations, it was suggested that oxygen transfer across the gills of rainbow trout is primarily perfusion limited (Daxboeck *et al.* 1982; Randall & Daxboeck *et al.* 1984); i.e., O_2 uptake is limited by the rate at which blood flows through the gills. This was consistent with the findings of some previous studies which indicated that the Pa_{O_2} did not change during exercise and therefore that the diffusion capacity increased in step with $\dot{V}_{O_2}$ (Stevens & Randall, 1967b; Kiceniuk & Jones, 1977).

Recent studies on salmonids have reported a drop in Pa_{O_2} at high swimming speeds (Thomas *et al.* 1987; Butler *et al.* 1992; Gallaugher *et al.* 1992). Nevertheless, in all these studies the blood O_2 content was unchanged and therefore cardiovascular O_2 delivery may not have decreased in spite of the reduced Pa_{O_2}. Thus, even though there is a diffusion limitation at gills, it does not normally appear to limit O_2 supply to tissues.

Cardiovascular O_2 transport and internal O_2 convection.

In the following section, the key concepts of cardiovascular O_2 transport are defined and discussed.

The cardiovascular O_2 transport ($\dot{T}_{O_2}$) is proportional to cardiac output ($\dot{Q}$) and O_2 content of arterial blood (Ca_{O_2}):

$$\dot{T}_{O_2} = \dot{Q} \cdot Ca_{O_2}$$

The $\dot{V}_{O_2}$ which is supported by cardiovascular O_2 transport, is always less than the $\dot{T}_{O_2}$, since not all the O_2 in the blood is extracted in tissues. The amount of O_2, which is extracted in tissues is described by the extraction ratio (E_{O2}):

$$E_{O2} = \dot{V}_{O_2} / \dot{T}_{O_2}$$

It should be noted that the E_{O2} can also be calculated from the O_2 content of arterial and venous blood:

$$E_{O2} = (Ca_{O_2} - Cv_{O_2}) / Ca_{O_2}$$

The internal O_2 convection is the O_2 consumed by the fish per unit time, and delivered by the cardiovascular system. The internal O_2 convection can be described as:

$$\dot{V}_{O_2} = \dot{T}_{O_2} \cdot E_{O2}$$

or by the Fick equation as:

$$\dot{V}_{O_2} = \dot{Q} \cdot (Ca_{O_2} - Cv_{O_2})$$

where $\dot{Q}$ is cardiac output and Ca_{O_2} and Cv_{O_2} are arterial and venous blood O_2 content, respectively. The internal O_2 convection in exercising salmonids increases because both $\dot{Q}$ and E_{O_2} increase (Kiceniuk & Jones, 1977).

Cardiac output.

The resting $\dot{Q}$ ($\dot{Q}_{rest}$) of fish has been measured in many species and reported values range from 6.2 mL·min^{-1}·kg^{-1} to 132 mL·min^{-1}·kg^{-1} (see Farrell & Jones, 1992). A large fraction of this variability can be accounted for by temperature (Cech *et al.* 1976; Farrell, 1986; Barron, 1987; Kolok *et al.* 1993; Kolok & Farrell, 1994) although different methodologies may also have contributed significantly to the variance (Farrell & Jones, 1992). At temperatures between 8 and 12° C, which was the ambient temperature of the fish in these study, $\dot{Q}_{rest}$ in teleost fish ranges between 8.7 and 39.4 mL·min^{-1}·kg^{-1} (see Farrell & Jones, 1992; Axelsson & Farrell, 1993; Kolok *et al.* 1993). More active fishes such as the coho salmon, tend to have higher $\dot{Q}_{rest}$ (39.4 mL·min^{-1}·kg^{-1}; Axelsson & Farrell, 1993) than more sluggish fish such as the eel (*Anguilla anguilla*), the largescale sucker (*Catostomus macrocheilus*), the lingcod (*Ophiodon elongatus*), and the sea raven (*Hemitripterus americanus*) which have $\dot{Q}_{rest}$ between 8.7 and 14.6 mL·min^{-1}·kg^{-1} (Hughes *et al.* 1972; Farrell, 1981; Axelsson *et al.* 1989; Kolok *et al.* 1993).

Although the cardiovascular physiology of salmonids, especially rainbow trout, has been studied extensively, the $\dot{Q}$ in exercising salmonids has never been measured directly, but only estimated with indirect methods. The $\dot{Q}$ in freely swimming rainbow trout has been estimated in two studies based on the Fick principle (Stevens & Randall, 1967b; Kiceniuk & Jones, 1977). In the study of Stevens & Randall (1967) blood O_2 content was not measured but estimated from blood P_{O_2}. Where all the required variables were

measured, a three-fold increase in $\dot{Q}$ from rest to maximum prolonged exercise was observed (Kiceniuk & Jones, 1977).

These estimates of $\dot{Q}$, based on the Fick principle, have been criticized because of errors that are unavoidable when this method is used for fish (Johansen & Petterson, 1981; Metcalfe & Butler, 1982; Daxboeck *et al.* 1982). These errors stem from two sources, namely the portion of $\dot{V}_{O_2}$, which is not supported by internal convection, and arterial to venous shunts in the gill or pre-gill circulation (Randall, 1985).

A certain proportion of the total $\dot{V}_{O_2}$ of fish is not supported by internal convection, but through direct diffusion of O_2 from the water to skin and gills. The exact contribution of this direct diffusion to $\dot{V}_{O_2}$ is not known, but it is possible to estimate it by combining values for gill and skin O_2 uptake from various sources. Based on studies of perfused trout preparations Daxboeck *et al.* (1982) suggest that the proportion of gill O_2 consumption supported by direct diffusion is 27% of resting metabolic rate. However, this is likely an over-estimate (Randall, 1985) because the $\dot{V}_{O_{2\,rest}}$ of their fish was low (16 $\mu mol \cdot min^{-1} \cdot kg^{-1}$) and their experimental setup probably measured external diffusion to gills and skin. The total O_2 consumption of the gill tissue of Atlantic cod (*Gadus morhua*) was estimated as 6.7% of resting metabolic rate (Johansen & Petterson, 1981) and the metabolic capacity of the gill tissues in Atlantic salmon (*Salmo salar*) was estimated to be only 5% of resting metabolic rate (McCormik *et al.* 1988). Moreover, 58% of the gill O_2 consumption may be supported by cardiovascular O_2 delivery (Johansen & Petterson, 1981) and therefore it is likely that less than 5% of total O_2 consumption is supported by external diffusion at the gills.

In fresh water, the cutaneous O_2 uptake in rainbow trout has been estimated as 13% of resting metabolic rate (Kirsch & Nonotte, 1977) and 8% for brook trout (*Salvelinus fontinalis)* (Nonnotte, 1981). Similar values are obtained for fish in seawater (Nonnotte

& Kirsch, 1978). This uptake is not likely to contribute to internal O_2 convection, since it only matches cutaneous O_2 consumption.

Neither the cutaneous O_2 consumption (Takeda, 1993) nor the direct diffusion of O_2 at the gills are likely to increase in step with $\dot{V}_{O_2}$ in swimming fish. Therefore, external diffusion will probably become a smaller proportion of $\dot{V}_{O_2}$ as swimming speed increases. If internal O_2 convection supports 85-90% of the total O_2 consumption of resting salmonids and there is a five- to nine-fold increase in $\dot{V}_{O_2}$ from rest to maximum (Kiceniuk & Jones, 1977; Webb, 1971b), the proportion of $\dot{V}_{O_{2max}}$ which is supported by direct diffusion may be less than 3%.

Arterial to venous shunts or plasma skimming at the gills create a second source of error for Fick estimates of $\dot{Q}$ and these have been reported to be 7-8% of $\dot{Q}_{rest}$ in fish (Ishimatsu *et al.* 1988; Sundin & Nilsson, 1992). Shunts or plasma skimming at the gills will cause underestimates of $\dot{Q}$, based on the Fick principle. Fortuitously, shunts or plasma skimming at the gills will tend to cancel the effect of direct diffusion of O_2 (Randall, 1985). Therefore, the the Fick method may give reliable estimates of $\dot{Q}$, particularly at $\dot{V}_{O_{2max}}$ when the contributions of the two sources of errors may be insignificant. This is supported by the findings of Neuman *et al.* (1983) who showed an agreement between estimates of $\dot{Q}$ based on the Fick principle and other methods of measuring $\dot{Q}$ (dye dilution) in fish with high $\dot{V}_{O_2}$.

In recent years miniature ultrasonic flow probes have become available, which allow measurements of $\dot{Q}$ in swimming fish. In this study, flow probes will be used to make the first direct measurements of $\dot{Q}$ in a swimming salmonid. These flow probes are mainly of two types, pulsed Doppler and Transonic. The pulsed Doppler flow probes allow only measurements of relative changes in blood flow. They are smaller than the transonic flow probes and are therefore better suited for measurements of blood flow in

swimming fish, especially salmonids, where limited space makes it difficult to place large flow probes around the ventral aorta. The transonic flow probes are larger, but they give instantaneous measurements of absolute blood flow. The two types of flow probes will be used in combination: Doppler for measuring relative changes in blood flow and the transonic to calibrate the Doppler probes.

The maximum $\dot{Q}$ ($\dot{Q}_{max}$) of fish during prolonged exercise also increases with temperature (Kolok *et al.* 1993; Kolok & Farrell, 1994) as does $\dot{Q}_{rest}$. Furthermore, active fish also have a higher $\dot{Q}_{max}$ than sluggish fish (Farrell & Jones, 1992; Kolok *et al.* 1993; Kolok & Farrell, 1994). However, there is no clear difference in the relative increase in $\dot{Q}$ ($\dot{Q}_{max}/\dot{Q}_{rest}$) between active and sluggish fish during exercise. The relative increase in $\dot{Q}$ in fish considered sluggish or at least not fast swimmers was as follows: eels (*Anguilla australis schmidtii)* no increase (Davie & Forster, 1980); 47-57% in Atlantic cod (Axelsson, 1988; Axelsson & Nilsson, 1986); 64% in the sea raven (Axelsson *et al.* 1989); and 51-284% in the largescale sucker, depending on temperature (Kolok *et al.* 1993). In more active fish, the following values have been reported: 148-174% in the northern squawfish (*Ptytocheilus oregonensis*; Kolok & Farrell, 1994), 199% in the rainbow trout (Kiceniuk & Jones, 1977), and 100% in skipjack tuna (*Katsuwonus pelamis*; Brill & Bushnell, 1991). In elasmobranchs, a 70% increase in $\dot{Q}$ has been measured (Piiper *et al.* 1977; Lai *et al.* 1990). Thus, active fish tend to have higher $\dot{Q}_{rest}$ and $\dot{Q}_{max}$ than sluggish fish, but the relative increase in $\dot{Q}$ is quite variable among the few species in which it has been measured.

Blood O_2 content.

The O_2 content of blood depends on haemoglobin concentration ([Hb]), the P_{O_2} of the blood, and the affinity of haemoglobin for O_2. The [Hb] is proportional to haematocrit (Hct), which is the red blood cell fraction in the blood, and the mean corpuscular

haemoglobin concentration (MCHC = [Hb] / Hct). In blood, most of the O_2 is carried bound to Hb and only 4-6% of the total O_2 content is dissolved in plasma (Boutilier *et al.* 1984). Because the affinity of Hb for O_2 varies, depending on the number of O_2 molecules bound to the Hb, the curve for O_2 saturation of haemoglobin as a function of P_{O_2} is sigmoidal. The affinity of fish Hb for O_2 is affected by pH, $[CO_2]$, intracellular nucleotide triphosphates, and temperature (Randall & Daxboeck, 1984). Reduced pH causes a a right shift of the O_2 saturation curve (Bohr shift), and reduces the O_2 binding capacity of Hb (Root shift). Furthermore, adrenaline can affect intracellular pH and nucleotide triphosphate levels of red blood cells and thus modulate the O_2 affinity of haemoglobin (Nikinmaa, 1982).

During aerobic exercise, the Ca_{O_2} of salmonids remains constant (Kiceniuk & Jones, 1977; Thomas *et al.* 1987; Butler *et al.* 1992; Gallaugher *et al.* 1992). In recent studies, the Hct of fresh water rainbow trout has been reported to increase as swimming speed increases (Thomas, 1987; Butler *et al.* 1992; Gallaugher *et al.* 1992), because both red blood cells are released from the spleen and plasma volume is reduced (Gallaugher *et al.* 1992). The increased Hct does not change Ca_{O_2} from resting values, partly because Pa_{O_2} decreases simultaneously (Thomas, 1987; Butler *et al.* 1992; Gallaugher *et al.* 1992). Similarly, Ca_{O_2} was constant in the leopard shark (*Triakis semifasciata*) (Lai *et al.* 1990) and the dogfish (*Scyliorhinus stellaris*) (Piiper *et al.* 1977) during aerobic exercise. An entirely different pattern is seen in swimming lemon shark (*Negaprion breviostris*); Ca_{O_2} increases during exercise (Bushnell *et al.* 1982).

Unlike the Ca_{O_2}, the Cv_{O_2} decreases by more than 50% during exercise (Kiceniuk & Jones, 1977). Therefore, E_{O_2} in trout increases from 32% at rest to 86% at maximum swimming speed (Kiceniuk & Jones, 1977). A similar pattern of increased E_{O_2} is seen in the leopard shark (Lai *et al.* 1990), but there was no significant change in E_{O_2} of exercising dogfish (*Scyliorhinus stellaris*; Piiper *et al.* 1977).

Limits to $\dot{V}_{O2max}$ in salmonids.

There is some controversy over what limits the maximum O_2 consumption of animals. In principle, it could be determined by any step in the O_2 cascade, which consist essentially of four levels in fish: gill gas exchange, cardiovascular O_2 transport, tissue gas exchange and mitochondrial oxidative phosphorylation. Furthermore, substrate availability or transport of ATP to the sites of utilization could also limit $\dot{V}_{O2max}$ (Wagner, 1993). There is a wealth of evidence to indicate that $\dot{V}_{O2max}$ increases with increased O_2 supply to tissues (Cerretelli & di Prampero, 1987; Welch, 1987; Powers *et al.* 1989; Knight *et al.* 1992). Therefore, it is a widely held view that $\dot{V}_{O2max}$ of mammals is primarily limited by maximum O_2 supply (Saltin, 1985; Rowell *et al.* 1986; Wagner, 1991).

Cardiovascular O_2 transport ($\dot{T}_{O_2}$) is a function of cardiac output and arterial blood O_2 content. In humans, the maximum O_2 supply to tissues appears to be determined primarily by $\dot{T}_{O_2}$, particularly cardiac output (Horstman *et al.* 1976; di Pamprero, 1985; Saltin, 1985; Wagner, 1993). However, other factors also limit O_2 supply, and a diffusion limitation or some form of deficit in tissue O_2 transport must be present, since venous blood is never totally devoid of O_2 (Nelson *et al.* 1987; Roca *et al.* 1989; Hogan *et al.* 1990; Richardson *et al.* 1993). Moreover, there is not a proportional increase in the $\dot{V}_{O2max}$ of an individual when O_2 supply is increased (Turner *et al.* 1993) and a reduction in $\dot{T}_{O_2}$ may not lead to a corresponding reduction in $\dot{V}_{O_2}$, because of a compensatory increase in O_2 extraction from the blood (Nelson *et al.* 1987). Therefore, it has been hypothesized, that limitations of $\dot{V}_{O2max}$ may be a result of the interplay of numerous factors and that the O_2 cascade can be regarded as a series of resistors to the flux of O_2 from environment to mitochondria (Lindstedt *et al.* 1987; di Prampero & Ferretti, 1990). Similarly, the concept of symmorphosis (Taylor & Weibel, 1981) states that "functional design (of the respiratory system) is commensurate with functional demand" (Weibel *et*

al. et al. 1992) such that all structures within the respiratory system are approximately matched with the aerobic capacity of an animal.

Several lines of evidence suggest that the $\dot{V}_{O2max}$ of salmonids is limited by O_2 supply to tissues. The $\dot{V}_{O2max}$ of fish is reduced in hypoxic water (Bushnell *et al.* 1983). Similarly, the U_{crit} is reduced when water is hypoxic (Dahlberg *et al.* 1968; Jones, 1971; Bushnell *et al.* 1984), and when the O_2 capacity of the blood is reduced (Jones, 1971; Brauner *et al.* 1993). It is likely that a coronary ligation will reduce maximum cardiac output and therefore also $\dot{T}_{O2\,max}$. Farrell and Steffensen (1987a) found that the U_{crit} of coronary-ligated chinook salmon was reduced. Furthermore, the $\dot{V}_{O2max}$ of sockeye salmon increased when they swam in hyperoxic water (Brett, 1964). Perhaps, the strongest argument in favor of the hypothesis that O_2 supply limits $\dot{V}_{O2max}$ comes from a study by Gallaugher and co-workers (Gallaugher, Thorarensen & Farrell, in prep.). In that study the $\dot{T}_{O2\,max}$ of rainbow trout was varied by experimentally changing the Hct above or below normal values. The results showed clearly that varying Hct from normal values could lead to a near proportional change in $\dot{V}_{O2max}$.

If the $\dot{V}_{O2max}$ of fish is limited by $\dot{T}_{O2}$, then the two should be closely correlated. There are only three studies where $\dot{T}_{O2}$ and $\dot{V}_{O2}$ have been measured simultaneously in fish, one with large rainbow trout (Kiceniuk & Jones, 1977) and two with elasmobranchs (Piiper *et al.* 1977; Lai *et al.* 1990). Analysis of these studies suggests that $\dot{T}_{O2\,max}$ is indeed correlated with $\dot{V}_{O2max}$, providing further support for the hypothesis that $\dot{V}_{O2max}$ of rainbow trout is limited by $\dot{T}_{O2\,max}$. However, in all these studies $\dot{Q}$ was estimated, either from heart rate and a previous measurement SV_H in the same species or with the Fick principle. Thus, there are no measurements of $\dot{T}_{O_2}$ in fish that are derived from simultaneous and direct measurements of $\dot{Q}$ and Ca_{O_2}. One of the objectives of this research will be to obtain reliable measurements of internal O_2 transport in chinook salmonid, under various states.

It has been suggested that the $\dot{V}_{O2max}$ of swimming fish is limited by the metabolic demand of the locomotory muscles and not O_2 delivery (Smit *et al.* 1971; Webb, 1993). This suggestion is based on the observation that U_{crit} does not increase when ambient O_2 tension is increased (Brett, 1964; Dahlberg *et al.* 1968). However, $\dot{V}_{O2max}$ increases when the ambient P_{O2} increases and in this thesis, it will be shown that U_{crit} and $\dot{V}_{O2max}$ may vary independently. Reports of higher $\dot{V}_{O2max}$ in either high salinity (30‰) or low salinity (0‰) compared with isotonic water (e.g. Rao, 1968; Farmer & Beamish, 1969; Febry & Lutz, 1987; Péres-Pinzón & Lutz, 1991), have been cited to indicate that $\dot{V}_{O2max}$ is not limited by supply in swimming fish (Webb, 1993). However, a fundamental criticism of these studies is that $\dot{T}_{O2}$ was not measured. Therefore, they do not provide conclusive evidence for or against the hypothesis that $\dot{V}_{O2max}$ is limited by $\dot{T}_{O2\,max}$.

The $\dot{T}_{O2\,max}$ is clearly not the sole determinant of $\dot{V}_{O2max}$ in salmonids. Limitations to O_2 diffusion may exist at the gills, and in tissues. Changes in maximum metabolic demand may also affect $\dot{V}_{O2max}$ independent of $\dot{T}_{O2}$ by increasing extraction of O_2 from blood. If the analogy of resistors arranged in series is borrowed from the mammalian literature (Lindstedt *et al.* 1987; di Pamprero & Ferretti, 1990) and applied to fish, each of the factors that were mentioned above as potential limitations to $\dot{V}_{O2max}$, would represent individual resistors. However, the relative contribution of the 'resistors' to the total resistance to flow of O_2 through the system varies. Therefore, a unit change in any of the larger 'resistors', has a greater effect on O_2 flux than a proportional change in the smaller 'resistors'. Since changes in $\dot{T}_{O2\,max}$ of trout can have a substantial effect on $\dot{V}_{O2max}$ (Gallaugher, Thorarensen & Farrell, in prep.), $\dot{T}_{O2}$ must be one of the larger and possibly the largest 'resistors' of the system.

Intestinal blood flow

Intestinal blood flow in fish is 30-40% of resting cardiac output ($\dot{Q}_{rest}$) (Axelsson *et al.* 1989; Axelsson & Fritsche, 1991; Axelsson *et al.* in review), which is slightly higher than the 20-30% reported for mammals (Burton, 1972; Heller & Mohrmann, 1981; Kuwahira, 1993). These values for intestinal blood flow of fish were measured with ultrasonic flow probes. Attempts have also been made to measure intestinal blood flow in fish with radiolabeled microspheres, which are slightly larger than red blood cells. After injection into the blood stream, these spheres become lodged in the capillaries in proportion to the regional blood flow. Results from studies where this method has been used, indicate that intestinal blood flow is much lower than the direct measurement, being 2-17% instead of 30-40% of $\dot{Q}_{rest}$ (Kent, 1973; Cameron, 1975; Randall & Daxboeck, 1982; Neumann *et al.* 1983; Barron *et al.* 1987; White *et al.* 1988; Kolok *et al.* 1993). However, the microsphere method is likely to underestimate intestinal blood flow, partly because of the difficulty getting complete mixing of the microspheres in the dorsal aorta prior to reaching the opening of the coeliacomesenteric artery (Barron, 1987; Bushnell *et al.* 1992). Therefore, values obtained by measuring intestinal blood flow with ultrasonic flow probes must be considered more reliable than values from microsphere studies.

If the metabolic capacity of salmonids exceeds $\dot{M}_{O_2\,max}$, then cardiovascular O_2 delivery cannot support the maximum O_2 demands of all tissues simultaneously. Certainly, all tissue beds are never perfused fully at any one time. Consequently, one of the following must happen in fed fish that are swimming: (1) Intestinal blood flow is reduced during exercise, and therefore gut function is compromised, to increase delivery of O_2 to the locomotory muscles; (2) post-feeding intestinal blood flow is maintained during exercise, and therefore U_{crit} of fed fish is lower than that of unfed fish because less blood flow is available to the locomotory muscles; or (3) a combination of the above, and both U_{crit} and gut function are compromised.

Blood flow that is redistributed from the intestines in exercising fish may contribute significantly to muscle blood flow. During exercise gut blood flow in Atlantic cod is reduced by 23% (Axelsson & Fritsche, 1991). The blood flow that is redistributed from the gut of the Atlantic cod during exercise is equivalent to 8% of blood flow going to tissues other than the gut. The results of Randall and Daxboeck (1982) suggest also that the intestinal blood flow may also be reduced in rainbow trout during exercise.

Following feeding, gut blood flow of fish increases by 58-100% (Axelsson *et al.* 1989; Axelsson & Fritsche, 1991; Axelsson *et al.* in review). Therefore, intestinal blood flow in fed fish is equal to 38-40% of $\dot{Q}_{max}$ in the sea raven and the Atlantic cod (Axelsson *et al.* 1989; Axelsson & Fritsche, 1991). Thus, gut blood flow in fed fish is a relatively large fraction of $\dot{Q}_{max}$. If intestinal blood flow in fed fish is maintained during exercise, it should significantly limit the blood flow available to other tissues, such as the locomotory muscles, and as a consequence, maximum swimming velocity should be reduced in fed fish. The sea raven, and the Atlantic cod are poor swimmers compared with salmonids. Moreover, salmonids are known for their ability to maintain relatively high swimming velocities without compromising growth. Therefore, it is to be expected that chinook are better able to maintain intestinal blood flow while swimming compared with the two other species of fish.

No reliable measurements of intestinal blood flow in fed or exercised salmonids exist. The measurements of blood flow distribution in rainbow trout (Randall & Daxboeck, 1982) were performed with microspheres, which at best show relative changes in organ blood flow from rest to exercise if $\dot{Q}$ is measured simultaneously. However, $\dot{Q}$ was not measured simultaneously in their study and therefore it is neither possible to quantify absolute intestinal blood flow nor the relative changes from rest to exercise, based on their results. In the present study, intestinal blood flow in resting, fed, and exercising

chinook salmon will be measured with flow probes to provide reliable measurements of organ blood flow.

Many mammals reduce visceral blood flow during exercise (Fronek & Fronek, 1970; Vatner *et al.* 1974; Vatner, 1978; Laughlin & Armstrong, 1982; Armstrong *et al.* 1987; Eriksen *et al.* 1990; McKirnan *et al.* 1991). The blood flow requirements for the locomotory muscles during exercise will likely depend on the O_2 content of arterial blood (Ca_{O_2}). Thus, the higher the Ca_{O_2}, the less blood flow is required to support the O_2 consumption of the muscles. Results from studies on dogs indicate that more blood flow is redistributed from the intestines of animals with reduced blood O_2 capacity during exercise (Vatner *et al.* 1971, 1972, 1974). Thus, the ability to maintain intestinal blood flow during exercise is likely limited by $\dot{T}_{O_2\,max}$ in mammals.

It is not known whether the same applies to fish, but in the present study the effects of $\dot{T}_{O_2}$ and blood O_2 capacity on intestinal blood flow will be examined. To this end, the direct effects of blood O_2 capacity on intestinal blood flow were studied (Chapter 7). Furthermore, exercise training of fish appears to affect $\dot{T}_{O_2}$ by increasing both blood O_2 capacity (Hochachka, 1963; Farmer & Beamish, 1978; Zbanyszek & Smith, 1984) and cardiac output (Farrell *et al.* 1991). Therefore, $\dot{V}_{O_2max}$ (Chapter 3), $\dot{Q}$, $\dot{T}_{O_2\,max}$ (Chapter 5), and intestinal blood flow (Chapter 7) will be examined in fish trained at different swimming velocities .

This thesis examines cardiovascular function in swimming chinook salmon (Chapter 5 and 7) and specifically the role of intestinal blood flow in cardiovascular dynamics during exercise (Chapter 8). The regulation of intestinal blood flow in exercising fish relative to $\dot{V}_{O_2}$ and $\dot{T}_{O_2}$ was studied (Chapter 5). The significance of changes in blood flow to the gut for O_2 transport to the locomotory muscles were investigated (Chapter 8). Furthermore, I evaluated whether the maximum swimming velocity that chinook salmon

can sustain, without compromising growth or maintenance functions, is limited by the ability to maintain intestinal blood flow while swimming (Chapter 9).

Specific objectives.

The experimental objectives of this thesis were to characterize some of the major cardiovascular changes which occur in response to the increased O_2 demands of swimming and/or feeding in chinook salmon (Chapter 3, 5, and 7).

The specific questions asked were:

1. What fraction of the total cardiac output is diverted to the intestines and how does it change in swimming or fed fish? (Chapter 7)

2. What is the benefit of blood flow redistribution from the splanchnic circulation for O_2 transport to the locomotory muscles, during periods of maximum aerobic exercise? (Chapter 8)

3. Does the maximum internal O_2 convection affect how much blood flow is redistributed between the viscera and the locomotory muscles? (Chapter 9)

4. Does this redistribution of blood flow compromise locomotory, digestive, or assimilatory functions, and are these constraints likely to limit the swimming velocity maintained by the fish during their feeding excursions in the ocean? (Chapter 9)

The following are the experiments performed to answer these questions. The order in which they are listed does not represent the chronological order in which they were performed.

Exp. I. The effect of feeding status on oxygen consumption and U_{crit}. This experiment was performed to define the O_2 demands placed on the cardiovascular system by the HI, and to examine the ability of chinook to support HI while swimming maximally. A group of fish was starved for one week and then swum to U_{crit} while $\dot{V}_{O_2}$

was measured. Two days later the same fish were fed and swum again to U_{crit}. To account for any confounding "training effects" of the repeated swim trials, these fish were swum for the third time 48 hours later. By comparing the $\dot{V}_{O_2}$ of either fed or starved fish, swimming at different velocities, it was possible to examine to what swimming speed the fish could maintain HI. Furthermore, the $\dot{V}_{O_{2max}}$ and U_{crit} of fed and unfed fish were compared, to examine whether a fixed $\dot{V}_{O_{2max}}$ ultimately limits to what degree digestion and locomotion can be maintained simultaneously. The results of this experiment are reported in Chapter 3.

Exp. II. The anatomy of the intestinal vasculature of salmonids. Before any studies could be performed on intestinal blood flow in salmonids, an examination of the vascular anatomy of the viscera was needed. This was done with corrosion casts of the arterial and venous circulations. The results were presented in Thorarensen *et al.* (1990), which is included as Appendix. III. A summary of these findings and an account of the general organization of the cardiovascular system in chinook salmon is given in Chapter 4.

Exp. III. Intestinal blood flow in swimming chinook salmon. This experiment was performed to measure intestinal blood flow and changes in intestinal blood flow in swimming fish. Exercise training may affect the ability of fish to maintain visceral blood flow while swimming. Therefore intestinal blood flow was measured in fish following eight months of continuous swimming at either high velocity (HS: 1.5 $bl \cdot s^{-1}$) or controls, at low velocity (LS1: 0.5 $bl \cdot s^{-1}$). The results from this experiment have been previously reported in (Thorarensen *et al.* 1993) and are also reported in Chapter 7.

Exp. IV. Oxygen delivery and $\dot{V}_{O_2}$ in swimming chinook salmon. This experiment was performed to measure $\dot{V}_{O_{2max}}$ in chinook salmon. The training regimes used in Exp. III had no significant effect on $\dot{V}_{O_{2max}}$ or U_{crit}. Therefore, one group of fish (TR) in this experiment was exposed to a more stringent training regime than was used in Exp. III. The fish were swum to U_{crit} on alternate days while otherwise maintaining 0.5 $bl \cdot s^{-1}$. A

second group of fish (LS2) swam continuously at a control velocity of 0.5 $bl \cdot s^{-1}$, as did the LS1 fish in Exp. III. The results are reported in Chapter 5.

Exp. V. Blood flow distribution in resting fish. Radiolabeled microspheres were used in an initial attempt to measure blood flow distribution in chinook salmon. It was hypothesized that when microspheres were injected into the dorsal aorta, they were not uniformly mixed in the blood stream as it passed the coeliacomesenteric artery. Therefore, the fish were cannulated upstream from the dorsal aorta, in the efferent branchial artery of the second gill arch, in an attempt to get better mixing of the microspheres with the blood, and to improve flow estimates. The results are presented in Chapter 6.

Exp. VI. Postprandial changes in intestinal blood flow. This experiment was performed to measure changes in intestinal blood flow following feeding. The results are presented in Chapter 7.

In addition to the experiments reported in this thesis, cardiac output and O_2 transport were also measured in rainbow trout, and since these data are referenced in the thesis, but have not been published, some of those results are presented in Appendices I and II.

Chapter 2

General methods.

Experimental animals.

Chinook salmon of Big Qualicum stock, were obtained from the West Vancouver laboratory of the Department of Fisheries and Oceans, and kept in outdoor tanks under natural photoperiod. The fish in Exp. III and IV were from an all-female (feminized) stock, while fish in other experiments were of both sexes. Ample supplies of sea water at ambient temperature (7-13 °C) and salinity (29-30‰) were provided (Table 1). Feeding rations and feeding frequency varied between experiments (Table 1), but food was presented at satiation levels whenever the fish were fed.

Training regimes.

The fish in Exp. III and IV were kept in annular swimming channels (outer diameter 3.35 m; inner diameter 2.90 m) which are described in detail in (Kiessling & Higgs, in review). Water velocity was measured in the centre of the flume and regular checks indicated that variability in cross-sectional flow was less than 10%.

In Exp. III the fish were exercise-trained for 8 months (December to July), swimming continuously at either 1.5 body-lengths·s^{-1} (bl·s^{-1}) (high speed: HS) or 0.5 bl·s^{-1} (low speed: LS1). The LS1 fish were regarded as controls to examine the effect of HS swimming. Experiments began after the training period and continued through August to November, during which time the training regimes continued. The water velocity in the channels was adjusted every month to maintain the appropriate training velocity (0.5 or 1.5 bl·s^{-1}) as the fish increased in size.

Table 1. Weight, length, feeding frequency, time of year when experiments were performed, and the temperature during the experiments. In all experiments the fish were fed to satiation each time food was offered†.

Experiment	Weight (g)	Length (cm)	Feeding freq.	Time of year	Temperature °C
Exp. I	520±30	33.7±0.5	thrice each week[a]	Nov.-Dec.	9-10°
Exp. II	300-1200	*	once daily[b]	Apr.	7-10°
Exp. III			twice daily[b]	Aug.-Nov.	8-11°
HS	362±15	30.3±0.3			
LS1	368±30	30.1±0.4			
Exp. IV			twice daily on alternate days[c]	Jan.-Mar.	8-10°
TR	380±20	31.4±0.3			
LS2	395±16	31.3±0.3			
Exp. V	313±23	*	once daily[a]	Dec.	7-8°
Exp. VI	200-350	*	once daily[a]	Nov.-Dec.	12-13°

†Weigth and length values are persented as MEAN±SEM

[a] Fish were fed dry pellets (Pacific Aquafoods, British Columbia).

[b] Fish were fed dry pellets (Biodiet dry, Oregon Bioproducts).

* Not measured

In Exp. IV, control fish (LS2) swam continuously at 0.5 bl·s^{-1}, i.e., the same regime as the LS1 fish in Exp. III. The trained fish (TR) also swam continuously at 0.5 bl·s^{-1}, but in addition they were swum to U_{crit} on alternate days by increasing the water velocity in the swim channel in steps of 10 cm·s^{-1} every 20 min until all fish had given up swimming against the current. The duration of each training session was about two hours. The mean daily swimming distance was 31% longer in the TR group than in the LS2 group, and the intensity of the TR training regime was higher than in either the HS group and the LS groups. Both the LS2 and TR fish were fed only on the days when the TR fish did not swim to U_{crit}. The fish were trained for two months (November to December). The measurements of $\dot{Q}$ and U_{crit} were performed from January to March, during which time the training regimes continued.

Critical swimming speed and respirometry.

U_{crit} and $\dot{V}_{O_2}$ were measured in a Brett-type swim-tunnel respirometer (Kiceniuk & Jones, 1977). The fish were allowed to recover for more than 5 hours in the respirometer before U_{crit} was determined. The water velocity in the tunnel was increased, first by about 0.5 bl·s^{-1} and then in steps of about 0.3 bl·s^{-1} until U_{crit} was approached, as indicated by frequent burst-activity. The final velocity increments prior to U_{crit} were about 0.2 bl·s^{-1}. Each velocity step was maintained for either 30 min or until the fish fatigued, i.e., when the fish could not swim off the rear grid. The fish were not induced to swim by applying electric shock to the rear grid. The velocity settings on the swim tunnel were calibrated regularly with a flow meter (Braystoke BFM002, Valeport Marine Scientific, Devon U.K.). U_{crit} was calculated as described by Brett (1964):

$$U_{crit} = U_L + \Delta U \cdot t / 30$$

where U_L is the highest velocity that the fish could maintain for 30 minutes, ΔU is the velocity increment, and t is the time that the fish could swim at the highest swimming

velocity. The swimming speed of the fish was corrected for the solid blocking effect (Bell & Terhune, 1970), since the cross sectional area of all the fish was more than 10% of that of the swimming chamber. The formula used was:

$$U_F = U_T \cdot (1 + \varepsilon_S)$$

where U_F is corrected velocity, U_T is measured velocity and ε_S is the proportional error due to solid blocking. ε_S is obtained as

$$\varepsilon_S = \tau \cdot \lambda \cdot (A_O \cdot A_t^{-1})^{1.5}$$

where $\tau = 0.8$, $\lambda = 0.5 \cdot$(fish length) $\cdot$ (fish thickness)$^{-1}$, A_O is maximum cross-sectional area of the fish, and A_t is the cross-sectional area of the test section. It is assumed that A_O is elliptical and calculated as:

$$A_O = 0.25 \cdot \pi \cdot d \cdot w$$

where d and w are maximum body depth and width respectively.

Oxygen tension of the water (Pw_{O2}) in the swim-tunnel was monitored continuously by an oxygen electrode (Radiometer E5046), which was thermostatically maintained at the experimental temperature. The O_2 electrode was connected to a PM71 unit (Radiometer, Copenhagen). Water from the swim-tunnel was drawn past the electrode by a roller pump and Pw_{O2} was recorded every second by a computer. $\dot{V}_{O_2}$ was measured by closing off the tunnel for 6 minutes while Pw_{O2} was recorded. A least squares procedure was used to calculate the slope or $\Delta Pw_{O2} / \Delta t$. Oxygen uptake was calculated as:

$$\dot{V}_{O_2} = V \cdot \Delta Pw_{O2} \cdot \alpha \cdot \Delta t^{-1} \cdot m^{-1}$$

where V is the volume of the tunnel (39.1 L), α is the solubility constant for O_2 at the experimental temperature and salinity (μmol O_2 L^{-1} water kPa^{-1}), and m is body mass. Between recordings, the tunnel was flushed with aerated seawater and O_2 saturation of the water in the tunnel was never allowed to decrease by more than 15% (Pw_{O2} of 18

kPa) from full saturation. The O_2 electrode was calibrated daily with air saturated water and by disconnecting the electrode from the PM71 module to obtain a zero reading.

Arterial cannulation for blood pressure measurements and blood sampling.

Fish were anaesthetized in a 1:2,000 solution of 2-phenoxyethanol (Sigma Chemical Co., St Louis MO) in sea water and anaethesia was maintained by irrigating the gills continuously with chilled 1:4,000 2-phenoxyethanol in sea water. To measure blood pressure and to obtain samples of arterial blood, a cannula (PE50) was inserted in the dorsal aorta (DA) with a method modified from Smith & Bell (1964). The cannula, was inserted with an indwelling steel trocar between the second and third gill arches and sutured to the roof of the mouth. The cannula was externalized in front of the nasal opening (Exp. III) or through the skin under the maxillary (Exp. IV) and filled with saline (0.9% NaCl containing heparin, 150 iu mL^{-1}). Finally, the cannula was anchored with silk suture (1-0) at a position anterior to the dorsal fin. The DA blood pressure (P_{DA}) was measured with a LDI5 transducer (Narco, Houston TX) connected to Grass preamplifier (Model 7P1J, Grass Instruments, Quincy MA). The pressure transducer was calibrated daily with a static water column and referenced to the surface of the water in the swim tunnel.

Flow probes.

Blood flow in the intestinal artery was measured with pulsed Doppler flow probes (DFP; TMI, Iowa City, IA) and Transonic flow probes (TFP) (Transonic Inc., Ithaca, NY). The DFP measures the velocity of the blood in the vessel, whereas the TFP measures absolute blood flow. However, the TFP are larger and have bulkier probe leads than the DFP, and therefore, they are not as well suited for measuring blood flow in swimming fish. Consequently, cardiac output was measured in Exp. IV by placing a DFP on the ventral

aorta. These flow probes were later calibrated *in situ* by placing a TFP flow probe on the bulbus arteriosus and simultaneously recording $\dot{Q}$ with both flow probes in the anaesthetized fish.

Placement of flow probes on the ventral aorta to measure $\dot{Q}$.

A DFP flow probe was placed around the ventral aorta (VA) just distal to the bulbus arteriosus (see Fig. 2, page 66b). The flow probes were made with rigid plastic collars selected to fit the VA snugly. The VA was accessed from the opercular cavity as it runs dorsad towards the gills and where it is readily visible when the operculum and gills are folded forward. The VA was teased free from the surrounding tissues without rupturing the pericardium or obstructing the coronary artery. The leads from the probe were sutured with silk thread (3-0) to the isthmus and to the side of the fish behind the cleithrum, just under the lateral line. The lead was anchored with the dorsal aorta cannula in front of the dorsal fin. The operation could be finished in less than 20 minutes.

This method of placing flow probes on the ventral aorta is an improvement over earlier techniques, where the pericardium had to be opened and the pectoral girdle severed in order to place the flow probes on the bulbus arteriosus (Wood & Shelton, 1980; Xu & Olson, 1993; Gamperl *et al.* 1994).

Placement of flow probes on the intestinal artery.

In separate experiments, either a DFP or a TFP were placed on the intestinal artery (IA) (Fig. 2) by modifying a technique developed for sea raven and Atlantic cod (Axelsson *et al.* 1989; Axelsson & Fritsche, 1991). A lateral incision was made in the body wall, beginning 2 cm ventral from the lateral line, 1-2 cm behind the cleithrum, and extending 4 cm ventrad, parallel to the myotomes. The skin was cut with a scalpel and the myotomes were carefully separated with blunt dissection. A short section of the intestinal artery was freed from the surrounding fascia and the probe cuff was placed on the vessel.

The incision was closed with 3-0 silk thread, using interrupted stitches through both muscles and skin. The probe leads were secured to the body surface and anchored dorsally with the DA cannula. The surgery was completed in less than 30 minutes. The fish were allowed to recover for at least 24 hours before experiments were performed.

Data acquisition and calculations.

Signals from flow meters, pressure transducers, and oxygen meters were amplified by a Grass chart recorder (Model 7PCP B, Grass Instruments, Quincy MA) and stored in a PC computer. The computer sampled signals for blood flow and blood pressure at a rate of 5 Hz. Variables were measured for 6 minutes and mean values were derived by averaging the values obtained during this period. Labtech Notebook software (Laboratory Technology Corp., Wilmington MA) was used to convert the signals to digital form, to process the signals, and to calculate f_H. When $\dot{q}_{IA}$ was measured in resting fish over extended periods, readings were taken automatically every hour, by recording the mean $\dot{q}_{IA}$ during a 10 minute period (Exp. VI). Extended measurements of mean $\dot{q}_{IA}$ in Exp. III were performed by recording the flow, averaged by a Butterworth low pass (0.1 Hz) filter, on the flow meter module once every minute.

Vascular resistances were calculated from:

$$R_{SYS} = P_{DA} / \dot{Q}$$

$$R_{SPLANC} = P_{DA} / \dot{q}_{IA}$$

where R_{SYS} is systemic vascular resistance, the combined resistance of all postlamellar vascular beds, and R_{SPLANC} is the combined vascular resistance of the splanchnic vascular beds and liver. The central venous pressure was not subtracted from the P_{DA} to obtain correct values for either resistance. In this study it was assumed, as is commonly done (e.g. Axelsson & Fritsche, 1991; Bushnell *et al.* 1992), that central venous pressure was small enough to be of little significance for calculations of resistance. Reported values

for central venous pressure in rainbow trout range between 0.2 to 0.3 kPa (Kiceniuk & Jones, 1977; Xu & Olson, 1993), approximately 9% of resting P_{DA}. Consequently resistance could be overestimated by as much as 9%.

Haematological measurements.

A 1 mL blood sample was removed from the fish to measure arterial P_{O2} (Pa_{O_2}), pH, oxygen content (Ca_{O_2}), haematocrit (Hct), and haemoglobin concentration ([Hb]). Samples to measure plasma lactate were taken at rest after the fish fatigued and following 1 hour of recovery. Pa_{O_2} was measured with a thermostatted electrode (E5046 Radiometer Copenhagen) connected to a PM71 unit (Radiometer Copenhagen). The pH of whole blood (in 100 μL samples) was measured in Exp. IV using a pH micro-electrode (G297/G2 Radiometer Copenhagen) connected to a PM71 unit. Blood Ca_{O_2} was measured in 30 μL blood samples by the method of Tucker (1967). Hct was measured in triplicate using 20 μL micropipettes spun for 3 minutes at 14,890 x g. Sigma diagnostic kits #525A and #826-UV (Sigma Chemical Co, St. Louis MI) were used to measure blood haemoglobin (using duplicate 20μL samples) and plasma lactate (in 100 μL samples), respectively. The blood that was used to measure Pa_{O_2} and any leftover blood from the initial 1 mL sample (total amount of blood 0.7-0.8 mL) was reinjected into the fish and enough saline (Exp. III) or blood from a donor fish (Exp. IV) was added to make a total volume of 1 mL. Before the fish fatigued, 3-4 blood samples were taken, resulting in approximately 1 mL of blood being replaced by saline (Exp. III) or blood from a donor fish (Exp. IV).

Mean corpuscular haemoglobin concentration (MCHC) was calculated as:

$$\text{MCHC} = \text{Hct} / [\text{Hb}]$$

The index of O_2 saturation (SAT) of Hb was calculated as:

$$\text{SAT} = (Ca_{O_2} - ((1\text{-Hct}/100) \cdot (\alpha \cdot Pa_{O_2})) / [\text{Hb}]$$

Where α is the solubility coefficient of O_2 in plasma in mL $O_2 \cdot kPa^{-1} \cdot 100$ mL^{-1} plasma (Boutilier *et al.* 1984). The units of SAT are mL $O_2 \cdot$ g Hb^{-1}.

Statistics.

Values are presented as mean ± S.E.M. throughout text and figures and the fiducial limit for accepting significance was $P<0.05$. Statistical analyses were performed using the General Linear Models and Correlation procedures in SAS® (Version 6, SAS Institute Inc., Cary NC). The $\dot{V}_{O_2}$ as a function of swimming velocity was modeled in Exp. III as a second order polynomial with different intercepts for individual fish. In Exp. I and IV, the $\dot{V}_{O_2}$ of fish swimming at similar velocities were combined and different groups were compared with a three-way analysis of variance (ANOVA) with swimming speed, treatment groups, and individuals at different swimming speeds as factors. Values for blood flow, blood pressure, vascular resistance, f_H, and haematological variables were also compared using three-way ANOVA. Values for U_{crit} and $\dot{V}_{O_{2max}}$ were compared with a one-way ANOVA, except in Exp. I, where repeated measures ANOVA was used to compare U_{crit} and $\dot{V}_{O_{2max}}$ values for unfed and fed fish. Other models are described as they are presented in the result sections.

Chapter 3

Oxygen uptake and swimming performance.

Introduction

In this chapter, the O_2 consumption of swimming and fed chinook salmon is reported to quantify the O_2 demands that are placed on the cardiovascular system during locomotion and feeding. Furthermore, the effects of exercise-training on maximum O_2 consumption and U_{crit} are reported. The U_{crit} and $\dot{V}_{O2max}$ of exercise-trained fish in Exp. III and IV was determined before they were operated on and these results are presented here; other results from these experiments on intestinal blood flow, $\dot{T}_{O2max}$, and $\dot{V}_{O2max}$ are presented in Chapters 5 and 7.

Locomotion.

The locomotion of fish has been broadly classified into three categories (Beamish, 1978). During *burst exercise*, fish swim at high velocities which can only be maintained for short periods (less than 20 s) and most of the energy used for locomotion is generated by anaerobic metabolism. A *sustained swimming* velocity can be maintained for long periods (greater than 200 min) without resulting in muscular fatigue and the energy is derived from aerobic metabolism. Between these two extremes is *prolonged exercise*, which is the swimming velocity that a fish can maintain for 20 seconds to 200 minutes. The prolonged swimming speed of fish is estimated in swim-tunnels, as the *critical swimming velocity* (U_{crit}), which is the maximum velocity that a fish can maintain over a given period of time (usually ten minutes to one hour).

There are two basic types of muscle fibers in the skeletal muscle of fish. About 66% of the body mass of salmonids is so-called white muscle (Stevens, 1968), which is

composed of glycolytic fibres with little myoglobin and few capillaries (Johnston, 1981; Egginton, 1992). These fibres have a low oxidative capacity and depend primarily on anaerobic metabolism (Webb, 1993). Red muscle is about 1-4% of the body mass of salmonids (Stevens, 1968; Webb, 1975) and it is visible as a thin dark layer just under the skin on each side of the fish. The red muscle consists of fibers with high mitochondrial density, abundant myoglobin and a rich capillary supply (Johnston, 1981; Eggington, 1992). At sustained swimming velocities, fish primarily use the red muscle fibres for swimming, although white fibres may be recruited at the beginning of exercise (Wokoma & Johnston, 1981). As swimming speed increases, increasing numbers of white fibres are recruited (Bone, 1978; Johnston & Moon, 1980b; Webb, 1993). It has been suggested that anaerobic metabolism does not contribute significantly to salmonid locomotion at swimming speeds less than 80% of U_{crit}. However, energy derived from anaerobic metabolism increases progressively as swimming speed approaches U_{crit} (Webb, 1971b).

The oxygen consumption of the locomotory muscles is not easily measured. However, the total O_2 uptake of swimming fish has been measured in a number of studies, and salmonids have received a particular attention (e.g. Brett, 1964, 1965; Brett & Glass, 1973; Kutty, 1968; Rao, 1968; Webb, 1971b). The oxygen uptake of swimming fish has generally been described by one of the following models:

$$\dot{V}_{O_2} = \mathrm{SMR} \cdot e^{\phi \cdot U} \text{ (Brett, 1964; Webb, 1971b)}$$

or

$$\dot{V}_{O_2} = \mathrm{SMR} + \phi \cdot U^{\gamma} \text{ (Kaufmann, 1990)}$$

where ϕ and γ are constants. The inherent assumption in both of these models is that oxygen uptake of swimming fish can be described as the cost of locomotion plus the standard metabolic rate (SMR), which is "..the minimum rate of oxygen consumption of an organism at rest in the postabsorptive stage and thermally acclimated" (Brett & Grove,

1979). By using these models it has been estimated that, for salmonids, the metabolic cost of swimming increases as a function of $U^{1.35\text{-}1.8}$ (Brett, 1964, 1965; Rao, 1968; Kutty, 1968; Webb, 1971b; Jones & Randall, 1978).

The increase in $\dot{V}_{O_2}$ from rest to maximum varies among fish species, fish size, and ambient temperature (Jones & Randall, 1978). In salmonids, the relative increase in $\dot{V}_{O_2}$ from SMR to maximum O_2 uptake may be more than 16-fold (Brett, 1965). However, the SMR is not measured, but estimated by extrapolating $\dot{V}_{O_2}$ curves of swimming fish down to a zero velocity. The SMR of fish is considered the equivalent of basal metabolic rate of mammals (Brett & Grove, 1979). The actual O_2 uptake measured in resting fish is called resting metabolic rate (Brett & Grove, 1979). The maximum O_2 uptake ($\dot{V}_{O_{2max}}$) is referred to as active metabolic rate and the difference between standard and active metabolic rate has been referred to as scope for activity (Fry, 1957). The scope for activity increases with size because the standard metabolic rate increases as a function of (body mass)$^{0.78}$ (bm$^{0.78}$), while the active metabolic rate increases as bm$^{0.97}$ (Brett, 1965). Therefore, $\dot{V}_{O_{2max}}$ in small fish may be only double the SMR, while the increase from SMR to $\dot{V}_{O_{2max}}$ may be more than 16-fold in large fish (Brett, 1965; Kaufmann, 1990). Because resting metabolic rate is usually higher than the calculated SMR, the scope for activity is somewhat less when comparing resting $\dot{V}_{O_2}$ and $\dot{V}_{O_{2max}}$. In large (0.9-1.5 kg) rainbow trout, the increase in $\dot{V}_{O_2}$ from rest to maximum was 8-fold (Kiceniuk and Jones, 1977) while in 300 g rainbow trout the increase was only 4.4 fold (Webb, 1971b).

Not all of the increase in $\dot{V}_{O_2}$ with swimming speed reflects the direct cost of locomotion, since the cost of cardiac and branchial (ventilatory) pumps changes with swimming velocity (Farrell & Steffensen, 1987b). However, as swimming speed increases the increased cost of the cardiac pump does not increase in step with $\dot{V}_{O_2}$ and the fish switch to the more efficient ram mode of ventilation. Therefore, the relative contribution of the

two pumps to the total $\dot{V}_{O_2}$ decreases to less than 7% as swimming speed approaches U_{crit} (Farrell & Steffensen, 1987b).

The cost of osmoregulation may contribute significantly to $\dot{V}_{O_2}$ and it has been suggested that the cost of osmoregulation may increase with swimming velocity (Rao, 1968; Jones & Randall, 1978; Febry & Lutz, 1987). This is consistent with the findings that ionic fluxes in rainbow trout in fresh water increase in proportion to $\dot{V}_{O_2}$ (Gonzalez & McDonald, 1992). Reported estimates of the metabolic cost of osmoregulation (summarized in Morgan & Iwama, 1991) are quite variable and do not necessarily show a significant increase in $\dot{V}_{O_2}$ with increased osmotic load (e.g., Muir & Niimi, 1972). This led Morgan & Iwama (1991) to conclude that the $\dot{V}_{O_2}$ of fish at different salinities did not necessarily reflect the variable cost of osmoregulation, which may be to small to detect with respirometry. This is in keeping with theoretical estimates, which suggest that of the metabolic cost of osmoregulation should be around 2% of resting metabolic rate (Eddy, 1982).

Protein synthesis may double in exercising rainbow trout (Houlihan & Laurent, 1987). The total cost of protein synthesis in Atlantic cod has been estimated as 24% of resting metabolic rate (Houlihan *et al.* 1988) and, if the same applies to the rainbow trout, protein synthesis in exercising fish could account for as much as 10% of $\dot{V}_{O_{2max}}$ in rainbow trout (Based on $\dot{V}_{O_{2max}}$ from Webb, 1971b). Therefore, the contribution of factors such as increased protein synthesis, osmoregulation, and the cost of the branchial and cardiac pumps could account for as much as 10 to 20% of $\dot{V}_{O_{2max}}$.

Most analyses of the $\dot{V}_{O_2}$ of swimming fish assume that SMR is constant at all swimming speeds and that the increase in $\dot{V}_{O_2}$ reflects the cost of locomotion (Brett, 1964, 1965; Webb, 1971b). However, Wieser (1989) suggested that the basal metabolism may reduced during periods of activity. This is consistent with the findings of Kaufmann & Wieser (1992), that the $\dot{V}_{O_2}$ of juvenile fish swimming in hypoxic water was lower at any

given swimming velocity than in normoxia. Therefore, the increase in $\dot{V}_{O_2}$ in swimming fish reflects a combination of factors, in addition to the net cost of locomotion, which may increase (e.g cost of ventilation and osmoregulation), or reduce (e.g. reduced basal metabolic rate) the apparent cost of locomotion.

Cost of digestion and food assimilation.

The maximum postprandial increase in $\dot{V}_{O_2}$ (HI) is typically double the $\dot{V}_{O_{2rest}}$ in fish that have low resting metabolic rate (Jobling, 1981). It has been suggested that this HI may decrease the scope for activity by 30-50% (Muir & Niimi, 1972; Beamish, 1974; Vahl & Davenport, 1979; Furnell, 1987), although the effect of feeding status on U_{crit} or $\dot{V}_{O_{2max}}$ has never been studied.

HI may be attributed to a number of factors, such as the mechanical cost of moving the digesta through the intestines, the cost of digestion, absorption, and the assimilation of macromolecules, mainly protein synthesis. Tandler and Beamish (1979) estimated that the 'mechanical component' of the HI was 10-30% depending on ration. However, Jobling and Davies (1980) were unable to detect any metabolic cost of intestinal peristalsis, suggesting that normally this component of digestion is small. Most of the increase in $\dot{V}_{O_2}$ appears to be due to processes that occur after nutrients have been taken up into the blood stream.

An increase in $\dot{V}_{O_2}$, similar to the HI, can be initiated by intra-arterial injections of amino acids (Coulson *et al.* 1978; Brown & Cameron, 1991b). The metabolic cost of excreting nitrogenous products is small in salmonids (Randall & Wright, 1987), since most of the nitrogen is excreted in the form of ammonia rather than urea (Brett & Zala, 1975). Therefore, most of the increase in HI following feeding of fish is likely due to a more rapid turnover of tissue proteins (Jobling, 1981; Brown & Cameron, 1991; Lyndon *et al.* 1992). Increased protein synthesis is observed in most tissues following feeding

(McMillan & Houlihan, 1988). Brown & Cameron (1991) estimated that the increase in O_2 consumption in the liver and white muscle of catfish were approximately equal. Similarly, Lyndon and coworkers (1992) found that the protein synthesis in the liver and the intestines was up to 40% of the total protein synthesis following feeding.

O_2 consumption in swimming and feeding fish.

If unfed fish reach $\dot{V}_{O2max}$ as they swim near U_{crit}, then fish should not be able to swim at maximum prolonged swimming speeds and simultaneously maintain intestinal function or the HI. The O_2 consumption of fish swimming during the postprandial stage has only been measured in two studies. Beamish (1974) swam fed largemouth bass (*Micropterus salmonides*) and observed no reduction of HI with increased swimming velocity. However, the bass were not swum maximally and therefore it is not known whether U_{crit} or $\dot{V}_{O2max}$ were affected. Furnell (1987) swam fed and unfed sablefish (*Anoplopoma fimbria*), up to 60% of U_{crit}. Contrary to the largemouth bass, the $\dot{V}_{O_2}$ of fed and unfed sablefish appeared to converge with increased swimming velocity, suggesting that less O_2 was allocated to the HI as swimming speed increased. Since the swimming speed that the fish were challenged with in both studies was relatively low it is not known what happens when fish swim maximally during the postprandial stage.

Exp. I was performed to examine what happens when chinook salmon are simultaneously faced with the demands of the HI and locomotion. A preliminary experiment had indicated that the U_{crit} was reduced by 9% in fed chinook salmon compared with unfed controls. Since this difference was relatively small, Exp. I was performed to confirm these results with a more rigid experimental design and to simultaneously measure $\dot{V}_{O_2}$. Therefore, a group of fish (n=11) was starved for one week and then swum to U_{crit} while measuring $\dot{V}_{O_2}$. Two days later the same fish were again swum to U_{crit}, 15 hours postprandially. This time was chosen because other studies indicated that HI has reached

a maximum at 10-15 hours postfeeding (Saunders, 1963; Muir & Niimi, 1972; Legrow & Beamish, 1986; Lyndon *et al.* 1992; see also Chapter 7). To account for any confounding effects due to repeated swim trials the fish were swum for a third time at 63 hours postprandial.

Effect of exercise-training on U_{crit} and $\dot{V}_{O_2max}$.

The maximum swimming speed and endurance of fish has been shown to increase by 9-20% in response to exercise-training (Brett *et al.* 1958; Farlinger & Beamish, 1978; Broughton *et al.* 1980; Besner & Smith, 1983; Leon, 1986; Houlihan *et al.* 1988b; Farrell *et al.* 1990). However, no change in maximum swimming speed was observed in other studies (Greer-Walker & Pull, 1973; Farrell *et al.* 1991). Although $\dot{T}_{O_2}$ has not been measured in trained fish it is known that blood O_2 capacity (Hochachka, 1961; Farlinger & Beamish, 1978; Zbanyszek & Smith, 1984) and $\dot{Q}_{max}$ (Farrell *et al.* 1991) increase in trained fish. Cardiovascular O_2 transport depends on $\dot{Q}$ and blood Ca_{O_2} and therefore, $\dot{V}_{O_2max}$ should increase in trained fish The effect of exercise-training on $\dot{V}_{O_2max}$ has not been measured before. However, the $\dot{V}_{O_2}$ of trained fish has been measured at intermediate swimming speeds and appears to be lower than in untrained controls (Nahhas *et al.* 1982; Woodward & Smith, 1985). The studies of trained fish were performed in order to examine the effect of exercise-training on $\dot{V}_{O_2max}$ and U_{crit}. It was predicted that $\dot{T}_{O_2max}$ would increase (Chapter 5 and 8) with training and therefore, $\dot{V}_{O_2max}$, U_{crit} and the ability to maintain intestinal blood flow should also increase (Chapter 7).

The fish were trained at the West Vancouver laboratory of the Department of Fisheries and Oceans by A.K. Kiessling and D. Higgs, who were performing studies on the effect of exercise-training on fish growth and feed utilization. Therefore, I was able to obtain fish for my study which had been expertly reared. Exp. III was the first of the two studies

and the HS training regime of Exp. III (continuous swimming at 1.5 bl·s^{-1}) proved to have little effect on $\dot{V}_{O2max}$ and U_{crit}. Therefore, the TR fish in Exp. IV were exposed to what was considered a more stringent regime in an attempt to obtain a group of fish that had significantly higher $\dot{V}_{O2max}$ and U_{crit}.

Methods

In Exp. I fish were starved for one week and then transferred to a swim-tunnel, where they were allowed 15 hours to habituate before being swum to U_{crit}. After this initial test the fish were returned to the holding tank. Two days later they were force-fed 2% of body-mass and placed in the swim-tunnel. Fifteen hours postprandial, U_{crit} and $\dot{V}_{O2max}$ were determined again. A final measurement of U_{crit} and $\dot{V}_{O2max}$ was performed two days after the fish had been swum the second time (i.e., 63 hours postprandial) to confirm that repeated swimming had no effect on the variables measured. The equivalent of 2% body-mass of dry pellets (Moor Clarke, Vancouver B.C.) was mixed with equal volume of water and pounded into a paste. The fish were lightly anaesthetized with 2-phenoxyethanol and the food paste was administered into the stomach through a tube adapted to a syringe. The fish were never seen regurgitating the food and therefore it was assumed that it was passed through the intestines. Before the first and the last swimming trials, the fish were 'sham-fed' by lightly anaesthetizing them and inserting a tube into the stomach before they were placed in the swim-tunnel.

All fish in Exp. III and IV had not been fed for at least 24 hours before U_{crit} and $\dot{V}_{O2max}$ were determined. The training protocol for Exp. III and Exp. IV and the procedures for measuring $\dot{V}_{O2}$ and U_{crit} are described in the General Methods section (Chapter 2).

Results

Effect of feeding status on U_{crit} and $\dot{V}_{O2max}$

HI increased resting metabolic rate by 33 μmol·min^{-1}·kg^{-1} (128%) at 15 h postprandial (Table 2). Moreover, the $\dot{V}_{O_2}$ of the fed fish was on the average 39 μmol·min^{-1}·kg^{-1} higher than that of fasted fish at all swimming velocities up to U_{crit}. Therefore, HI did not appear to change as swimming speed increased (Fig. 1a).

Originally, I attempted to analyze these data by fitting polynomial models to the $\dot{V}_{O_2}$ data at different swimming velocities. However, when the polynomial lines were compared to the original data it was evident that they did not describe the original data faithfully, especially for fed fish swimming at high velocities, where observed values tended to be higher than predicted values (Fig. 1a). Therefore, the data were analyzed by comparing fed and starved fish swimming at identical velocities. This was done in two sections. First, the $\dot{V}_{O_2}$ values of fed and starved fish swimming at the 5 lowest velocities were compared. Then the $\dot{V}_{O_2}$ of fed fish swimming at the four highest swimming velocities, before they fatigued, was compared to unfed fish swimming at the same speed. The different sections are connected by lines in Fig. 1a. Finally, the mean $\dot{V}_{O_2}$ of the starved fish swimming at the four highest swimming velocies was calculated and is shown in Fig. 1a as open boxes. The $\dot{V}_{O_2}$ of fed fish was significantly ($p<0.001$) higher than that of starved fish at all swimming velocities.

The HI had different effects on $\dot{V}_{O2max}$ and U_{crit}. The $\dot{V}_{O2max}$ was not significantly different in fed and unfed fish. However, the U_{crit} of fed fish was 10% lower than that of starved fish, confirming the results from the preliminary experiment where feeding caused a 9% decrease in U_{crit} (Table 2).

Table 2. The effect of feeding status on U_{crit}, $\dot{V}_{O2rest}$ and $\dot{V}_{O2max}$. Fish are from Exp. I* and a preliminary experiment.†

Experiment	Condition	n	$\dot{V}_{O_2}$ $\mu mol \cdot min^{-1} \cdot kg^{-1}$		U_{crit}	
			Rest	Max.	$bl \cdot s^{-1}$	$cm \cdot s^{-1}$
Preliminary	Starved	8			2.7(0.1 [a]	73.2(0.8 [a]
Preliminary	Fed	8			2.5(0.1) [b]	68.1(0.7) [b]
Exp. I	Starved	11	25(3) [a]	255(11)	2.2(0.1) [a]	74.6(2.6) [a]
Exp. I	15 h postprandial	11	57(3) [b]	249(11)	2.0(0.1) [b]	67.6(3.5) [b]
Exp. I	63 h postprandial	11	29(3) [a]	266(11)	2.2(0.1) [ab]	73.4(3.7) [ab]

†Numbers in parentheses are SEM.

*All fish in Exp. I swam three times: First starved (for one week), then 15 hours after they were fed 2% of body-mass, and finally 2 days post feeding.

[a,b] Variables from the same experiment denoted with different letters are significantly different ($p<0.05$; Tukey test).

The U_{crit} and the $\dot{V}_{O2max}$ of fish two days postprandial were not significantly different from values for fasted fish. This indicates that repeated swimming trials did not affect performance (Table 2) and that most of the food had been cleared from the gut 63 h (48 + 15 h) postprandial.

Effect of exercise-training on U_{crit} and $\dot{V}_{O2max}$.

As expected, the $\dot{V}_{O2max}$ and the U_{crit} of the LS1 and LS2 were similar, because both groups were exposed to the same training regime.

Exercise-training had little effect on U_{crit}. The mean U_{crit} values of the groups that were exposed to the more vigorous training regimes (HS and TR) were not significantly different from those of their respective controls (Table 3). The $\dot{V}_{O2max}$ of both HS and TR were higher than in the LS1 groups, but only the TR group was significantly higher than the LS2 group (Table 3). Further analysis of the data showed that the higher $\dot{V}_{O2max}$ in the TR group could partly be explained by the observation that the best swimmers in the TR group swam significantly faster than the best swimmers in the LS2 group (Fig. 1c). However, when fish swimming at the same speed are compared, the $\dot{V}_{O_2}$ of the TR was also significantly higher than in the LS2 fish (Fig. 1c).

Effect of swimming speed on O_2 uptake.

Most of the increase in $\dot{V}_{O_2}$ occurred as the fish were swimming at velocities faster than 50-60% of U_{crit} (Figs. 1a,b,c). At lower velocities, $\dot{V}_{O_2}$ was relatively constant. The following polynomial described $\dot{V}_{O_2}$ as a function of swimming speed in both the HS and LS1 groups of Exp. III (R^2= 0.86; Fig. 1b, Table 4a):

$$\dot{V}_{O_2} = I_f - 1.37 \cdot U + 0.043 \cdot U^2$$

(I_f is intercept for different fish; U is swimming velocity in $cm \cdot s^{-1}$). The curves for the two groups were not significantly different. As can be seen in Fig. 1b, there was

Table 3. U_{crit}, $\dot{V}_{O2rest}$ and $\dot{V}_{O2max}$ of fish exposed to different training regimes. Fish in Exp. III were exercise-trained by swimming continuously at either 0.5 bl·s^{-1} (LS1) or 1.5 bl·s^{-1} (HS) for eight months. Fish in Exp. VI were exercise-trained by swimming continuously at 0.5 bl·s^{-1} (LS2) or on alternate days additionally swimming to U_{crit} (TR).†

Exp.	Training regime	n	$\dot{V}_{O_2}$ μmol·min^{-1}·kg^{-1}		U_{crit}	
			Rest	Max.	bl·s^{-1}	cm·s^{-1}.
III	LS1	10	83(8)	290(15)	2.8(0.1)	85.5(1.9)
III	HS	10	88(8)	320(12)	2.9(0.1)	87.5(2.2)
VI	LS2	12	80(13)	296(19)[a]	2.6(0.1)	83.3(2.4)
VI	TR	12	71(13)	371(19)[b]	2.8(0.1)	86.3(2.4)

†Numbers in parentheses are SEM.

[ab] Variables denoted with different letters were significantly different.

considerable variability in $\dot{V}_{O_2}$ at each swimming velocity and this variability was also evident in other experiments. Individual variability accounted for a significant ($p<0.0001$) portion (16%) of the total variance and a better fitting model was obtained when each fish was assigned a different intercept (I_f) than when a common intercept was assumed for all fish (Table 4a).

The $\dot{V}_{O_2}$ in the two training groups of Exp. IV (Fig. 1c; Table 4b) was analyzed by comparing the $\dot{V}_{O_2}$ of fish that were swimming at a similar velocity (11 levels). This was done because the polynomial models did not adequately represent the data for the fish in Exp. IV. Here individual variability accounted also for a significant ($p<0.0001$) portion of the total variance (Table 4b).

Table 4a. Polynomial model for $\dot{V}_{O_2}$ as a function of swimming velocity (U; in cm·s^{-1}). Combined data from HS and LS1 groups of Exp. III ($R^2 = 0.86$). The analysis applies to the data presented in Fig. 1b.

Source of variance	Degrees of freedom	Mean square	F	P
Individuals	21	6092.4	4.65	0.0001
U	1	12118.1	9.24	0.0027
U^2	1	127300.7	97.08	0.0001
Error	198	1311.2		

Table 4b. ANOVA for $\dot{V}_{O_2}$ at different swimming velocities for TR and LS2 groups in Exp. IV ($R^2 = 0.86$). The analysis applies to the data presented in Fig. 1c.

Source of variation	Degrees of freedom	Mean Square	F	P
Individuals	19	5569.8	3.12	0.0001
Swsp.*	10	150078.9	84.02	0.0001
Train.†	1	16599.6	9.29	0.0027
Swsp x Train.‡	9	1410.1	0.79	0.6265
Error	203	1786.2		

* Swsp: Swimming speed. Classified as 11 categories depending on swimming velocity.

† Training regime (TR *vs* LS2).

‡ Interaction between swimming speed and training regime.

Figure 1. $\dot{V}_{O_2}$ as a function of swimming velocity. (a) Starved chinook salmon and fed 15 h postprandial (Exp I). The data was analyzed in several sections. First, the mean $\dot{V}_{O_2}$ of fed and starved fish swimming at the five lowest swimming speeds was compared. Then the $\dot{V}_{O_2}$ of fed fish swimming at the last four velocities before they fatigued, was compared to that of starved fish swimming at the same speed. The $\dot{V}_{O_2}$ of fed fish was significantly ($p<0.001$) higher than in the starved fish, but $\dot{V}_{O_{2max}}$ was not significantly different in the two groups. The dotted lines show polynomials fitted to the data for fed and starved fish. The figure is continued on the next page.

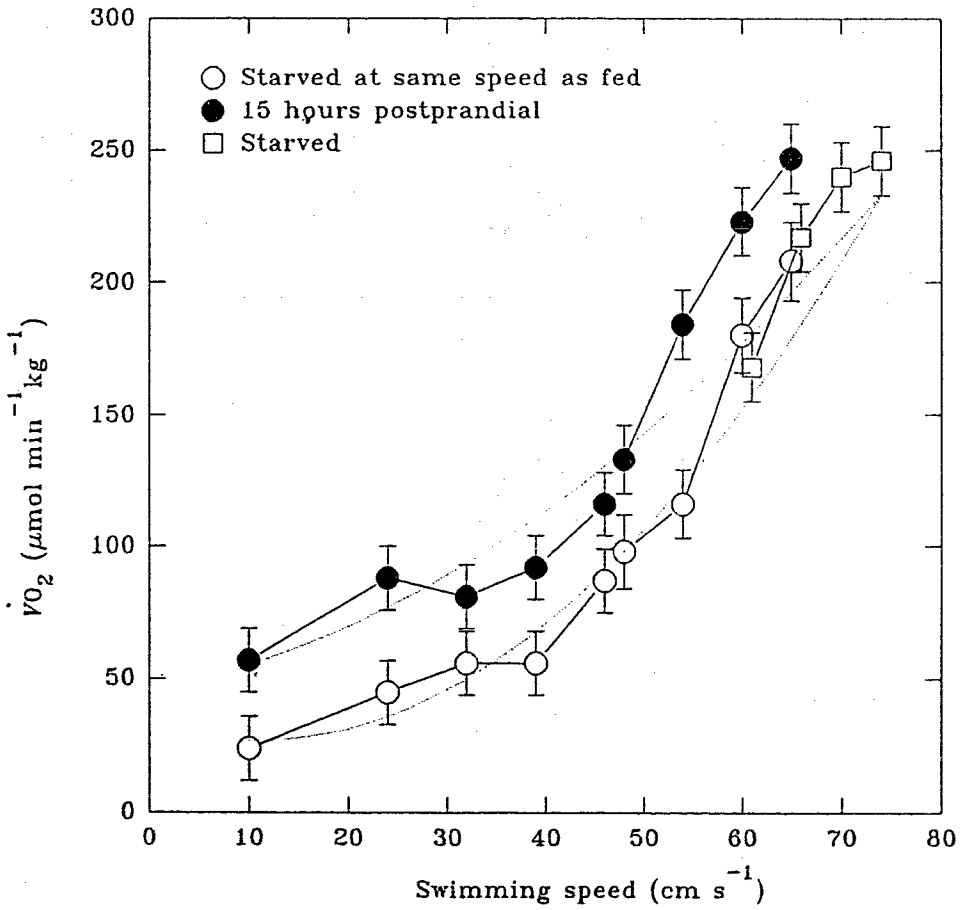

Starved at same speed as fed
15 hours postprandial
Starved
300
250
200
150
100
50
0
0
10
20
30
40
50
60
70
80
$\dot{V}O_2$ (μmol min^{-1} kg^{-1})
Swimming speed (cm s^{-1})

Figure 1 cont. b). HS and LS1 fish from Exp. III. $\dot{V}_{O_2}$ was not significantly different in the two groups.

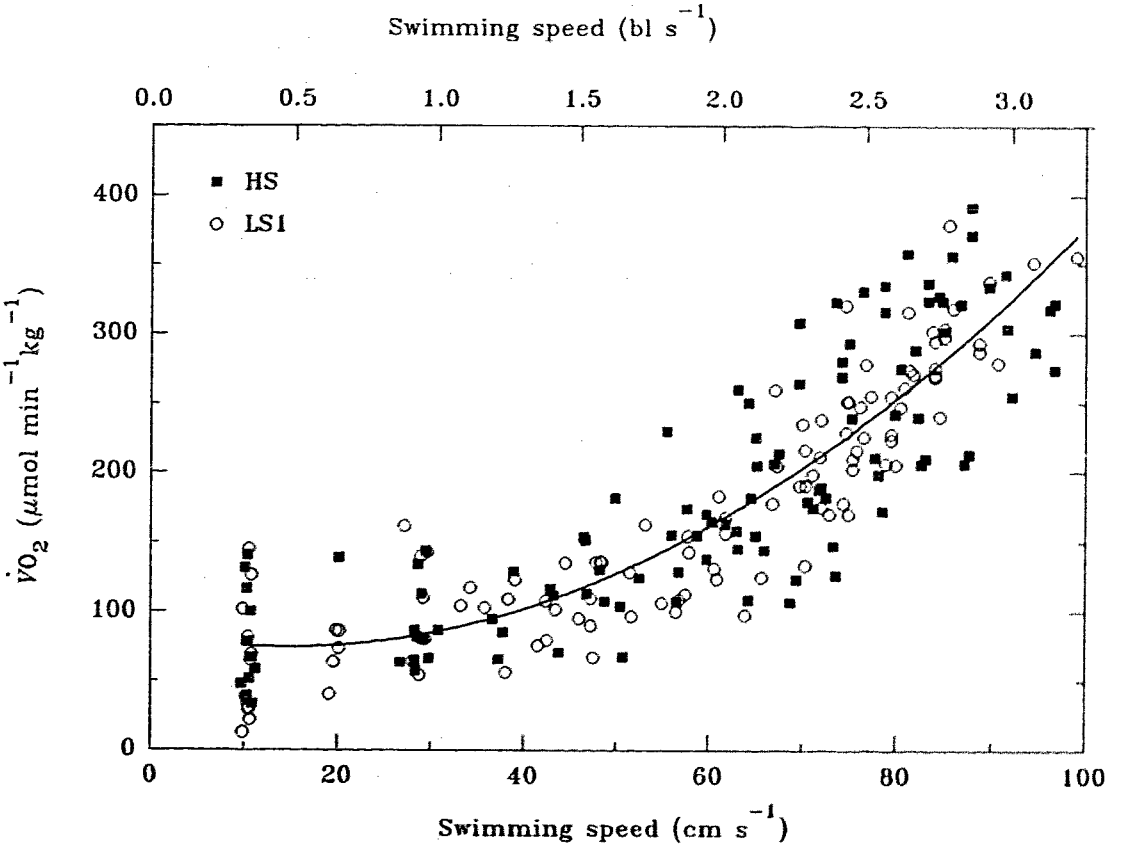
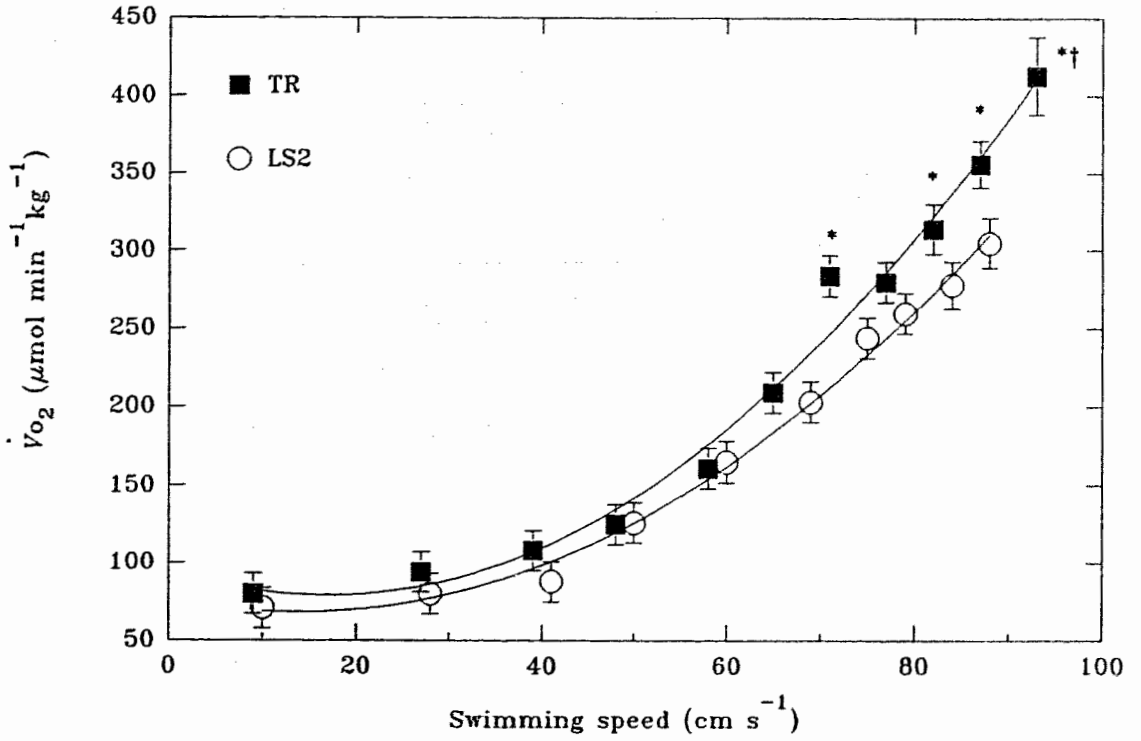

Swimming speed (bl s^{-1})
0.0
0.5
1.0
1.5
2.0
2.5
3.0
HS
LSI
400
300
200
100
0
$\dot{V}O_2$ (μmol min^{-1}kg^{-1})
0
20
40
60
80
100
Swimming speed (cm s^{-1})

Figure 1 cont. c) Trained (TR) and untrained (LS2) fish in Exp. IV. *denotes a significant difference in $\dot{V}_{O_2}$ at the same swimming velocity; †: the fastest swimmers in the TR group swam faster than the fastest swimmers in the LS2 group.


450
400
350
300
250
200
150
100
50
0
20
40
60
80
100
TR
LS2
*
*
*
*†
$\dot{V}o_2$ (μmol min^{-1}kg^{-1})
Swimming speed (cm s^{-1})

$\dot{V}_{O2max}$ and U_{crit} of operated fish.

The U_{crit} of operated fish was 21-23% lower than that of unoperated fish (Table 5). However, $\dot{V}_{O2max}$ of operated and unoperated fish was not significantly different in any group (Table 5). Still there was a tendency for trained fish (HS and TR) to better maintain their $\dot{V}_{O2max}$ ($\Delta\dot{V}_{O2max}$ = -1%) after the operation than the LS fish ($\Delta\dot{V}_{O2max}$ = -14% and -18%), although this was not statistically significant (Table 5).

Table 5. Mean $\dot{V}_{O2max}$ and U_{crit} of operated fish from Exp. III and Exp. IV and difference between the U_{crit} (ΔU_{crit}) and $\dot{V}_{O2max}$ ($\Delta\dot{V}_{O2max}$) between unoperated fish (Table 3) and operated fish. The operated fish in Exp. III swam with a Doppler flow probe on the intestinal artery and a cannula in the dorsal aorta. The operated fish in Exp. IV swam with a Doppler flow probe on the ventral aorta and a cannula in the dorsal aorta.

Experiment	Group	$\dot{V}_{O2max}$ $\mu mol \cdot min^{-1} \cdot kg^{-1}$	$\Delta\dot{V}_{O2max}$	U_{crit} $(cm \cdot s^{-1})$	ΔU_{crit}
Exp. III	HS	318(25)	-1%	68.8(3.1)	-21%
	LS1	250(22)	-14%	65.9(2.8)	-23%
Exp. IV	TR	366(31)	-1%	68.4(2.0)	-21%
	LS2	244(28)	-18%	65.6(2.1)	-21%

Discussion

The U_{crit} values of the fish in Exp. III and IV (2.6-2.9 bl·s^{-1}) were similar to the 2.65 bl·s^{-1} reported by (Randall *et al.*, 1987) for chinook salmon of the same size (30-35 cm) and at the same ambient temperature (9-11° C) The $\dot{V}_{O2max}$ of the fish (290-370 µmol·min^{-1}·kg^{-1}) was comparable to values (310-340 µmol·min^{-1}·kg^{-1}) for sockeye salmon (Brett & Glass, 1973). This suggests, that the fish in these experiments were in good condition and that they performed equally well or better than fish in other studies.

U_{crit} and $\dot{V}_{O2rest}$ of the fish in Exp. I were significantly lower than in the other two experiments, and $\dot{V}_{O2max}$ was 10-15% lower, albeit not significantly, than in the LS groups. A likely explanation for this is that the fish in Exp. I were fed less (see Table 1) than in the other two experiments. Reduced feeding or starvation are known to decrease $\dot{V}_{O2rest}$ in fish (Saunders, 1963; Beamish, 1964; Brett & Grove, 1978; Brett, 1983; Lyndon *et al.* 1992). Furthermore, a low feeding rate has been linked to lower aerobic and anaerobic metabolic capacity of tissues (Pelletier *et al.* 1994) and possibly reduced swimming performance (Kolok, 1992).

U_{crit} and $\dot{V}_{O2}$ during the postprandial stage.

This is the first time that results on the effect of feeding status on $\dot{V}_{O2max}$ and swimming performance are reported for any species of fish. On average, the $\dot{V}_{O2}$ of fed fish was 39 µmol·min^{-1}·kg^{-1} higher than in unfed fish and there was no indication that this difference decreased as swimming speed increased. This suggests that the metabolic processes behind the HI were not compromised at high swimming velocities, but in stead, the maximum locomotory function (U_{crit}) was reduced.

As in the present study, Beamish (1974) observed that the HI of largemouth bass was unchanged up to a velocity where $\dot{V}_{O2}$ was approaching $\dot{V}_{O2max}$, although the fish were not swum up to U_{crit}. Therefore, largemouth bass and chinook salmon appear to respond

to exercise in a similar way and the results of these experiments do not support the hypothesis that intestinal function, as measured by HI, is compromised during exercise. Moreover, Beamish (1974) found that velocity had no effect on the duration of the HI in the bass which further supports the hypothesis that the digestive process was not delayed during exercise as might be expected if intestinal function had been compromised by exercise. In contrast, Furnell (1987) reported that the HI of sablefish was reduced as swimming speed increased and suggested that sablefish had a physiological mechanism to reduce O_2 allocation to digestive functions. This may represent a species difference between sablefish, and chinook salmon and largemouth bass. However, close inspection of Furnell's results suggests that this not necessarily the case. First, Furnell measured only three fish and they were not swum to U_{crit}. Second, log-linear lines were fitted to the data. The results from the present study suggest that exponential or polynomial models may in some cases not reproduce the original data faithfully. In fact, when polynomial models were fitted to the data in Exp. I the curves for fed and starved fish converged in a manner similar to that observed by Furnell (Fig. 1a). However, since these polynomial curves diverged from the original data at the highest velocities, I rejected this approach. Finally, there were only a few data points for $\dot{V}_{O_2}$, in Furnell's study, at velocities where the curves converged. Therefore, his conclusion that the curves converge is largely based on the projection of fitted lines. Whether the responses of sablefish are different from those of chinook salmon and largemouth bass remains clouded at this time.

In small (body mass 11 g) rainbow trout, the peak levels of HI were 60% of the predicted $\dot{V}_{O_{2max}}$ (Legrow & Beamish, 1986) and in 20g sockeye salmon, peak levels of HI were about 50% of $\dot{V}_{O_{2max}}$ (Brett, 1983). This apparent difference between the salmonid species could be related to size, although it is not known exactly how the HI affects the scope for activity ($\dot{V}_{O_{2max}}$ - $\dot{V}_{O_{2rest}}$) of fish of different sizes. Scope for activity does increase with size (Brett, 1965) while the relative increase in $\dot{V}_{O_2}$ from $\dot{V}_{O_{2rest}}$ appears to

be consistently 1.6- to 2.0-fold (Jobling, 1981), suggesting that scope for activity would be reduced less by HI in large fish than in small fish. Therefore, comparison of the results from present experiment with other salmonid studies is hampered by the fish being of dissimilar size and at different temperatures.

The postprandial $\dot{V}_{O_2}$ was 23% of the $\dot{V}_{O_{2max}}$ of a chinook salmon, while in Atlantic cod the postprandial $\dot{V}_{O_2}$ is actually greater than the maximum $\dot{V}_{O_2}$ of swimming fish (Soofiani & Priede, 1985). Thus the HI affects the scope for activity much less in chinook salmon than in Atlantic cod. Clearly, the scope for activity in fed chinook salmon is clearly greater than in other less active species such as the Atlantic cod.

The results of this experiment are consistent with the hypothesis that $\dot{V}_{O_{2max}}$ is limited by maximum O_2 delivery. The $\dot{V}_{O_{2max}}$ was the same in fed and unfed fish which is what would be predicted by the hypothesis. Furthermore, the reduced U_{crit} of the fed fish compared to unfed fish is consistent with the idea that more blood flow is allocated to meet the O_2 demand of the HI during the postprandial stage while less blood flow is available to the locomotory muscles. Thus, at $\dot{V}_{O_{2max}}$ there may be a dynamic competition among different tissues for a limited arterial supply of O_2, a prediction examined in subsequent chapters by measuring changes in gut blood flow in fed, unfed, and exercising fish.

Effects of training on $\dot{V}_{O_2}$ and maximum swimming velocity.

There was little or no improvement in swimming performance, as measured by U_{crit}, with either training regime. Although, there was some indication that a few fish in the TR group had responded to training with a slight increase in U_{crit}, the TR training regime in Exp. IV was more stringent and therefore more likely to improve performance than the HS regime. Some studies have reported a 9-20% increase in swimming performance after exercise-training (Brett *et al.* 1958; Farlinger & Beamish, 1978; Broughton *et al.* 1980; Bessner & Smith, 1983; Leon, 1986; Houlihan *et al.* 1987; Farrell *et al.* 1989) while other studies have failed to show significant effects of exercise-training on U_{crit} (Greer-Walker & Pull, 1973; Farrell *et al.* 1991). The large individual variability in the swimming performance of fish makes it difficult to distinguish the relatively small changes in U_{crit}. This difficulty may partly explain the disparity between experiments. A number of other factors may also contribute to these inconsistent results, such as different control and holding conditions. Different rearing conditions may also affect results in training experiments. Keeping salmonids in low or no water currents and at a high loading density may be stressful for fish and therefore levels of stress hormones may increase (Woodward & Smith, 1985). In still water, fish are more aggressive which may curtail growth (Christiansen *et al.* 1991) and possibly swimming performance. In fact, Greer-Walker & Pull (1973) reported a reduced swimming performance of control fish over time, while performance of trained fish was unchanged.

The present experiments show a clear dichotomy between U_{crit} and $\dot{V}_{O_{2max}}$. The $\dot{V}_{O_2}$ of the TR fish was significantly higher than that of the LS2 fish, when they were swimming near U_{crit} (Fig. 1c) and the same trend was evident in Exp. III in that the $\dot{V}_{O_{2max}}$ of the HS fish was higher than that of the LS1 fish, while U_{crit} was similar. Other studies have also demonstrated a similar disparity between swimming velocity and $\dot{V}_{O_2}$. Brett (1964) found that sockeye salmon swimming in hyperoxic water had higher $\dot{V}_{O_{2max}}$ but the same U_{crit} as

fish swimming in normoxic water. The results of Gallaugher *et al.* (in prep.) show that anaemic fish consume less O_2 than polycythemic fish while swimming at the same speed (see also Fig. 10). These results clearly demonstrate that the increase in $\dot{V}_{O_2}$ in swimming fish is only partly due to increased cost of locomotion. Therefore, U_{crit} is a poor predictor of $\dot{V}_{O_{2max}}$ and *vice versa*. The residual $\dot{V}_{O_2}$ must be consumed by basal metabolism and supportive functions, such as osmoregulation or protein turnover, which may be in increased demand as swimming speed increases. It is therefore suggested, that the higher $\dot{V}_{O_2}$ in the TR reflects an increased ability to maintain basal and supportive functions while swimming. Thus, the fish that were exposed to intensive training regimes (HS and TR) appear to have acclimated to training by increasing their ability to support these basal functions rather than by increasing U_{crit}.

There is substantial variance in $\dot{V}_{O_2}$ of fish swimming at the same velocity (Fig. 1b). Not all this variability is random error and can be accounted for by consistent individual differences (Table 4a, b). For example, fish with high $\dot{V}_{O_2}$ at one velocity are also likely to have high $\dot{V}_{O_2}$ at other swimming speeds. This source of variance can be introduced into polynomial (Table 4a) or log-linear models by assigning different intercepts for each fish. Similarly, ANOVA with "repeated measure" design (Table 4b) can be used to account for this variability. I am not aware of other studies where this approach has been used for $\dot{V}_{O_2}$ data of swimming fish, but the use of these models is recommended since it increases the sensitivity of statistical analyses considerably.

Polynomial, and particularly, exponential models have been used widely for the analysis of O_2 consumption in swimming fish (e.g. Brett, 1964, 1965; Webb, 1971b; Kiceniuk & Jones, 1977; Furnell, 1987). The results of this study suggest that these models should be treated with caution, in that they may hide significant nuances of the original data (Fig. 1a). While polynomial models can describe the $\dot{V}_{O_2}$ as a function of swimming velocity of the fish in Exp. III, with a good measure of statistical confidence, they did not

adequately represent the data in Exp. I and IV. This is likely because of the complex changes in efficiency of muscles, mechanical efficiency of the propeller mechanism, cost of ventilation, and possibly reduced basal metabolism with increased swimming speed. Therefore, there is no reason to assume that a simple exponential function can describe the data adequately.

The $\dot{V}_{O_2}$ of the unoperated fish from Exp. III and IV was measured to validate the results obtained from heavily cannulated fish in the same experiment. The U_{crit} of the operated fish in Exp. III and IV was reduced by 21-23% compared with that of unoperated fish (Table 5). This reduction in swimming speed is to be expected, since the extra drag of the cannula and the leads from the flow probes will reduce U_{crit}. In other studies, U_{crit} has been reduced by up to 70%, depending on the extra drag load placed on the fish (Webb, 1971a; Kiceniuk & Jones, 1977). Even though U_{crit} was reduced, the fish appeared to be performing the same maximum work since the $\dot{V}_{O_2max}$ of the operated fish proved not to be significantly different from that of unoperated fish (Table 5). If the $\dot{V}_{O_2max}$ was not significantly affected by the operation, it seems unlikely that the flow probes compromised cardiac output or cardiovascular O_2 transport.

Chapter 4

Vascular anatomy of salmonids.

Introduction.

The literature pertaining to the blood vasculature of teleosts is diverse (for review see Harder, 1975) and the general morphology of the salmonid vascular system has been described (Gorkiewicz, 1947; Smith & Bell, 1976). However, specific knowledge on the salmonid gut vasculature is mainly derived from dissections of rainbow trout (Grodzinski, 1938; Koniar, 1947), and only limited information is available for other species (Smith & Bell, 1976). Therefore, it proved necessary to perform an anatomical study of the gastrointestinal vasculature of chinook salmon, to identify arterial and venous connections to the gut vasculature. The complete study, published as Thorarensen *et al.* (1991), is presented in Appendix III. In this chapter, the findings relevant to the thesis are summarized along with some additional observations on the anatomical arrangement of the systemic circulation in chinook salmon.

Methods.

In short, vascular corrosion casts were made of the arterial and venous vasculature by injecting an acrylic casting material (Batson's No 17 from Polysciences PA, or Mercox from Ladd Research Industries Inc. VT) into arteries and veins and allowing the resin to polymerize. Tissues were digested away from the acrylic casts of the vessels in 30% KOH (3 days) followed by 5% HNO_3 (1 day) after which the casts were rinsed and dried for later observation. Full details of the methods are found in Appendix III.

Complementary observations on the gut vascular network were performed (10 fish) where the vasculature was revealed by dissection and injected with India ink.

Results and Discussion.

Central systemic circulation.

Blood passes from the gills *via* the efferent branchial arteries to the dorsal aorta and to other smaller diameter vessels, which branch from the efferent branchial arteries to supply the head region (Fig. 2). The afferent pseudobranchial arteries branch ventrally from the efferent branchial arteries of the first gill arches and deliver oxygenated blood to the pseudobranch and then to the eyes *via* the efferent pseudobranchial artery. The efferent branchial arteries from the second gill arches join ventrally to form the hypobranchial artery, which supplies blood to the coronary circulation. The carotid arteries branch off the efferent arteries of the first gill arch and supply blood flow to the cerebral circulation. It is not known how large fraction of cardiac output passes through these cephalic vessels, but based on results from isolated head preparations it may be around 5% (Gardaire *et al.* 1991). The venous drainage from the gills is 7-8% of $\dot{Q}_{rest}$ (Ishimatsu *et al.* 1988; Sundin and Nilsson, 1992), and therefore 85-90% of cardiac output must flow through the dorsal aorta at rest.

The segmental arteries which supply blood flow to the muscles branch regularly along the length of the dorsal aorta. However, the largest vessel branching from the dorsal aorta is the coeliacomesenteric artery (CMA).

Venous blood from the trunk and the tail returns to the heart primarily through the posterior cardinal veins, which run through the kidney. Blood from the tail area flows through the caudal vein, which forms the renal portal system as it enters the kidney. The kidney also receives venous blood from the segmental veins of the trunk, which also enter the renal portal system (Moore, 1933; Smith & Bell, 1976). Blood flows from the kidney through the posterior cardinal vein. Venous blood from the head region returns primarily through the anterior cardinal (jugular) veins. The posterior and anterior veins unite and

form the *ductus cuvieri* which empties into the *sinus venosus* of the heart Venous blood from the liver flows directly into the *sinus venous* through the hepatic veins.

Intestinal circulation.

The CMA originates just caudal to the origin of the DA in salmonids (Fig. 2). The CMA passes through the head kidney and bifurcates near the septum to form the gastrointestinal artery (GA; names as in Grodzinski, 1938) and intestinal artery (IA). The IA is the larger of these two vessels. Judging by the relative vessel diameters, the IA carries at least 85% of the total blood flow to the gut. In this thesis, flow probes were placed on the IA and it was assumed that blood flow in the IA accurately reflected total blood flow to the digestive tract.

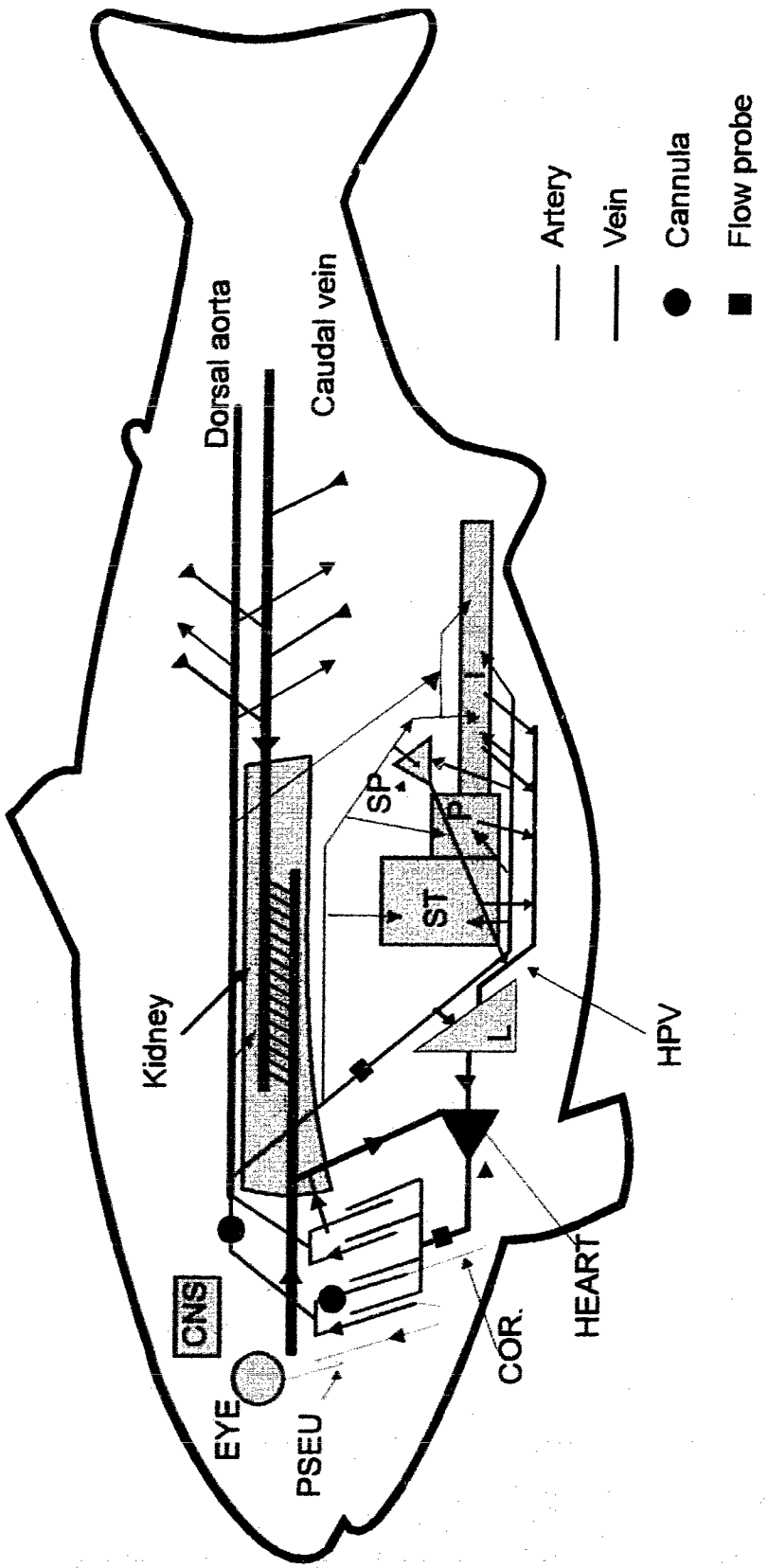
The IA, through its various branches, supplies blood to the liver, the pyloric caecae, spleen, intestine and ventral parts of the stomach. The GA sends off branches to dorsal aspects of the stomach, gonads, spleen and dorsal portion of the intestine. Since more than 85% of total gut blood flow is likely carried in the IA, changes in blood flow in other vessels, such as the GA, are not likely to produce a large error in estimates of total gut blood flow.

To some extent the GA isanalogous to the mesenteric artery, and the IA to the coeliac artery of Atlantic cod (Jensen *et al.* 1991) and Red Irish Lord (Axelsson *et al.* in review). The mesenteric and coeliac arteries are of similar size in Atlantic cod and Red Irish Lord and during the postprandial stage blood flow does not change concurrently in the two vessels (Axelsson & Fritsche, 1991; Axelsson *et al.* in review). However, in chinook salmon, the IA supplies blood to most of the same organs as does the GA. Therefore, errors due to variability in GA flow, independent of IA flow, probably amount to an underestimate of total visceral blood flow of no more than 15%.

Two or three other minor vessels also connect the posterior DA and the posterior intestinal vasculature, but because of a small diameter their contribution to total intestinal blood flow is probably insignificant compared to the IA .

Blood from the digestive tract, the gonads and the spleen flows to the liver through the hepatic portal system. Thus, the liver receives both venous blood and arterial blood through a branch of the IA.

Figure 2. Schematic outline of the cardiovascular system of chinook salmon. The flow probes on the ventral aorta and the intestinal artery are shown as green squares and the insertion points for the cannulae in the dorsal aorta and the efferent branchial artery of the 2nd gill arch are shown as green circles. COR: coronary artery; CNS: central nervous system; HPV: hepatic portal vein; I: intestine; L: liver; P: pyloric region; PSEU; pseudobranch; SP: spleen; ST: stomach.


Artery
Vein
Cannula
Flow probe
Dorsal aorta
Caudal vein
Kidney
SP
ST
L
HPV
CNS
EYE
PSEU
COR.
HEART

Chapter 5

Cardiac output and oxygen supply in swimming chinook salmon.

Introduction

The $\dot{V}_{O2max}$ of the TR fish in Exp. IV proved to be 27% higher than in the control LS2 group (See Chapter 3) and in this chapter the basis for this difference will be examined by measuring cardiovascular O_2 transport ($\dot{T}_{O_2} = \dot{Q} \cdot Ca_{O_2}$) and internal O_2 convection ($D_{O2} = \dot{Q} \cdot (Ca_{O_2} - Cv_{O_2})$ the two groups of fish.

In the General Introduction, an analogy was drawn between the O_2 cascade and an electrical circuit with 'resistors' arranged in series. In spite of over-simplifications, such as not taking the sigmoidal shape of the O_2 dissociation curve into account, this conceptual model can be of aid in understanding the complicated interactions of the various steps in the O_2 cascade. Thus each step in the O_2 flux, ventilation, gill diffusion, cardiovascular O_2 transport, diffusion from capillaries, and O_2 consumption by mitochondria, can be considered as 'resistors'. The 27% increase in $\dot{V}_{O2max}$ of the TR fish must either be caused by a concerted reduction in the 'resistance' of all the resistors or by changes in one of the larger 'resistors', since changes in one of the smaller resistors would have little effect on total resistance.

Mitochondrial enzyme activity in the muscles of both groups of experimental fish was similar (A.K. Kiessling & A.S. Kolok pers. com.). Moreover, the increase in $\dot{V}_{O2max}$ in the TR group was not associated with a corresponding increase in U_{crit} (Fig. 1c), indicating that the O_2 demand of the swimming muscles was unchanged. Instead, the higher $\dot{V}_{O2max}$ in the TR group may be accounted for by other functions being maintained at higher levels while the fish were swimming.

It was suggested in the General Introduction that the $\dot{V}_{O2max}$ is primarily limited by $\dot{T}_{O_2}$. Therefore, the higher $\dot{V}_{O2max}$ in the TR group could be associated with increased $\dot{Q}_{max}$ and (or) Ca_{O_2}. A second significant limiting factor is an apparent diffusion limitation in tissues which affects extraction of O_2 from blood as it passes through tissues. The O_2 content of mixed venous blood (Cv_{O_2}) in rainbow trout is 14% of Ca_{O_2} (Kiceniuk & Jones, 1977). The E_{O2max} of chinook salmon is not known, but it is likely that $\dot{V}_{O2max}$ could be elevated by increasing the extraction of O_2 from the blood as it passes through the capillaries.

In the present experiment, $\dot{Q}$, Ca_{O_2}, and $\dot{V}_{O2max}$ of chinook salmon were measured. Exercise-trained fish were used for this study, because it is known that exercise-training affects various components of the O_2 cascade. Increased heart size (Hochachka, 1961; Greer-Walker & Emerson, 1978; Farrell *et al.* 1990), enhance cardiac performance (*in vitro*) (Farrell *et al.* 1991) and increased haemoglobin content of blood (Hochachka, 1961; Farlinger & Beamish, 1978; Zbanyszek & Smith, 1984) have been reported in exercise-trained fish. All these changes could affect $\dot{T}_{O2max}$ and therefore $\dot{V}_{O2max}$. Furthermore, increases in capillary density have been observed in trained fish (Davie *et al.* 1986; Sänger, 1992). Increased capillary density may increase E_{O_2} by reducing diffusion distance between capillaries and mitochondria or by increasing the residence time of blood in capillaries.

The extraction ratio of O_2 from the blood in tissues was estimated with the equation:

$$E_{O_2} = \dot{V}_{O_2} / \dot{T}_{O_2}$$

which is obtained by rearranging the Fick equation (see General Introduction, Chapter 1). As explained in the General Introduction (Chapter 1), the Fick principle can reliably predict E_{O_2} in salmonids (Neuman *et al.* 1983; Appendix I; Appendix II).

The objectives of this experiment were to measure oxygen transport in chinook salmon

and to examine the effect of exercise-training on cardiovascular O_2 delivery.

Methods

The training regime and husbandry for the fish in this experiment were described in the General Methods (Chapter 2). Briefly, the TR fish were swum to U_{crit} on alternate days. Otherwise they swam continuously at 0.5 bl·s^{-1}. The LS2 group served as control and swam continuously at 0.5 bl·s^{-1}. Both groups were only fed on the days when the TR group did not swim to U_{crit}.

In this study, pulsed Doppler flow probes were used to measure relative changes in $\dot{Q}$ in swimming fish. These flow probes were later calibrated *in situ* by placing a Transonic flow probe on the bulbus and recording flow simultaneously with both probes. The Transonic flow probes measure absolute blood flow, but because of their rather larger size, they may affect significantly the ability of small fish to swim and to maintain position when swimming against a strong current. Since the fish in this study were relatively small, Doppler probes were chosen to measure $\dot{Q}$ while the fish were swimming. A detailed description of the implantation of flow probes, of procedures for swimming the fish and of collection of data are given in the General Methods (Chapter 2).

The Doppler flow probes on the ventral aorta of six fish from each group were successfully calibrated after they had swum to U_{crit} and these results are presented here. Many more fish swam without being successfully calibrated or without another variable being successfully measured, such as $\dot{V}_{O_2}$ or Ca_{O_2}. Thus, for consistency, only fish that had all variables measured successfully were included. Variables measured in fish that were not included in the analysis were in general agreement with the results presented here.

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Results

The $\dot{Q}_{rest}$ was 35.8 mL·min^{-1}·kg^{-1} and 33.6 mL·min^{-1}·kg^{-1} for the LS2 and TR groups, respectively. There was no significant difference between the $\dot{Q}$ of trained and control LS2 fish in Exp. IV (Fig. 3a, Table 6) at any swimming velocity. Over 60% of the increase in $\dot{Q}$ occurred as the fish started swimming (Fig. 3a). $\dot{Q}$ increased by 94% and 83%, to a maximum of 65.5 and 65.1 mL·min^{-1}·kg^{-1} in the LS2 and TR groups, respectively. $\dot{Q}_{max}$ was recorded at swimming velocities of 90±6% and 94±1% of U_{crit}, respectively. The increase in $\dot{Q}$ from rest to $\dot{Q}_{max}$ was brought about by a 61-65% increase in SV_H and a 10-22 % increase in f_H (Table 6).

While $\dot{Q}_{rest}$ and $\dot{Q}_{max}$ were the same in both groups, the means of achieving the increase in $\dot{Q}$ were different. As the TR fish started swimming, they relied more on SV_H (Fig. 3e) to increase $\dot{Q}$, whereas, the LS2 fish increased f_H (Fig. 3d). Moreover, the f_H was significantly lower in the TR group, at the $p<0.055$ level, at rest, and significantly ($p<0.05$) higher at U_{crit} (Fig 3d).

The $\dot{V}_{O2max}$ of the trained and the control fish was significantly different, independent of whether the fish were unoperated (Table 3) or operated (Table 6). The $\dot{V}_{O2max}$ of the operated fish was not significantly different compared with the unoperated fish. The U_{crit} of the operated fish was 21% lower than that of the unoperated fish (Table 5).

The $\dot{V}_{O_2}$ did not increase significantly until the fish were swimming at a velocity close to 60-80% of U_{crit}. However, more than 50% of the maximum increase in $\dot{Q}$ and $\dot{V}_{O_2}$ occurred as soon as the fish started swimming. Therefore, E_{O2} must have decreased during the initial phases of swimming.

There was no significant change in P_{DA} with increased swimming speed in the TR fish while blood pressure increased significantly in the LS2 group (Fig. 3). Furthermore, a comparison of the mean P_{DA} in the two groups with ANOVA indicated that pressure was

significantly ($p<0.006$) higher in the LS2 group. There was no significant change in R_{sys} in the LS2 group, whereas R_{sys} decreased significantly in the TR group.The Hct was significantly higher in the TR group than in the LS2 groups. However, Ca_{O_2}, was not significantly different in the two groups (Table 7). In both groups Pa_{O_2} decreased significantly as swimming speed increased, but O_2 saturation of Hb did not change significantly as swimming speed increased in spite of the decrease in Pa_{O_2}. Because the O_2 saturation of Hb was 16% higher ($p<0.0004$) in the LS2 group than in the TR group.

After 1h of recovery, $\dot{V}_{O_2}$, $\dot{Q}$, and $\dot{T}_{O_2}$ were not significantly different from pre-exercise values. However, f_H was significantly higher and P_{DA} was significantly lower that pre-exercise values in the TR group.

Figure 3. (A) Cardiac output, (B) O_2 delivery, (C) $\dot{V}_{O_2}$. The figure is continued on the next page.

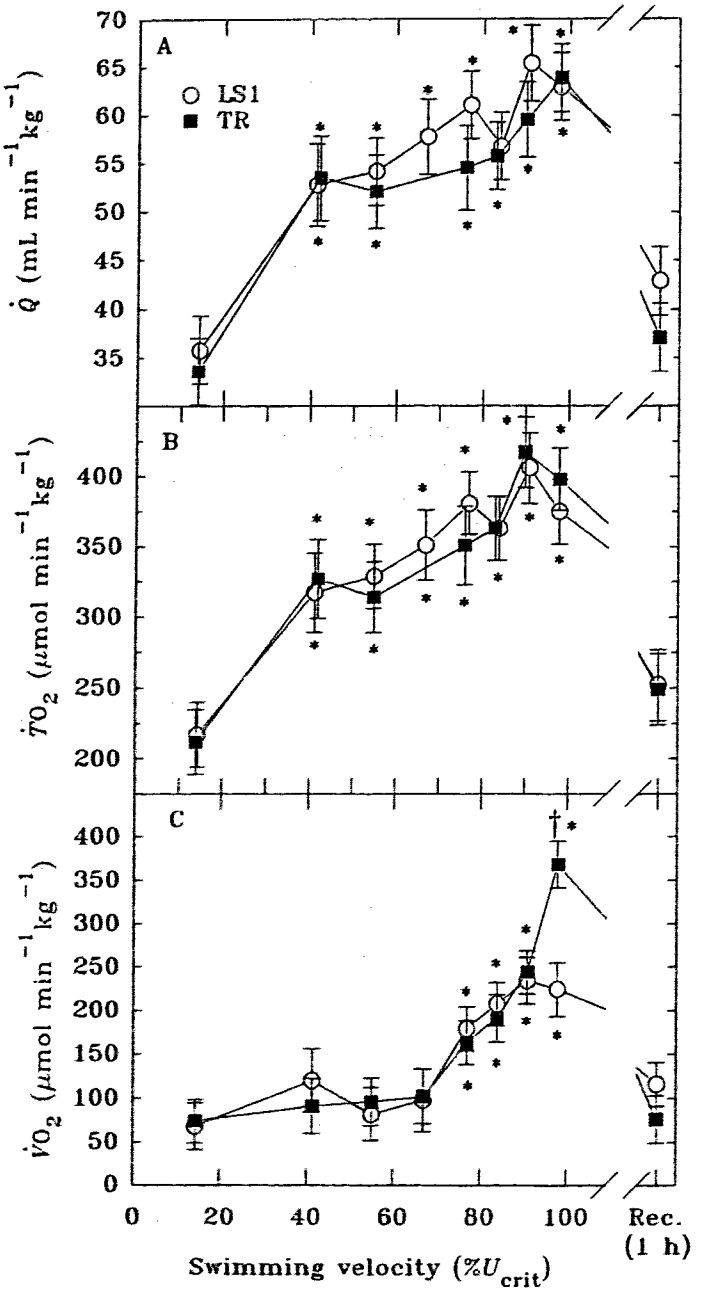

A
B
C
LS1
TR
$\dot{Q}$ (mL min^{-1} kg^{-1})
$\dot{T}O_2$ (μmol min^{-1} kg^{-1})
$\dot{V}O_2$ (μmol min^{-1} kg^{-1})
70
65
60
55
50
45
40
35
400
350
300
250
200
150
100
50
0
0
20
40
60
80
100
Rec.
(1 h)
Swimming velocity (%U_{crit})

Figure 3 cont. (D) heart rate, (E) stroke volume, (F) systemic vascular resistance, and (g) dorsal aortic blood pressure in fish from Exp. III. * indicates a significant increase from resting levels. † indicates a significant ($p<0.05$) difference between TR and LS2 fish. Symbols are the same as in Fig. 3a.

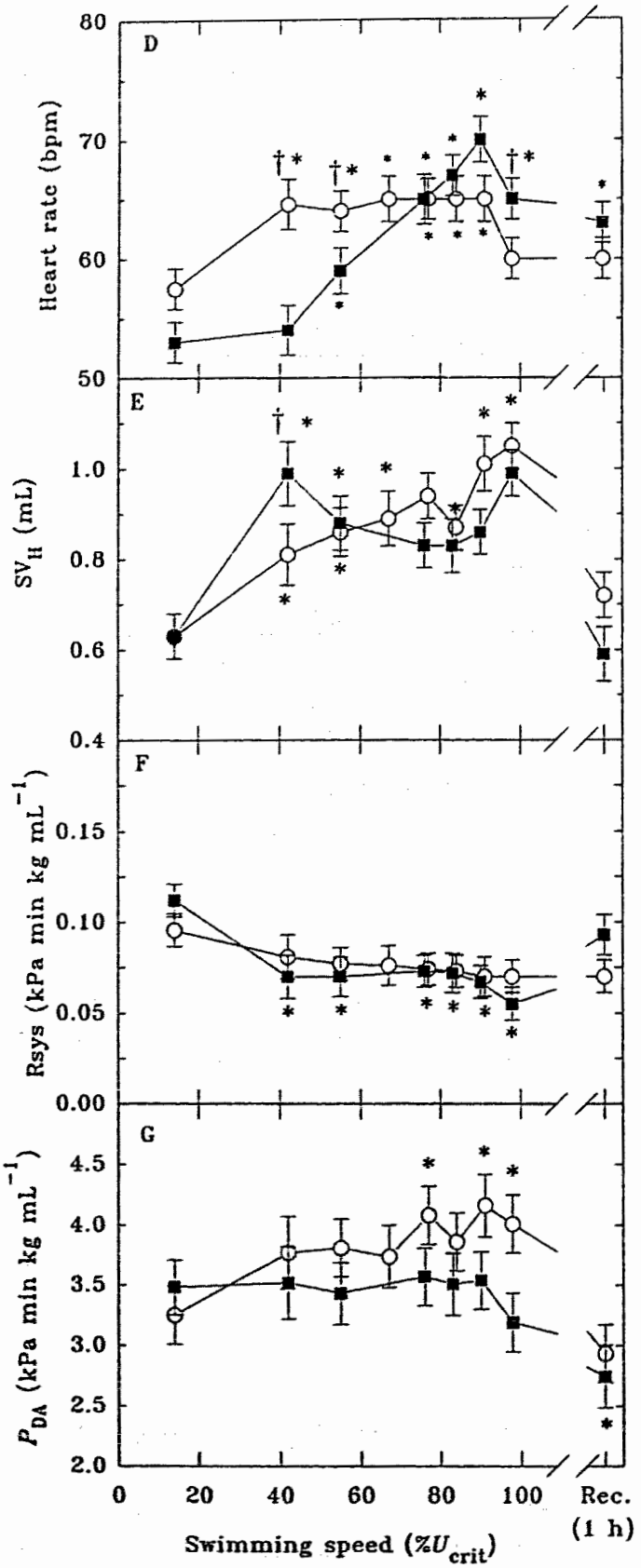

D
Heart rate (bpm)
80
70
60
50
E
SV_H (mL)
1.0
0.8
0.6
0.4
F
Rsys (kPa min kg mL^{-1})
0.15
0.10
0.05
0.00
G
P_{DA} (kPa min kg mL^{-1})
4.5
4.0
3.5
3.0
2.5
2.0
0
20
40
60
80
100
Rec.
(1 h)
Swimming speed (%U_{crit})

Table 6. Cardiorespiratory variables from fish in Exp. IV (n=6 for each group).

	LS2		TR	
	Rest	$\dot{Q}_{max}$	Rest	$\dot{Q}_{max}$
$\dot{Q}$ ($mL \cdot min^{-1} \cdot kg^{-1}$)	35.8(4.5)	65.6(7.3) *	33.6(3.6)	65.1(7.9) *
SV_H (mL)	0.63(0.07)	1.04(0.11) *	0.63(0.04)	0.97(0.13) *
f_H (bpm)	57(4)	63(±2) *	53(2)	64(2) *
P_{DA} (kPa)	3.2(0.2)	4.0(0.2) *	3.5(0.3)	3.6(0.3) †
R_{SYS} ($kPa \cdot min^{-1} \cdot kg \cdot mL^{-1}$)	0.095(0.010)	0.071(0.015)	0.112(0.016)	0.058(0.014) *
$\dot{M}_{O_2}$ (mL $O_2 \cdot min^{-1} \cdot kg^{-1}$)	217(20)	393(39) *	213(30.9)	408(50) *
$\dot{V}_{O_2}$ (mL $O_2 \cdot min^{-1} \cdot kg^{-1}$)	63(13)	244(28) *†	74(13)	366(28) *†

* Significant change from rest

† Significant difference between groups

Table 7. Haematological variables of chinook salmon from Exp. IV.

		Rest	Swimming			Recovery
			56% U_{crit}	88% U_{crit}	U_{crit}	+ 1 h
Hct (%)*	LS2	29.8±1.2	29.0±1.0	31.5±1.3	30.0±1.2	29.2±1.1
	TR	33.0±1.2	33.8±1.2	35.7±1.5	34.3±1.2	32.5±1.3
C_{aO_2} (mL $O_2 \cdot dL^{-1}$)	LS2	13.8±0.6	14.1±0.6	14.5±0.6	13.5±0.6	13.8±0.6
	TR	13.9±0.5	13.9±0.4	15.5±0.7	14.1±0.5	14.7±0.7
O_2 saturation of Hb*						
mL $O_2 \cdot g^{-1}$Hb	LS2	1.16±0.21	1.28±0.21	1.21±0.17	1.184±0.2	1.13±0.22
	TR	0.96±0.18	1.02±0.21	1.00±0.12	1.03±0.27	1.20±0.25
P_{aO_2} (kPa)	LS2	13.0±0.6	13.2±0.6	11.3±0.6†	10.1±0.7†	11.7±0.6
	TR	14.1±0.5	13.4±0.5	11.5±0.7†	8.5±0.6†	11.5±0.6
pH	LS2	7.93±0.02	7.90±0.02	7.85±0.02†	7.82±0.02†	7.80±0.02†
	TR	7.91±0.02	7.89±0.02	7.87±0.02	7.79±0.02†	7.82±0.02†

* Values form the two groups were compared with ANOVA and were found to be significantly different ($p<0.05$)

† Significant change from rest ($p<0.05$).

Discussion

This is the first study to report direct measurements of $\dot{Q}$ in a swimming salmonid. To examine the validity of these measurements and to confirm that the cardiovascular performance of the operated fish was similar to that of unoperated fish the $\dot{V}_{O_2max}$ of unoperated fish was measured (See Chapter 3). The $\dot{V}_{O_2max}$ of operated and unoperated fish was not significantly different (Table 5). Therefore, it is concluded that the flow probes did not significantly affect maximum cardiovascular performance.

The $\dot{Q}_{rest}$ of chinook salmon (LS2: 35.8 mL·min^{-1}·kg^{-1}; TR: 33.6 mL·min^{-1}·kg^{-1}) was similar to the 39.4 mL·min^{-1}·kg^{-1} reported for coho salmon (Axelsson & Farrell, 1993). However, the values for $\dot{Q}_{rest}$ were substantially higher than the $\dot{Q}_{rest}$ of rainbow trout, for which reported values range between 17 and 25 mL·min^{-1}·kg^{-1} (Cameron & Davis, 1970; Davis & Cameron, 1971; Kiceniuk & Jones, 1977; Xu & Olson, 1993; Gamperl *et al.* 1994; Appendix I; Appendix II), and in other species of fish (11-19 mL·min^{-1}·kg^{-1}) recorded at a similar temperature (9-11° C) (see Farrell & Jones, 1992). The $\dot{Q}$ of the rainbow trout reported in Appendices I and II was 20 and 26 mL·min^{-1}·kg^{-1}, respectively, and was measured in the same way, and under the same conditions, as in the present study. Therefore, it is concluded that the $\dot{Q}_{rest}$ of coho and chinook salmon is higher than that of rainbow trout.

The $\dot{Q}_{max}$ of chinook salmon (65 mL·min^{-1}·kg^{-1} in both groups) was 20-30% higher than the 48 mL·min^{-1}·kg^{-1} (Appendix I) and 52.6 mL·min^{-1}·kg^{-1} (Kiceniuk & Jones, 1977) reported for $\dot{Q}_{max}$ in rainbow trout. In addition, the $\dot{Q}_{max}$ of the chinook was higher than has been recorded in any other species of fish, at 9-11° C (see Farrell & Jones, 1992).

The E_{O_2max} was calculated and not measured directly. As the fish started swimming, $\dot{T}_{O_2}$ increased by more than 50% while $\dot{V}_{O_2}$ did not change significantly and therefore the E_{O_2} must have been reduced initially. At U_{crit}, the E_{O_2max} was 90% and 62% for the TR and LS2 fish, respectively. These extraction ratios are in line with the values reported for

rainbow trout (Kiceniuk & Jones, 1977; Appendix II), where extraction increases from 40% at rest to 65-86% at $\dot{V}_{O2max}$. The E_{O2max} in mixed venous blood of mammals is typically 60-80% (Taylor *et al.* 1987; Jones *et al.* 1989; Longworth *et al.* 1989; Piiper, 1990), although E_{O2max} can be as high as 80-90% in muscles for relatively short periods of intense exercise (13-15 min; Richardson *et al.* 1993). Therefore, the E_{O2max} attained by the TR fish and the rainbow trout from the study of Kiceniuk & Jones (1977) is equal to or greater than that reported for mammals.

Many factors may have contributed to the higher E_{O2max} of the TR fish as compared with the LS2 fish. The increase in E_{O2max} in the TR group could be a result of more capillaries being perfused simultaneously, either because capillarization increased in the TR fish or because more tissues were perfused simultaneously. This would increase the surface area available for diffusion from capillaries and reduce the mean distance between capillaries and mitochondria, both of which would increase O_2 conductance (Weibel *et al.* 1992). Moreover, when a greater number of capillaries are perfused while $\dot{Q}$ remains the same, the mean transit time of blood cells in capillaries increases and therefore more time is available for the unloading of O_2. Transit time has been implicated as one of the limitations to O_2 extraction from blood in mammals (Saltin, 1985). The greater reduction in systemic resistance and the lower P_{DA} at U_{crit} in the TR group than in the LS2 group, are consistent with more capillaries being perfused simultaneously, either because more tissues are perfused simultaneously or because capillarization of various tissues has increased as a result of training. Furthermore, if more tissues were being perfused simultaneously in the TR group, $\dot{V}_{O_2}$ at any given swimming speed should have been higher than in the LS2 group and this is in fact what was found (Fig. 1b and Fig. 3c). Capillarization is known to increase by 27% in red muscle and 95% in white muscle of rainbow trout in response to exercise-training (Davie *et al.* 1986) and by 40 and 77% in the red muscle of two cyprinid species (Sänger, 1992). Further studies are needed to

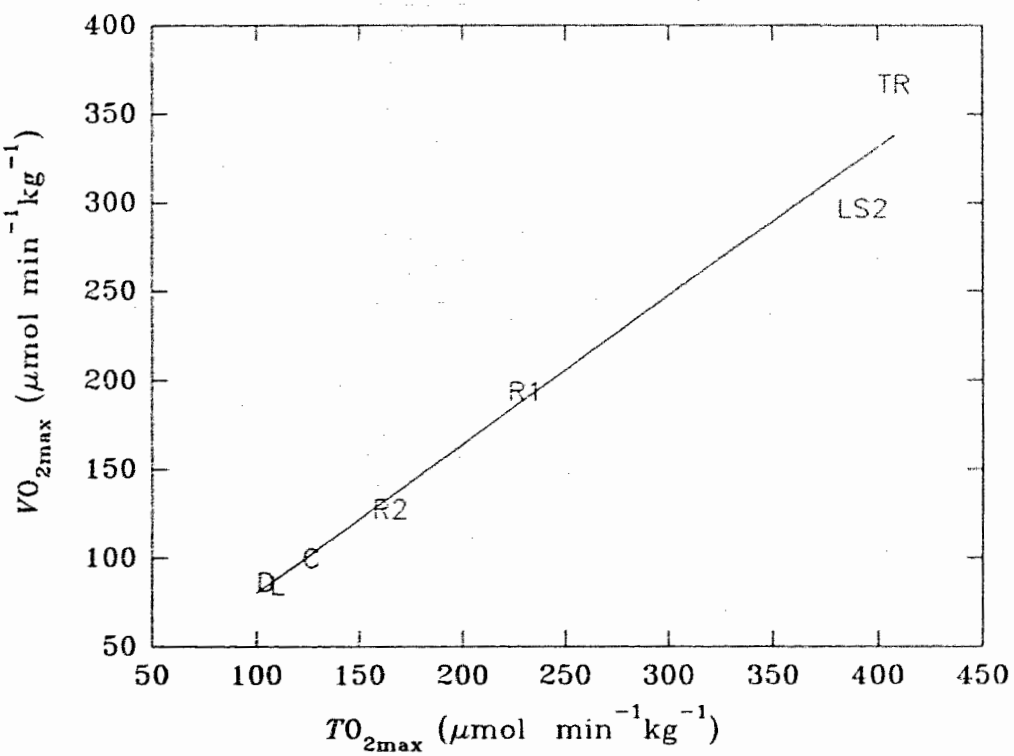
demonstrate a linkage between E_{O2max}, P_{DA}, R_{SYS}, and capillary perfusion. Another factor contributing to the increased E_{O2max} in trained fish could be myoglobin content of muscles, which is know to increase in exercise-trained fish (Love *et al.* 1977), and facilitate O_2 transport within the muscle fibres (Gayeski *et al.* 1985; Bailey & Driedzic, 1986).

The chinook salmon have a higher $\dot{T}_{O2max}$ than has been measured in any other species of fish apart from tuna (Brill & Bushnell, 1991b). The $\dot{T}_{O2max}$ of chinook salmon is more than 75% higher than in rainbow trout (Kiceniuk & Jones, 1977; Appendix I) and results from another study (Appendix II), where the $\dot{T}_{O2max}$ of rainbow trout was measured the same way as in Appendix I, suggest that, in some instances, this difference may be as much as 125%. Similarly, the $\dot{T}_{O2max}$ of chinook salmon was 275% higher than in dogfish (at 18° C; Piiper *et al.* 1977) and in leopard shark (at 14-24° C;Lai *et al.* 1990). The $\dot{T}_{O2max}$ of Atlantic cod has not been measured, but it is possible to estimate a value using a literature value for $\dot{Q}_{max}$ (Axelsson, 1988) and assuming that Ca_{O_2} was similar to the LS1 fish in Exp. III (See chapter 6) which had a similar Hct to Atlantic cod (P. Gallaugher pers. com.). Such a calculation reveals that the $\dot{T}_{O2max}$ of chinook salmon is about 270% higher than in a Atlantic cod.

The high $\dot{T}_{O2max}$ of chinook salmon is also reflected in a higher $\dot{V}_{O2max}$ than in other fish. Interestingly, there is a remarkably good correlation between $\dot{V}_{O2max}$ and $\dot{T}_{O2max}$ in various fish (Fig. 4). Thus, the O_2 transporting capacity of the cardiovascular systems of fish is closely matched to their maximum O_2 uptake. A similar relationship between $\dot{T}_{O2max}$ and $\dot{V}_{O2max}$ is also seen in mammals (Lindstedt *et al.* 1987). $\dot{T}_{O2max}$ in humans has been implicated as the primary limitation to $\dot{V}_{O2max}$ (di Prampero, 1985; Wagner, 1993) and therefore any changes in maximum O_2 consumption are necessarily correlated with transport. The tight correlation ($R^2 = 0.99$) between $\dot{T}_{O2max}$ and $\dot{V}_{O2max}$ underlines the importance of $\dot{T}_{O2max}$ in determining $\dot{V}_{O2max}$ of fish and may suggest that $\dot{V}_{O2max}$ of fish is

limited primarily by $\dot{T}_{O2max}$. Increases in the capacity of a single step in the O_2 cascade can only have limited effects on $\dot{V}_{O2max}$ since other steps in the O_2 cascade will take over as primary limitations for O_2 transport. The range of $\dot{V}_{O2max}$ shown in Fig. 4 is more than four-fold and therefore, metabolic capacity and other steps in the O_2 cascade must also change with $\dot{T}_{O2max}$ to produced this range in $\dot{V}_{O2max}$.

Figure 4. $\dot{V}_{O2max}$ as a function of total cardiovascular oxygen supply in chinook salmon from Exp. IV (LS2 and TR), dogfish (D) (Piiper *et al.* 1977), leopard shark (Lai *et al.* 1990), rainbow trout (R1 from Kiceniuk and Jones (1977) and R2 from Appendix I), (C), based on values from Axelsson (1988) for $\dot{Q}_{max}$, Soofiani & Priede (1985) for $\dot{V}_{O2max}$, and Gallaugher (pers. com) for Ca_{O2}. $\dot{V}_{O2max} = 0.84 \cdot \dot{T}_{O2max} - 3.61$ ($R^2 = 0.99$).


400
350
300
250
200
150
100
50
50
100
150
200
250
300
350
400
450
TR
LS2
R1
R2
C
D
VO_{2max} (μmol min^{-1}kg^{-1})
TO_{2max} (μmol min^{-1}kg^{-1})

The basis for the higher $\dot{T}_{O2max}$ in the chinook as compared to other teleost species is explained both by a higher $\dot{Q}_{max}$ and Ca_{O_2}. The $\dot{Q}_{max}$ was 25-83% higher than in rainbow trout (Kiceniuk & Jones, 1977; Appendix, I and II) and Ca_{O_2} in the TR group was as much as 48% higher than in rainbow trout (Kiceniuk & Jones, 1977; Appendix, I) and 63% higher than in Atlantic cod. The $\dot{V}_{O2max}$ of skipjack tuna may be more than four times larger than in chinook salmon (Gooding *et al.* 1981). The Ca_{O_2} of resting skipjack tuna has been reported as 19 $mL \cdot dL^{-1}$ (Brill & Bushnell, 1991a) or 37% higher than in chinook salmon, but Ca_{O_2} may reach 25 $mL \cdot dL^{-1}$ during exercise (Brill & Bushnell, 1991b), which is 77% higher than in chinook salmon. Given this $\dot{V}_{O2max}$ and Ca_{O_2} it is possible to estimate $\dot{Q}_{max}$ of the skipjack tuna as 150-200 $mL \cdot min^{-1} \cdot kg^{-1}$ (Brill & Bushnell, 1991b). Therefore, in all teleost species studied so far, a high $\dot{T}_{O2max}$ is associated with both a high $\dot{Q}_{max}$ and a high Ca_{O_2}.

The $\dot{Q}_{max}$ of the chinook salmon was 37% lower than in dogfish and 14% higher than in the leopard shark (Lai *et al.* 1990). Therefore, the low $\dot{T}_{O2max}$ in the sharks is mainly a function of low Ca_{O_2}, which was 80% (dogfish) and 70% (leopard shark) lower than in chinook salmon.

The high $\dot{Q}_{max}$ of chinook salmon are explained by their ability to simultaneously maintain a high f_H and a high SV_H (Fig. 5), although SV_{Hmax} and f_{Hmax} are not exceptionally high by themselves. Most fish appear to have difficulty with expressing their maximum SV_H when f_H is also at maximum (Farrell 1984; 1985; Farrell *et al.* 1989; Farrell & Jones, 1992). Results from *in vitro* studies of cardiac performance in rainbow trout indicate, that both resting and maximum f_H and SV_H are negatively correlated (Farrell *et al.* 1989), and the same has been observed in *in vivo* experiments (Appendix I). This phenomenon is also evident when f_H and SV_H in the LS2 and TR groups are compared at different swimming speeds (Fig. 3d,e) For example, in the TR group, as f_H began to increase with swimming speed SV_H fell considerably (Fig. 3d, e).

To illustrate this point further, existing data has been plotted in Fig. 5 where SV_H and f_H of various species of fish are shown with isopleths of $\dot{Q}_{max}$. $\dot{Q}$ is a function of f_H and SV_H and the isopleths in Fig. 5 show the $\dot{Q}$ given by any combination of f_H and SV_H. In principle, $\dot{Q}_{max}$ can be increased by any combination of SV_H and f_H. The most effective way of increasing $\dot{Q}_{max}$ would be to change f_H and SV_H in such a manner that $\dot{Q}_{max}$ would increase perpendicular to the isopleths, assuming that the capacity to increase SV_H or f_H is not limited. Thus, it is not surprising to find that the Antarctic fish (P in Fig. 5) regulate $\dot{Q}$ during exercise by varying f_H. Most of the teleosts (Ch, C, E, Ns R, and S in Fig. 5) are clustered in a range where $\dot{Q}_{max}$ could be increased equally well by increasing SV_H or f_H. However, increased f_H in the same species of fish is invariably associated with a reduction of SV_H and therefore, $\dot{Q}_{max}$ does not change in direct proportion to the increase in f_H. Moreover, high f_H and SV_H appear to be mutually exclusive.

Most likely this negative relationship between SV_H and f_H is associated either with problems of maintaining contractility at high f_H or with insufficient filling of the heart at high f_H (Farrell, 1984; Farrell & Jones, 1992). The end diastolic-volume of the ventricle is the primary determinant of SV_H in rainbow trout and cardiac filling is primarily determined by filling time and the pressure differential from the central veins to the atrium (Farrell, 1991). The higher $\dot{Q}_{max}$ of chinook salmon, compared with other species of fish, may be related to greater contractility of the heart which would reduce the duration of the contractile phases and thus leave more time for cardiac filling. The relative size of chinook hearts (0.10 - 0.12% of body-mass) is within the range of what is reported for rainbow trout hearts although it is near the upper limits for heart size in immature fish (0.08-0.13; Farrell *et al.* 1988). The best performance of rainbow trout hearts *in vitro* are better than *in vivo* (Farrell *et al.* 1991; Appendix I). The *in vitro* performance of trout hearts matches that of chinook salmon *in vivo*. Thus, the higher *in vivo* $\dot{Q}_{max}$ of chinook salmon compared with rainbow trout can be as much a result of the

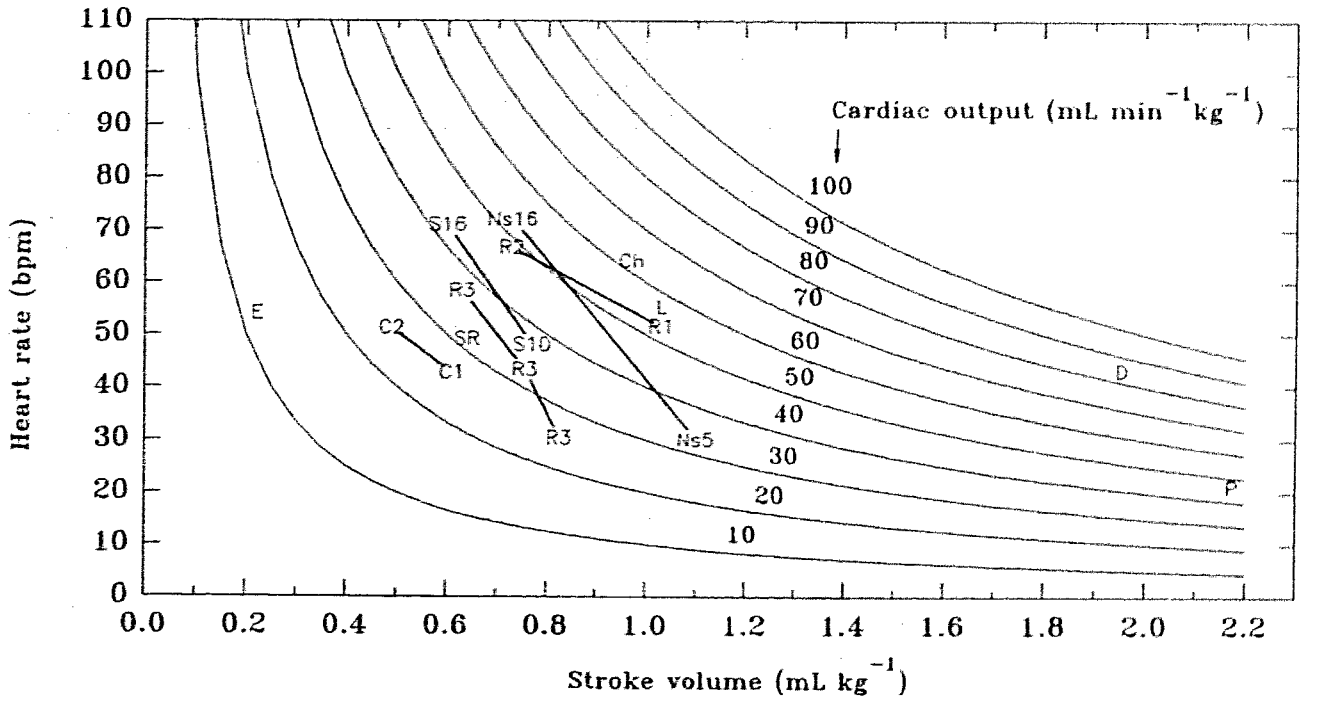
conditions under which the hearts function *in vivo* as it is to the intrinsic properties of the heart. The high $\dot{Q}_{max}$ of chinook salmon may therefore be related to their ability to maintain a high filling pressures for the heart while swimming maximally, possibly by shifting blood volume from peripheral and splanchnic veins to the central veins (Satchell, 1992).

The reduction in Pa_{O_2} as swimming speed increased (Table 7, Table 10, Appendix I) has been observed in more recent studies of swimming salmonids (Thomas *et al.* 1987; Butler *et al.* 1991; Gallaugher *et al.* 1992). This decrease in Pa_{O_2} can be caused either by a disproportionate increase in ventilation volume and $\dot{V}_{O_2}$ or by some form of diffusion limitation for O_2 at the gills. Since ventilation volume increases in step with $\dot{V}_{O_2}$ (Stevens & Randall, 1967; Piiper *et al.* 1977; Kiceniuk & Jones, 1977; Appendix III), it is unlikely to cause a reduction in Pa_{O_2}. However, the total conductivity for O_2 at the gills may not increase in step with $\dot{V}_{O_2}$, and that would cause reduced Pa_{O_2}. It is also possible that O_2 binding to haemoglobin is not at equilibrium with plasma O_2 as the blood passes through the gill lamellae, and therefore, Pa_{O_2} decreases as the arterial blood leaves the gills and O_2 levels equilibrate.

Although the Pa_{O_2} decreased from from rest to U_{crit}, Ca_{O_2} and saturation (Table 7) did not change significantly. Therefore, cardiovascular O_2 transport was not compromised. However, the reduced Pa_{O_2} could affect the mean driving pressure for O_2 from capillaries to mitochondria. The saturation curve for O_2 as a function of P_{O_2} is sigmoidal and since O_2 saturation did not change significantly between 8.5 and 14.1 kPa, those points must be on the upper, flat portion of the saturation curve. The mean driving pressure for O_2 out of the capillaries is proportional to the mean capillary P_{O_2}. Because of the sigmoidal shape of the O_2 dissociation curve and because 8.5 kPa is still on the flat portion of the curve, a reduction in Pa_{O_2} from 14.1 to 8.5 kPa will only have a minimal effect on the driving pressure for O_2. Therefore, it is concluded that the reduction in Pa_{O_2} is of little

significance for O_2 delivery under normoxic conditions because $\dot{V}_{O_2}$ and mean driving pressure for O_2 out of capillaries is not affected appreciably.

Figure 5. Stroke volume and heart rate at $\dot{Q}_{max}$ for different species of fish. Superimposed on the graph are isopleths for $\dot{Q}$. Ch: chinook salmon (Present experiment); C1: Atlantic cod (Axelsson, 1988); C2: Atlantic cod (Axelsson & Nilsson, 1986); D: dogfish (Piiper, *et al.* 1977); E: Eel (*Anguilla australis schmidtii*; Davie & Forster, 1980); L: Leopard shark (Lai *et al.* 1989) Ns5, Ns16: Northern squawfish at 5 and 16° C respectively (Kolok & Farrell, in press); P: *Pagothenia borchgrevinki* (0-1° C) (Axelsson *et al.* 1992); R1: rainbow trout (Kiceniuk & Jones); R2: rainbow trout (Appendix 1); R3: isolated heart preparation in Farrell *et al.* 1989; S10, S16: Largescale suckers at 10 and 16° C respectively (Kolok *et al.* 1993); SR: sea raven (*Hemitripterus americanus*) (Axelsson *et al.* 1989).


Heart rate (bpm)
Stroke volume (mL kg^{-1})
Cardiac output (mL min^{-1} kg^{-1})
110
100
90
80
70
60
50
40
30
20
10
0
0.0
0.2
0.4
0.6
0.8
1.0
1.2
1.4
1.6
1.8
2.0
2.2
E
C2
C1
SR
S16
Ns16
R3
S10
R3
R3
R3
Ch
L
R1
Ns5
D
P

Chapter 6

Blood flow distribution in chinook salmon

Introduction

In this experiment (Exp. V), radiolabeled microspheres were used in an attempt to measure blood flow distribution in fish under various conditions. This method has been used previously to measured blood flow distribution in fish (Kent, 1973; Cameron 1975; Daxboeck, 1981; Neumann *et al.* 1983; Barron *et al.* 1987; White *et al.* 1988; Kolok *et al.* 1993). The microspheres are injected into the circulation and are subsequently lodged in the capillaries in proportion to blood flow distribution to various tissues.

There are difficulties involved in applying the microsphere method to fish. When used with mammals, the microspheres are injected into the heart where they mix with the blood and are then distributed throughout the cardiovascular system. In fish this approach does not work, because the microspheres would be trapped in the gill lamellae. Therefore, the microspheres have to be injected, either into the efferent branchial arteries or into the dorsal aorta. This means that tissues that are perfused by vessels which do not branch off the dorsal aorta, such as the cephalic circulation and the coronary circulation (See Chapter 4), are excluded from the estimates of blood flow distribution. Furthermore, if the microsphere method is to work properly, the microspheres have to be uniformly mixed with the blood. When the microspheres are injected into the dorsal aorta of fish, there is an obvious problem of adequate mixing of microspheres with the blood stream prior to some of the first arterial branches of the dorsal aorta, such as the CMA, which also happens to be the major branch of the systemic circulation.

Inadequate mixing of microspheres may be one of the reasons why intestinal blood flow is consistently underestimated as being 2-21% of cardiac output (Kent, 1973; Cameron

1975; Daxboeck, 1981; Barron *et al.* 1987; White *et al.* 1988; Kolok *et al.* 1993) compared with studies where intestinal blood flow is measured with flow probes (Axelsson *et al.* 1989; Axelsson & Fritsche, 1991; Axelsson, Thorarensen, Nilsson & Farrell, in review). The reason for this is not clear, but it has been suggested that either the cannulae protrude beyond the opening of the coeliacomesenteric artery or that the microspheres are incompletely mixed in the blood as they pass the opening of the coeliacomesenteric artery (Barron *et al.* 1987; Bushnell *et al.* 1992).

In an attempt to resolve the problem of the under representation of gut blood flow, I tried to inject radiolabeled microspheres into a point, upstream of the dorsal aorta, i.e., in the efferent branchial artery of the 2nd gill arch (see Fig. 2). If this approach worked, the method could have been used to measure not only gut blood flow, but also relative distribution of blood flow to other tissues in a less invasive manner than by using flow probes. Still, the cephalic circulation would have been underestimated, but this is likely a small fraction of total cardiac output and therefore the error involved would be relatively small. The results of this study on the blood flow distribution in chinook salmon (Exp. V) are presented here.

Methods

Preparation of fish: Chinook salmon (200-460g) were anaesthetized as described in the General Methods (Chapter 2). A needle (26 g) was bent at a 20° angle and fitted to the tip of a cannula made from PE50 tubing that had previously been pulled over heat to the appropriate diameter. The needle was inserted dorsad into the second gill arch just under and perpendicular to the gill filaments, parallel to the gill arch. The position of the cannula was confirmed by the presence of freely flowing blood under pressure in the cannula. The cannula was sutured to the gill arch and then to the dorsal side of the fish. The fish were placed in a black holding box and allowed to recover for 24 hours.

The microspheres (Du Pont, Wilmington DE) were 15 μm in diameter and labeled with either Sc^{46} (Specific activity 12.22 $mCi \cdot g^{-1}$) or Sn^{113} (Specific activity 12.52 $mCi \cdot g^{-1}$). Stock solutions were made up by suspending $1.2 \cdot 10^6$ microspheres per mL of saline (0.9% NaCl). To ensure proper mixing, the stock solutions were vortexed and sonicated before they were injected into the fish. For injection, 0.5 mL of the stock solution was drawn into a syringe and the syringe was shaken constantly with a mechanical shaker while the solution was injected over a period of 45-60 sec. The microsphere solution was cleared from the cannula with 0.5 mL of saline.

Preparation and counting of tissues samples.

The tissue samples were weighed and placed in test tubes for counting. Eyes, opercular muscle and ventricular muscle were sampled whole. The liver, spleen, pyloric region, midgut and hindgut were placed in one or more test tubes, depending on organ size. Kidney and gills were weighed and duplicate subsamples taken. The fish were filleted and six subsamples were taken of white muscle, red muscle and skin, three on either side, one pair behind the pectoral fins, a second pair behind dorsal fish and a third pair of samples at the tail. The total weight of white and red muscle in each fillet was obtained

by separating and weighing the white and red muscle from the entire fillet. After all samples had been collected, the remainder of the fish was homogenized with a measured volume of water. Subsamples of the homogenate were counted to estimate the total amount of label injected.

The radioactivity of the samples were counted in a multichannel gamma counter at the Radiology Lab. at the British Columbia Institute of Technology. The Sn^{113} activity was counted in a window between 380-420 KeV and Sc^{46} from 800-2000 KeV and both labels were counted for 2 minutes. Samples were corrected for background radioactivity by counting activity in blank samples.

Fractional blood flow distribution was calculated as:

Total count of label in tissue / Total counts of label in fish.

The total counts of a label in a tissue were obtained either as direct counts if the whole tissue was counted, or if subsamples were counted as:

counts in subsample · total weight of tissue / weight of subsample

Total counts in the fish were estimated from the mean count of the subsamples of the homogenate of the residual tissues.

Total counts in fish = (Mean count in homogenated sub samples · ((weight of tissue homogenated) + (weight of water added to the homogenate)) / (weight of subsample)) + Total counts of samples from all tissues sampled.

Results

The relative weights of different tissues in chinook salmon (Table 8) were similar to those reported for rainbow trout (Stevens, 1968). However, the relative mass of the red aerobic muscles was five times greater in chinook salmon than in the rainbow trout. Both estimates refer only to the distinct lateral mass of red muscle and do not include red fibres that may be found mixed in with the white muscle mass, or in other body locations.

Blood flow distribution was measured with microspheres in resting unfed chinook salmon (n=12) (Table 9). The blood flow distribution was, similar to what has been observed in rainbow trout (Daxboeck, 1981; Barron, 1987). Most of the microspheres (48%) were trapped in the white or mosaic muscle mass. Entrapment of microspheres in the gut (13%) was similar to rainbow trout and within the range (2-21%) of values reported for fish (Cameron, 1975; Daxboeck 1981; Barron *et al.* 1987; White *et al.* 1988; Kolok *et al.* 1993). There appeared to be less blood flow to the kidney and more blood flow to the skin in chinook salmon than in rainbow trout. There was more blood flow to the gonads in Daxboeck's fish, since they were maturing, while chinook salmon were all immature.

I also attempted to measure blood flow distribution in fed fish 3, 6, 12 and 24 hours postprandial with a second injection of microspheres with a different radiolabel. However, only few of the second injections of the microspheres were successful and therefore these results were inconclusive.

Table 8 Relative weight of different tissues in chinook salmon and rainbow trout*.

	Chinook	Rainbow trout
Eyes	0.8	
Opercular muscles	0.7	
Brain	0.2	
Ventricle	0.12	0.2
Kidney	1.2	
Liver†	1.4	1.4
Spleen†	0.2	0.3
Stomach†‡	0.2	
Pyloric caecae†‡	2.4	
Midgut†‡	0.1	
Hindgut†‡	0.2	
Gut total†‡	2.9	5.1
Gonads†	0.2	2.6
Swim bladder†	0.2	
Total supplied by CMA	4.3	
Gills	2.7	3.9
White (mosaic) muscle	57.1	66.0
Red muscle	5.4	1.0
Skin	6.0	4.0
Other	19.8	5.1
Head		10.3

* Values from Stevens (1968).
Values indicated by † are included in Total supplied by CMA
Values indicated by ‡ are included in Gut total.

Table 9. Blood flow distribution (%) in resting unfed chinook salmon (at 7-8° C) and rainbow trout (10-12°C).

Tissue	Chinook	Rainbow trout	
		Daxboeck.*	Barron†
White (mosaic) muscle	48.3	49.0	55.7
Red muscle	11.3	11.2	2.4
Tissues perfused by the CMA‡	12.7	13.0	21.3
Liver	3.13		2.4
Stomach	0.52		3.8
Pyloric caecae	4.58		8.8
Intestines	1.14		5.2
Spleen	3.20		1.1
Swimbladder	0.10		
Kidney	1.37	4.5	4.6
Skin	15.7	8.3	
Gonads ‡‡	0.02	10.0	
Gills	3.02		
Brain	0.07		
Opercular muscles	0.01		
Ventricle	0.08		<0.02
Eyes	<0.01		

* From Daxboeck 1981 (at 10° C).
† From Barron *et al.* 1987 (at 12° C).
‡ CMA: Coeliacomesenteric artery supplies most of the blood flow to the intestines.
‡‡ Strictly speaking the gonads should be included with the CMA. However, only in Daxboeck's study was there appreciable blood flow to the gonads and therefore they are listed separately to facilitate comparison of total gut blood flow in all studies.

Discussion

The relative weight of the red lateral muscle mass in chinook salmon is about five times larger than in rainbow trout (Table 8). This is consistent with the greater aerobic capacity of chinook salmon. At rest, the blood flow to the red muscle was 11% in chinook salmon and comparable to red muscle blood flow as estimated by Daxboeck (1981) for rainbow trout. However, Barron *et al.* (1987) estimated red muscle blood flow as 2% of cardiac output and that would suggest that blood flow per g red muscle was the same in chinook salmon and rainbow trout. However, the large variation of these data makes comparisons difficult.

The microspheres were injected into the efferent artery of the 2nd gill arch and therefore the entrapment in the eyes, the opercular muscles, the brain and the heart does not reflect a true distribution of cardiac output. In fact, the low entrapment in these tissues suggests that there was very little recirculation of microspheres and that essentially all the microspheres were trapped during the first passage through the capillaries. Therefore, entrapment of microspheres in the liver and in the kidney will only reflect arterial blood supply to these tissues and does not include blood flow through their respective portal systems.

The entrapment of the microspheres in this study was for the most part similar to that observed in rainbow trout (Table 9). A similar proportion (48-55%) of the microspheres was lodged in the white muscles in all studies. Slight differences in blood flow to the kidney and the skin between sea water chinook salmon and fresh water rainbow trout may reflect different osmotic challenges of the two environment. Seawater fish must retain water and minimise urine production, while fresh water fish must excrete water as hypotonic urine. Therefore, chinook salmon are likely to have reduced kidney function compared with fresh water rainbow trout (Hickman & Trump, 1969).

Even though the microspheres were injected into the efferent artery of the 2nd gill arch, the entrapment of microspheres in the viscera was comparable (12.7%; Table 12) to that reported in other studies where the microspheres were injected directly into the dorsal aorta (Kent, 1973; Cameron 1975; Daxboeck, 1981; Barron *et al.* 1987; White *et al.* 1988; Kolok *et al.* 1993). The estimates in rainbow trout were 13% (Daxboeck, 1981) and 21.5% (Barron *et al.* 1987). These values are clearly much lower than 34% of $\dot{Q}_{rest}$ reported in Chapter 7 and the 30-40 % of $\dot{Q}$ reported in other studies where flow probes have been used to measure intestinal blood flow (Axelsson *et al.* 1989; Axelsson & Fritsche, 1991; Axelsson, Thorarensen, Nilsson & Farrell, in review). Therefore, the attempt to improve microsphere mixing with blood by injecting them into the efferent branchial artery was unsuccessful. Primarily, because the cephalic circulation is missed, the intestinal blood flow is underestimated. However, the microsphere method may have value in fish to measure blood flow distribution between red and white trunk muscles.

Apart from incomplete mixing of microspheres with blood, other reasons may also contribute to the discrepancy between the blood flow distribution measured with the microsphere method and with flow probes. It is possible that the injection of the microspheres could induce a stress response involving a reduction in gut blood flow. This response is evident in Fig. 6 where the intestinal blood flow of sham-fed fish is reduced for the first few hours after they were disturbed. Furthermore, blood from the third and fourth gill arches could be preferably diverted to the intestines given the close proximity of the epibranchial artery from the two posterior gill arches to the opening of the CMA (Fig. 2). Therefore flow in the coeliacomesenteric artery is underestimated when the microspheres are injected into the efferent branchial arteries from the first and second gill arches. . Thus, the cardiovascular anatomy of fish and other problems such as potential stress responses and incomplete mixing may result in underestimates of gut blood flow when the microsphere method is used.

Since the microsphere method proved to be inappropriate for measuring blood flow distribution in chinook salmon, blood flow distribution was measured with flow probes (Chapter 7). The only vessels which are accessible for implanting flow probes in fish are the ventral aorta and the bigger arteries and veins of the gut circulation. Therefore, blood flow to the gut of chinook salmon was measured in this thesis to estimate, among other, the relevance of redistribution of blood flow during exercise for O_2 delivery to muscles. Blood flow to muscles were estimated in Chapter 8 using .

Chapter 7

Intestinal blood flow.

Introduction.

In this chapter are presented results from Exp. III and VI. Exp. III was performed to measure changes in intestinal blood flow in swimming fish, and to examine how gut blood flow in unfed fish changed relative to other variables such as $\dot{V}_{O_2}$. Exp. III also examined if training affected $\dot{T}_{O_2}$ and $\dot{q}_{IA}$ during exercise. Exp. VI was performed to measure postprandial changes in intestinal blood flow.

Methods.

Chinook salmon were exercise trained for 8 months, swimming at either 0.5 bl·s^{-1} (LS1) or 1.5 bl·s^{-1} (HS). Holding tanks and husbandry were as was described in the General Methods (Chapter 2). Fish from both training groups were cannulated in the dorsal aorta and a Doppler flow probe was placed on the IA. The fish were given at least 24 hours to recover before experiments were commenced. The operated fish were then swum to U_{crit} while blood flow in the IA ($\dot{q}_{IA}$), $\dot{V}_{O_2}$, and various haematological variables were measured. Food was withheld from the fish for at least 24 hours before the operation.

To measure absolute blood flow in the IA and to assess the effect of blood O_2 capacity on $\dot{q}_{IA}$, a Transonic flow probe was placed on the IA in a separate group of LS1 fish. The fish were placed in black holding boxes and given 24 hours to recover before experiments began. The $\dot{q}_{IA}$ was recorded by a computer every minute overnight. Between 04:00 h and 08:00 h $\dot{q}_{IA}$ was the most stable, so these values were averaged to represent resting blood flow. Following this period, Hct and Ca_{O_2} were measured and 1 mL of blood removed from the fish and replaced with saline to reduce Hct. Hct was reduced in a progressive fashion by repeating the same procedure one to three times at 24 h intervals

and the relationship between Hct and absolute values for $\dot{q}_{IA}$ was established for individual fish. A few attempts were made to increase Hct with a transfusion of packed red blood cells, but only two attempts were successful.

The measurements of postprandial changes in $\dot{q}_{IA}$ were performed on a separate group of fish which had not been exercise-trained but kept in circular outdoor tanks and fed daily to satiation. The fish were not fed 24 hours prior to the operation. A flow probe was implanted on the IA of anaesthetized fish as described above and in the General Methods (Chapter 2) and they were placed in black holding boxes. The fish were given at least 24 hours to recover before experiments began. The $\dot{q}_{IA}$ of unfed fish was recorded for 10 minutes every hour for 24 hours before the fish were fed. The recordings during the last 5 hours were averaged and used as resting value. Thus, the fish had not been fed for 43 hours prior to the recording of the resting values. The results of this experiment showed that this is adequate time for $\dot{q}_{IA}$ to return to resting values after the fish have been fed (Fig. 6). Eight fish were force-fed the 2 % body mass as described in Chapter 3 and placed back in the black box. The $\dot{q}_{IA}$ was then recorded as before for 40 hours. Control fish (n=5) were sham-fed and treated the same way as fed fish, except food was not introduced into the stomach.

Results.

Postprandial changes in gut blood flow.

Following feeding, $\dot{q}_{IA}$ increased by a maximum of 81±9.3% (Fig. 6). After 30 hours $\dot{q}_{IA}$ had returned to resting levels and by 36 hours postprandial the $\dot{q}_{IA}$ of the fed fish was not significantly different from that of control fish. There was some variability in when peak flow was reached, but on the average it was recorded 23±1 hours postprandial. Because of this variability in when peak flow was recorded in individual fish, the mean peak flow was higher than the mean flow of all fish at 23 hours postprandial (Fig. 6).

Gut blood flow in unfed fish during exercise.

The relative changes in $\dot{q}_{IA}$ were linearly (r^2=0.83) related to $\dot{V}_{O_2max}$ in both HS and LS1 groups (Fig. 7). Thus, as the O_2 demand of the locomotory muscles increases blood flow to the gut is reduced in direct relationship with the increased $\dot{V}_{O_2}$.

Since $\dot{V}_{O_2}$ increased exponentially with swimming speed, the reduction in $\dot{q}_{IA}$ was also exponential. As swimming speed increased up to 50-60% of U_{crit}, the relative $\dot{q}_{IA}$ did not change significantly (Fig 8a), consistent with $\dot{V}_{O_2}$ not changing at these velocities. At higher velocities $\dot{q}_{IA}$ decreased abruptly. By U_{crit}, $\dot{q}_{IA}$ was reduced by 50-70% compared with resting values.

The $\dot{q}_{IA}$ was reduced because splanchnic resistance increased (Fig. 8b). The splanchnic resistance was significantly ($p<0.004$) greater in the LS1 group (334%) than in the HS group (128%) at the mean U_{crit}. f_H increased significantly with swimming speed in both groups, but P_{DA} did not change with swimming speed. Neither the f_H nor the P_{DA} were significantly different in the two groups.

Figure 6. Postprandial increase in blood flow in chinook salmon (n=8) after they were force-fed paste made from equal volumes of dry feed (2% body-mass) and water. Control fish (n=5) were sham fed. The mean maximum flows for all fish is shown at the mean time postprandial when it occurred. * denotes significantly different from prefeeding levels and † denotes significant difference between fed fish and controls.

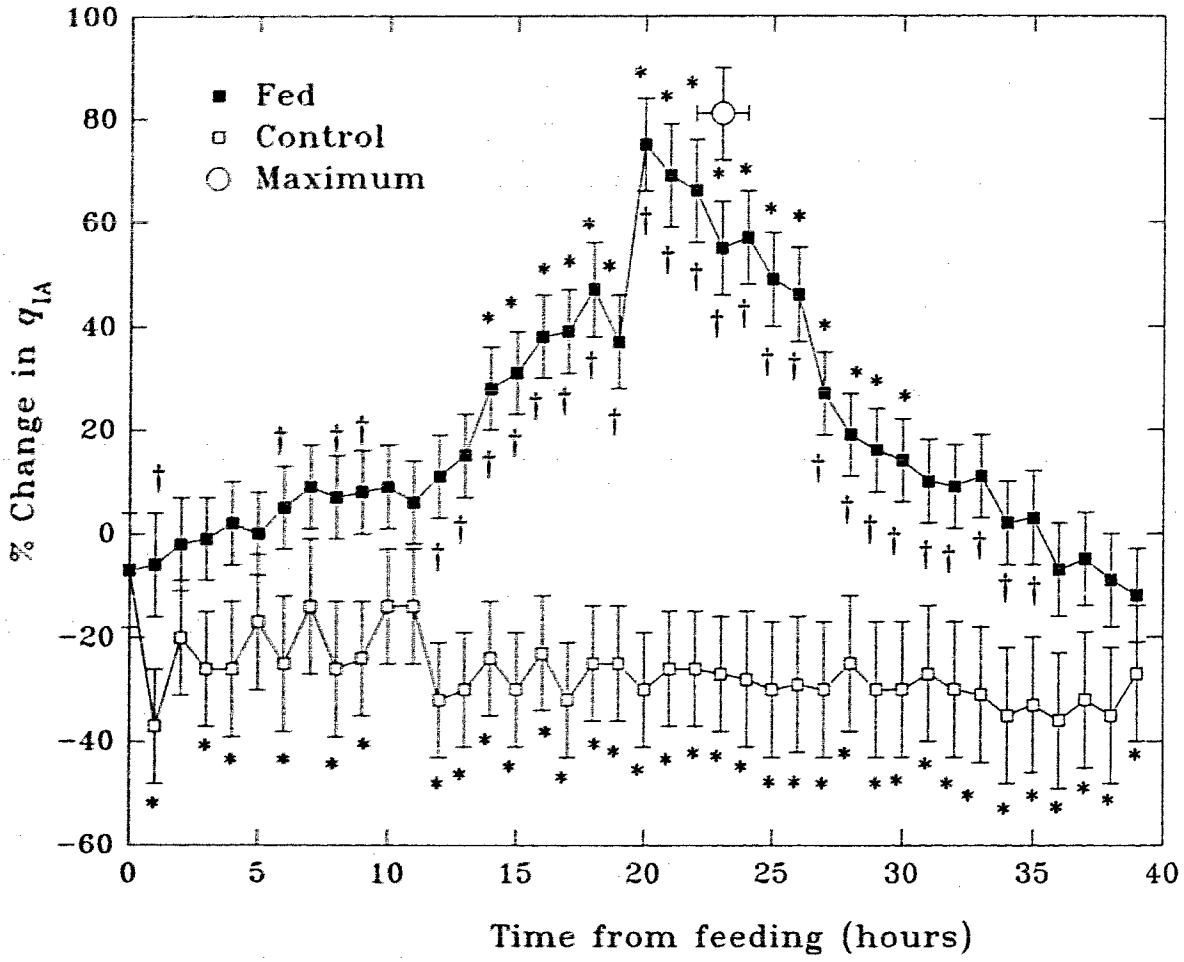
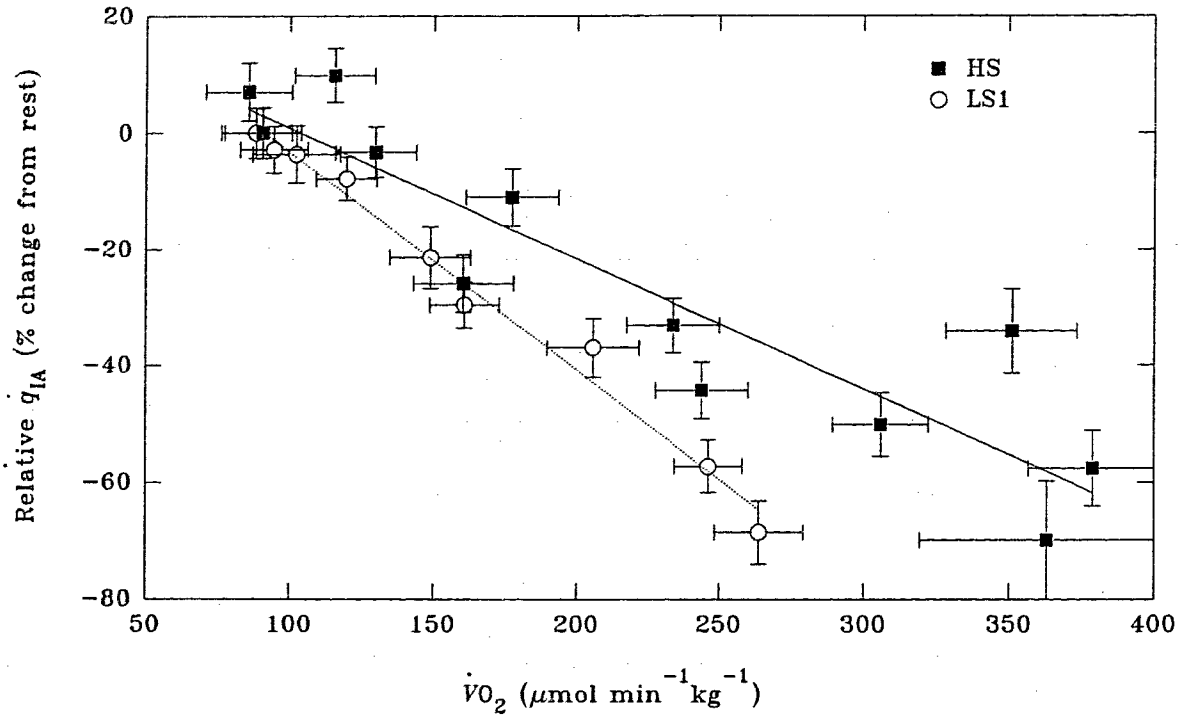

Fed
Control
Maximum
% Change in g_{IA}
Time from feeding (hours)
100
80
60
40
20
0
−20
−40
−60
0
5
10
15
20
25
30
35
40

Figure 7. Relative changes in intestinal blood flow in fish from Exp. III. as a function of $\dot{V}_{O_2}$. The curves are: HS: $\dot{q}_{IA} = 23.246 - 0.224 \cdot \dot{V}_{O_2}$ ($R^2 = 0.920$); LS1: $\dot{q}_{IA} = 34.3 - 0.376 \cdot \dot{V}_{O_2}$ ($R^2 = 0.992$). The slopes of the two curves are not significantly different.


20
0
−20
−40
−60
−80
50
100
150
200
250
300
350
400
HS
LS1
Relative $\dot{q}_{IA}$ (% change from rest)
$\dot{V}O_2$ (μmol min^{-1}kg^{-1})

Training effects on gut blood flow.

The HS-trained fish maintained intestinal blood flow better as swimming speed increased. A comparison (Two-way ANOVA with repeated measures) showed that blood flow was reduced less ($p<0.0003$) in this group than in the LS1 group. There was some variability in the maximum swimming speed of fish from both groups and fewer fish contributed to each mean value as swimming speed increased (Fig. 8). Therefore, the statistical analysis for the data presented in Fig. 8 only included values that were equal to or lower than the mean U_{crit} in both groups. The best swimmers in the HS group swam faster and were treated separately at the highest swimming speeds (values are shown in Fig 8a but not connected with a line). These good swimmers, which were better able to maintain gut blood flow than the other fish (Fig. 8a), give further support to the observation that trained fish could better maintain gut blood flow while swimming.

Haematology: As in Exp. IV, the mean Hct of the fish which were exposed to the more stringent training regime (HS) was significantly ($p<0.0001$) higher than in the LS1 fish (Table 10). However, unlike Exp. IV this difference in Hct was reflected in the significantly ($p<0.0001$) higher [Hb] and Ca_{O_2} (Table 10). The Pa_{O_2} of both groups was reduced significantly as in Exp. IV, but was not different in the two groups. Although the Ca_{O_2} was higher in the HS group, the $\dot{V}_{O_{2max}}$ in the groups were not significantly different. However, Hct had a significant effect on $\dot{V}_{O_2}$ as indicated by the following relationship ($R^2=0.71$):

$$\dot{V}_{O_2} = -75.8 + \ln_{Hct} + \ln_{Hct} \cdot U^2$$

which suggests that fish with high Hct had a higher $\dot{V}_{O_2}$ at all swimming velocities.

Figure 8. Cardiovascular changes during exercise in chinook salmon, swimming with a DA cannula and a Doppler flow probe on the intestinal artery. Individual points represent mean values for 2-3 fish of the group that swam faster than the remainder. a: Blood flow in the intestinal artery expressed as the percentage change from the resting level. There is significant difference between groups ($p<0.0003$) and significant changes in both groups as swimming speed increases ($p<0.0001$). b: The relative resistance of the splanchnic vascular bed. Resistance increases significantly with swimming speed in both groups ($p<0.0001$) and resistance increased significantly ($p<0.004$) more at lower swimming velocoities in the LS1 group than in the HS group. The figure continues on the next page.

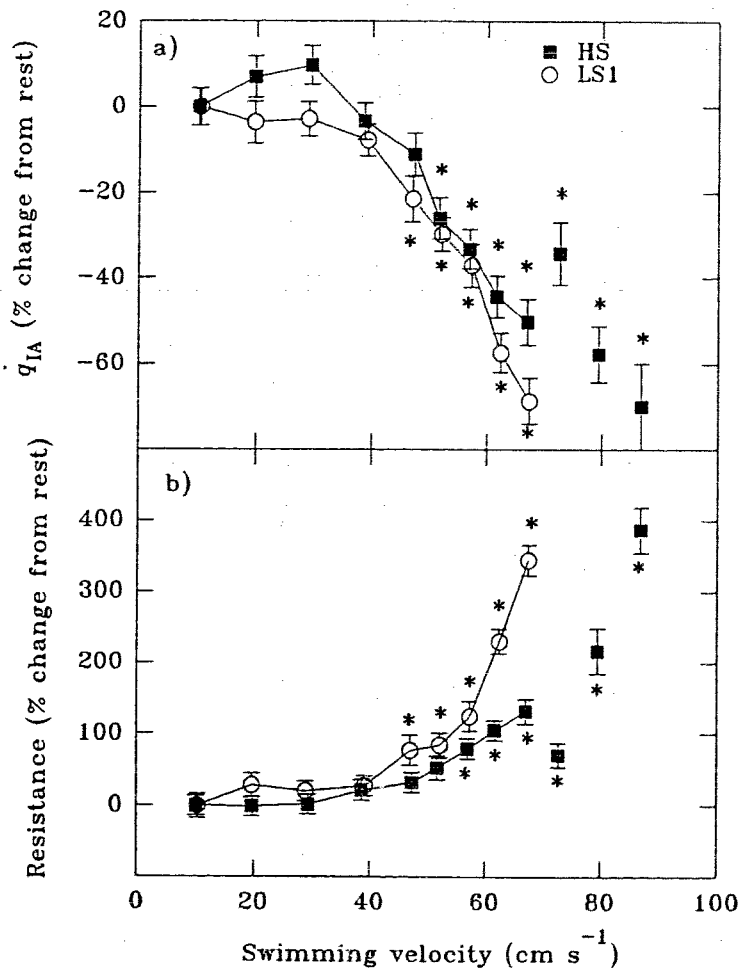

a)
b)
HS
LS1
20
0
−20
−40
−60
400
300
200
100
0
0
20
40
60
80
100
$\dot{q}_{IA}$ (% change from rest)
Resistance (% change from rest)
Swimming velocity (cm s^{-1})

Figure 8 cont. c: Pressure in the dorsal aorta. The results from the two groups are not significantly different and there are no significant changes with swimming speed. d: Heart rate shows a significant increase with swimming speed ($p<0.0001$). but heart rate in the two groups was not significantly different. .

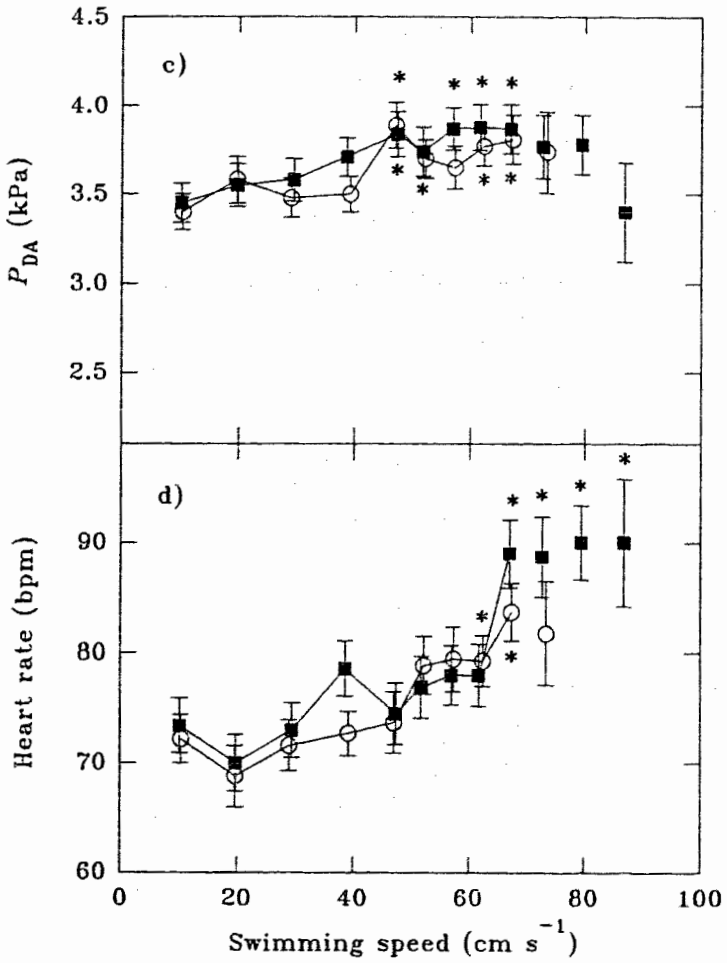

c)
d)
P_{DA} (kPa)
Heart rate (bpm)
Swimming speed (cm s^{-1})
4.5
4.0
3.5
3.0
2.5
90
80
70
60
0
20
40
60
80
100

Table 10. Haematocrit (Hct), blood O_2 content (Ca_{O_2}), haemoglobin concentration ([Hb]), arterial P_{O2} (Pa_{O_2}), mean cell haemoglobin concentration (MCHC) and plasma lactate concentration in chinook salmon trained at either 0.5 bl·s^{-1} (LS1; N=15) or 1.5 bl·s^{-1} (HS; N=11).

		Swimming		Recovery			
	Tr. speed	Rest	1 bl·s-1	85% U_{crit}	U_{crit}	1 h	Mean***
Hct	LS1	23.3(0.9)	23.8(0.8)	24.4(0.6)	24.5(0.9)	22.1(1.0)	23.3 †
	HS	27.1(1.0)	27.2(0.9)	28.4(1.0)	28.3(1.0)	26.7(1.0)	27.6
Ca_{O_2}	LS1	8.3(0.5)	9.3(0.4)	9.1(0.3)	8.5(0.5)	8.3(0.5)	8.7 †
(vol%)	HS	10.9(0.6)	11.0(0.5)	11.5(0.4)	10.5(0.6)	10.6(0.5)	10.9
[Hb]	LS1	7.16(0.3)	6.98(0.3)	7.22(0.2)	7.21(0.3)	6.57(0.3)	7.0 †
(g·dl^{-1})	HS	8.61(0.3)	8.24(0.3)	8.61(0.2)	8.37(0.4)	8.07(0.3)	8.4
Pa_{O_2}	LS1	15.9(0.5)	15.0(0.5)	13.1(0.4)	11.3(0.5)	15.3(0.5)	14.1 *
(kPa)	HS	15.7(0.6)	14.8(0.5)	12.2(0.4)	10.4(0.6)	15.2(0.6)	13.6
MCHC	LS1	304(5)	290(4)	294(3)	295(5)	297(5)	296 ‡
(g·L^{-1})	HS	312(6)	303(5)	305(5)	295(6)	303(5)	304
Lactate	LS1	0(0.5)			1.5(0.4)	2.6(0.3)	1.4 *
(mmol·L^{-1})	HS	0.1(0.5)			1.7(0.4)	2.1(0.4)	1.3 *

* Indicates significant difference as swimming speed increases

** Mean value for group at all swimming velocities and during recovery.

† Indicates significant difference between HS and LS1 groups.

‡ Indicates significant difference between mean responses of the two training groups.

Intestinal blood flow as a function of Hct.

Since the Hct was different in the two groups of fish in Exp. III, and the measurements of $\dot{q}_{IA}$ in swimming fish were made with pulsed Doppler flow probes which measure only relative changes in blood flow $\dot{q}_{IA}$, it was of interest to calibrate absolute $\dot{q}_{IA}$ and to examine whether Hct affected $\dot{q}_{IA}$. Therefore $\dot{q}_{IA}$ was measured at rest in a separate group of fish with a Transonic flow probe, while the Hct of the fish was adjusted to reflect the broad physiological range (20-36%) seen in the HS and LS1 fish.

Absolute $\dot{q}_{IA}$ was inversely related to Hct ($p<0.0002$) for all individual fish ($n=10$). A linear regression of $\dot{q}_{IA}$ *versus* Hct that assumed the same slope for all fish but with different intercepts explained 86% of the total variance:

$$\dot{q}_{IA} = I_f - 0.57 \cdot Hct$$

where I_f is the intercept for individual fish. The mean intercept for all fish was 27.5±1.5% and the range of Hct used for this analysis was from 20% to 36%. There was a considerable variability among individuals in the absolute level of $\dot{q}_{IA}$ and I_f ranged from 22 to 36%. There was no significant ($p<0.2$) difference among the slopes of individual fish, which ranged from 0 to -1.2.

Discussion

Postprandial gut blood flow.

This is the second study to measure intestinal blood flow continuously over a period of time postprandially. The postprandial increase in gut blood flow was transient and peaked after 23 hours. By 35 hours postprandial, the blood flow had returned to resting levels. The 81% postprandial increase in gut blood flow observed in chinook salmon is in keeping with the 60-110% increase observed in sea raven (Axelsson *et al.* 1989), Atlantic cod (Axelsson & Fritsche, 1991) and red Irish lord (Axelsson, Thorarensen, Nilsson & Farrell, in review). In addition to the maximum relative increase in postprandial gut blood flow being similar in chinook salmon and red Irish lord, the absolute increase gut blood flow in chinook salmon (about 10 $mL \cdot min^{-1} \cdot kg^{-1}$) was similar as in the Red Irish Lord (about 8 $mL \cdot min^{-1} \cdot kg^{-1}$). The peak values in the coeliac artery blood flow of the Red Irish Lord and the $\dot{q}_{IA}$ of chinook salmon were also reached at approximately the same time, but gut blood flow remained elevated for much longer time in the red Irish lord (Axelsson, Thorarensen, & Farrell, in review). Blood flow in the coeliac artery plateaued after 24 hours and then remained elevated for over three days, and blood flow in the mesenteric artery plateaued after 48 hours and had not decreased four days later. The different duration of the elevated postprandial blood flow are likely due to the amount of food ingested. The red Irish lord was fed 10-15% of body mass of cut up salmon, while chinook salmon were fed 2% body mass of dry pellets ground into paste.

The finding in Exp. I, that $\dot{V}_{O_2}$ had returned to pre-feeding levels two days postprandial, concurs with the results of this study, that $\dot{q}_{IA}$ had returned to resting levels 35 hours postprandial. However, in Exp. I the $\dot{V}_{O_2}$ of fed fish was increased 15 hours postprandial, but as is evident from Fig. 6 that intestinal blood flow is only beginning to increase at that

time. It is possible that the combined effect of the operation and force-feeding delayed the increase in $\dot{q}_{IA}$. The decrease in $\dot{q}_{IA}$ of the control fish, after they were sham fed, may suggest that they were stressed and this may have contributed to a delay in the increase of $\dot{q}_{IA}$.

Exercise.

This is the first study to report changes in intestinal blood flow as a function of swimming velocity and also the first to measure intestinal blood flow in salmonids with flow probes.

The results of this study clearly show that the pumping capacity of chinook heart is not enough to allow the fish to maintain maximum aerobic swimming velocity without redistributing blood flow away from the viscera to the locomotory muscles. At maximum swimming velocity intestinal blood flow was reduced by 60-70%. A 30% reduction in gut blood flow has also been reported for Atlantic cod (Axelsson & Fritsche, 1991). This is the first time that the reduction in gut blood flow during exercise has been shown to be linearly related to $\dot{V}_{O_2}$. This indicates that at swimming velocities over 50-60% of U_{crit} gut blood flow is regulated relative to the metabolic demands of the locomotory muscles. The exact control mechanism for this is unknown. However, the resistance of the intestinal vasculature of fish increases in response to adrenergic, cholinergic and peptidergic stimulation (Holmgren & Nilsson, 1974; Axelsson *et al.* 1989; Olson & Meisheri, 1989; Axelsson & Fritsche 1991; Holmgren *et al.* 1992; Xu & Olson, 1993; Thorarensen & Farrell, unpubl. obs.)

Based on the relationship that was established between $\dot{q}_{IA}$ and Hct, it was possible to predict that the resting $\dot{q}_{IA}$ in unfed fish should have been 14.2 $mL \cdot min^{-1} \cdot kg^{-1}$ and 12.1 $mL \cdot min^{-1} \cdot kg^{-1}$ for the LS1 and HS fish respectively. As described in Chapter 4, $\dot{q}_{IA}$ is likely 85% of total blood flow to the gut. This would mean that total gut blood flow was

14-17 mL·min^{-1}·kg^{-1}. This range is comparable to values reported for blood flow in the hepatic portal vein in anaesthetized rainbow trout (13 mL·min^{-1}·kg^{-1}; McLean & Ash, 1989), but considerably higher than the total gut blood observed in more sluggish species of fish: 5.8 mL·min^{-1}·kg^{-1} in sea raven (Axelsson *et al.* 1989; Axelsson, 1990); 7.6 mL·min^{-1}·kg^{-1} the Atlantic cod (Axelsson & Fritsche, 1991); 9.0 mL·min^{-1}·kg^{-1} in the red Irish lord (Axelsson, Thorarensen, Nilsson & Farrell, in press). All these measurements were performed between 9 and 12° C and therefore this difference in gut blood flow, which can be up to three-fold, may be an important species difference.

The proportion of $\dot{Q}_{rest}$ that is diverted to the gut can be calculated for the fish in Exp. IV for which the cardiac output and Hct are known. The intestinal blood flow in the LS2 fish (Hct 30%) in Exp. IV should have been about 12 mL·min^{-1}·kg^{-1} (based on the relationship established between $\dot{q}_{IA}$ and Hct. This means that the total intestinal blood flow was approximately 34% of $\dot{Q}_{rest}$. This is a similar proportion of $\dot{Q}_{rest}$ as was reported for the sluggish fish (30-40%; Axelsson *et al.* 1989; Axelsson & Fritsche, 1991; Axelsson, Thorarensen, Nilsson & Farrell, in press). Therefore, the proportion of $\dot{Q}_{rest}$ that is diverted to the gut may be similar in fish even though absolute levels differ. The proportion of $\dot{Q}_{rest}$ diverted to the gut in fish is slightly higher than what has been reported for mammals which ranges between 20-30% of $\dot{Q}_{rest}$ (Burton 1972; Kuwahira *et al.* 1993).

It is not clear why gut blood flow is higher in chinook salmon, but it is undoubtedly related to their exceptionally high growth rate (Brett, 1983). Moreover, chinook salmon were the smallest of the fish used in these gut blood flow studies, only 365 g (Table 1), while the sea ravens were 670-2,300 g and the Atlantic cod were 550-1,050 g. Both weight-specific resting metabolic rate and growth rate of fish decrease with size (Brett, 1964,1965, 1979; Kaufmann, 1991). Finally, the resting metabolic rate of chinook salmon (83-88 μmol·min^{-1}·kg^{-1}) was high compared with the resting metabolic rate in

Atlantic cod (30-55 μmol·min^{-1}·kg^{-1}; Saunders, 1963; Soofiani & Priede, 1985) and rainbow trout (25 μmol·min^{-1}·kg^{-1}) because the fish had been fed intensively and they had been growing well before the experiment started. Thus, their high gut blood flow may be associated with a high growth rate, a high resting metabolic rate, and possibly small body size.

At rest, the intestinal blood flow was variable among individuals, but $\dot{q}_{IA}$ of all fish increased as Hct and blood O_2 carrying capacity were reduced. This indicates that blood flow to the intestines in resting fish is adjusted to maintain O_2 transport to the intestines. This conclusion is in keeping with the observations that $\dot{Q}_{rest}$ increases in rainbow trout when Hct and the O_2 capacity of the blood is reduced (Cameron & Davis, 1970; Wood & Shelton, 1980; Gallaugher, Thorarensen & Farrell, in prep.). Similar changes in intestinal blood flow have also been seen in mammals when blood O_2 capacity is varied (Vatner *et al.* 1972; Fan, *et al.* 1980; Davis & Hohimer, 1991), possibly involving some form of autoregulation, either metabolic or myogenic (Sheperd, 1977; 1980; Granger & Norris, 1980). There are some indications that autoregulatory responses also exist in fish (Canty & Farrell, 1985; Axelsson and Fritsche, 1991). I therefore suggest that chinook salmon may autoregulate gut blood flow when O_2 capacity of blood is reduced.

Training effects.

To examine the effect of exercise training on absolute intestinal blood flow in swimming fish, the relative changes in $\dot{q}_{IA}$ (Fig. 8) were estimated from the relationship that was established between $\dot{q}_{IA}$ in resting fish and Hct. The predicted $\dot{q}_{IA}$ is shown in Fig 9a. In addition, O_2 transport to the intestines was estimated by multiplying $\dot{q}_{IA}$ by Ca_{O_2} (Fig. 9b). By increasing Hct , the HS trained fish maintained the same O_2 transport to the intestines swimming at 1.5 bl·s^{-1}as the LS1 fish at 0.5 bl·s^{-1} (Fig. 9b). The increased Hct elevated the O_2 carrying capacity of the blood, and thus O_2 transport to the intestines was

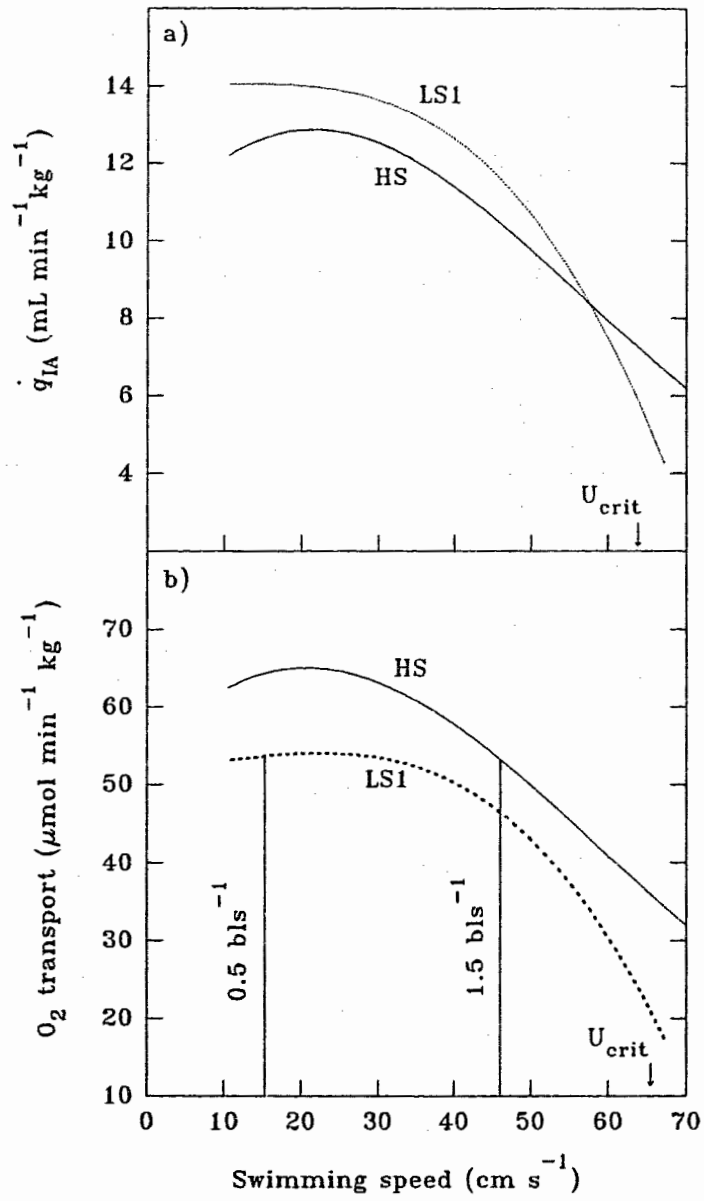
higher in the HS-trained group at all swimming speeds (Fig. 9b), in spite of a lower $\dot{q}_{IA}$ at rest (Fig. 9A). Hence any loss in intestinal O_2 transport which would otherwise have occurred because of reduced intestinal blood flow was compensated for by the increased Hct.

An additional advantage of an elevated Hct is that less blood flow is required to meet the O_2 demands of the locomotory muscles and thus there is less need to redistribute blood flow away from the viscera as swimming speed is increased (Fig 9A). This is consistent with our finding that $\dot{q}_{IA}$ was reduced less in the HS-trained group as swimming velocity was increased (Fig. 8a). The elevated Hct may also produce a greater scope for increasing intestinal blood flow postprandially without compromising oxygen transport to the locomotory muscles. I suggest, therefore, that a significant role of the elevated Hct for the HS group was to maintain O_2 transport to the intestines, and consequently normal intestinal function, while swimming continuously at a relatively high velocity. Further support for this suggestion is given by the relationship that was found between Hct and $\dot{V}_{O_2}$, which shows that at any given swimming velocity the $\dot{V}_{O_2}$ was higher in fish with higher Hct. This indicates that fish with high Hct are indeed able to support more auxiliary functions while swimming

Likelwise, a similar trend was also observed in Exp. IV (see Chapter 3). I suggested that the TR fish were better able to support other functions while swimming, because $\dot{V}_{O_2max}$, but not U_{crit} was higher than in the controls. The more stringent training regime (TR) had higher Hct. However, the higher Hct but not Ca_{O_2} as was the case in Exp. III. Instead, O_2 delivery to tissues in the TR group was elevated by increasing E_{O_2max}. Thus, internal O_2 convection increased in both training regimes through different mechanisms. This increased O_2 convection appears to have allowed the fish to support more basal maintenance functions while swimming rather than increasing U_{crit}.

The LS2 group in Exp IV and the LS1 group in the present study were exposed to the same training regime and the fish came from the same stock. As expected, both U_{crit} and $\dot{V}_{O2max}$ in the two groups were not significantly different. However, the mean Hct of the LS1 fish was 22% lower than in the LS2 group and the maximum blood O_2 capacity was 34% lower in the LS1 group (9.1 %vol.) than in the LS2 group (14.5 %vol.). This difference could be due to an inherent difference between the two groups, possibly as a result of seasonal differences in Hct: Exp IV was performed in winter while Exp. III was conducted in the fall. However, it is also possible that the different Hct in the two groups was caused by the more invasive surgery used in Exp. III.

Figure 9. Predicted O_2 transport and blood flow in the intestinal artery ($\dot{q}_{IA}$) of swimming chinook salmon. (a) Predicted blood flow in the intestinal artery and (b) Predicted O_2 transport ($\dot{q}_{IA} \cdot Ca_{O_2}$). Dotted line; fish trained at 0.5 bl·s^{-1}; Solid line: fish trained at 1.5 bl·s^{-1}. The vertical lines show the training velocity in the two groups.


a)
LS1
HS
U_{crit}
$\dot{q}_{IA}$ (mL min^{-1}kg^{-1})
14
12
10
8
6
4
b)
HS
LS1
0.5 bls^{-1}
1.5 bls^{-1}
U_{crit}
O_2 transport (μmol min^{-1} kg^{-1})
70
60
50
40
30
20
10
0
10
20
30
40
50
60
70
Swimming speed (cm s^{-1})

Potential effects of feeding on gut blood flow in swimming fish.

By using a model fish similar to the LS2 fish in Exp. IV (Hct = 30%) with a resting gut blood flow of 12 mL·min^{-1}·kg^{-1}, it is possible to calculate that the 81% increase in $\dot{q}_{IA}$ should have resulted in maximum postprandial gut blood flow of about 22 mL·min^{-1}·kg^{-1}. This is higher than the 12-18 mL·min^{-1}·kg^{-1} reported for the sea raven, the Atlantic cod and the red Irish lord (Axelsson *et al.* 1989; Axelsson & Fritsche, 1991; Axelsson, Thorarensen, Nilsson & Farrell, in review). Again this may reflect the higher metabolic and growth rates of chinook salmon.

The estimated maximum postprandial gut blood flow in chinook salmon (22 mL·min^{-1}·kg^{-1}) corresponds to 34% of $\dot{Q}_{max}$. If this intestinal blood flow was maintained in fed fish while they exercised, the maximum blood flow available for the locomotory muscles would be significantly reduced in fed fish. This is consistent with the findings of Exp. I that the U_{crit} of the fed fish was 10% lower than that of starved fish. The maximum gut blood flow in fed Atlantic cod (12 mL·min^{-1}·kg^{-1} Axelsson & Fritsche, 1991) is about 40% of $\dot{Q}_{max}$ (Axelsson, 1988) and the maximum gut blood flow is probably a similar proportion of $\dot{Q}_{max}$ in the sea raven (Axelsson *et al.* 1989). This suggests, that proportionately the cardiovascular changes associated with feeding in all these fish species are similar. However, because $\dot{Q}_{max}$ is higher in chinook salmon than in the other fish, the residual blood flow ($\dot{Q}_{max}$ - gut blood flow) during the postprandial stage is 40-45 mL·min^{-1}·kg^{-1} in chinook salmon and only 18 mL·min^{-1}·kg^{-1} in the Atlantic cod and the sea raven. Moreover, after the blood flow reserved for the central nervous system, heart and other essential functions has been subtracted, the difference in blood flow available to muscles will likely be even more pronounced. It is therefore concluded that the greater capacity of the cardiovascular system in chinook salmon will allow them to sustain much higher swimming speeds while feeding, than sea raven and Atlantic cod.

Chapter 8.

The significance of the redistribution of blood flow from the gut of chinook salmon during exercise.

Introduction

In the preceding chapters I measured $\dot{V}_{O_2}$, $\dot{Q}$, $\dot{T}_{O_2}$, and $\dot{q}_{IA}$ in swimming fish as well as $\dot{V}_{O_2}$ and $\dot{q}_{IA}$ in starved, fed and exercising fish. In this chapter these values will be used to estimate the significance of blood flow redistribution from the gut of swimming fish.

The results presented in earlier chapters clearly demonstrate that blood flow is redistributed away from the gut directly in relation to the O_2 demand of the locomotory muscles as measured by the increase in $\dot{V}_{O_2}$ in exercising fish. The significance of this reduction in gut blood flow in terms of increased O_2 transport to the locomotory muscles, and other possible benefits is the focus of this chapter.

It has been suggested that a reduction in gut blood flow in swimming fish may be necessary to increase $\dot{Q}$ (Satchell, 1992). The reasoning behind this is as follows. Most fish, including chinook rely on SV_H to regulate $\dot{Q}$ during exercie, and SV_H is in part determined by the filling pressure of the heart (Farrell, 1991). The filling pressure of the heart is primarily determined by the central venous pressure. Satchell (1992) suggested that, in a manner analogous to mammals, fish could shift blood volume both from systemic venules and veins (with muscle pumps) and from the splanchnic circulation to the central veins to increase the filling pressure of the heart. His suggestion has not been experimentally verified, but there is some circumstantial evidence in support of this. A small increase in central venous pressure is seen in exercising rainbow trout (Kiceniuk & Jones, 1977), which is consistent with blood being shifted from peripheral or splanchnic veins. Moreover, after cholinergic and ß-adrenergic blockade in Atlantic cod, most of the

increase in $\dot{Q}$ during exercise can still occur, possibly as a result of increased filling pressure of the heart caused by the 'muscle pump' (Axelsson, 1988).

In large mammals, the hepatic portal vein and its tributaries hold as much as 30% of the total blood volume (Brooksby & Donald, 1972; Bennett & Rothe, 1981). When resistance in the splanchnic arterioles increases, gut blood flow decreases. There is resistance to blood flow across the liver, so when blood flow in the hepatic portal vein decreases blood pressure in the hepatic portal vein decreases also. When the transmural pressure in the veins is reduced, blood is expelled as a result of passive recoil of the veins (Caldini *et al.* 1974; Green & Jackman, 1984).

In fish, the appropriate conditions appear to exist that would allow significant shifts of blood volume from the gut to occur during exercise. The hepatic portal venous system in salmonids is large (Thorarensen *et al.* 1989; Appendix III) and estimates of the volume of blood in these vessels suggest that they may contain in excess of 23% of total blood volume (Stevens, 1968; Olson, 1992). Furthermore, blood pressure in the hepatic portal vein is higher than in the *sinus venosus* (Stevens & Randall, 1967a; Kiceniuk & Jones, 1977; Wood & Shelton, 1980) because there is resistance to blood flow across the liver. This would allow the pressure (and therefore the volume) in the hepatic portal vein to be regulated by flow*. Finally, the results of Exp. III show that intestinal blood flow is significantly reduced in exercising fish. Therefore, all required conditions exist to allow shifts in blood volume from the splanchnic veins. However, the only measurements of blood pressure in the splanchnic veins of rainbow trout (Stevens & Randall, 1967a) suggest that blood pressure does not decrease, but rather increases. It is possible, but

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* Pressure in the hepatic portal vein = blood flow · liver resistance;

blood volume in the hepatic portal vein = pressure ·C; (C = capacitance of of the system (ΔVolume/Δ Pressure).

untested, that the splanchnic veins are contracted during exercise and expel part of the blood volume contained within them. This may be needed, because central venous blood pressure must increase to increase $\dot{Q}$ (Farrell, 1991).

A second potential benefit from reducing gut blood flow is to maintain arterial blood pressure during exercise. The importance of maintaining P_{DA} is two fold. First, a back pressure on the gills is required to maintain blood pressure in the ventral aorta in exercising fish, since the increased O_2 conductance of the gills depends to a large extent on an increase in blood pressure. The pressure in the ventral aorta P_{VA} is approximately equal to:

$$P_{VA} = \dot{Q} \cdot (R_{GILLS} + R_{SYS})$$

where R_{SYS} and R_{GILLS} are the resistance in the gills and the systemic circulation. Second, adequate P_{DA} is important to maintain the diameter of blood vessels, which depends in part on the transmural pressure. Since the resistance to flow in a vessel is inversely proportional to the radius to the fourth power, vascular resistance depends on blood pressure (Burton, 1972), provided wall tension in the vessel is not changed to compensate for changes in pressure. If pressure drops below the critical closing pressure, the vessel collapses and blood flow stops entirely. Studies on perfused tail preparations of fish (Wood & Shelton, 1974; Canty & Farrell, 1985) suggest that pressure may affect vascular resistance in fish at low perfusion pressures (< 2.0-2.5 kPa) although the critical closing pressure for a perfused rainbow trout preparation was less than 0.1 kPa.

As swimming speed increases, parallel vascular circuits in the skeletal muscles open up and systemic resistance decreases. Total resistance in these circuits is:

$$1 / R_{TOTAL} = 1 / R_1 + 1 / R_2 + 1 / R_3 \ldots\ldots 1 / R_n$$

where R_1 to R_n are the resistances in the various systemic circuits. The more circuits that open up, the lower R_{TOT} becomes. In chinook salmon, there is almost a four-fold increase

in muscle blood flow during exercise (Table 13) and total systemic resistance decreases (Fig. 3f) despite an increase in splanchnic resistance (Fig. 8b). It is obvious that blood pressure would fall if splanchnic resistance had not increased, but the extent of this is unclear. If the peripheral circulation is defined as the combined systemic circulation apart from the splanchnic vascular bed, then:

$$R_{SYS} = R_{PERIPH} \cdot R_{SPLAN} / (R_{PERIPH} + R_{SPLAN})$$

where R_{PERIPH} and R_{SYS} are the resistance's of the peripheral and splanchnic vascular beds, respectively. Information on $\dot{Q}$, intestinal blood flow and blood pressure, allow R_{SYS}, R_{PERIPH} and R_{SPLAN} to be calculated and the effect that increased R_{SPLAN} has on blood pressure in exercising fish can be modeled.

Methods

For these calculations a model fish was defined (Table 11) similar to the LS2 fish from Exp. IV for $\dot{V}_{O2max}$, U_{crit}, Hct, $\dot{Q}_{rest}$ and $\dot{Q}_{max}$. Relative changes in gut blood flow during exercise and postprandial are from Exp. III and the resting gut blood flow is calculated based on the formula derived in Exp. III using an Hct of 30%. The cost of swimming is assumed to be proportional to U^2, in accordance with Exp. III and other studies (Weihs, 1973).

Table 11. Parameters used in calculations for the model fish .

Parameter	Value
$\dot{V}_{O2max}$	290 μmol·min^{-1}·kg^{-1}
U_{crit}	83 cm·s^{-1}
Hct	30%
$\dot{Q}_{rest}$	35 mL·min^{-1}·kg^{-1}
$\dot{Q}_{max}$	65 mL min^{-1}·kg^{-1}
Gut blood flow at rest	12 mL·min^{-1}·kg^{-1}
Max. postprandial gut blood flow	22 mL·min^{-1}·kg^{-1}
Min. gut blood flow during exercise	5 mL·min^{-1}·kg^{-1}
Cost of swimming	proportional to U^2

Results and discussion.

Before the importance of blood flow redistribution from the gut to the muscles can be assessed, muscle blood flow in exercising fish has to be estimated.

Estimation of skeletal muscle blood flow.

Having measured intestinal blood flow in resting and swimming chinook salmon, muscle blood flow was estimated by correcting the microsphere data from Chapter 6 with values from the literature for blood flow to tissues that are underestimated by the microsphere method.

The biggest verifiable error with the microsphere method is an underestimate of intestinal blood flow. About 13% of the microspheres were lodged in the viscera instead of 34% predicted from direct measurements of $\dot{q}_{IA}$ (Exp. III). In addition, gill venous return, which is reported as 6-7% of $\dot{Q}$, is not measured (Ishiwata *et al.* 1987; Sundin & Nilsson, 1992). Blood flow in the coronary artery of coho salmon and rainbow trout is around 1% of $\dot{Q}$ (Axelsson & Farrell, 1993; Gamperl *et al.* in press). Blood flow to the remaining regions of the head has never been measured *in vivo*. However, *in vitro* measurements (Gardair *et al.* 1991) suggest that cephalic portion of arterial blood flow from the gills is 5% of $\dot{Q}$. Thus, around 33% of $\dot{Q}_{rest}$ is not properly represented in microsphere estimates of blood flow distribution. The regional blood flow used to correct the microsphere data is shown in Table 12.

The estimate of skeletal muscle blood flow in resting chinook salmon (Table 12) was obtained as follows. The relative blood flow distribution to the gut, coronaries, cephalic circulation, and gill venous return was added up (46%). Second, the residual blood flow (54%) was allocated to muscles, kidney and skin in the same proportion as estimated with the microsphere method.

The estimate for blood flow distribution in chinook salmon at U_{crit} (Table 12) is based on the values for $\dot{Q}$ from Exp. IV which increased by 86% and changes in $\dot{q}_{IA}$ from Exp. III which was reduced by 60%. Furthermore, it was assumed that, as in mammals (Heller & Mohrman, 1981), the absolute cephalic blood flow was unchanged during exercise. In the absence of data, absolute gill venous return in exercising fish was assumed to remain the same. The relative changes in distribution of blood flow to the kidney and skin in exercising fish were assumed to be in the same proportions as observed by Daxboeck (1981). Muscle blood flow was estimated as the residual blood flow (82%) after the above had been accounted for (18%).

These calculations indicate that, during exercise, total blood flow to the muscles increases nearly four-fold (Table 13). Furthermore, at least 77% (Table 13) of cardiac output is diverted to the muscles at U_{crit}. In the next chapter these values will be used in model calculation of cardiovascular dynamics in chinook salmon.

Table 12. Corrected blood flow distribution in chinook salmon. Resting muscle, kidney and skin blood flow at rest was corrected with values from the literature (boxed) and results from Exp. III. Expected distribution at U_{crit} was estimated by assuming a 86% increase in $\dot{Q}$ (Exp. IV). Other assumptions are explained in footnotes.

Tissue	Observed resting values‡	Corrected resting chinook values		Expected chinook values at U_{crit}	
From microspheres					
Muscle total	59	42%		82	†
Red	11%	8%			
White	48%	34%			
Kidney	2%	1%		1%	††
Skin	16%	11%		3%	††
From other sources					
Gut	34%	34%		7%	
Coronaries	1%	1%	*	1%	‡
Cephalic.	5%	5%	**	3%	‡‡
Gill venous ret.	6%	6%	***	3%	‡‡
Total	46			18	
Total		100%		100%	

* Based on Axelsson & Farrell (1992).
** Based on Gardair *et al.* 1991..
*** Based on Ishiwata *et al.* (1987) and Sundin & Nilsson, 1992.
† Total muscle blood flow estimated as residual flow after boxed values have been subtracted from 100%.
†† Assuming the same proportional change from rest as was observed by Daxboeck (1981) for exhausted fish.
‡ Assuming same proportional increase as for $\dot{Q}$.
‡‡ Assuming constant blood flow at rest and at U_{crit}.

Table 13 Estimates of muscle blood flow (mL·min^{-1}·kg^{-1} body-mass), at rest and U_{crit} in chinook salmon. It is assumed that $\dot{Q}_{rest}$ is 35 mL·min^{-1}·kg^{-1} and $\dot{Q}_{max}$ is 65 mL·min^{-1}·kg^{-1} in accordance with the results of Exp. IV.

	Rest	U_{crit}	Factorial change
Muscle	14	54	3.9x
Splanchnic	12	5	0.4x
Other tissues	9	6	0.7x

The significance of blood flow redistributed from the gut during exercise for muscle O_2 delivery.

According to the model, resting blood flow to the gut (12 mL·min^{-1}·kg^{-1}; Table 11) is reduced to 5 mL·min^{-1}·kg^{-1} as the fish swim maximally. Since estimated muscle blood flow at U_{crit} is 54 mL·min^{-1}·kg^{-1} (Table 13), 7 mL·min^{-1}·kg^{-1} is diverted from the gut as the fish swim maximally and contributes 13% to muscle blood flow at U_{crit}.

If the cost of swimming is proportional to velocity squared, the 13% of O_2 delivery to muscles gained by redistributing blood flow from the gut should increase U_{crit} by 6%, (i.e. $\sqrt{1.13}$). This means that if there were no constraints other than O_2 delivery, blood flow would not have to be redistributed from the gut until the fish were swimming at 94% of U_{crit}. However, the results from Exp. III show that blood flow from the gut is reduced already at 50-60% of U_{crit}. This could mean that blood flow is gradually redistributed from various tissues, in addition to the gut, to the locomotory muscles during exercise. For example, Randall & Daxboeck (1982) suggested that blood flow was redistributed from white to red muscles in exercising rainbow trout. Alternatively, there may be some other significance to redistributing blood flow from the gut when the fish swim maximally than just to increase O_2 delivery to the muscles .

The importance of reduced gut blood flow during exercise for maintaining blood pressure.

The significance of reducing gut blood flow during exercise for maintaining blood pressure was examined by estimating what would happen if R_{SPLANC} did not increase during exercise (Table 14). It is possible to perform these calculations based on values obtained from Exp. III and IV, as shown in Table 14a along with calculated R_{SPLANC} and R_{PERIPH} at rest and during exercise.

Four scenarios where examined. The first scenario examined was: what would P_{DA} be at U_{crit} if R_{SPLANC} did not change during exercise and how would this P_{DA} affect q_{SPLANC} and q_{PERIPH} (Table 14b-1)? These calculations suggest that if R_{SPLANC} had not changed during exercise, while all other variables were equal (Table 14b-1), then P_{DA} would have been be reduced by 13% from 4.0 kPa to 3.5 kPa. Since the P_{DA} in the TR group was similar (3.5 kPa) at U_{crit}, this change in blood pressure is not likely to be of any significance for vascular function.

If R_{SPLANC} did not increase during exercise, q_{SPLAN} would increase from 12 mL·min^{-1}·kg^{-1} at rest to 13 mL·min^{-1}·kg^{-1} at U_{crit} because of a slight increase in blood pressure above the resting value (Table 14b-1). If the difference between this new q_{SPLAN} and q_{SPLAN} under normal conditions at U_{crit}, caused a corresponding reduction in muscle blood flow (Table 13), then U_{crit} should be reduced by 8% (Table 14b-1).

The changes in P_{DA} at U_{crit} that would result from the hypothetical situation of constant R_{SPLANC} are probably not significant for cardiovascular function in the fish. However, they limit U_{crit}. In order to reach normal U_{crit} muscle blood flow has to be restored to a normal value of 54 mL·min^{-1}·kg^{-1} (Table 13). This can either be accomplished by increasing Q_{max} or by reducing R_{PERIPH}. The second scenario examined (Table 14b-2) was: if R_{SPLANC} did not increase, what would P_{DA} be if q_{PERIPH} was restored to normal levels by reducing R_{PERIPH}? If R_{PERIPH} is reduced enough to restore muscle blood flow to 54 mL·min^{-1}·kg^{-1}, but with a hypothetical constant R_{SPLANC}, P_{DA} would be reduced even further to 1.4 kPa (Table 14b-2). This is a condition of severe hypotension which would likely impair lamellar recruitment. Clearly an increase in R_{SPLANC} is of critical importance in preventing hypotension during maximal swimming..

Scenario three (Table 14b-3): how much would $\dot{Q}_{max}$ have to increase to restore P_{DA} to 4 kPa while R_{SPLANC} and R_{PERIPH} were the same as in Table 14b-1? Given the conditions in Table 14b-1, the P_{DA} could be restored to 4.0 by either increasing $\dot{Q}_{max}$ by 15% to 75

$mL \cdot min^{-1} \cdot kg^{-1}$ (Table 14b-3). This could increase q_{PERIPH} enough to allow the fish to maintain normal U_{crit}.

Scenario four (Table 14b-4): what would R_{PERIPH} have to be to restore P_{DA} to 4 kPa while $\dot{Q}_{max}$ and R_{SPLANC} were the same as in Table 14b-1. This P_{DA} could also be increase to 4.0 kPa by increasing R_{PERIPH}. However, this would decrease q_{PERIPH} even further. If this reduction in q_{PERIPH} came from muscle blood flow, then it is possible to estimate that the U_{crit} would be reduced by 10%. Interestingly, a 10% reduction in U_{crit} as was also observed in the fed fish of Exp. I, which indicates that similar conditions could have existed in the fed fish as are shown in Table 14b-3. Under these conditions gut blood flow is increased by 25% from resting levels (Table 14b-3) at the expense of muscle blood flow. The consistency between these model calculations and observed changes in U_{crit} of fed fish, lends further support to the suggestion put forward in Chapter 3, that U_{crit} was indeed reduced because intestinal blood flow was maintained, while muscle blood flow decreased.

These calculations suggest that as much as 2/3 of gut blood flow in resting fed fish was maintained as they swam maximally. It is not known how critical intestinal blood flow is for digestion and absorption and therefore the effect of this reduction in gut blood flow cannot be estimated. However, when considered in conjunction with the results of Exp. I which show that the HI is not reduced at as swimming speed increased, it seems likely that most of gut function remained intact.

Possible shift of blood volume from the splanchnic circulation during exercise.

By examining simultaneous changes in cardiac output and intestinal blood flow in swimming fish, it is possible to examine the potential significance of the reduced visceral blood flow for increasing cardiac output. Over 60% of the increase in $\dot{Q}$ in chinook salmon occurred as the fish started swimming (Fig. 2a), but gut blood flow was not

reduced until they swam at velocities over 50-60% of U_{crit} (Fig. 8a). Therefore, expulsion of blood from the splanchnic circulation by passive recoil of the veins is not likely to have contributed to the initial increase in $\dot{Q}$. However, the reduction in gut blood flow coincides with the second phase of the increase in $\dot{Q}$ (Fig. 8a). These observations suggest, that in addition to directly increasing O_2 delivery to muscles the redistribution of blood from the intestines may also be of importance for creating the filling pressure required for the heart to reach $\dot{Q}_{max}$ although chinook salmon can attain most of the maximum increase in $\dot{Q}$ without changing gut blood flow. It is also possible that some blood was expelled from the gut vasculature as a result of active contraction of the splanchnic veins (Stevens & Randall, 1967a) and the results of the present experiments warrant further investigation of this topic.

Table 14. The significance of increased splanchnic resistance for pressure in the dorsal aorta and for systemic resistance ($1 / R_{SYS} = 1 / R_{SPLAN} + 1 / R_{PERIPH}$) in exercising fish.

a) Premises and calculated resistance in the splanchnic (R_{SPLAN}) and peripheral (R_{PERIPH}) vascular beds and blood flow (q_{SPLAN}, q_{PERIPH}) in normal fish.

Normal condition (extension of Table 11)	$\dot{Q}$ mL·min^{-1}·kg^{-1}	P_{DA} kPa	R_{PERIPH} kPa·mL^{-1}·min·kg	R_{SPLAN} kPa·mL^{-1}·min·kg	q_{SPLAN} mL·min^{-1}·kg^{-1}	q_{PERIPH} mL·min^{-1}·kg^{-1}
Rest	35	3.3	0.143	0.275	12	23
Max.	65	4.0	0.067	0.800	5	60

b) Estimates of the consequences of not increasing R_{SPLAN} in exercising fish. Estimated values for each condition are shown boxed

Conditions	$\dot{Q}$ mL·min^{-1}·kg^{-1}	P_{DA} kPA	R_{PERIPH}	R_{SPLAN}	q_{SPLAN} mL·min^{-1}·kg^{-1}	q_{PERIPH} mL·min^{-1}·kg^{-1}	Expected change in U_{crit}
1. What would P_{DA} be if R_{PERIPH} and $\dot{Q}$ were the same as in normal fish at U_{crit} while R_{SPLAN} was the same as at rest.	65	3.5**	0.067	0.275	13	52§	8%*
2. What would P_{DA} be if R_{PERIPH} was reduced to make q_{PERIPH} 60 mL·min^{-1}·kg^{-1}?‡	65	1.4‡‡	0.023‡‡‡	0.275	5	60	0
3. What would $\dot{Q}$ have to be to restore P_{DA} to 4.0 kPa while other conditions were the same as in 1?	75***	4.0	0.067	0.275	15	60	0
4. What would R_{PERIPH} have to be to restore P_{DA} to 4.0 kPa while other conditions were the same as in 1?	65	4.0	0.079†	0.275	15	50	10%

* Reduction in U_{crit} is calculated by assuming that all the reduction in q_{PERIPH} comes from the muscles which have a maximum flow of 54 mL·min^{-1}·kg^{-1} (Table 13) and that the cost of swimming is proportional to U^2.

$\Delta U_{crit} = \sqrt{((MBF-(q_{SPLAN}-nq_{SPLAN}))/MBF)}$; MBF: normal muscle blood flow (54 mL·min^{-1}·kg^{-1}; Table 13); nq_{SPLAN}: gut blood flow at U_{crit} in a normal fish (5 mL·min^{-1}·kg^{-1}; Table 11).

§ $q_{SPLANC} = P_{DA} / R_{SPLANC}$ $q_{PERIF} = P_{DA} / R_{PERIPH}$

** $P_{DA} = \dot{Q} \cdot (R_{PERIPH} \cdot R_{SPLAN} / (R_{PERIPH} + R_{SPLAN}))$

*** $\dot{Q} = 4 \cdot (R_{PERIPH} + R_{SPLAN}) / (R_{PERIPH} \cdot R_{SPLAN})$

† $R_{PERIPH} = R_{SPLANC} \cdot R_{TOTAL} / (R_{SPLANC} - R_{TOTAL})$; $R_{TOTAL} = P_{DA} / \dot{Q} = 4 / 65$.

‡ P_{DA} and R_{PERIPH} were calculated with an iterative procedure in LOTUS 123.

‡‡ $P_{DA} = \dot{Q} \cdot (R_{PERIPH} \cdot R_{SPLANC}) / (R_{PERIPH} + R_{SPLANC})$

‡‡‡ $R_{PERIPH} = P_{DA} / 60$

Chapter 9

General summary

The general function of the cardiovascular system of chinook salmon

A number of novel findings have been reported in this thesis which have been discussed in detail in previous chapters. Summarized here are the contributions of this thesis to the understanding of the general function of the cardiovascular system of salmonids. Finally, the implications of these findings for the ability of chinook salmon to feed and swim are examined.

The findings of this study are consistent with the hypothesis that maximum oxygen uptake of chinook salmon is limited by maximum internal O_2 supply. The $\dot{V}_{O2max}$ of fed fish was the same as in starved fish in spite of the increased O_2 demand due to the HI in the fed fish. That suggests that $\dot{V}_{O2max}$ is limited by maximum O_2 supply rather than the metabolic capacity of the mitochondria. Based on results from rainbow trout (Gallaugher, Thorarensen & Farrell, in prep.), $\dot{T}_{O_2}$ should increase with Hct and therefore the relationship that was found in Exp. III between $\dot{V}_{O_2}$ and Hct ($\dot{V}_{O_2} = -75.8 + \ln_{Hct} + \ln_{Hct} \cdot U^2$) suggests that $\dot{V}_{O_2}$ increases as a function of $\dot{T}_{O_2}$. It is therefore suggested that at $\dot{V}_{O2max}$, demand for O_2 is greater than maximum supply. However, factors other than $\dot{T}_{O_2}$ can affect $\dot{V}_{O2max}$ in chinook salmon such as diffusion from capillaries to mitochondria. This was demonstrated in Exp. IV. Training allowed fish to increased $\dot{V}_{O2max}$ by increasing E_{O2max}. Finally, the reduction in Pa_{O_2} as swimming speed increases, is indicative of some form of diffusion limitation at the gills, even though, this diffusion limitation does not appear to impair O_2 delivery significantly under normoxic conditions. Therefore, it is suggested that $\dot{T}_{O_2}$ is the primarily limit to $\dot{V}_{O2max}$ in chinook salmon, but other steps in the O_2 cascade also affect the flux of O_2 from environment to mitochondria.

The contribution of each step in the O_2 cascade of chinook salmon to the overall resistance to O_2 flux from environment to mitochondria is not known exactly. In the introduction, an analogy was drawn between the O_2 cascade and a electric circuit, with resistors arranged in series. The good correlation between $\dot{T}_{O2max}$ and $\dot{V}_{O2max}$ of different species of fish (Fig. 4) suggests that $\dot{T}_{O_2}$ may be the principal limiting factor (major resistor) under normoxic conditions. Under other conditions, such as environmental hypoxia, the relative contribution of each step may differ.

Since the maximum O_2 demand exceeds maximum delivery, different tissues compete with the locomotory muscles for a limited O_2 supply as swimming speed increases. The observation that the U_{crit} of fed fish decreases is a new finding, and suggests that fed fish sacrifice locomotory ability in favor of supporting digestive and assimilatory processes. Therefore, leverage in the competition for the limited O_2 supply appears to be in part determined by the relative O_2 demands of these tissues.

Nonetheless, gut blood flow in exercising fish is tightly regulated relative to the O_2 demand of the locomotory muscles. The blood flow that is redistributed away from the gut may contribute about 13% of the total O_2 delivered to the locomotory muscles at U_{crit}. Furthermore, it is important to increase splanchnic resistance to provide adequate blood flow to the locomotory muscles without compromising blood pressure at U_{crit}. Finally, the reduction in gut blood flow may shift blood from the splanchnic venous system to the central veins and thus increase the filling pressure of the heart. Thus, a certain amount of redistribution of blood flow away from the gut must occur in maximally in exercising fish.

The internal O_2 convection ($D_{O2max} = \dot{T}_{O2max} \cdot E_{O2max}$), depends both on maximum cardiovascular O_2 transport and on maximum extraction of O_2 in tissues. Since blood flow has to be redistributed from the gut and possibly other tissues during exercise to meet the O_2 demand of the locomotory muscles, the question arises: Is the redistribution

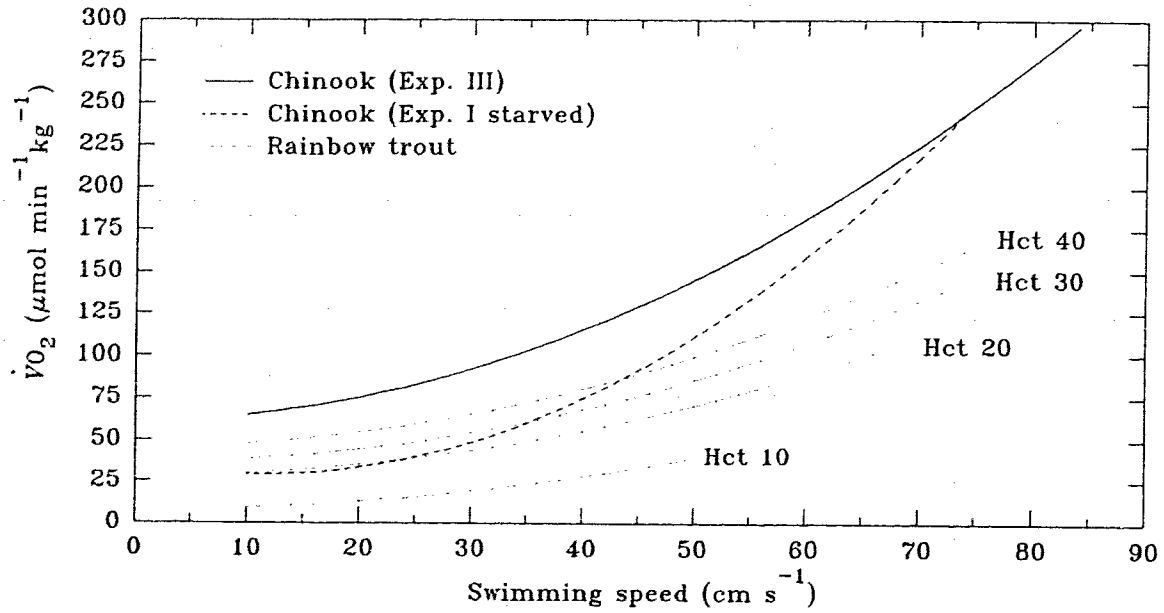
affected by $\dot{D}_{O_2max}$ and do fish with a high $\dot{D}_{O_2max}$ have to redistribute less blood flow to the locomotory muscles at U_{crit}? The results from these experiments suggest that this is the case. First, gut blood flow and O_2 delivery to the viscera was significantly better maintained during swimming in the HS fish in Exp. III which had a higher Hct. Second, fish (from Exp. III) with high Hct, had higher $\dot{V}_{O_2}$ at any given swimming velocity than fish with low Hct, again indicating that fish with higher $\dot{D}_{O_2max}$ are able to support more functions while swimming than fish with low $\dot{T}_{O_2max}$. Further support for this comes from studies of rainbow trout, which show that as Hct increased, so did $\dot{T}_{O_2}$ and that fish with higher $\dot{T}_{O_2}$ had higher $\dot{V}_{O_2}$ at any given swimming velocity (Fig. 10; based on Gallaugher, Thorarensen & Farrell, in prep), similar to what was found in Exp. III. Third, at the highest swimming speeds, the $\dot{V}_{O_2}$ of the TR fish in Exp. IV was higher than in the LS2 group (Fig. 1), which suggests that the greater $\dot{D}_{O_2max}$ allowed the TR fish to maintain more basal functions than the LS2 fish. Fourth, $\dot{V}_{O_2}$ is higher at all swimming speeds in chinook salmon than in rainbow trout (Fig. 10) and chinook salmon have a significantly higher $\dot{T}_{O_2max}$ than rainbow trout (Fig. 4). Interestingly, Fig. 10 bears a striking resemblance to Figure 2 in Jones & Randall (1978) which shows the $\dot{V}_{O_2}$ of sockeye salmon (Brett, 1965) as being higher than that of rainbow trout (Rao, 1968), either at all swimming speeds or increasing more with swimming speed. The difference between the rainbow trout in Fig. 10 and chinook salmon may be due to size, because $\dot{V}_{O_2}$ at any given swimming velocity is higher in small than in large fish (Brett, 1965; Dabrowski *et al.* 1988; Kaufmann, 1992), and the rainbow trout were almost 50% larger (542g *vs* 365g) than chinook salmon. However, size can neither explain the difference in the $\dot{V}_{O_2}$ of rainbow trout with different Hct (Fig. 10) nor between the TR and LS2 fish in Exp. IV (Fig. 1). Therefore, the higher the $\dot{D}_{O_2max}$, the more basal functions appear to be maintained while the fish are swimming. Evidence for this is given by the higher $\dot{V}_{O_2}$ at

any swimming velocity (Fig. 10) and the results of Exp. III, which suggest that this is due to better O_2 supply to tissues other than the locomotory muscles in fish with high $\dot{D}_{O_2max}$.

Based on the results of Exp. I, it was suggested that there was a dynamic competition for O_2 delivery among different tissues. Moreover, the relative O_2 demand of tissues appeared to affect blood flow distribution. Thus, less blood flow appeared to be diverted to locomotory muscles and more to the gut in fed than in starved fish. Based on what was said above about the influence of $\dot{D}_{O_2max}$ on blood flow distribution, it is now possible to state that the results of this thesis suggest that blood flow distribution in fish is regulated with reference to both the relative O_2 demands of different tissues and to the overall ability of the cardiovascular system to deliver O_2.

When $\dot{D}_{O_2max}$ increases, the added O_2 delivery appears to be distributed among various tissues in addition to the locomotory muscles. Therefore, the effects of increased $\dot{D}_{O_2max}$ on maximum swimming performance may be limited. This would explain why Brett (1964) found that the $\dot{V}_{O_2max}$ of sockeye salmon increased when they swam in hyperoxic water, while U_{crit} did not change. The results of Gallaugher *et al.* (in prep) for rainbow trout also show clearly (Fig. 10) that an increase in $\dot{V}_{O_2max}$ does not lead to a corresponding increase in U_{crit}. Even though an increased $\dot{D}_{O_2max}$ may have a limited effect on U_{crit}, it ultimately affects the swimming velocity that the fish can maintain over days or weeks by enhancing the ability of the fish to support basal maintenance functions, digestive functions and growth. Thus, rainbow trout may have to defer a number functions until after they stop swimming while chinook salmon can support these same functions while swimming. This may explain why the sustainable swimming velocity of sockeye salmon is higher than in rainbow trout (Brett, 1964).

Figure 10. $\dot{V}_{O_2}$ as a function of swimming speed in chinook salmon from Exp. III and starved fish in Exp. I, and rainbow trout with different pre-set haematocrit (based on Gallaugher, Thorarensen & Farrell, in prep.). The $\dot{V}_{O_{2max}}$ of chinook salmon is higher than in the rainbow trout, and the $\dot{V}_{O_{2max}}$ of the rainbow trout increased as a linear function of Hct. The lines for the different Hct levels are isopleths from the model $\dot{V}_{O_2} = 25.6 \cdot \ln_{Hct} + 0.0056 \cdot \ln_{Hct} \cdot U^2 - 48.4$, which was fitted to a dataset from swimming fish with adjusted Hct ($R^2 = 0.73$).


Chinook (Exp. III)
Chinook (Exp. I starved)
Rainbow trout
Hct 40
Hct 30
Hct 20
Hct 10
$\dot{V}O_2$ (μmol min^{-1} kg^{-1})
Swimming speed (cm s^{-1})
0
25
50
75
100
125
150
175
200
225
250
275
300
0
10
20
30
40
50
60
70
80
90

The capacity of chinook salmon to swim, maintain gut function, and grow.

The maximum swimming velocity that chinook salmon can sustain without compromising growth must be set by their maximum rate of energy intake, the cost of swimming and possibly physiological constraints such as gut blood flow, which limit the capacity of the fish to swim and feed simultaneously.

The exercise-training experiments (Kiessling & Higgs, in review) demonstrated that chinook salmon can maintain a swimming velocity of at least 1.5 bl·s^{-1} without compromising growth. Further support for this is given by the observation that the growth of other fish from the same study (Kiessling & Higgs, in review), that were fed 25% less while swimming at 1.5 bl·s^{-1}, was not reduced compared with fish fed the same amount while swimming at 0.5 bl·s^{-1}. The energetic cost of maintaining a swimming speed of 1.5 bl·s^{-1} was 40-50% higher than to swim at 0.5 bl·s^{-1} (Fig. 1). Fish in the HS group, that were fed full ration, met this by increasing food intake by 20% compared with the LS1 group, while fish on restricted ration consumed equal amounts regardless of swimming velocity (Kiessling & Higgs, in review). This suggests that the fish swimming continuously at 1.5 bl·s^{-1} were able to increase the utilization of ingested energy compared to the fish that swam at 0.5 bl·s^{-1}. Increased metabolic efficiency has also been reported in exercised-trained brook rainbow trout (*Salvelinus fontinalis*) (Leon, 1986). Thus, chinook salmon could likely maintain a swimming velocity higher than 1.5 bl·s^{-1} without compromising growth. Rainbow trout have lower sustainable swimming speed than do salmon (Brett, 1964; Webb, 1975). Rainbow trout (100-600g) maintained a swimming velocity of 1 bl·s^{-1} without compromising growth (Davie *et al.* 1986; Farrell *et al.* 1989), but not at 1.6 bl·s^{-1} (fish 600g; Farrell *et al.* 1990). In other studies performed on smaller fish (5-70g) which have much higher U_{crit} values (in bl·s^{-1}) (Brett & Glass, 1973; Daxboeck, 1982), rainbow trout, coho salmon and Arctic charr maintained a

continuous swimming velocity of over 2 bl·s^{-1} without reducing growth rate (Nahhas *et al.* 1982; Bessner & Smith, 1983; Christiansen *et al.* 1991).

The ability of chinook to maintain relatively high swimming velocities without compromising growth is partly related to the fact that the HI was only 17% of the aerobic scope ($\dot{V}_{O2max}$ - $\dot{V}_{O2rest}$), which is much less than the 30-50% observed in some other species of fish (Jobling, 1982). Thus, in relative terms, the cost of digestion and assimilation may be much less for chinook than other species of fish with a lower aerobic scope. This high scope for activity in chinook salmon is a result of higher $\dot{V}_{O2max}$ than in most other species of temperate fish (Fig. 4). Moreover, the high $\dot{Q}_{max}$ of the chinook allows them to maintain intestinal blood flow while simultaneously swimming at higher velocities than is possible for less active fish such as the Atlantic cod and the sea raven with a lower $\dot{Q}_{max}$ (Chapter 7). Therefore, chinook salmon are able to sustain a higher swimming velocity while simultaneously supporting the metabolic cost of digestion and growth better than most other temperate fish.

These experiments provided no evidence for reduced digestive function in exercising fed fish (Chapter 3). Instead, maximum swimming performance of fed fish is reduced. It was estimated that the maximum gut blood flow in fed swimming fish was only a third lower than in fed resting fish (See Chapter 8), which is apparently adequate to support near full gut function in swimming fed fish, since HI was unchanged. The uptake of nutrients from the intestines was not measured, but the HI may be a reliable indirect measure of uptake. The HI of fish appears to reflect mostly the energetic cost of growth, primarily as increased protein synthesis (Jobling, 1983; Brown & Cameron, 1991b; Lyndon *et al.* 1992). Although the mechanism is not completely understood, the protein synthesis appears to be stimulated by increased availability of substrate (Peres-Sala *et al.* 1987). This is supported by the observation that following a meal, there is a transient increase in plasma concentration of amino acids (McLean & Ash, 1989) which parallels

the time course for the HI (Brown & Cameron, 1991a; Lyndon *et al.* 1992). Therefore, the results of these experiments suggest that the maximum sustainable swimming speed of chinook salmon, that is the velocity that can be sustained indefinitely, is not limited by their ability to maintain intestinal blood flow. However, the sustainable swimming velocity could be limited by the ability to support intestinal and muscle blood flow simultaneously, or by the maximum energy intake.

The energy content of the food ingested daily by the fish in the HS group was 49 Kcal·kg^{-1} body mass·day^{-1} (Kiessling & Higgs, in review). By applying an oxycalorific coefficient of 3.42 cal·mg^{-1} O_2 consumed, (Brett, 1983), it is possible to estimate the maximum swimming speed that the fish could sustain if they were able to ingest this ration at any velocity. However, not all the ingested energy is available for metabolism, since some is lost as fecal material, and nonfecal material (e.g. ammonia):

Metabolizable energy = Ingested energy - Fecal energy - Nonfecal energy loss

(Brett & Grove, 1978). The metabolizable energy of the diet was calculated from the food composition (Beamish *et al.* 1986; D. Higgs, pers com.) as 80% of the ingested energy. Thus, with 39 Kcal of metabolizable energy per day, the HS fish could have sustained a $\dot{V}_{O_2}$ of 208 μmol·min^{-1}·kg^{-1} after the HI has been subtracted. This would have allowed the fish to maintain a swimming velocity of about 71 cm·s^{-1} (Fig 1b) or 2.4 bl·s^{-1}. Therefore, the maximum sustainable swimming velocity of chinook salmon (300-500g) may lie between 1.5 and 2.4 bl·s^{-1} (54-83% of U_{crit}) and it is possible that even higher swimming velocities could be sustained if the feed utilization increases further.

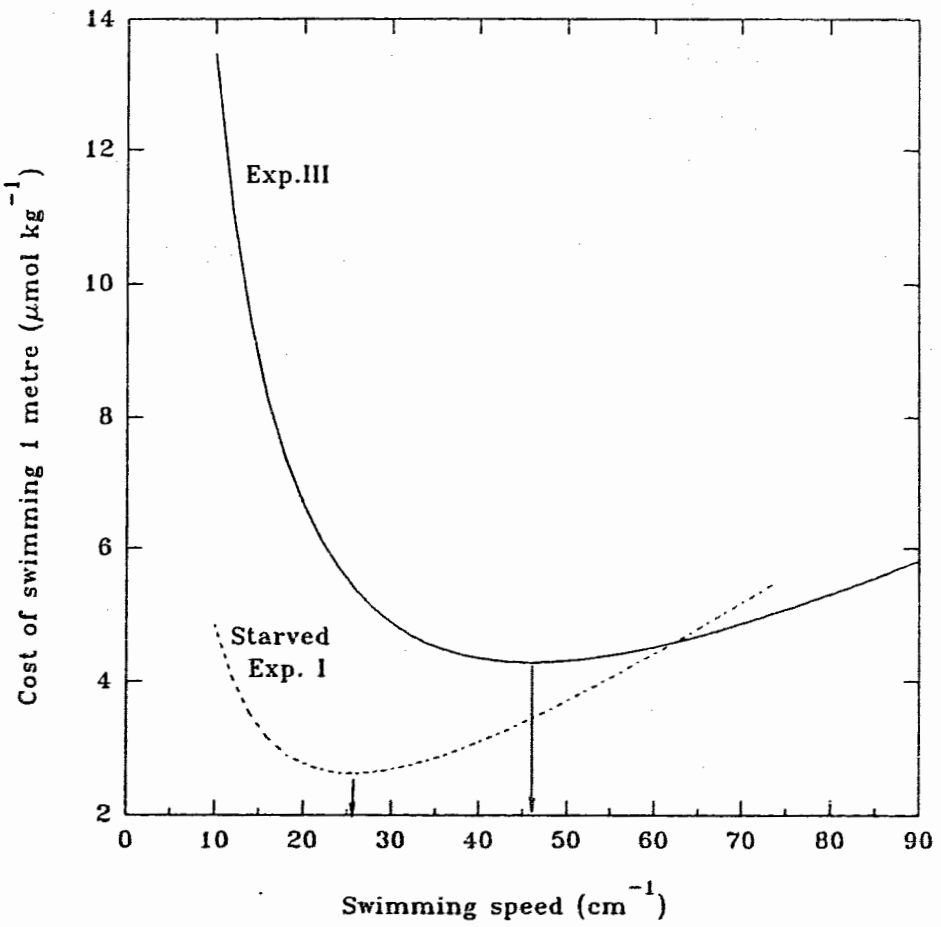
It is difficult to estimate the cost of routine activity of wild fish. Spontaneous routine swimming activity under natural conditions may be more expensive than suggested by the cost of steady swimming in laboratory swim-tunnels (Webb, 1991; Boisclair & Tang, 1993). This is mainly because the routine swimming of fish is not steady and rectilinear as in swim-tunnels, but interrupted by accelerations and powered turns (Webb, 1991).

The net cost of routine swimming may be three- to six-fold higher than for steady swimming (Webb, 1991; Boisclair & Tang, 1993). Therefore, the sustainable swimming velocity estimated here, based on maximum energy intake, may be considerably higher than the routine velocity which can maintained in the wild with the same energy intake.

The few measurements that are available for routine swimming velocity of salmon in the ocean suggest that they typically maintain a swimming speed near 1 $bl \cdot s^{-1}$ (See Quinn, 1988). Moreover, wild salmon are able to maintain a similar swimming speed while feeding (Brett, 1983). It has been suggested that the cruising speed of salmonids is similar to the optimum swimming speed (U_{opt}) (Weihs, 1973; Brett, 1983), which is the velocity that maximizes distance traveled for a unit energy expended. The optimum swimming speed of the fish in Exp. III was just over 1.5 $bl \cdot s^{-1}$ (Fig. 11) which is higher than the 0.8 $bl \cdot s^{-1}$ for the starved fish in Exp. I and 0.8 $bl \cdot s^{-1}$ for sockeye that were larger than chinook in the present study (Brett, 1983). If foraging success is proportional to distance covered there may be substantial gains to be had from swimming at U_{opt} compared with lower velocities (Fig. 11). Similarly, during migration there is a considerable energetic advantage in maintaining U_{opt}. The difference in U_{opt} among the fish in Exp. I, Exp. III and Brett's sockeye salmon can be attributed to differences in resting metabolic rate. The resting metabolic rate of the starved fish in Exp I (Table 2) was lower than in fish from Exp. III (Table 3) and the difference between Brett's fish and those of Exp. III can also be explained by the lower standard metabolic rate of larger fish (Brett, 1965). This also confirms conclusions of the theoretical analysis by Weihs (1973) which suggested that U_{opt} should change with the resting metabolic rate. Moreover, this is also consistent with the finding that the cruising speed of *Euthynnus affinis* was lower after periods of food deprivation than in actively feeding animals (Magnuson, 1970; Cahn, 1972).

In conclusion, based on the maximum energy intake of the fish in Exp. III and the maximum $\dot{V}_{O_2}$ supported by this intake, the chinook salmon could maintain a swimming velocity as high as 83% of the U_{crit} of unfed fish. Moreover, there is no indication that gut function is compromised in exercising fish. Instead U_{crit} is reduced. The routine swimming velocity of wild salmon is only 36-45% of the U_{crit} of the chinook salmon in Exp. I, III and IV, and similar to the U_{opt}. This may suggest that maximum routine activity of salmonids is dictated more by the economy of energy utilization rather than physiological limitations such as intestinal blood flow and the maximum capacity of the gut to process food, which appear to be able to support a higher $\dot{V}_{O_2}$ than is required for swimming and feeding at U_{opt}. The caveat is that, the actual cost of routine activity may be considerably higher than indicated by measurements of the cost of steady swimming in a swim-tunnel (Fig. 1a, b, c) and may actually approach the maximum sustainable $\dot{V}_{O_2}$, set by the maximum energy intake or other physiological limitations.

Figure 11. Calculated cost of swimming one meter at different velocities for fish in Exp. (HS and LS1) and starved fish in Exp. I. The arrows indicate the U_{crit}, which were 0.8 and for the starved fish from Exp. I and 1.5 $bl \cdot s^{-1}$ for fish from Exp. III.


14
12
10
8
6
4
2
0
10
20
30
40
50
60
70
80
90
Exp.III
Starved
Exp. I
Cost of swimming 1 metre (μmol kg⁻¹)
Swimming speed (cm⁻¹)

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Appendix I

Cardiac output in swimming rainbow trout, *Oncorhynchus mykiss*.

Helgi Thorarensen, Patricia Gallaugher and Anthony P. Farrell

Summary

This is the first study to report absolute measurements of cardiac output ($\dot{Q}$) in rainbow trout (body mass 401-1025g), swimming in seawater (10±1° C), measured with Transonic flow probes. Simultaneous measurements of Hct and arterial blood oxygen content in the fish, indicated that the fish were in a good physiological condition, while low resting heart rate, arterial blood pressure and systemic resistance suggested relatively low levels of stress. As swimming speed increased, $\dot{Q}$ increased by 84% from 26.6 mL·min^{-1}·kg^{-1} at rest to a maximum of 48.7 mL·min^{-1}·kg^{-1} at 97.3% of U_{crit}. This $\dot{Q}_{max}$ is similar to that observed in fresh water. The increase in $\dot{Q}$ was brought about by a 38% increase in heart rate (f_H) and a 25% increase in stroke volume (SV_H).

Correlations of different cardiac variables showed that individual variability in $\dot{Q}_{max}$ was mainly due to variability in SV_H, since maximum f_H was relatively invariant among fish. At rest, SV_H and f_H were negatively correlated. Combined, these, findings suggest that, f_H can only be effective in changing $\dot{Q}$ insofar as SV_H can be maintained. Neither $\dot{Q}_{max}$ nor SV_H were correlated with plasma osmolality. However, f_H was negatively correlated with plasma osmolality at U_{crit}.

Introduction

As salmonids increase swimming speed up to the maximum prolonged swimming velocity (U_{crit}; Hoar and Randall, 1978), the transfer of gases between the environment and the locomotory muscles increases as a result of a host of concurrent adjustments to the cardio-respiratory system. An important component of these adjustments is increased cardiac output ($\dot{Q}$). Only two papers have reported $\dot{Q}$ in swimming rainbow trout (Stevens *et al.* 1967; Kiceniuk and Jones, 1977). In both studies $\dot{Q}$ was not measured directly, but was instead estimated with the Fick principle (i.e. $\dot{Q} = \dot{V}_{O_2} / (Ca_{O_2} - Cv_{O_2})$; $\dot{V}_{O_2}$= Oxygen consumption; Ca_{O_2}, Cv_{O_2}= oxygen content of arterial and venous blood, respectively). The use of the Fick principle, to estimate $\dot{Q}$ in fish, has been criticized because of some inherent errors (Johansen and Petterson, 1981; Metcalfe and Butler, 1982; Daxboeck *et al.* 1983; Randall, 1985). Furthermore, Stevens *et al.* (1967) only estimated blood oxygen content from blood P_{O_2}, adding yet another source of error. Kiceniuk and Jones (1977) measured all the necessary variables, but they did not use the same group of fish to estimate $\dot{Q}_{rest}$ and $\dot{Q}_{max}$ and their estimate of $\dot{Q}_{max}$ was based on only four fish. Therefore, our current knowledge of cardiovascular dynamics in swimming rainbow trout is at best fragmentary. The objective of this study was to provide direct measurements of $\dot{Q}$ and other cardiovascular variables at different swimming velocities up to the critical swimming speed (U_{crit}) in a single set of fish.

Various methods have been used to measure $\dot{Q}$ in either resting or restrained rainbow trout. These include flow probes (Wood and Shelton, 1980a,b; Xu & Olson, 1993; Gamperl *et al.* in press), dye dilution techniques (Neuman *et al.* 1983; Barron *et al.* 1987) and the Fick principle (Holeton *et al.* 1967; Stevens *et al.* 1967b; Cameron and Davis, 1970; Davis and Cameron 1971; Kiceniuk and Jones, 1977; Neuman *et al.* 1983). These studies have generated a broad range of $\dot{Q}$ values for resting or restrained rainbow trout ranging from 16-65 $mL \cdot min^{-1} \cdot kg^{-1}$ (see Farrell and Jones, 1992). Some of this

variability could be accounted for by the different temperatures at which these studies were performed (5-18° C), but methodological differences are also likely to have contributed to the variability.

The recent development of ultrasonic flow probes has allowed continuous measurements of blood flow in swimming fish (Axelsson and Nilsson, 1986; Axelsson *et al.* 1989; Axelsson and Fritsche, 1992; Thorarensen *et al.* 1993; Kolok *et al.* in press). One type of ultrasonic flow probes, the Transonic is particularly useful because it gives readings of absolute blood flow as well as zero flow signal, a feature not shared by either Doppler or electromagnetic flow probes. Our laboratory has developed techniques to use these flow probes to measure cardiac output in swimming fish (Kolok *et al.* 1983; Kolok & Farrell, 1994). Moreover, the flow probes have little effect on U_{crit}, and the rank order of swimming performance within a group of fish was maintained after implantation of the flow probes (Kolok & Farrell, in review). Thus, Transonic flow probes provide a more reliable measurements of $\dot{Q}$ in fish than previously were possible. Resting cardiac output was measured in rainbow trout with Transonic flow probes, implanted on the ventral aorta after they had opened the pericardiu (Xu & Olson, 1993). Since *vis a fronte* filling of the heart can only occur if the pericardium is intact (Farrell *et al.* 1988) cardiac performance may be altered. In the present experiment a novel method was used to place a Transonic probe on the ventral aorta, which did require the pericardium to be opened. This method was used to obtain, for the first time, absolute measurements of cardiac output in a salmonid. Furthermore, by looking for correlates of maximum cardiac performance, we also attempted to shed further light on cardiovascular dynamics and the determinants of maximum cardiac performance during aerobic swimming.

Materials and methods

Experimental animals.

Rainbow trout, *Oncorhynchus mykiss*, of both sexes (mean weight 610g, range 401-1025g) were transported from West Creek Trout Farm, Aldergrove, British Columbia to the West Vancouver Laboratory (Department of Fisheries and Oceans), where the experiments were performed. The experiments were conducted during November and December, but prior to this the fish were acclimatized to seawater (salinity 30 ppt; 9-11° C) for two months or longer . During the acclimation period, the fish were kept outdoors in circular tanks that were supplied with an ample flow of seawater. The fish were fed satiation levels of dry pellets (Moore Clarke, British Columbia) once daily. Before surgery the fish were starved for at least 24 h.

Surgery.

The fish were anaesthetized in a 1:2,000 solution of 2-phenoxyethanol (Sigma Chemical Co., St. Louis, MO) in seawater and the anaesthesia was maintained by continuously irrigating the gills with a 1:4,000 solution of 2-phenoxyethanol in seawater. A cannula (PE 50) was inserted into the dorsal aorta (DA) as described by Thorarensen *et al.* (1993) to allow measurements of blood pressure (P_{DA}) and to obtain blood samples. P_{DA} was measured with LDI5 pressure transducers (Narco, Houston, TX).

To measure $\dot{Q}$, a Transonic flow probe (Transonic Inc., Ithaca, NY) was placed around the ventral aorta (VA) of the trout, just distal to the bulbus arteriosus. The VA was accessed from the opercular cavity, where it is readily visible as it runs dorsad immediately ventral to the gills, when operculum and gills are folded forward. This site has been used previously for the cannulation of the *VA* with PE tubing, for ligation of the coronary artery , and for measurement of blood flow in the *VA* (Farrell and Steffensen, 1987a) and for measurements of coronary and *VA* blood flow (Axelsson & Farrell, 1992).

The *VA* was exposed by gently teasing apart overlying skin, without rupturing the pericardium or obstructing the coronary artery, so the flow probe could be placed around the *VA*. The flow probe was secured in place with crosswise stitches (3-0 silk suture) that were tied to the probe, and this ensured that the flow probe would not move while the fish were swimming. The leads were anchored with a 1-0 silk suture near the pectoral fin, and to the back just anterior to the dorsal fin. Two sizes of flow probes were used depending on the size of the fish; the larger probe was 9 mm long had a 14.8 mm^2 square sensing window and the smaller probe was 8 mm long with a 4 mm^2 window. The surgery was completed in less than 30 minutes and the fish were allowed to recover for 18-24 hours before experiments commenced.

Experimental procedures

For these experiments we used the same swim tunnel as is described in Kiceniuk and Jones (1977). All experiments were performed at 10±1° C. Prior to experiments, the fish were allowed to habituate to the tunnel for at least 12 h. While the fish were still resting $\dot{Q}$ and P_{DA} were recorded and an initial blood sample taken to measure haematocrit (Hct), haemoglobin concentration ([Hb]), partial pressure of oxygen (Pa_{O_2}) and Ca_{O_2} in the arterial blood. Swimming velocity was then increased to about 1 body-length s^{-1} ($bl \cdot s^{-1}$) and the same variables recorded again. Subsequently, swimming speed was increased in steps of 0.25 $bl \cdot s^{-1}$, each step being maintained for 30 min or until the fish fatigued. At each step, a stable level of $\dot{Q}$ and P_{DA} representative of that swimming speed were recorded 10 minutes after the velocity was increased. When the fish began to show early signs of fatigue, blood samples were also taken after every velocity increment until the fish reached U_{crit}. After the fish had recovered for 1 hour after fatigue, a final blood sample was taken. Plasma lactate was measured in blood the samples taken at rest, at fatigue, and during recovery. Each time a blood sample was taken 1 mL of blood was drawn from the fish. Some of that blood was used for analysis, but the remainder was

reinjected into the fish along with enough saline to replace the blood used for the haematological measurements. In total, 4-6 blood samples were taken from the fish while they swam resulting in a total volume of 1-1.5 mL of blood being replaced with saline prior to the final sample. Replacing this amount of blood with saline has minimal effect on Hct in rainbow trout (Gallaugher *et al.* 1992).

Analysis of blood samples.

Blood $PO_2$was measured with a thermostatted electrode (E5046, Radiometer, Copenhagen) maintained at the experimental temperature and connected to a PM71 unit. Ca_{O_2} was measured with the method of Tucker (1967). Duplicate samples for Hct were spun in micropipettes (10 µl). Blood [Hb] and plasma [lactate] were analyzed with Sigma kits 525A and 826-UV, respectively. The saturation of haemoglobin in arterial blood was calculated by subtracting the O_2 dissolved in plasma (Boutilier *et al.* 1984) from the Ca_{O_2} and by assuming that 1g of haemoglobin can bind 1.38 mL of oxygen.

Data acquisition and statistical analyses.

The signals for P_{DA} and $\dot{Q}$ were recorded directly on a Grass chart recorder (model 7PCP B, Grass Instruments, Quincy, MA) and relayed to a computer for storage and on-line processing with Labtech Notebook (Laboratory Technology Corp., MA). At each velocity step, the computer sampled the signals for P_{DA} and $\dot{Q}$ for 6 minutes at a rate of 5 Hz and calculated heart rate from the flow pulses. The mean values for the 6 minute periods were used for analysis. Systemic resistance R_{sys} was calculated from mean P_{DA} and $\dot{Q}$ at each step ($R_{sys} = P_{DA} / \dot{Q}$), with the assumption that changes in venous pressure were negligible relative to arterial pressure (Farrell, 1991).

Statistical comparisons were performed using the General Linear Models Procedure and the Correlation Procedure in SAS (Version 6, SAS Institute). Data for variables recorded at different swimming velocities were compared using an analysis of variance (ANOVA)

with repeated measures. Individual means were compared with Tukey's tests. To examine the variability in the cardiovascular variables the coefficients of variation (CV) were calculated (CV = 100·standard deviation / mean).

Results

Absolute $\dot{Q}$ *as a function of body mass.*

Most commonly, $\dot{Q}$ is normalized per kg body mass (bm) and expressed in units of $mL \cdot min^{-1} \cdot kg^{-1}$. For this to be legitimate, $\dot{Q}$ must scale isometrically with bm, i.e., a plot of $\dot{Q}$ and bm must be a linearly related and the line must intercept the y-axis at zero (Packard and Boardman, 1988). If these conditions are not met, normalizing $\dot{Q}$ for groups of large and small fish will not be comparable. Both $\dot{Q}_{max}$ and $\dot{Q}_{rest}$ of the rainbow trout (expressed as $mL \cdot min^{-1}$) increased linearly with body mass and the intercepts were not significantly different from zero. The relationships between $\dot{Q}$ and body mass (in $mL \cdot min^{-1}$) was ($R^2 = 0.78$, both at rest and maximum):

$$\dot{Q}_{max} = 0.142 + 48.53 \cdot bm$$

$$\dot{Q}_{rest} = 3.647 + 20.39 \cdot bm$$

Since both relationships appear to be linear and the intercepts are low, particularly for $\dot{Q}_{max}$, the use of $\dot{Q}$ normalized for bm is justified. In fact the $\dot{Q}_{max}$ values predicted from the equation for the smallest and largest fish used (401 and 1025g) are within 0.2 $mL \cdot min^{-1} \cdot kg^{-1}$ from the mean of $\dot{Q}$ normalized for bm. There is slightly more error introduced at rest where the predicted values for the largest and the smallest fish differ as much as 2.8 $mL \cdot min^{-1} \cdot kg^{-1}$ from the mean, but for the sake of simplicity of presentation all values are normalized for weight. This approach is not likely to have affected our results since none of the variables were correlated with bm after they had been normalized.

Cardiac variables.

As the fish increased swimming speed, $\dot{Q}$ increased (Fig 1a) from 26.6±2.4 $mL \cdot min^{-1} \cdot kg^{-1}$ at rest to a $\dot{Q}_{max}$ of 48.7±4.0 $mL \cdot min^{-1} \cdot kg^{-1}$. The mean swimming speed when $\dot{Q}_{max}$ was

reached was 97.3%±1.3 of U_{crit}; U_{crit} was 82 cm·s^{-1} or 2.35 bl·s^{-1}. The 82% increase in $\dot{Q}$ was a result of a 38% increase in f_H (48.4±3.3 to 66.9±2.1 bpm) and a 25% increase in mean stroke volume (0.58±0.06 to 0.73±0.05 mL·kg^{-1}; Fig. 1a). P_{DA} increased by 25% (3.29±0.20 kPa to 4.12±0.19 kPa), while R_{SYS} fell by 29% (Fig. 1b) (0.126±0.008 to 0.090±0.007 kPa·mL^{-1}·min·kg).

Following 1 h of recovery after fatigue, $\dot{Q}$ had returned to 29.3±2.15 mL·min^{-1}·kg^{-1}, while stroke volume (0.483 mL·kg^{-1}) and P_{DA} were significantly lower and f_H was significantly higher than at rest. The 12% change in plasma osmolality from rest to U_{crit} was not significant (Table 1).

Correlations of cardiac variables.

There was a significant correlation between SV_H and $\dot{Q}$ both at $\dot{Q}_{rest}$ ($P<0.01$) and at $\dot{Q}_{max}$ ($p<0.0005$) (Fig. 2a). However, neither resting nor maximum f_H was correlated with $\dot{Q}_{rest}$ or $\dot{Q}_{max}$ (Fig. 2b). There was a significant ($p<0.002$) negative correlation between SV_H and f_H at rest (Fig. 2d). The variance in maximum SV_H (CV=23.6%) was significantly ($p<0.01$) higher than the variance in maximum f_H (CV= 9.8%). At rest the variance in SV_H was also higher (CV= 33.6%) than the variance in f_H (CV= 21.8%), but the two were not significantly different. The fish with the largest resting SV_H also tended to have the largest SV_H at $\dot{Q}_{max}$ ($p<0.01$) (Fig. 2c) as well as the highest $\dot{Q}_{max}$ ($p<0.01$). The fish with the largest resting SV_H also had the greatest scope to increase f_H (Fig. 2d, e). However, the scope for increasing SV_H did not change significantly with the size of the resting SV_H. Plasma osmolality at U_{crit} was negatively correlated with f_H ($p<0.003$), but osmolality was neither correlated with $\dot{Q}_{max}$ or SV_H. Except for osmolality at U_{crit}, none of the variables in Table 1 correlated with swimming performance or $\dot{Q}$.

Haematological variables.

The following changes occurred in blood and plasma as the fish swam to U_{crit} (Table 1). Pa_{O_2} was significantly reduced, but Ca_{O_2} and oxygen saturation of haemoglobin were not significantly altered (Table 1). A 9% increase in Hct was associated with a corresponding reduction in MCHC, indicating that the increase in Hct was caused by swelling of red blood cells rather than by haemoconcentration (Table 1). Following one hour of recovery, [Hb] was significantly reduced compared with resting values. Plasma lactate was elevated in fish swimming at U_{crit}, and even more so after 1 h of recovery.

Discussion

This is the first study to report direct measurements of $\dot{Q}$ in swimming rainbow trout. Furthermore, we employed a novel method to measure $\dot{Q}$ and therefore the condition and performance of these fish must be compared to previous studies, to establish the credibility of this method for measurement of $\dot{Q}$ in salmonids.

Even though the fish in this study were burdened with the extra drag of the cannula and the lead from the flow probe, their swimming performance was similar to or better than what has been observed in other comparable studies. The U_{crit} of the fish in this experiment (2.35 bl·s^{-1}) was considerably higher than the U_{crit} (0.5-1.5 bl·s^{-1}) of the rainbow trout in the study of Kiceniuk and Jones (1977). In fact, it compares favorably with the 1.74-2.65 bl·s^{-1} reported in some recent studies of uncannulated salmonids, of similar size, swimming in both fresh water and seawater (Duthie, 1983; Randall *et al.* 1987; Farrell *et al.* 1990, 1991; Pearson and Stevens, 1991; Butler *et al.* 1992; Gallaugher *et al.* 1992). This suggests that the flow probes appeared to have little effect on maximum swimming performance.

Haematological variables (Table 1) were comparable to those in other studies of swimming salmonids (Thomas *et al.* 1987; Butler *et al.* 1992; Gallaugher and Axelsson, 1992; Thorarensen *et al.* 1993). The mean Hct of the fish in this study (28-30%) was within the range of that reported in similar studies (Kiceniuk and Jones, 1977; Thomas *et al.* 1987; Gallaugher and Axelsson, 1992) and higher than the 15-25% reported in previous studies where skeletal muscle has been dissected through to implant flow probes on the *VA* to measure $\dot{Q}$ in rainbow trout (Wood & Shelton, 1980a; Gamperl *et al.* in press). This suggests that the fish did not suffer excessive bleeding due to the surgery. The resting lactate levels were low, and comparable to those has previously reported for resting fish (Milligan and Wood, 1986; Tang *et al.* 1989), indicating that the fish were not fatigued prior to the experiment. As in some recent experiments, Pa_{O_2} fell significantly

as the fish swam up to U_{crit} (Thomas *et al.* 1987; Butler *et al.* 1992; Gallaugher *et al.* 1992; Thorarensen *et al.* 1993). However, Ca_{O_2} and haemoglobin saturation remained constant and thus it appears as if the drop in Pa_{O_2} is of little significance for the oxygen carrying capacity of arterial blood.

The cardiac variable that can be most accurately measured is heart rate and therefore it is a valuable means for comparing different studies. The resting f_H of the fish in this study (48.4 bpm) was lower than reported in most other studies (53 to 56 bpm) at similar temperature (10-15° C) where the fish were equipped only with a dorsal aorta cannula (Smith, 1977; Wood *et al.* 1979; Butler *et al.* 1986). Similarly the maximum f_H observed in this study (66.9 bpm) is in keeping with what has been observed in other studies, 63 bpm (Smith, 1977) and 70 bpm (Butler *et al.* 1986). Both resting and maximum f_H were greater than those reported by Kiceniuk & Jones (1977; 37.8 and 51.4 bpm, respectively). The reasons for this difference in f_H are not apparent, but could be related to size, since the fish used by Kiceniuk and Jones (1977) were at least two or three times larger than the fish in the studies mentioned above and in the present study. In both mammals, and some invertebrates f_H is known to scale negatively with body mass (Schmidt-Nielsen, 1984) and that could also apply to trout. However, we were unable to find any correlation between f_H and body mass, although the size range of our fish may have been to narrow for this relationship to show up.

Intact pericardium is essential for development of $\dot{Q}_{max}$ (Farrell *et al.* 1988). The method used in the present study to implant the flow probe on the VA does not require that the pericardium is ruptured, as is commonly done when the $\dot{Q}$ of fish is measured (Wood & Shelton, 1980a,b; Kolok *et al.* 1994; Xu & Olson, 1993; Gamperl *et al.* in press). In fact, the good swimming performance of the fish in this study suggests that probe placement did not appreciably affect $\dot{Q}_{max}$.

The $\dot{Q}_{rest}$ was higher in the present study (26.6 mL·min^{-1}·kg^{-1}) than in that of Kiceniuk and Jones (17.6 mL·min^{-1}·kg^{-1}). It is unlikely that this difference is due to excessive stress in our fish, since P_{DA} and R_{SYS} were lower than those reported by Kiceniuk & Jones (1977). The $\dot{Q}_{max}$ (48.7 mL·min^{-1}·kg^{-1}) of the rainbow trout in this study, which were in seawater, was similar to the 52.6. mL·min^{-1}·kg^{-1} reported by Kiceniuk and Jones (1977) for rainbow trout in fresh water. The consistancy between $\dot{Q}_{max}$ determined by Fick estimates and direct measurements, concurs with the suggestion of Randall (1985) that the errors inherent in the Fick estimate tend to cancel out. Moreover, Neuman *et al.* (1983) found good agreement between Fick estimates of $\dot{Q}$ in rainbow trout and direct measurements with other methods when $\dot{V}_{O_2}$ was high.

Correlates of cardiac performance.

The correlations between cardiac performance and various cardiac variables (Fig. 2) reveal some new information, and confirm older findings about cardiac dynamics in rainbow trout. The first is that variability in both $\dot{Q}_{rest}$ and $\dot{Q}_{max}$ is primarily determined by SV_H and not f_H. There was a positive correlation between SV_H and $\dot{Q}$ (Fig 2a), while there was no significant correlation between $\dot{Q}_{rest}$ and f_H (Fig. 2b). In fact, maximum f_H was relatively invariable among individuals whereas maximum SV_H was not.

The negative correlation between f_H and SV_H at rest (Fig 2b, d), may be explained by the time available for atrial filling (Farrell, 1991), which is inversely related to f_H. This correlation concurs with results from other studies that show only a tenuous relationship between $\dot{Q}_{rest}$ and f_H (Cameron and Davis, 1970; Wood and Shelton, 1980b). This negative correlation between SV_H and f_H and the lack of correlation between f_H and $\dot{Q}$, suggests that $\dot{Q}$ is regulated primarily through changes in stroke volume, and confirms that f_H can only change $\dot{Q}$ insofar as SV_H is maintained (Farrell, 1985).

For individual fish, the relative contributions of SV_H and f_H to increases in $\dot{Q}$ depend on the resting conditions of the fish. The scope for increase in f_H is highly dependent on f_H at rest (Fig. 2e). This and the negative relationship between SV_H and f_H at rest set the framework for how $\dot{Q}$ is increased. Fish with high resting SV_H and consequently low f_H relied relatively more on f_H to increase $\dot{Q}$ than fish with high resting f_H which had little scope to increase f_H (Fig. 4e). The fish with the lowest f_H, which also had the greatest scope to increase f_H and the largest SV_H reached the highest $\dot{Q}_{max}$. Thus, the effect of SV_H on $\dot{Q}$ appears to be both direct and indirect, in that the fish with high SV_H will have high $\dot{Q}_{max}$ and the largest scope to increase f_H during exercise. These results are the first to show the complexity of interactions among $\dot{Q}$, SV_H and f_H in intact fish and that the ability to reach a high $\dot{Q}_{max}$ is primarily determined by their capacity to generate high SV_H.

Comparisons of the cardiac performance in intact trout and *in situ* preparations (Farrell *et al.* 1986) can be revealing and may offer insight into how close the heart is to its maximum potential at $\dot{Q}_{max}$. The highest $\dot{Q}$ observed for an *in situ* trout heart is 63-74 $mL \cdot min^{-1} \cdot kg^{-1}$ (Farrell *et al.* 1991), varying depending on temperature, training state and level of adrenergic stimulation (Farrell *et al.* 1991; Franklin and Davie, 1992). The $\dot{Q}_{max}$ of the rainbow trout of the present *in vivo* study (48 $mL \cdot min^{-1} \cdot kg^{-1}$) was lower, largely due to the lower SV_H (0.729 $mL \cdot min^{-1}$) *in vivo* compared with the *in situ* preparations (0.9-1.1 $mL \cdot kg^{-1}$). Thus the trout do not appear to exploit the full flow potential of their heart *in vivo*, either due to the inability of the fish to maintain venous pressure at the level required to generate maximum SV_H or that adrenergic stimulation of the heart is not maximal.

In this study we have, for the first time, measured cardiac output in a swimming salmonid. The results confirm the importance of SV_H as a determinant of $\dot{Q}$ in rainbow

trout and that cardiac scope depends to a large extent on a fishes ability to maintain a large SV_H.

App. I. Table 1. Haematological variables recorded at different swimming velocities and after 1h. of recovery. N=10 for all means except osmolality, where N=6.

	U_{crit} %	Pa_{O2} torr	CaO_2 % vol.	Hct %	Hb $g \cdot dL^{-1}$	Sat. %	Lact. mmol	Osm mosm	MCHC
Mean	6.8	104.4	10.3	27.6	8.2	87.4	0.62	285.2	0.297
SEM	0.2	4.1	0.5	1.5	0.4	2.5	0.11	20.4	0.004
Mean	48.1	100.1	10.4	26.7	7.7	94.6	-	-	0.286
SEM	1.2	3.9	0.3	1.2	0.4	2.7	-	-	0.005
Mean	86.3	73.9*	10.3	28.4	8.3	87.1	-	-	0.296
SEM	1.3	7.1	0.4	1.4	0.3	2.1	-	-	0.011
Mean	97.7	55.6*	10.1	30.2*	8.1	88.8	1.95*	320.7	0.272*
SEM	0.6	6.2	0.4	2.0	0.4	0.4	0.36	18.3	0.008
After 1 h. recovery									
Mean	-	104.5	9.7	25.4	7.1*	93.7	2.98*	319.8	0.280
SEM	-	6.0	0.5	1.4	0.3	1.9	0.63	24.3	0.004

* Values are significantly ($p<0.05$) different from resting values.

Figure 1. Cardiovascular variables at different swimming speeds and after 1 h. of recovery. a) Cardiac output (■), stroke volume (O) and heart rate (◊). b) Blood pressure in the dorsal aorta and systemic resistance ($P_{DA}/\dot{Q}$).

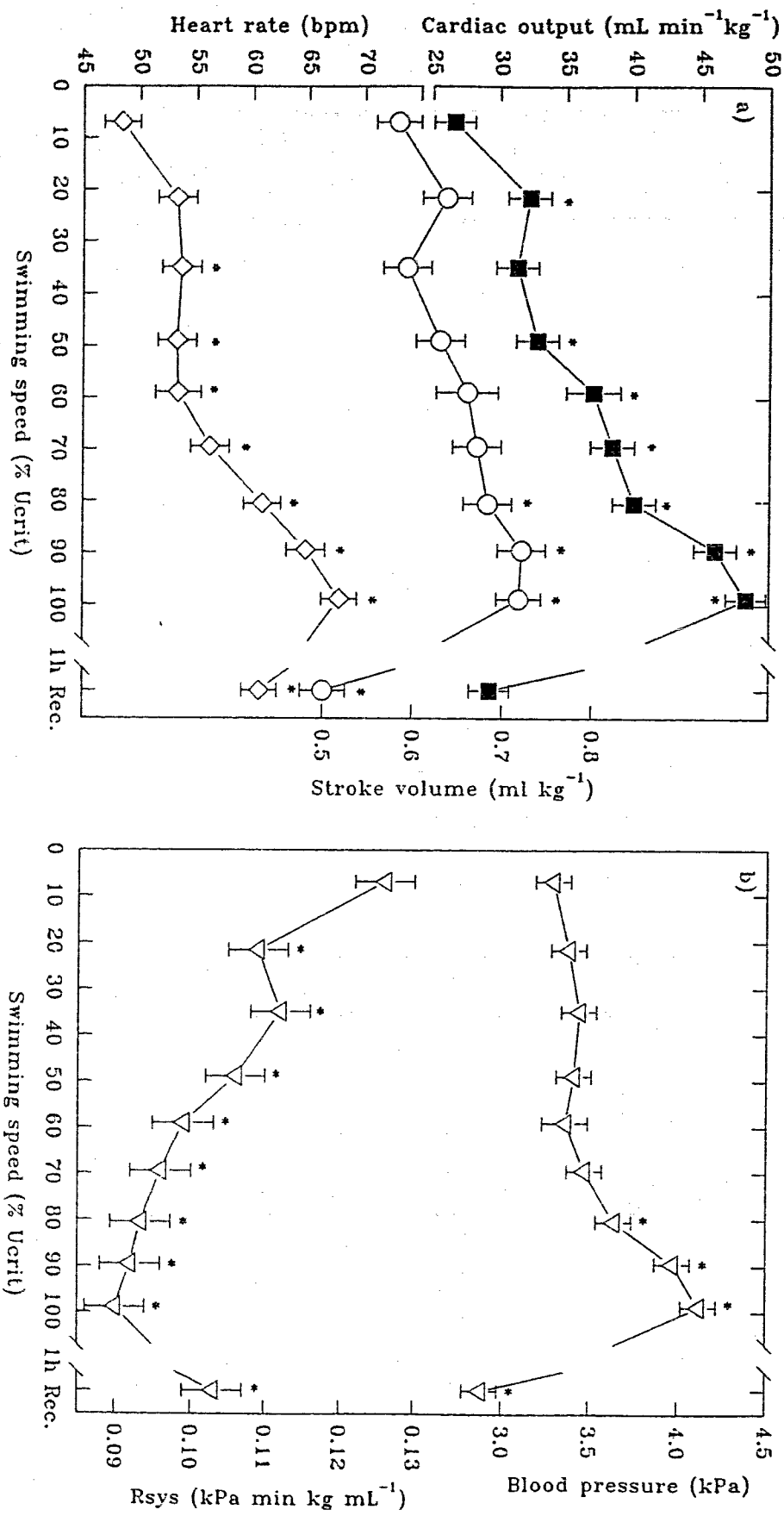
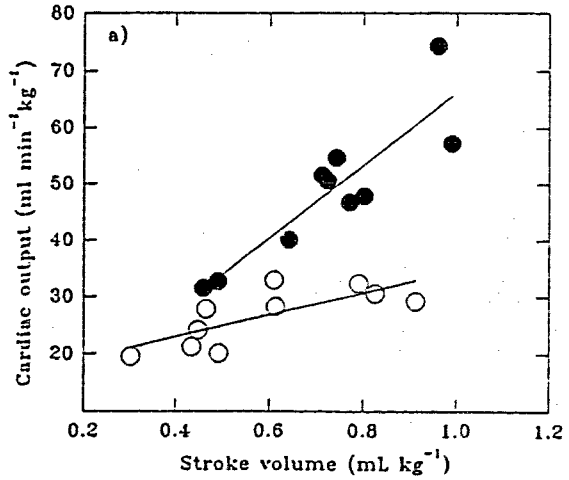
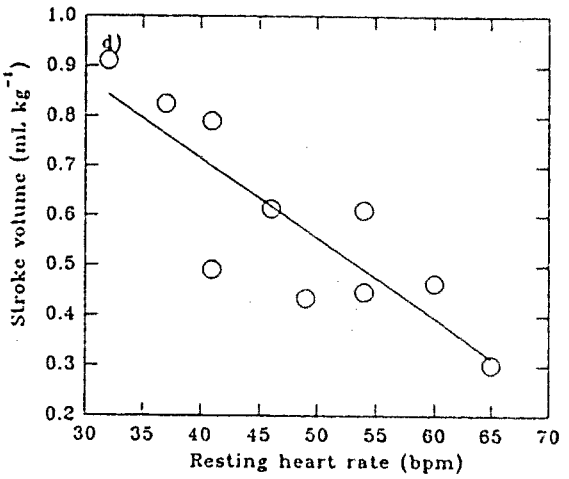
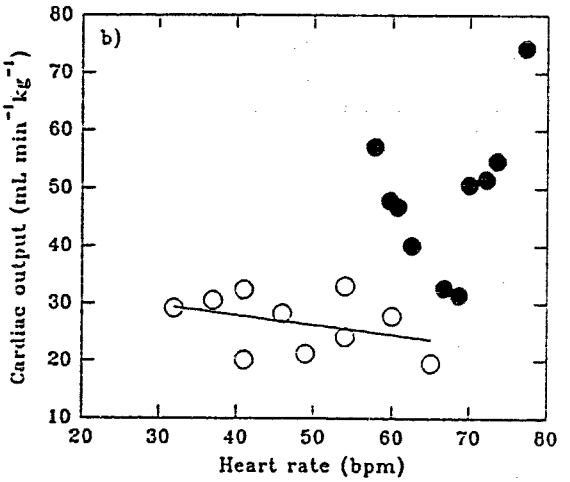
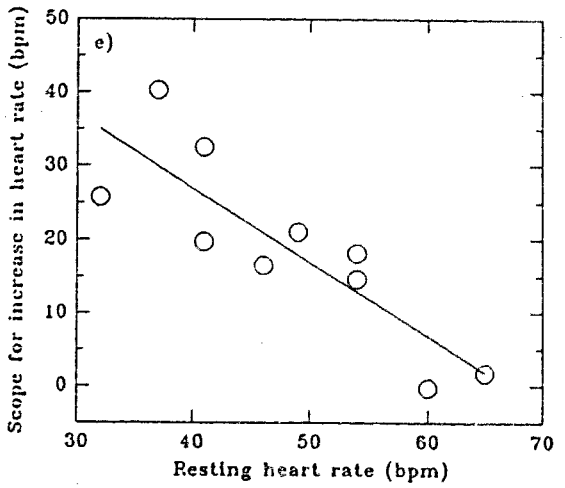

Heart rate (bpm)
Cardiac output (mL min^{-1}kg^{-1})
a)
Swimming speed (% Ucrit)
1h Rec.
Stroke volume (ml kg^{-1})
b)
Rsys (kPa min kg mL^{-1})
Blood pressure (kPa)

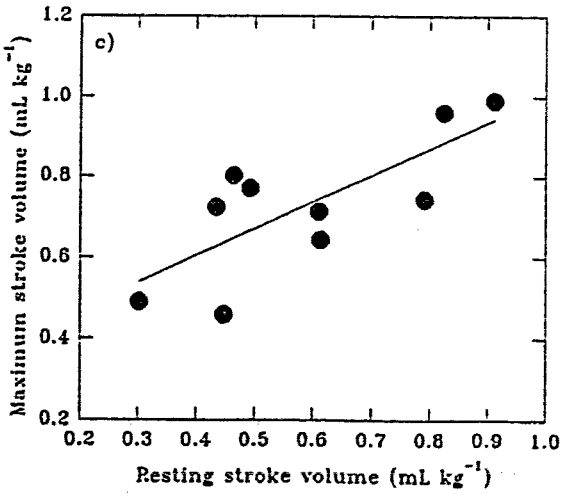
Figure 2. Relationships between various cardiovascular variables in fish that were swum to U_{crit}. The significance of correlations is shown in parentheses. a) Stroke volume and cardiac output at rest (O) ($p<0.01$), and maximum cardiac output (●) ($p<0.0005$). b) Heart rate and cardiac output at rest (O) (ns), and maximum cardiac output (●) (ns). c) Resting and maximum stroke volume ($p<0.01$). d) Resting heart rate and stroke volume (0.002). d) Resting heart rate and scope for increase in heart rate (0.002).


a)
Cardiac output (ml min^{-1}kg^{-1})
Stroke volume (mL kg^{-1})


d)
Stroke volume (mL kg^{-1})
Resting heart rate (bpm)


b)
Cardiac output (mL min^{-1}kg^{-1})
Heart rate (bpm)


e)
Scope for increase in heart rate (bpm)
Resting heart rate (bpm)


c)
Maximum stroke volume (mL kg^{-1})
Resting stroke volume (mL kg^{-1})

Appendix II

Cardiovascular O_2 delivery in rainbow trout.

Introduction

Here are presented some results from an unpublished study which is referenced in this thesis. Relative changes in cardiac output in swimming rainbow trout were measured with Doppler flow probes and then calibrated at $\dot{V}_{O2max}$ with Fick estimates of $\dot{Q}$. These results are compared to $\dot{Q}$ values measured with Transonic flow probes on the same group of rainbow trout. Furthermore, the ventilation volume ($\dot{V}_g$) of the rainbow trout was measured.

Methods

Rainbow trout (mean weight 576g) were split into two groups. One group of fish was fitted with a Doppler flow probe and a dorsal aorta cannula as described in the General Methods section (Chapter 2). In addition, the afferent gill artery of the second gill arch was cannulated to sample venous blood. The cannula for the afferent gill artery was made from PE50 tubing, which had previously been drawn to a fine tip. The cannula was inserted with a trocar. Expired water from the gills was sampled for measurements of $\dot{V}_g$ as described by Holton & Randall (1967).

A second group of fish was cannulated in the dorsal aorta and a transonic flow probe was placed on the ventral aorta as described in Appendix I.

Fish from both groups were swum to U_{crit} as described in the General Methods section, while cardiorespiratory variables were measured. The ambient temperature during the experiments was 9±1 °C .

Results and discussion

The major findings of this study which are relevant to this thesis are presented in Table 1. There was no significant difference between resting or maximum $\dot{Q}$ values measured, with with transonic flow probes, or Doppler flow probes calibrated with Fick estimates at $\dot{V}_{O2\,max}$. This confirms that Fick estimates give reliable measurements of $\dot{Q}$ when $\dot{V}_{O_2}$ is high.

The increase in $\dot{V}_g$ was greater than the increase in $\dot{V}_{O_2}$ from rest to maximum. This is similar to what was observed by Kiceniuk & Jones (1977).

AII. Table 1. Cardiorespiratory variables measured in swimming chinook salmon fitted Doppler (n=9) or Transonic (n=6) flow probes.* In fish fitted with Doppler flow probes, gill ventilation volume ($\dot{V}_g$) and $\dot{Q}_{max}$ were estimated with the Fick principle.

	$\dot{Q}$ measured with a Doppler flow probe			$\dot{Q}$ measured with a transonic flow probe	
	$\dot{Q}$ mL blood·min^{-1}·kg^{-1}	$\dot{V}_{O_2}$ µmol O_2·min^{-1}·kg^{-1}	$\dot{V}_g$ mL water·min^{-1}·kg^{-1}	$\dot{Q}$ mL blood·min^{-1}·kg^{-1}	$\dot{V}_{O_2}$ µmol O_2·min^{-1}·kg^{-1}
Resting	21.4(3.0)	25.6(2.0)	193(31)	19.3(2.0)	30.4(6)
Maximum	35.1(4.7)	126.5(7.8)	1200(309)	34.8(2.5)	121.3(11.6)

* Number in parentheses are SEM.

Appendix III

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The blood vasculature of the gastrointestinal tract in chinook, *Oncorhynchus tshawytscha* (Walbaum), and coho, *O. kisutch* (Walbaum), salmon

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The venous and arterial vasculature of the chinook and coho salmon gastrointestinal tract were examined using corrosion casts and India ink injection techniques. Observations derived from 28 individuals of various sizes and of both sexes were used to construct simplified venous and arterial plans. Examination of the blood vasculature revealed the presence of a variety of anastomoses hitherto undescribed in teleosts.

Key words: *Oncorhynchus tshawytscha; Oncorhynchus kisutch*; blood vasculature; gastrointestinal tract; morphology; corrosion casts; chinook salmon; coho salmon.

I. INTRODUCTION

Imperative to an understanding of physiological aspects of energy acquisition in fishes, are measurements of post-prandial blood flow and nutrient net absorption. In order to select appropriate vessels for cannulation and flow measurements a detailed knowledge of the gastrointestinal vasculature is required. Literature pertaining to the blood vasculature of teleosts is diverse (for review see Harder, 1975) and the general morphology of the salmonid circulatory system has been described (Gorkiewicz, 1947; Smith & Bell, 1975). However, knowledge on salmonid gut vasculature is mainly derived from dissections of rainbow trout, *Oncorhynchus mykiss* (Walbaum), (Grodzinski, 1938; Koniar, 1947) and only limited information is available for other species. Accordingly, the present study was undertaken to provide a comprehensive account of the gastrointestinal vasculature in coho salmon, *O. kisutch* (Walbaum), and chinook salmon, *O. tshawytscha* (Walbaum).

II. MATERIALS AND METHODS

Coho and chinook salmon (300–1200 g wet wt; $n = 18$) were used to make corrosion casts from both venous and arterial vasculature. Fish were fasted for 48 h prior to cast preparation. All animals were anaesthetized in sea water containing 0·04% 2-phenoxyethanol (Syndel, Vancouver, B.C., Canada). A cannulae was inserted into the aorta (a.) dorsalis and the fish was injected, via the cannula, with 1000 iu heparin/kg in 0·9% saline. Five minutes later the a. ventralis was severed, and isotonic saline (0·9% NaCl with 10–7 papaverin and 10 iu heparin) flushed through the a. dorsalis cannula under a pressure of 60 cm H_2O, until no blood was present in the washout. For casts of the venous vasculature,

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an additional cannula was placed in the vena (v.) intestinalis ventralis or the v. splenica and flushed in a similar manner although at lower pressure.

Corrosion casts were prepared according to the techniques described in Gannon (1978). The vascular system was casted using Batson's No. 17 anatomical corrosion compound (Polysciences, U.S.A.). To produce a slowly polymerizing mix the components of the corrosion compound were combined in the following quantities: monomer 40 ml, catalyst 3 ml, promoter 0·2 ml and methyl methacrylate 10 ml. Best results were obtained when the corrosion compound was injected under pulsatile pressure (10–60 cm H_2O) by manually pressing the piston of a 30 ml syringe. Less pressure was used during preparation of venous casts. With the exception of the renal tissue, the resin did not pass capillary beds. Infusion of the casting mixture was terminated following its passage through the a. ventralis.

The compound was polymerized by leaving fish in a 70° C water bath overnight. Tissues were digested in 30% KOH (3 days) followed by 5% HNO_3 (1 day) after which the resultant casts were gently removed, rinsed and dried for later observation.

In addition to corrosion casting, complimentary observations of the arterial and venous vasculature were undertaken in 10 fish. In these studies, the vasculature was revealed by dissection and injection of India ink (Singh, 1960).

Simplified vascular plans for both coho and chinook salmon were constructed by combining observations from castings and morphological studies. Each of the three techniques were employed to mutually confirm the existence, and interconnections of various vessels. However, observations relating to vessels obscured by surrounding tissues (e.g. the splenic and hepatic vascular architecture) were derived exclusively from cast preparations. All recorded descriptions were drawn to scale. The final diagrams depicted in Figs 1 and 2 represent a conglomerate, derived from observations of the vasculature of 28 (14 coho and 14 chinook) individuals of both sexes.

III. RESULTS

No between-sex or species variation in the vasculature of coho or chinook salmon was observed. The following descriptions therefore, are presented in general terms.

THE ARTERIAL VASCULATURE

The arterial vasculature of the coho and chinook salmon is depicted in Fig. 1. All organs within the body cavity were supplied by the a. coelomesenterica. The a. coleomesenterica branched from the a. dorsalis, passing caudoventrally at a 60° angle shortly after the branchial arteries fused, and immediately posterior to the aa. suclaviae. The a. coleomesenterica passed through the head kidney to the right of the oesophagus, and bifurcated near the septum to form the a. intestinalis and a. gastrointestinalis. The a. gastrointestinalis branched further forming the aa. genitales, which supplied the gonads; the a. gastrica which fed the dorsal surface of the stomach; the a. gastrosplenica which serviced the stomach and spleen; and the a. intestinalis dorsalis which supplied the ventral aspect of the stomach and the dorsal mid-and hindgut, sending branches to the pyloric region. The a. intestinalis supplied the liver via the a. hepatica; the pyloric caecae and spleen via the a. duodenosplenica; and the ventral region of the intestine and pyloric caecae via the a. intestinalis ventralis. The arterial and capillary network was most dense within the pyloric caecal region of the gut. At least two other unpaired arteries connected the a. dorsalis to the a. intestinalis dorsalis. These were of lesser diameter than the a. coelomesenterica. The larger of the vessels supplied the hindgut, joining the a. intestinalis dorsalis in the pelvic region, while the other, which ran close to the ureter, supplied the hindgut and rectum. The a. intestinalis dorsalis therefore exhibited at

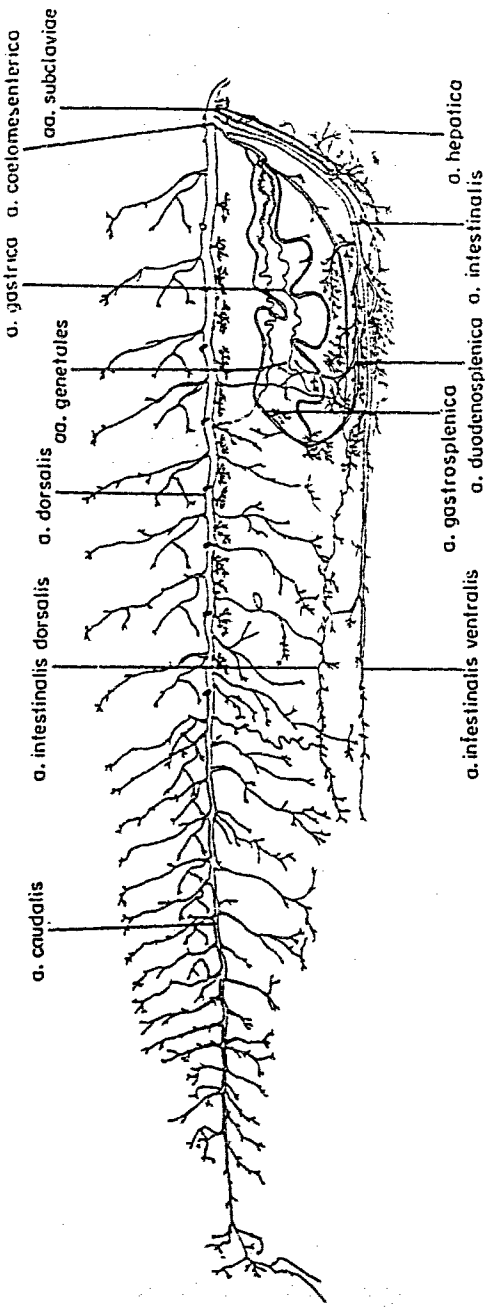

FIG. 1. Arterial vasculature of the chinook and coho salmon. The drawing represents a composite of observations made upon 14 individuals using corrosion casts, India ink injection and direct observation. a., Arteria; aa., arteriae.

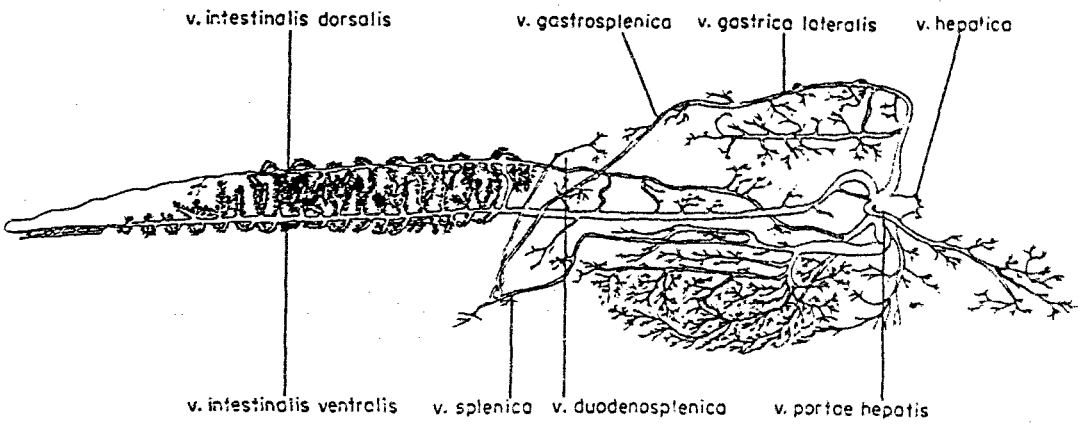

FIG. 2. Venous vasculature of the chinook and coho salmon. The diagram represents a composite of observations made upon 14 individuals using corrosion casts, India ink injection and direct observation. v., Vena; vv., venae.

least three different connections to the a. dorsalis. There were also numerous small connections between the a. intestinalis dorsalis and the a. intestinalis ventralis.

The a. duodenosplenica and a. gastrosplenica connected within the spleen proper. Furthermore, branches of the a. gastrica were found to couple to the a. duodenosplenica. The posterior gut was supplied by branches from the a. dorsalis and a. intestinalis. Thus, the a. intestinalis, a. gastrointestinalis and the posterior branches from the a. dorsalis were all anatomically connected. Some interior segmental arteries were observed to send off small vessels into the mesentery which ultimately connected with the gonadal and swimbladder vasculature.

Several casts exhibited a single vessel lying above the vertebral column (not shown in Fig. 1). The vessel was dorsoventrally flattened and of approximately 1 mm external diameter in a 30 cm individual. This vessel formed a capillary network around the insertions of the dorsal fin, and ran craniad until it split, on either side of the vertebral column, in the proximity of the first a. segmentalis dorsalis. Further connections could not be confirmed using casting techniques, India ink or dissection procedures.

THE VENOUS VASCULATURE

The venous vasculature of coho and chinook salmon are depicted in Fig. 2. The hepatic portal system demonstrated a certain degree of variation in terms of the tortuous paths taken by its supplying vessels. These included the v. gastrosplenica, v. duodenosplenica, v. intestinalis ventralis, v. intestinalis dorsalis, and a branch of the renal portal system.

The v. gastrica lateralis passed over the surface of the stomach wall, to ultimately form the v. gastrosplenica. The v. gastrosplenica passed directly into the spleen, wherein it anastomosed with the v. splenica, to form a venous loop to the v. portae hepatis. The v. splenica branched multitudinously, forming an intricate capillary network which mirrored the size and form of the spleen. Two major vessels fed into the v. duodenosplenica. These originated from the caecal mass, and branched in an elaborate manner to provide the form of the pyloric caeca. Ultimately, the v. duodenosplenica drained into the v. portae hepatis, thereby completing a

gastroduodeno-splenic loop. Also, the venous drainage of the caecae and stomach anastomosed profusely. The gall bladder and bile duct were serviced by a single vessel which was drained by the v. portae hepatis.

The v. intestinalis ventralis and v. intestinalis dorsalis drained the ventral and dorsal sections of the gut respectively. The v. intestinalis ventralis received blood from the ventral body wall via seven vessels, which ran in a ventro-craniad direction. Both the v. intestinalis ventralis and v. intestinalis dorsalis formed an interconnecting loop, which adjoined at the rectum. The v. intestinalis ventralis/v. intestinalis dorsalis loop was supplemented by a further 15 minor, and two major vessels which anastomozed, along the entire length of the gut. These vessels formed a complex interdigitating system of feather-like venuoles. While the v. intestinalis dorsalis remained in close association with the gut, ultimately passing directly into the v. portae hepatis, the v. intestinalis ventralis diverted away from the gut wall before draining into the v. portae hepatis. Both v. intestinalis ventralis and v. intestinalis dorsalis received various branches from the stomach wall and caecal mass. Upon all of the above vessels converging, the v. portae hepatis proper was formed. After a short union, however, the v. portae hepatis branched multitudinously to form a diffuse network of vv. hepaticae, ultimately giving rise to capillary beds which, in casting, were of the general form of the liver itself.

IV. DISCUSSION

Most of the blood that enters the splanchnic circulation is carried by the a. coelomesenterica. This is seen in a number of other species of teleost (Godsil, 1954; Nawar, 1955; Singh, 1960; Petukat, 1965). In essence, the observations of the present study conform to those of Grodzinski (1938) and Koniar (1947) on the rainbow trout. But, the degree of anastomoses as described herein have not been previously described in salmonids. However, interconnections between the a. dorsalis and the hind gut have been reported in other *Oncorhynchus* (Smith & Bell, 1975; Olson & Meisheri, 1989). Comparisons between the three techniques used in the present study determine that corrosion casting provided a more detailed picture of the vasculature than either direct *in situ* dissection or India ink injection. It is likely therefore that the sheer number of anastomoses, and the presence of the offshoots from the a. dorsalis, which supplied the dorsal aspect of the intestine, were simply missed in earlier studies.

The anastomoses recorded among the a. gastrosplenica, a. gastrica lateralis and the a. duodenosplenica converging in the spleen, along with corresponding inosculations on the venous side, have not been reported previously for fish but similar connections exist in mammals (Miller, 1964). The small posterior arteries that supply the dorsal regions of the intestine and the a. coelomesenterica were connected via anastomoses between the dorsal and ventral aspects of the posterior intestine, and through the a. intestinalis dorsalis. Such connections have been described in a number of other teleost species (Nawar, 1955; Petukat, 1965).

Both arterial and venous casts indicated that the densest and most extensive capillary beds were those of the pyloric region. This is consistent with the finding that most of the absorptive surface of the gut in salmonids is found in the pyloric caecae (Buddington & Diamond, 1987). Preliminary results from injections of radiolabeled microspheres also indicate that more than half of the gastrointestinal

blood flow may be diverted to the pyloric caecae during the postprandial stage (H. Thorarensen and A. P. Farrell, unpubl. obs.).

No signs of lymphatic vessels associated with the digestive tract were found in any cast, although vessels—such as the large vessel located above the vertebral column—that have traditionally been considered elements of the lymphatic system (Kampmeier, 1970) were consistently filled. In extensive studies on chinook salmon, Greene (1913), as in the present investigation, was unable to characterize lymphatics associated with the gastrointestinal tract and Robinson & Mead (1973) were unable to characterize lacteals in rainbow trout. A variety of recent studies question the existence of a lymphatic system proper in teleosts. Instead it has been suggested that bony fishes possess a secondary circulation which exhibits different anatomical links to the systemic circulation (Vogel & Clariez, 1981; Vogel, 1985; Steffensen *et al.*, 1986). In the present study, vessels corresponding to secondary arteries were visualized running on either side of the a. dorsalis, but such vessels were not apparent in the splanchnic circulation, which may be a common feature in teleosts (Vogel & Clariez, 1981). Should lymphatics and secondary circulation be absent from the gastrointestinal tract, all material absorbed by the gut would pass via the hepatic portal vein.

Net absorption of nutrients can be measured if their concentration in the a. coelomesenterica and the hepatic portal vein are known along with gastrointestinal blood flow (McLean & Ash, 1989). It is possible to measure changes in post-prandial blood flow by placing a flow probe on the a. intestinalis, which carries most of the blood supplying the liver and the main absorptive surfaces of the gastrointestinal tract. Aortic concentrations of absorbed substances can be measured in blood obtained from a cannula inserted into the a. dorsalis. Blood from all parts of the gastrointestinal tract drains into the v. portae hepatis which forms a sinus as it enters the liver. Samples of hepatic portal blood can be obtained by cannulating the v. intestinalis ventralis non-occlusively and running a catheter into the hepatic sinus. In combination such techniques should provide a reasonably accurate indication of (a)nutrient net absorption.

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