

FISH CARDIOVASCULAR PHYSIOLOGY: PUSHING THE LIMITS
OF A LIFE SUPPORT SYSTEM

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Introduction

The circulatory system has limits to what it can and cannot do. These limits vary considerably between species and even within a species. As the first organ system to develop embryologically, the form and function of the circulatory system will undoubtedly be shaped by a variety of environmental factors, i.e., phenotypic plasticity. The purpose of this talk is to overview recent experiments in my laboratory that were intended to explore how plastic the salmonid circulatory system was with respect its upper limit for tissue oxygen delivery.

End products of phenotypic plasticity are evident for rainbow trout (*Oncorhynchus mykiss*) when comparing so fairly basic cardiovascular variables. For example, acclimation to cold temperature can induce a 50% increase in ventricular mass (e.g., Graham and Farrell, 1989). Ventricular mass also shows a sexual dichotomy of a similar magnitude; mature female rainbow trout can have a considerably (30-40%) smaller ventricle (e.g., Graham and Farrell, 1992). Clearly, changes of this magnitude represent a substantial cardiac remodeling. Farrell et al. (1988) suggested that myocardial hyperplasia and hypertrophy were both involved in this cardiac remodeling, and in this regard the fish heart differs from the mammalian heart where myocardial growth after birth is through muscle hypertrophy. Thorarensen et al. (1996) suggested that the sexual dimorphism for ventricular mass in rainbow trout reflected myocardial growth in males in response to elevated endogenous 11-keto testosterone.

While other cardiovascular differences are evident from comparative studies, the cause-effect relationships are largely unknown because controlled experiments have not been performed. For this reason, Helgi Thorarensen and Pat Gallagher performed a comprehensive and collaborative study of the plasticity of the salmonid circulatory system in response to exercise-training as part of their Ph.D. theses (Thorarensen, 1994; Gallagher, 1994). Some of this work has been published (Gallagher et al., 1992; Thorarensen et al., 1993; Gallagher et al., 1995; Thorarensen and Farrell, 1996). The following draws together the major findings from these published and unpublished and from other exercise training studies performed in my laboratory. Collectively the research did not confirm our working hypothesis, that exercise-training produces a better circulatory system to support a higher critical swimming speed (U_{crit}).

Circulatory responses to exercise-training in salmonids

Hatchery-reared rainbow trout were exercise-trained for 18 h per day for 28 days at water velocities up to 60% of their U_{crit} . At the end of this training period, U_{crit} was the same in control and exercise-trained fish despite significant improvements to maximum cardiac performance in the exercise-trained fish (Farrell et al., 1991). Exercise-training produced around a 25% improvement in maximum cardiac pumping capacity as measured *in vitro*. There were significant increases in maximum stroke volume and myocardial power output, but maximum heart rate and adrenergic responsiveness were unaltered. In addition, there were significant increases in the maximal activities of mitochondrial enzymes in cardiac and especially red skeletal muscle.

The absence of improved swimming performance despite overt improvements to cardiac pumping capacity was puzzling. Although the results pointed to the lack of a tight linkage between maximum prolonged swimming performance and maximum cardiac performance, we had other concerns that included: (a) the results might be peculiar to the inbred strain of hatchery-reared rainbow trout, (b) the fish's ability to eat and digest food satisfactorily during exercise-training, and (c) the lack of *in vivo* measures of cardiac performance.

To satisfy these concerns, two exercise-training studies with a single stock of juvenile chinook salmon were performed (Thorarensen, 1994; Gallagher, 1994). The first study (low speed training) compared the effect of a continuous swimming for 8 months at 0.5 body lengths per second (bl/s) versus 1.5 bl/s. The second study (high speed training) used a more intense and shorter training regime (a swim to U_{crit} on alternate days over a 2 month training period). At the end of the training regimes, *in vivo* cardiovascular performance was assessed while the individual fish swam to U_{crit} in a Brett-type respirometer.

Neither training regime had any significant effect on U_{crit} , confirming the earlier findings for rainbow trout. However, high speed training significantly increased (by 27%) maximum oxygen uptake at U_{crit} (low speed training also increased maximum oxygen uptake by 11%, but this change was not statistically significant). Even so, the 27% increase in maximum oxygen uptake was not associated with a significant increase in either the arterial oxygen content or the maximum cardiac output measured at U_{crit} . Therefore, a greater oxygen extraction by the tissues (i.e., a lower venous oxygen content) was the most important cardiovascular response for this training regime. An additional outcome was that exercise-trained fish appeared to swim less "efficiently", at least as measured by a higher oxygen cost for the same swimming speed. It was also clear that erythropoietic activity was not stimulated by exercise training and this observation raised the possibility that, in line with the optimal hematocrit hypothesis, the number of circulating red blood cells was constrained by blood viscosity. Both possibilities were subsequently examined (Thorarensen, 1994; Gallagher, 1994).

Can increases in hematocrit and blood oxygen content improve maximum oxygen uptake and swimming performance?

The short answer to this question is yes. The experimental support for this comes from blood doping experiments in rainbow trout in which arterial oxygen content was experimentally manipulated as a direct function of transfused/removed red blood cells (Gallagher et al., 1995). In these experiments hematocrit (Hct), Hb concentration and arterial oxygen content were the experimental variables, whereas U_{crit} and maximum oxygen consumption (among others) were the measured variables.

As expected, experimental anemia resulted in marked reductions in both U_{crit} and maximum oxygen consumption. This observation confirmed earlier work (Jones, 1971) and reaffirmed the idea of a tight linkage between U_{crit} and maximum oxygen consumption. The novel finding was that, U_{crit} and maximum oxygen consumption both increased as Hct was experimentally elevated above the normocythemic range. Of course, this finding is contrary to the optimal hematocrit hypothesis, which predicts that benefit to oxygen transport of elevating Hct above the resting level is outweighed by the costs of increased blood viscosity. Equally important was the observation that the gains in U_{crit} and maximum oxygen consumption per unit change in Hct were rather small for polycythemia when compared with those for anemia. Clearly, the tight linkage between U_{crit} and maximum oxygen consumption that exists under anemic conditions breaks down. In fact, given the high level of individual variability superimposed on the shallow slopes for polycythemic fish, it is hardly surprising that earlier investigators failed to show any benefit to swimming from small increases in Hct (Jones, 1971; Gallagher et al., 1992). Gallagher et al. (1995) suggested that this shallow slope in polycythemic fish is related to a **reduced** ability of polycythemic blood to load oxygen at the gills rather than a viscosity-imposed reduction in cardiac pumping capacity. This is a novel suggestion and warrants further study.

Multi-tasking - A possible explanation for divergence between U_{crit} and maximum oxygen consumption in exercise-trained fish?

In the training studies with chinook salmon, maximum oxygen consumption increased without any change in U_{crit} . In contrast, the blood doping experiments showed that U_{crit} continued to increase with Hct values up to 55% whereas maximum oxygen consumption plateaued at a Hct of 42%. Both observations point to a significant alteration in the tight linkage between U_{crit} and maximum oxygen consumption as seen under anemic conditions. To explain this shift, it was proposed that fish with additional potential for tissue oxygen delivery used this potential to integrate more tasks while swimming to their U_{crit} . Our first clue that exercise-trained fish might perform more things at once (multi-tasking) rather than swimming much faster came from an experiment that compared oxygen consumption during swimming in fed and unfed chinook salmon (Thorarensen and Farrell, 1996).

The maximum oxygen consumption of chinook salmon forced to swim to U_{crit} 15 h post-prandially (i.e., when the gut blood flow is elevated) was identical to that in unfed fish. However, U_{crit} was significantly reduced in the fed fish. Furthermore, at all swimming speeds up to their respective U_{crit} values, fed fish had a higher oxygen consumption (around 39 $\mu\text{mol}/\text{min}/\text{kg}$) compared with unfed fish (Thorarensen and Farrell, 1996). Our interpretation of these findings is that the upper limit to oxygen delivery by the circulatory system is reached certainly during the swimming challenge and that the oxygen cost of digestion (the heat increment) diverts a significant amount of oxygen away from the locomotory muscles, resulting in a lower U_{crit} .

Our second observation in support of the multi-tasking hypothesis came from the relationship between swimming speed and maximum oxygen consumption. At low velocities, the polynomial equation describing this relationship showed that at low velocities there was no appreciable oxygen cost associated with swimming faster (Thorarensen et al., 1993). This means that at low swimming speeds either swimming muscles become more efficient or oxygen resources for routine functions are diverted to the swimming muscles. Given the present knowledge base we felt that blood flow redistribution during exercise was the more important of these possibilities. For

example, while as much 40% of cardiac output goes to the gut circulation in resting fish, gut blood flow is reduced during swimming, presumably being diverted to the swimming muscles (Randall and Daxboeck, 1982; Thorarensen et al., 1993). In chinook salmon, there was a linear correlation between the percentage reduction in gut blood flow and the increase in oxygen consumption that occurs during swimming (Thorarensen et al., 1993). Moreover, exercise-trained chinook salmon defended their gut blood flow during exercise better than control fish. At a given level of oxygen consumption, gut blood flow was higher in exercise-trained compared with control fish (Thorarensen et al., 1993). In fact, the constriction of the gut circulation, being greater in control fish, was reflected in a higher systemic vascular resistance and dorsal aortic blood pressure. Therefore, it appears that the additional capacity for oxygen transfer in exercise-trained chinook salmon is in part directed to the additional oxygen costs of **maintaining digestive functions during swimming** rather than swimming faster.

Although we have not studied other physiological functions in any depth, it seems very likely that other physiological systems, in addition to the digestive system, may benefit from exercise-training. This conjecture is supported by the observation that exercise-trained fish had a smaller osmotic disturbance when swum to U_{crit} (Gallaughan, 1994). Since it is well known that swimming causes significant osmotic and ionic disturbances, being able to better regulate these disturbances **during** swimming (as a result of exercise training) will have benefits for recovery. The oxygen cost of improved iono- and osmoregulation during swimming is presumably reflected in the elevated maximum oxygen consumption at U_{crit} in exercise-trained chinook. In hindsight, multi-tasking of integrated physiological functions may be the wiser oxygen investment strategy given the exponential cost of swimming faster.

Conclusions

By using exercise-training as a tool to experimentally explore the plasticity of the salmonid circulatory system, we expected trained fish to swim faster. This was not the case. Increased cardiac pumping capacity as a result of exercise training was observed in vitro, but this training effect may not be expressed during swimming to U_{crit} in vivo. Likewise, while benefits to swimming performance and maximum oxygen uptake can accrue from an increased Hb concentration, it was a greater oxygen extraction by the tissues that accounted for the elevated maximum oxygen consumption at U_{crit} in exercise-trained chinook. Furthermore, the suggestion is advanced that the improved capacity for oxygen delivery in exercise-trained fish supports a higher level of integrated physiological functioning than normal. As such, oxygen cost per unit swimming speed breaks down as a simple measure of swimming efficiency. The idea of multi-tasking still needs much refinement and research. In this regard, individual variability could be used to better advantage in the future since the physiological responses to exercise training were rarely more than 50% and individual variability is usually much greater than this.

References

- Farrell, AP, Hammons, AM, Graham, MS, and Tibbits, GF 1988 Cardiac growth in rainbow trout, *Oncorhynchus mykiss*. Can. J. Zool. 66: 2368-2373.
- Farrell, AP, Johansen, JA, and Suarez, RK 1991 Effects of exercise training on cardiac performance and muscle enzymes in rainbow trout, *Oncorhynchus mykiss*. Fish Physiol. Biochem. 9: 301-312.

Gallaugh, P 1994 The role of haematocrit in oxygen transport and swimming in salmonid fishes. Ph.D. Thesis pp. 1-248. Simon Fraser University, Burnaby.

Gallaugh, P, Axelsson, M, and Farrell, AP 1992 Swimming performance and haematological variables in splenectomized rainbow trout, *Oncorhynchus mykiss*. J. Exp. Biol. 171: 301-314.

Gallaugh, P, Thorarensen, H, and Farrell, AP 1995 Hematocrit in oxygen transport and swimming in rainbow trout (*Oncorhynchus mykiss*). Respir. Physiol. 102:279-292.

Graham, MS, and Farrell, AP 1989 The effect of temperature and adrenaline on the performance of a perfused trout heart. Physiol. Zool. 62: 38-61.

Graham, MS, and Farrell, AP 1992 Environmental influences on cardiovascular variables in rainbow trout, *Oncorhynchus mykiss* (Walbaum). J. Fish Biol. 41:851-858.

Jones, DR 1971 The effect of hypoxia and anemia on the swimming performance of rainbow trout (*Salmo gairdneri*). J. Exp. Biol. 55:541-551.

Randall, DJ and Daxboeck, C 1982 Cardiovascular changes in the rainbow trout (*Salmo gairdneri* Richardson) during exercise. Can. J. Zool. 60: 1135-1140.

Thorarensen, H 1994 Gastrointestinal blood flow in chinook salmon (*Oncorhynchus tshawytscha*). Ph.D. Thesis pp. 1-193. Simon Fraser University, Burnaby.

Thorarensen, H, and Farrell, A.P. 1996. The effect of routine and maximum metabolic rate on prolonged swimming speed of chinook salmon (*Oncorhynchus tshawytscha*). J. Exp. Biol. in review.

Thorarensen, H, Gallaugh, P, Kiessling, A, and Farrell, AP 1993 Intestinal blood flow in swimming chinook salmon, *Oncorhynchus tshawytscha* : the effect of hematocrit on blood flow distribution. J. Exp. Biol. 179: 115-129.

Thorarensen, H, Young, G, and Davie, P 1996. 11-ketotestosterone stimulates growth of heart and red muscle in rainbow trout. Can. J. Zool. in press.