DIFFERENTIAL RECRUITMENT OF ANAEROBIC MUSCLE FIBRES OF RAINBOW TROUT (*ONCORHYNCHUS MYKISS*) AND SOCKEYE SALMON (*ONCORHYNCHUS NERKA*) DURING EXHAUSTIVE EXERCISE

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Introduction

Exhaustive exercise in fish requires the recruitment of white muscle fibres, which results in the production of lactate due to anaerobic metabolism. The lactate accumulates within the white muscle and is subsequently metabolized by *in situ* glycogenesis during recovery (Milligan and Wood, 1986). Generally, muscle samples for the purpose of monitoring recovery metabolism are taken from the mid-region of the fish. There has been some indication that during exercise white muscle fibres are recruited, depending on the type of exercise and acclimation temperature (Rome, 1990); detection of this activity may be contingent on where along the length of the body activity is monitored. The pattern of muscle fibre recruitment appears to differ between species (Jayne and Lauder, 1994), and this may be due to differences in the innervation of axial muscle fibres (Bone, 1978).

Phosphocreatine is utilized during the production of ATP within white muscle prior to the initiation of anaerobic metabolism. This reaction proceeds quickly and is not sufficient to support long term burst exercise. The depletion of phosphocreatine in the white muscle is followed by the production of lactate and protons through anaerobic glycolysis. This depletion of phosphocreatine and accumulation of lactate and protons can be monitored. Subsequently, the lactate levels can be used to estimate the amount of anaerobic metabolism that occurred during the exercise bout.

Maximal aerobic swimming exercise (Ucrit) is the maximum sustainable swimming speed that a fish can maintain. At and below Ucrit, white (anaerobic) muscle fibres are recruited to support swimming (Brill and Dizon, 1979; Johnston and Moon, 1980). Thus, estimates of the extent of anaerobic metabolism that occurs at sub-maximal swimming speeds may be an important component in energy budgets for fish. The anaerobic component of energy budgets for spawning migration is unknown, but may be a substantial factor where fish have arduous migrations, such as the Fraser River (Hinch and Rand, 1996, see conference proceedings). The anaerobic capacity of wild salmon is unknown, and a comparison of the accumulation of lactate during migration with experimentally exhausted, farm-raised sockeye is presented. The accumulation of lactate in different areas along the body was examined to determine if differential recruitment occurred during exercise.

Methods

Rainbow trout (*Oncorhynchus mykiss*) and sockeye salmon (*O. nerka*) were obtained from West Creek Trout Farm, Abbotsford, British Columbia. Fish were subsequently exercised in a Brett-style respirometer (Gehrke *et al.*, 1990). Resting fish were placed in a black plexiglass box with flow-through water supply for a minimum of 24 hours prior to sampling. Muscle samples were then analysed for lactate.

Wild migrating sockeye salmon were caught in August 1994, after completing the Hell's Gate fishway, approximately 200 km from the mouth of the Fraser River. Both the fishing and the subsequent lactate analysis of the wild sockeye muscle samples were performed by A. Kiessling (Dept. of Agriculture and Dept. of Food Science, Swedish University of Agricultural Sciences. Box 7024, S-750 07 Uppsala, Sweden).

Results

Rainbow trout that were exercised to exhaustion accumulated more lactate in the anterior white muscle mass than in the posterior muscle mass (Figure 1). Significantly greater quantities of lactate accumulated in the white muscle following exhaustion than samples taken from resting rainbow trout. Maximum critical aerobic swimming resulted in lower lactate levels than following exhaustive exercise; this is significant at the anterior and posterior of the body. There were no significant differences between the level of lactate in any portion of the body following Ucrit. At rest, there was a significant difference in the lactate levels between the mid and posterior region.

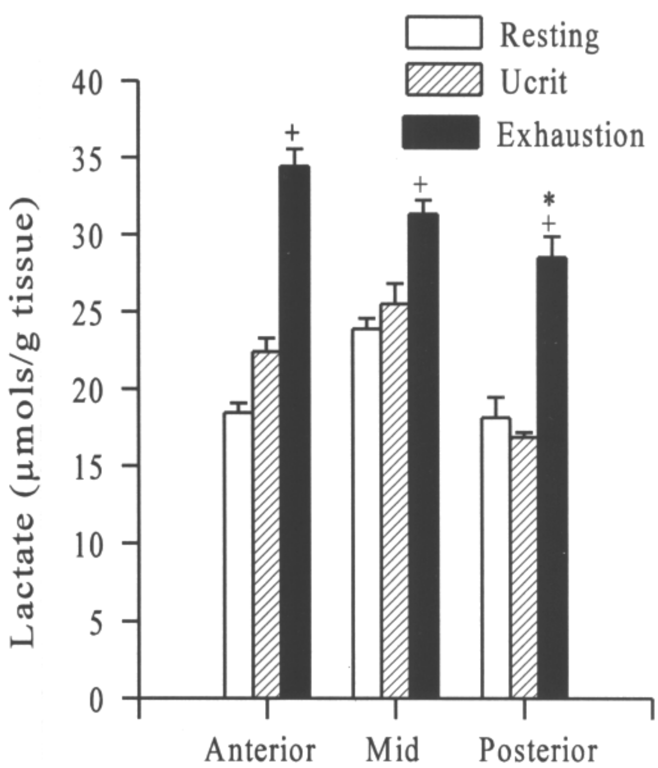

Figure 1. Rainbow trout muscle lactate levels at rest and following exercise. + Significant difference within the section, compared to resting values (P<0.01). *Signifcant difference within treatment, compared to anterior section (P<0.01).

Farm-raised sockeye salmon exhibited a slightly different trend; exhausted fish had lactate levels in the anterior white muscle mass which were not significantly different from resting levels. In the posterior muscle mass, lactate levels following exhaustion were significantly higher than the resting levels. Additionally, the resting lactate levels in the posterior muscle mass were significantly lower than the in the anterior muscle mass (Figure 2). No significant difference was observed in the lactate levels in the mid-region of the resting sockeye when compared to the anterior or posterior muscle masses. The lactate that accumulated in the mid muscle mass was less than in the anterior muscle mass of resting sockeye. No significant difference was observed between the different sections following exhaustive exercise.

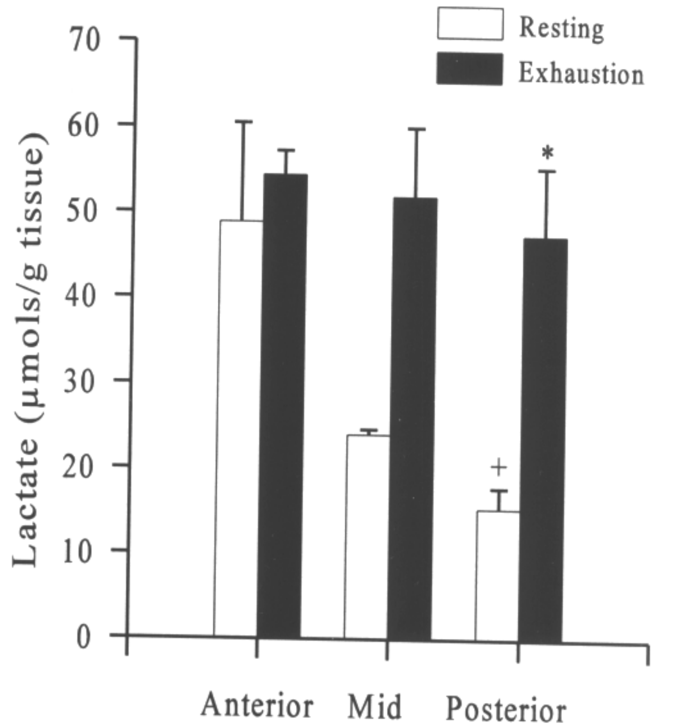

Figure 2. Farm-raised sockeye salmon muscle lactate levels at rest and after exhaustive exercise. *Significant difference from resting values in that section (P<0.05). + Significant difference within treatment, from anterior section (P<0.05).

Lactate accumulation in wild caught, migrating sockeye salmon that had just completed Hell's Gate were significantly lower than lactate levels in farm-raised, exhausted sockeye. No difference between the resting levels of lactate and those measured in wild sockeye were found in the anterior muscle mass. In the posterior muscle mass, both the exhausted and post Hell's Gate sockeye had lactate levels which were significantly higher than the resting lactate concentration (Figure 3).

Lactate levels within the muscle of resting rainbow trout, compared to resting sockeye salmon were significantly different in the anterior muscle mass. No differences were observed in the mid and posterior regions (Figure 4).

Experimentally exhausted fish, both rainbow trout and sockeye salmon were compared to lactate levels found in migrating sockeye. The accumulation of lactate in the anterior muscle mass was significantly greater in farm-raised, exhausted salmon than in either rainbow trout or in wild, migrating salmon. Additionally, in the mid and posterior muscle mass, rainbow trout accumulated less lactate, than exhausted sockeye and wild migrating sockeye (Figure 5).

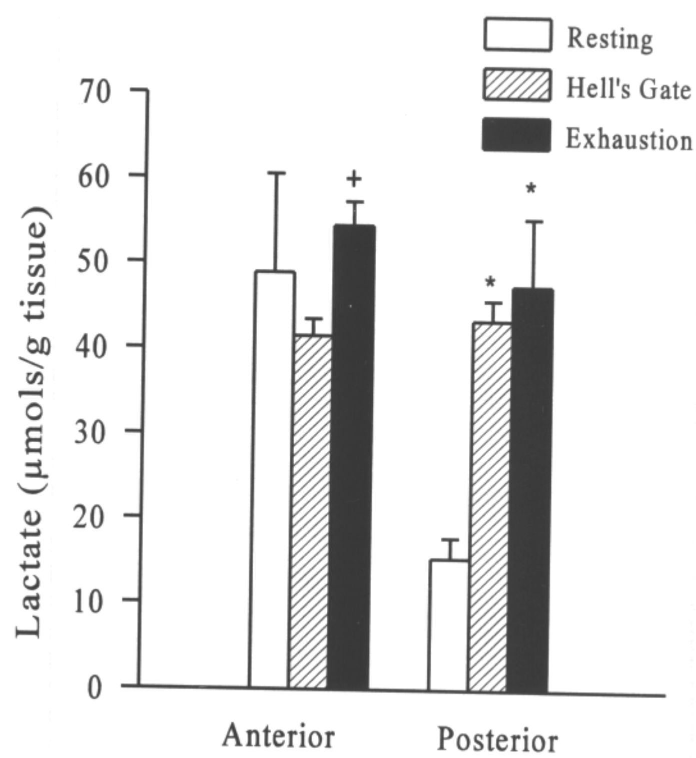

Figure 3. Muscle lactate levels in farm-raised resting and exhausted sockeye and wild, migrating sockeye (Hell's Gate). *Significant difference from resting levels in that section (P<0.01). +Significant difference from Hell's Gate levels (P<0.05).

Discussion

The muscle samples that were obtained and analysed for lactate were of comparable size, with no difference in connective tissue, bone or skin content in the samples. In the anterior sections, more red muscle mass may have been included in the analysis. This would result in a conservative estimate of the concentration of lactate in the white muscle mass in the anterior of the fish. Thus, any differences found in glycolytic fibre recruitment between the anterior and posterior musculature may be more pronounced.

Muscle lactate in resting fish

Rainbow trout and sockeye salmon had similar resting muscle lactate in the posterior and mid muscle mass. In the anterior musculature, sockeye had significantly higher levels of lactate. This discrepancy may be due to unrecorded activity which may have occurred prior to sampling. All fish (rainbow trout and sockeye) that struggled during the sampling procedure (i.e. with an escape response) were allowed to return to a resting state for a minimum of 24 hours, prior to any subsequent attempt at sampling for the purpose of obtaining muscle samples from resting fish. The level of lactate measured in the muscle mass of resting fish in this experiment were consistently higher than previously reported values. Literature values for resting rainbow trout range from 0.71 mmol l^{-1} intracellular fluid (Tang and Boutilier, 1991) to 14.79 mmol kg^{-1} wet mass (Johnston, 1975). These values are obtained using various experimental designs. The lower resting lactate values are obtained from anaesthetized fish. The higher values of lactate reported in the literature were obtained from fish in which the entire body was placed into liquid nitrogen (Johnston, 1975). Anaesthetization was not used in obtaining samples for this study because a comparison between experimentally obtained lactate accumulation and the lactate concentrations measured in wild, migrating sockeye salmon was desired. As anaesthetization of fish swimming in the Fraser River was impossible, it was decided that experimental animals would also not be anaesthetized. Although consistently higher than literature values, the lactate measured in both sockeye and rainbow trout muscle (resting fish) were not significantly different from the upper lactate values in

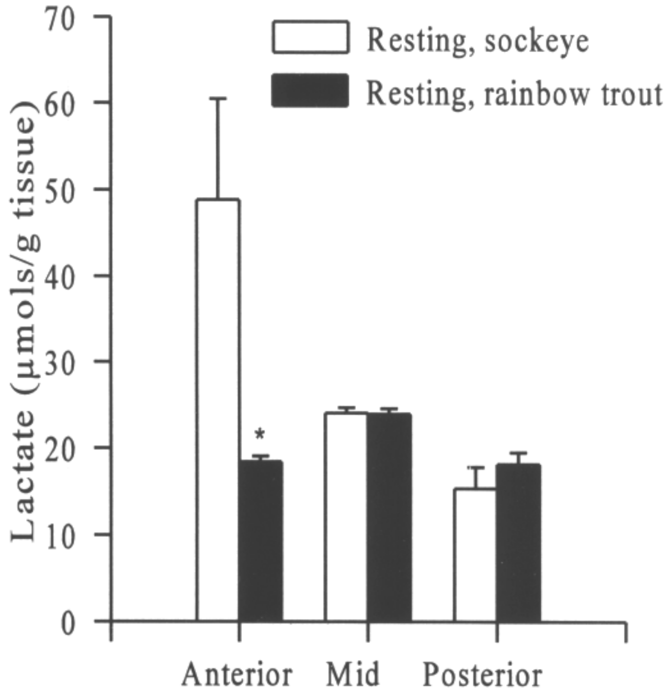

Figure 4. Resting muscle lactate levels in farm-raised sockeye and rainbow trout. *Significant difference between fish in the section (P<0.05).

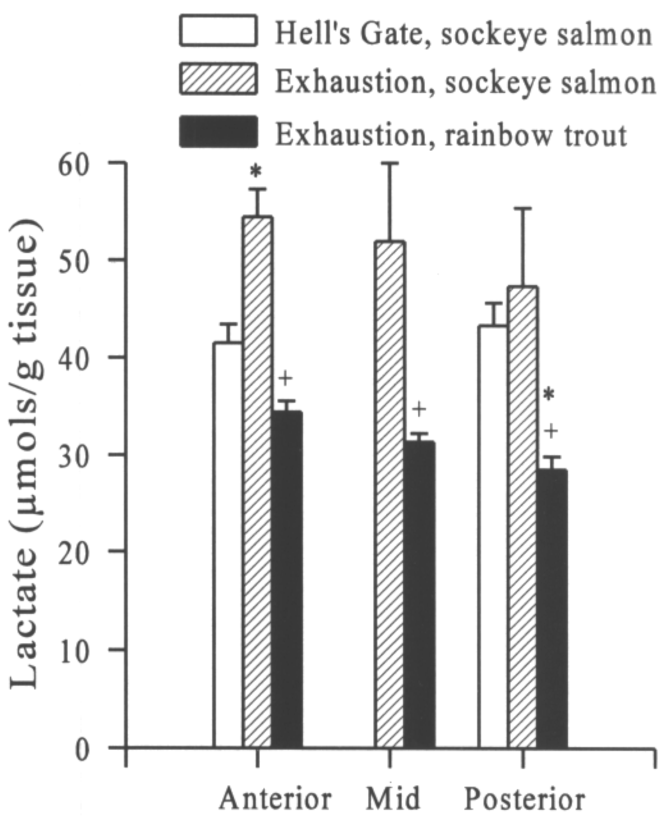

Figure 5. Muscle lactate levels in wild, migrating sockeye (Hell's Gate) and exhausted, farm-raised sockeye and rainbow trout. *Significant difference from wild sockeye (P<0.05). +Significant difference from farmed sockeye (P<0.05).

the literature for resting rainbow trout, with the exception of the sockeye salmon anterior muscle mass.

Muscle lactate accumulation following Ucrit

Ucrit, although not exhaustive, does require the recruitment of anaerobic fibres, as illustrated by the accumulation of lactate (Figure 1). That there was no significant difference from the resting values may be an artifact of a small sample size, or may indicate that the role that anaerobic metabolism plays in supporting sustained critical swimming is minimal. Alternatively, because of the high resting lactate levels that were recorded in this study, the increase in lactate within the white muscle due to anaerobic metabolism to support exercise may be masked.

Muscle lactate accumulation following exhaustive exercise

In farm-raised rainbow trout, lactate accumulation following exhaustive exercise was not uniform across the length of the fish's body. This result indicates that differential recruitment of glycolytic muscle fibres may be occurring during exhaustive swimming. The amount of lactate that accumulated in the white muscle, when compared to literature values, is within the range of exhausted values, 22.19 mmol kg^{-1} (Tang and Boutilier, 1991) and 41.72 μmols g^{-1} (Schulte *et al.*, 1992). Although not shown here, phosphocreatine (PCr) was measured in the same regions. There appears to be greater PCr stores in the posterior muscle mass, which were depleted to a greater extent following exhaustive exercise. This may explain why greater quantities of lactate were measured in the anterior muscle fibres. PCr is depleted initially during white muscle recruitment, and if it is available in larger quantities in the posterior muscle mass, this may result in reduced accumulation of lactate.

Rainbow trout that have been exercised to exhaustion show various levels of accumulated lactate within the musculature. The data that have been collected from sockeye salmon do not show any significant differences along the length of the body; although a slightly decreasing trend, from the anterior to the posterior regions may be present. To confirm this trend, additional subjects would be required. Additional experiments monitoring the accumulation of lactate following maximal aerobic swimming in both rainbow trout and sockeye salmon are also required to assist in the estimation of the extent of anaerobic metabolism that occurs during elevated swimming velocities.

River migrating salmon vs. experimentally exercised salmon and rainbow trout

The accumulation of lactate in the anterior musculature of wild, migrating salmon was similar to the unusually high resting values in farm-raised sockeye, but was lower than the experimentally exhausted sockeye. In the posterior musculature, this result is reversed. The accumulation of lactate is not different after exhaustive exercise from lactate measured in sockeye that had just passed through the Hell's Gate fishway (Figure 3). The migrating sockeye and exhausted sockeye had greater lactate accumulation in the posterior musculature than found in the resting salmon muscle. This indicates that anaerobic metabolism is required during migration through Hell's Gate fishway; more so, it appears that they are approaching exhaustion during this passage.

Sockeye salmon, both farm-raised and wild migrating salmon, accumulated more lactate within the white muscle fibres than did rainbow trout. This difference in lactate accumulation following exhaustive exercise (experimentally and during spawning migration) indicate that sockeye salmon have a greater anaerobic capacity than rainbow trout.

Differential recruitment of glycolytic fibres

The pattern of lactate accumulation that was seen following exhaustive exercise in rainbow trout and in the resting muscle of sockeye salmon indicate that differential recruitment of muscle fibres occurred. Prior to sampling, any of the resting fish may have been active; this activity would be unknown, and the increased lactate levels in the anterior musculature would be the only evidence of this activity. If this is the cause of the elevated lactate values observed from resting sockeye, this would indicate that glycolytic fibres were preferentially utilized in the anterior portion of musculature. White muscle activity at anterior and posterior longitudinal positions in the bluegill sunfish (*Lepomis macrochirus*) during swimming episodes have been investigated (Jayne and Lauder, 1994). Electromyogramy has revealed that the duration of white muscle fibre activation is longer anteriorly, and the onset time is earlier than in the posterior white muscle fibres during swimming at intermediate speeds. The results obtained in our experiments compliment these results; recruitment of anterior glycolytic fibres is more extensive than posterior glycolytic fibres during exercise.

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