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Adaptive Significance of Lactate Dehydrogenase B2 Isozymes in Rainbow Trout, *Salmo gairdneri* and a Biochemical Genetic Comparison of Cutthroat Trout (*Salmo Clarki*) Populations

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ADAPTIVE SIGNIFICANCE OF LACTATE DEHYDROGENASE B^2 ISOZYMES IN RAINBOW TROUT, SALMO GAIRDNERI AND A BIOCHEMICAL GENETIC COMPARISON OF CUTTHROAT TROUT (SALMO CLARKI) POPULATIONS

by

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Gerald T. Klar

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Adaptive Significance of Lactate Dehydrogenase B^2 Isozymes in Rainbow Trout, Salmo gairdneri and a Biochemical Genetic Comparison of Cutthroat Trout (Salmo Clarki) Populations

by

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Utah State University, 1978

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Rainbow trout lactate dehydrogenase (LDH) $B^{2'}$ $B^{2'}$, $B^{2'}$ $B^{2''}$ and $B^{2''}$ $B^{2''}$ phenotypes were tested under a variety of conditions for swimming endurance, oxygen consumption rates, and blood pH, pO_2, and lactate. The $B^{2''}$ $B^{2''}$ phenotype exhibited a lower swimming endurance, under a limited oxygen supply, than the $B^{2'}$ $B^{2'}$ and $B^{2'}$ $B^{2''}$ phenotypes. Blood pH at fatigue did not differ among the LDH phenotypes that swam under low oxygen conditions (2 mg/l). Blood pO_2 at fatigue was significantly higher for females than for males under low oxygen conditions. Oxygen consumption rates were significantly higher for males than for females at dissolved oxygen concentrations of 3 mg/l to 8 mg/l. The depression in oxygen consumption rates at low oxygen levels was greater for males than for females. The increase in blood lactate with swimming under low oxygen concentrations and static exposure to low oxygen depended on LDH phenotype. The adaptive significance of the LDH $B^{2'}$ and $B^{2''}$ alleles was discussed.

Thirteen populations of Intermountain cutthroat trout were sampled for biochemical genetic variation. Variation was observed in muscle aspartate aminotransferase for all populations which would fit a genetic model of two loci and three alleles. Unique muscle A group lactate dehydrogenase variation was observed in Snake Valley cutthroat trout which suggested the presence of several variant alleles. Allele frequencies for tetrazolium oxidase changed during three consecutive years in Bear Lake cutthroat trout that were trapped and spawned artificially. The possible causes of those changes were discussed. No variation was observed in alphaglycerophosphate dehydrogenase, isocitrate dehydrogenase, eye lactate dehydrogenase or muscle malate dehydrogenase in cutthroat trout.

(100 pages)

INTRODUCTION

Biochemical adaptation was described by Hochachka and Somero (1973) as biochemical changes that are adaptive at the level of basic metabolic function. They described three mechanisms of biochemical adaptation where: (1) the types of molecules may be changed; (2) the concentrations of molecules may be altered; and (3) the function of the molecules may be regulated. The first mechanism may be effected by the acquisition of new genetic material or by the activation of genes (isozymes) already present within the organism. The second mechanism may be effected by control of protein synthesis within the cell. The third mechanism is based on the structure of the protein molecule and its sensitivity to modulators. The first two mechanisms are slow, requiring from hours to generations, and provide a "coarse" adjustment in metabolic function. The third mechanism can occur almost instantaneously and provides a "fine" metabolic adjustment.

The most striking example of biochemical adaptation is the acquisition of the complex biochemical pathways found in present day organisms. Ohno et al. (1968) proposed that major events in evolution were marked by gene duplication. Markert et al. (1975) have traced the evolution of the gene coding for lactate dehydrogenase through many periods of gene duplication and tissue specialization. It is probable that, through the process of gene duplication and mutation, organisms were able to add the enzymes (and isozymes) necessary for biochemical pathways such as the Krebs cycle and electron transport.

Adaptive Significance of Isozymes

Isozymes are defined as multiple molecular forms of an enzyme that catalyze the same reaction. Isozymes differ structurally which often creates a charge difference and allows their separation by electrophoretic techniques. The structural difference may also alter the kinetic function such that two isozymes may catalyze the same reaction under different conditions. Isozymes may be coded by genes at two different loci or may be in the form of alleles at a single locus that are inherited by Mendelian principles (Allendorf 1975).

Hochachka and Somero (1968) reported that the thermal optima (temperature with minimal Km) for brook trout (Salvelinus fontinalis) and lake trout (Salvelinus namaycush) A_4, B_4, C_4 and muscle LDH (lactate dehydrogenase) fell within the physiological range for the species. However, the thermal optimum for muscle LDH was 15-20 C and did not change during cold acclimation while A_4, B_4 and C_4 isozymes increased in activity during cold acclimation and showed lower thermal optima. They suggested that isozymes activated during cold acclimation (A_4, B_4, C_4) had adaptive significance in that catalytic rates are maintained at low temperatures by the activated isozymes. Somero and Hochachka (1969) reported that King Crab (Paralithodes camtschatica) muscle has two kinetic forms of LDH, a low Km LDH active at 10-15 C and a high Km LDH that makes no significant contribution to metabolism at 10-15 C. However, as the temperature decreases the Km of the "high Km form" decreases sharply which activates the isozyme at low temperature (5 C). Somero and Hochachka (1969) stated: "In the two isoenzyme system examined, the Km changes which occur as the temperature is lowered appears to activate certain isoenzymes which, at physiological

substrate concentrations, are inactive at higher temperatures. Isoenzyme systems therefore appear to be of importance in short-term temperature compensation as well as in long-term temperature acclimatization." Somero (1969b) reported that two forms of pyruvate kinase occurred in King Crab (Paralithodes camtschatica), a cold form with minimal Km near 5 C and a warm form with minimal Km near 10-12 C. Somero suggested that, with this system, pyruvate kinase would function well over the range of habitat temperatures of the King Crab. Further evidence of biochemical adaptation was presented by Somero and Hochachka (1968), Somero (1969a), Hochachka and Lewis (1970), Somero and Hochachka (1971). Johnson (1971), Baldwin and Hochachka (1970), Moon and Hochachka (1971), and Somero (1973). Various isozymes were demonstrated to have altered kinetics with adaptive significance.

Lactate Dehydrogenase Isozymes

Numerous studies have reported the electrophoretic patterns of LDH isozymes in fish (Markert and Faulhaber 1965; Massaro and Markert 1968; Bailey and Wilson 1968; Morrison and Wright 1966; Odense 1969; Wuntch and Goldberg 1970; Shaklee et al., 1973). The LDH isozyme pattern in fish is complex with multiple loci and many different systems of nomenclature for the loci.

Allendorf (1973) reported that there were five loci coding for LDH in rainbow trout (Salmo gairdneri). The system of nomenclature (confirmed by starch gel electrophoresis) for salmonid fish LDH used in this study is as follows: The slow migrating group of five isozymes found in skeletal muscle is labeled the A group, with the slowest (most cathodal) isozyme corresponding to the A^1 locus and the fastest isozyme

of this group corresponding to the A^2 locus. A second group of five isozymes migrating faster than the A group and found in liver, heart, and brain is termed the B group. Following the terminology of Utter and Hodgins (1972) the fastest migrating isozyme of the B group is labeled the $B^{2'}$ locus. The slowest migrating isozyme of the B group is labeled the B^1 locus. The single isozyme migrating faster than the B group and found in the eye is labeled the C locus.

Williscroft and Tsuyuki (1970) reported a variant LDH isozyme in the liver of rainbow trout, which they labeled C and suggested that it was an allelic form of C'. Utter and Hodgins (1972) reported the same variant in steelhead trout (anadromous rainbow trout), called the variant $B^{2''}$ and suggested that it was an allele at the $B^{2'}$ locus. Inheritance studies by Allendorf (1973) of steelhead trout and Stillings (1974) of the Beity strain of rainbow trout confirmed that $B^{2''}$ and $B^{2'}$ were alleles at the same locus.

Northcote et al. (1970) reported that in a stream population of rainbow trout the LDH $B^{2''}$ allele was more frequent upstream from a waterfalls than downstream. They suggested that $B^{2''}$ allele was associated with conditions of higher water velocities above the falls. Tsuyuki and Williscroft (1973) investigated some kinetic parameters of the LDH $B_4^{2''}$ isozyme and found that it had a three fold higher rate of lactate turnover when compared to the $B_4^{2'}$ isozyme. They suggested that this could confer higher swimming endurance to fish with $B_4^{2''}$ isozymes and that $B^{2''}$ phenotypes are associated with high water velocities. Stalnaker et al. (1973) reported that the $B^{2''}$ allele was rare in inland hatchery rainbow trout, ranging in frequency from 0.00 to 0.0346. Utter and Hodgins (1972) reported that the frequency of the $B^{2''}$ allele was 0.17 in one population of steelhead trout and 0.00 in three populations

of inland rainbow trout. Huzyk and Tsuyuki (1974) reported that the frequency of the $B^{2''}$ allele in rainbow trout from 7 British Columbia streams ranged from 0.395 to 0.800 and that the frequency of the $B^{2''}$ allele from 7 steelhead populations ranged from 0.00 to 0.467. They stated: "Preliminary evidence from this laboratory indicates a direct correlation of the B"B" (sic $B^{2''}$ $B^{2''}$) phenotype of rainbow trout with superior swimming endurance." Allendorf (1975) reported that the frequency of the $B^{2''}$ allele in 32 populations of steelhead trout ranged from 0.00 to 0.708 and from 0.00 to 0.045 in 6 populations of resident rainbow trout. From the evidence reviewed it appears that the distribution of the LDH $B^{2''}$ allele is varied but that it generally occurs in higher frequency in steelhead trout and resident stream rainbow trout as opposed to hatchery rainbow trout.

Kao (1977) isolated the LDH $B_4^{2'}$ and $B_4^{2''}$ isozymes from rainbow trout and studied their kinetic parameters. The Km for lactate of the $B_4^{2''}$ (18.3 mM) isozyme was slightly higher than for the $B_4^{2'}$ (12.8 mM) isozyme. The Km for pyruvate of the $B_4^{2''}$ (0.090 mM) isozyme was also higher than for $B_4^{2'}$ (0.062 mM) isozyme. The temperature-Km relationship for both isozymes was similar showing an increase in Km with temperature. The effect of pH on enzyme activity differed between the two isozymes. Both showed a pH optimum of 7.1 but the activity of the $B_4^{2''}$ isozyme dropped more rapidly on the acid side. Using lactate as a product inhibitor of pyruvate reduction, Kao reported that the $B_4^{2'}$ isozyme exhibited the expected linear, noncompetitive inhibition pattern. However, lactate had an apparent product activation effect on the $B_4^{2''}$ isozyme below 20 mM lactate with a maximum activation of 12 percent at 10 mM lactate. The $B_4^{2'}$ isozyme showed an ordered bi-bi reaction mechanism with NADH binding

to the enzyme first followed by pyruvate, similar to other LDH's. However, Kao also reported that pyruvate would bind to the $B_4^{2''}$ isozyme in the absence of NADH and suggested a random kinetic mechanism for $B_4^{2''}$ where either pyruvate or NADH could bind independently. Kao and Farley (1977) reported that the $B_4^{2''}$ isozyme converted lactate to pyruvate about four times faster than the $B_4^{2'}$ isozyme, which agreed with Tsuyuki and Williscroft (1973).

The B^2 group of LDH isozymes is found primarily in the more aerobic tissues of rainbow trout such as liver and heart. The kinetic results of Kao (1977) suggest the following hypothesis: that the $B^{2''}$ $B^{2''}$ phenotype would have a greater metabolic flexibility than the $B^{2'}$ $B^{2'}$ phenotype under conditions of a limited oxygen supply. When oxygen becomes limiting the lactate induced activation characteristic of the $B_4^{2''}$ isozyme could stimulate anaerobic metabolism in tissues possessing that isozyme and thus maintain metabolic activity in those tissues. For the $B_4^{2'}$ isozyme, NADH levels must increase before pyruvate reduction can occur to any great extent because NADH must bind to the isozyme first. Since the $B_4^{2''}$ isozyme can bind pyruvate in the absence of NADH, NADH levels need not increase greatly before the isozyme can carry out pyruvate reduction. This characteristic may act as a primer for pyruvate reduction and give the $B^{2''}$ $B^{2''}$ phenotype more flexibility in anaerobic metabolism.

Biochemical Genetics

One approach to the problem of determining genetic variation within a species is the electrophoretic analysis of enzymes and other protein components in various tissues. The strength of this approach is

its capacity to visualize proteins directly through histochemical staining. This approach allows the detection of multiple gene products that catalyze the same reaction. According to the one gene-one enzyme hypothesis, the amino acid sequence of each enzyme is a direct translation of the nucleotide sequence in a segment of genetic material. Hubby and Lewontin (1966, p. 579) stated: "Since enzymes and proteins are, as far as we know, made up of polypeptides, from one or sometimes two different structural genes, then we can expect that electrophoretic differences in enzyme protein will segregate as single mendelizing genes. Thus, if we survey a large number of isozymes and other proteins and if we determine the electrophoretic mobility of such proteins from single individuals, it should be possible to detect variability from individual to individual at single loci."

Electrophoretic Analysis of Fish Proteins

Extensive electrophoretic analyses have been conducted on fish proteins. The literature on LDH in fish has been cited earlier. Utter et al. (1970) reported transferrin polymorphism in coho salmon (Oncorhynchus kisutch). Utter and Hodgins (1970) reported phosphoglucomutase polymorphism and Hodgins et al. (1969) reported LDH polymorphism in sockeye salmon (Oncorhynchus nerka). Avise and Smith (1974) compared Centrarchid populations over a wide area in several protein systems. Bailey and Wilson (1970) reported that several forms of malate dehydrogenase exist in salmonids. Utter and Hodgins (1972) described variation for alphaglycerophosphate dehydrogenase, lactate dehydrogenase, malate dehydrogenase, tetrazolium oxidase, phosphoglucomutase and serum transferrin in rainbow trout. Allendorf (1975) described an extensive survey of 32 loci in rainbow trout.

Cutthroat Trout Biochemical Genetics

Comparatively little data are available on the biochemical genetics of cutthroat trout (Salmo clarki). Utter et al. (1973) analyzed a total of 16 individuals from two locations. They observed no variation in malate dehydrogenase, lactate dehydrogenase, phosphoglucomutase or fast muscle protein. They observed variation in tetrazolium oxidase and reported a frequency of 0.90 for the B allele and 0.10 for the A allele. Reinitz (1974) conducted an electrophoretic survey of six protein systems in cutthroat trout. However his unique system of nomenclature makes interpretation of loci and alleles somewhat difficult. Allendorf and Utter (1976) reported that aspartate aminotransferase in cutthroat trout was coded by two loci with three alleles present.

Cutthroat trout provide an excellent opportunity for biochemical genetic analysis in that several "pure" native populations exhibiting considerable variation in morphological characters exist in the Intermountain West (Behnke 1968, 1973, 1975, 1976). These populations exist in a wide variety of habitats from high mountain lakes to desert headwater streams. In contrast many rainbow trout populations have been displaced from their native habitat and, in many cases, their genetic makeup may reflect an adaptation to the artifical and relatively homogeneous environment present under most hatchery situations. Cutthroat trout still occur in their many and diverse native habitats, even though in many cases their range and abundance have been considerably reduced. Unique evolutionary events in biochemical adaptation may therefore still be evident in the native cutthroat trout isozyme complement.

Objectives

The objectives of this study were to further understanding of the adaptive significance of isozymes in fishes by:

1. Examining the hypothesis that the LDH $B^{2''}$ $B^{2''}$ phenotype provides greater metabolic flexibility than the $B^{2'}$ $B^{2'}$ phenotype as suggested by the studies of Kao (1977), Kao and Farley (1977) and Tsuyuki and Williscroft (1973), through a comparison of the performance, both physical and physiological, of rainbow trout $B^{2''}$ $B^{2''}$, $B^{2''}$ $B^{2'}$ and $B^{2'}$ $B^{2'}$ LDH phenotypes under limited oxygen availability.
2. Examining the hypothesis that uncontaminated "wild" populations of cutthroat trout exhibit high biochemical variability offering unique isozymes indicative of adaptation to widely varying habitat conditions, through an electrophoretic survey of native cutthroat trout populations.

MATERIALS AND METHODS

Physiological Studies of LDH Phenotype

Fish stocks

Fish used in the experiments were of the Beity strain of rainbow trout spawned and reared during an earlier study (Stillings 1974). The Beity brood stock were obtained by the Utah Division of Wildlife Resources from Valley, Washington and reared at the Mantua Fish Hatchery, Mantua, Utah. In December 1972, thirteen brood fish of the LDH $B^{2'}$ $B^{2''}$ phenotype were transferred to the Utah Water Research Laboratory, Utah State University. Those fish were spawned in December 1972 and January 1973, producing fish for this study.

The fish were reared at the Water Research Laboratory in round tanks 1.5 meters in diameter and 0.46 meters in depth. Logan River water was supplied to the tanks at a rate of 12 to 16 liters per minute. Water temperature ranged from a high of 12 C in late August to a low of 2 C in January. The fish were maintained on a diet of commercial dry trout pellets.[1]

Swimming experiment one, oxygen at saturation and temperature at 8 C

All handling prior to the actual experiment was with fish that were anesthetized with a synergic mixture of 20 ppm MS-222 (tricaine methane-sulfonate) and 5 ppm quinaldine. All fish were handled twice during the month preceeding the experiment, once to obtain a blood

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[1]Moore Clark Inc., Salt Lake City, Utah

sample and apply a cold brand and a second time to weigh, measure and segregate the fish into groups according to their LDH phenotype ($B^{2'}$ $B^{2'}$, $B^{2'}$ $B^{2''}$, $B^{2''}$ $B^{2''}$). Ten days elapsed between the last day of handling and the beginning of the experiment.

During the separation procedure, 0.5 ml of blood was obtained from each fish by cardiac puncture. Serum was separated by low speed centrifugation and applied to a Ridgway et al. (1970) starch gel and electrophoresed for two hours at 15 volts per centimeter. After staining for LDH each fish was classified as phenotype I ($B^{2'}$ $B^{2'}$), II ($B^{2'}$ $B^{2''}$) or III ($B^{2''}$ $B^{2''}$). This classification is used throughout this report.

Stamina tests were conducted in a stamina tunnel similar to that described by Burrows (1960). Logan River water was supplied to the stamina tunnel at the rate of six exchanges per hour. Temperature remained at a constant 8 C throughout the experiment. Water pO_2 ranged from 107 to 120 mmHg and pCO_2 from 5.9 to 8.2 mmHg, but did not vary more than ±0.1 mmHg during the course of a stamina run. Fish were removed from feed 24 hours prior to testing.

Groups of three to four fish were placed into the exercise tube of the stamina tunnel. Fish were acclimated to swimming inside the exercise tube at velocities of one body length per second for five minutes. During the sixth minute the water velocity was increased to two body lengths per second. Electrodes were positioned at the rear of the exercise tube so that a DC electric field could be created in the rear 12 cm region of the tube by actuating a rheostat on a DC power supply. Ten to 20 volts DC from one to two seconds were used to encourage fish that drifted to the rear of the tube to swim in the

main current as long as possible. The electric field had no visible effect on fish that were not in the 12 cm region of the tube.

At the point of fatigue the time was recorded and the fish were removed from the stamina tunnel. A fish was considered fatigued when it lost equilibrium and orientation in the exercise tube. Twenty-two phenotype I, 23 phenotype II and 18 phenotype III fish were tested. A cardiac blood sample was obtained from a random sample of 10 fish of each phenotype within 30 seconds after removal from the stamina tunnel. Fish were removed from the stamina tunnel in a net, placed on their back and the blood sample taken. No anesthetic was used or required. Wydoski et al. (1976) reported that the type of anesthetic used affects the blood pH, pO_2 and pCO_2. Blood pH, pO_2 and pCO_2 were determined on each blood sample with an IL Model 213 Blood Gas Analyzer.[2] Blood samples were equilibrated to 8 C and read.

Since there was a wide range in total lengths (23 to 37 cm), the rainbow trout were divided into size groups (Table 1). Mean lengths

Table 1. The mean lengths of rainbow trout groups tested at two body lengths per second and oxygen saturation (swimming experiment one).

Size Group		Phenotype I	Phenotype II	Phenotype III
1.	Mean length (cm)	32.8	33.1	32.7
	Range (cm)	30-37	30-37	30-36
2.	Mean length (cm)	26.6	27.1	26.5
	Range (cm)	23.5-29.5	24-29	23-29

[2]Instrumentation Laboratory Inc., Lexingon, Mass.

and ranges were similar for each phenotype. Water velocity in the stamina tunnel was maintained at 66 cm/sec and 54 cm/sec (two body lengths per second) for groups one and two respectively.

Swimming experiment two, declining oxygen and temperature at 10 C

Approximately one year before the experiment began, the fish were segregated into groups according to LDH phenotype. All handling during segregation was done with fish anesthetized with a synergic mixture of 20 ppm MS-222 and 5 ppm quinaldine. Each fish was cold branded (using a mixture of dry ice and acetone) with a number and 0.5 ml blood obtained by cardiac puncture. Serum was electrophoresed as described earlier and each fish was classified as phenotype I ($B^{2'}$ $B^{2'}$), II ($B^{2'}$ $B^{2''}$) or III ($B^{2''}$ $B^{2''}$). All fish were then separated into groups and rearing conditions continued as described in the section on "Fish Stocks."

Sixteen days before the beginning of swimming experiment two the fish were anesthetized, weighed, measured and transferred to three circular tanks 1.5 meters in diameter with a water depth of 0.5 meters. Each tank was divided into three equal compartments by wire screens and 12 fish of a specific LDH phenotype were placed into each compartment; therefore, all three phenotypes were represented in each tank. Logan River water was supplied to the acclimation tanks through a temperature control system consisting of two head tanks and three mixing tanks. One head tank was supplied with Logan River water at 3-5 C, and the second head tank was supplied with Logan River water at 3-5 C which was heated to 15-16 C with electrical immersion heaters. Heated water was delivered to each of the three mixing tanks with inflow controlled by float valves.

Cold water inflow to each mixing tank was supplied by a solenoid valve controlled by a thermoregulator. Thus when the temperature in the mixing tank rose above the set point of the thermoregulator, the solenoid valve would open allowing cold water to enter the mixing tank. Water was delivered from the mixing tanks to the acclimation tanks at a flow rate of 9-10 liters per minute. Fish were placed into the acclimation tanks at 3 C (their original holding temperature) and allowed 6 hours to recover from the effects of the anesthetic. The temperature was increased to 10 C during the next 24 hours and maintained at 10 ± 0.5 C for the duration of the experiment. Air stones were placed into each compartment of the acclimation tanks and dissolved oxygen was maintained at 90-95 percent of saturation. The fish were fed a diet of dry pelleted trout food once each day at a rate of 0.8 percent body weight per day. Fish were removed from feed 12-24 hours prior to testing.

The second swimming experiment was carried out in the active respirometer described by Dickson and Kramer (1971). That apparatus consisted of a 15 cm plexiglass tube fastened within a 30 cm plexiglass tube with a total volume of 27.5 liters. A propeller inserted into the inner tube drew water through the inner tube where the fish was placed and circulated the water between the two tubes. The respirometer was submerged in a 400 liter water bath. Temperature was maintained at 10 ± 0.5 C by flushing water through the bath from the mixing tanks that were described earlier. Dissolved oxygen in the respirometer was monitored with a YSI[3] oxygen electrode and meter that were connected to a Sargent Welch Model SRG chart recorder.

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[3]Yellow Springs Instrument Co., Yellow Springs, Ohio.

A single fish was netted from the acclimation tank, placed in a bucket containing 6 liters of water and transferred within 30 seconds to the respirometer. Each fish was acclimated inside the respirometer for 15 minutes at one body length per second water velocity and 8 mg/l dissolved oxygen. During the 16th minute the water velocity was increased to two body lengths per second, the chart recorder switched on and the respirometer sealed. Each fish was allowed to swim to the point of fatigue with the respirometer sealed. Fatigue was defined as that point when the fish lost equilibrium and orientation inside the exercise tube and was impinged against the rear retaining screen. Fatigued fish were removed from the respirometer, a blood sample taken by cardiac puncture, then they were weighed, measured and placed in a holding tank at 3-5 C. The blood sample was immediately analyzed for pH and pO_2 with an IL Model 213 Blood Gas Analyzer. The sample chamber of the blood gas analyzer was equilibrated to 10 C. Electrophoretic verification of LDH phenotype was determined from the blood serum sample. From the recorder charts respiration rates at 8 mg/l through 3 mg/l oxygen concentration and the point of fatigue, total swimming time and oxygen concentration at fatigue were calculated. A total of 18 fish of each of the three LDH phenotypes were tested (Table 2).

Swimming experiment three, oxygen at 2 mg/l and temperature at 10 C

Fish used in this experiment were acclimated under the same conditions as those used in experiment two. This experiment was also carried out in the active respirometer at 10 ± 0.5 C. Dissolved oxygen was maintained at 2 ± 0.1 mg/l by bubbling nitrogen into the 400 liter water

Table 2. Sizes of rainbow trout tested at two body lengths per second and declining oxygen (swimming experiment two).

	Phenotype					
	I		II		III	
Size of Fish	Males	Females	Males	Females	Males	Females
Mean length (cm)	29.1	29.8	27.7	31.5	29.9	32.4
Range (cm)	26-32	24-33	26-31	29-34	26-33	29-34
Mean wt (g)	295	337	243	332	332	381
Range (g)	159-379	159-437	138-433	270-385	206-430	279-437
Number of fish	8	10	7	11	10	8

bath. Nitrogen inflow was controlled by a solenoid valve-relay system connected to a YSI oxygen meter. During testing the respirometer was left open to water circulation from the bath. The dissolved oxygen electrode used in the nitrogen control system was positioned to record dissolved oxygen in the respirometer discharge. To insure adequate circulation, water was pumped at a rate of 5 l/min from the bath into the respirometer.

At the beginning of each day of testing, the water bath was brought from dissolved oxygen saturation to equilibrium at 2 mg/l dissolved oxygen. When the relay set point of 2 mg/l was reached, a water sample was taken for dissolved oxygen titration and necessary adjustments were made in electrode calibration at that time. Dissolved oxygen titrations were made periodically during testing.

Fish were removed from feed 12-24 hours prior to testing. A single fish was netted from the acclimation tank, placed in a bucket containing

6 liters of water at 2 mg/l oxygen and transferred to the respirometer within 30 seconds. When the fish entered the respirometer a timer was started and the water velocity adjusted to one body length per second. At the end of ten minutes at one body length per second the water velocity was increased to 1.5 body lengths per second and the fish allowed to swim to fatigue. At the fatigue point the fish was removed from the respirometer, the total time recorded, a cardiac blood sample taken, and the fish then weighed and measured.

The first six fish on each test day were placed in a covered holding tank at 10 C and dissolved oxygen saturation. A portion of the blood sample at fatigue from those six fish was removed for blood lactate determination. All blood samples taken at fatigue were analyzed for pH and pO_2 and electrophoretic verification of LDH phenotype. Eight hours after fatigue each of the six fish in the holding tank was anesthetized with MS-222-quinaldine and a cardiac blood sample taken for blood lactate determination. To free the holding container for tests the next day, all six fish were sampled for a blood lactate determination at the same time the morning following fatigue. The time of that sample was recorded to the nearest half hour and ranged from 17 to 23 hours post fatigue. Blood lactate concentration was determined by the method of Harrower and Brown (1972).

A total of 18 fish of phenotypes I and III and 17 fish of phenotype II were tested for total swimming time, blood pH and pO_2 (Table 3). Sample sizes for blood lactate tests are indicated in Table 4.

Table 3. Sizes of rainbow trout tested at 1.5 body lengths per second and 2 mg/l oxygen (swimming experiment three).

	Phenotype					
	I		II		III	
Size of fish	Males	Females	Males	Females	Males	Females
Mean length (cm)	30.5	32.1	27.9	30.5	31.1	33.2
Range (cm)	28-34	29-35	26-30	24-34	28-35	30-37
Mean wt. (g)	337	398	272	325	368	401
Range (g)	247-470	260-482	224-316	139-427	271-480	281-549
Number of fish	9	9	6	11	10	8

Table 4. Number of fish sampled for blood lactate in the third swimming experiment (swimming speed at 1.5 body lengths per second and oxygen at 2 mg/l).

	Phenotype		
Time	I	II	III
At fatigue	14	11	13
8 hrs post fatigue	12	9	12
21 hrs post fatigue[a]	11	9	11

[a]Mean time of sample on the morning following testing.

Static low oxygen experiments

The first static low oxygen experiment was carried out at 10 C with a group of Beity rainbow trout reared and segregated as described earlier. The experiment was carried out in a 400 liter tank supplied with Logan River water. Oxygen was removed from the water by bubbling nitrogen directly into the experimental tank and maintained at a constant 2 ± 0.1 mg/l with the solenoid control system described earlier. Equilibrium at 2 mg/l dissolved oxygen was established daily and one LDH phenotype group was tested on each of three days. Equilibrium and electrode calibration were checked periodically by titrating water samples from the experimental tank. Fish were removed from feed 24 hours prior to testing. Fish were transferred from the holding tanks by net to the experimental tank. Seven fish of one LDH phenotype were placed into the experimental tank for 60 ± 4 minutes, followed by seven fish for 45 ± 4 minutes, four fish for 30 ± 2 minutes and finally four fish for 15 ± 2 minutes. At the end of each time period the fish were removed from the experimental tank, anesthetized with MS-222-quinaldine and a cardiac blood sample was obtained for blood lactate determination. The fish were placed in holding containers and mortality to 24 hours recorded.

The second static low oxygen experiment was similar to the first with the following exceptions; the fish used were the same fish tested in the second swimming experiment and seven fish of each phenotype were tested for 60 ± 4 minutes, four fish for 30 ± 2 minutes, and four fish for 15 ± 2 minutes.

Electrophoretic Analysis of Cutthroat Trout

Populations sampled

Cutthroat trout were sampled in streams, lakes and a hatchery in Utah, Colorado, Wyoming and Nevada (Table 5).

Table 5. Cutthroat trout populations that were sampled for electrophoretic analysis of protein systems

Cutthroat trout population	Number of fish
Goshute Creek, Nevada--1973	28
Goshute Creek, Nevada--1976 SVCT	30
Pine Creek, Nevada SVCT	13
Trout Creek, Utah SVCT	17
Birch Creek, Juab County, Utah SVCT	31
Water Canyon Creek, Utah BCT	13
Asay Creek, Utah BCT	20
Birch Creek, Beaver County, Utah BCT	29
One Mile Creek, Utah Rainbow hyb	29
Little West Fork Creek, Utah CRCT	35
Forest Canyon Creek, Colorado	10
Yellowstone Lake, Wyoming	51
Bear Lake, Utah--1974	48
Bear Lake, Utah--1975	29
Bear Lake, Utah--1976	28
Auburn Hatchery, Wyoming	29

Fish were collected from the populations by electrofishing, angling or netting. Specimens were killed immediately and the liver was removed and placed in a separate container. Fish carcass and liver samples were cooled on ice if transportation time to Utah State University was 10 hours or less, at which time they were frozen at -20 C. For transportation times greater than 10 hours, samples were frozen in the field on dry ice.

Sample preparation

Livers tested were ground to a paste in an equal volume of distilled water with a glass stirring rod. Supernatant was separated by low speed centrifugation for five minutes. White skeletal muscle tested was removed from below the dorsal fin and above the lateral line of the frozen carcass and ground as described for livers. One eye was removed intact from the frozen carcass, cleaned of extraneous tissue, placed in a test tube and minced in three drops of distilled water. Supernatant was separated by low speed centrifugation.

Electrophoresis

Starch gel electrophoresis followed the procedures of Allendorf (1975). The buffer systems used in the analyses were:

1. Described by Ridgway et al. (1970). Gel buffer (pH 8.5) consisted of 29.7 mM tris, 49.5 mM citric acid, 0.6 mM lithium hydroxide, and 3.0 mM boric acid. Electrode buffer consisted of 60.0 mM lithium hydroxide and 300.0 mM boric acid. A potential of 15 volts per centimeter was applied to the gel for two hours.
2. Described by Markert and Faulhaber (1965). Gel buffer consisted of 45.0 mM tris, 25.0 mM boric acid and 1.0 mM disodium ethylenediamine tetraacetate. Electrode buffer consisted of 150.0 mM tris, 100.0 mM boric acid and 4.0 mM disodium ethylenediamine tetraacetate. A potential of 20 volts per centimeter was applied to the gel for two hours.

3. Described by Wolf et al. (1970). Electrode buffer was 0.1 M phosphate, pH 6.5 and diluted 1:10 for the gel preparation. A potential of 8 volts per centimeter was applied to the gel for two hours.

Gels were prepared using 14 percent starch[4] following the methods of Kristjansson (1963) in that three-fourths of the buffer was heated to boiling and added to the starch suspended in the remainder of the buffer. After boiling, the mixture was degassed under vacuum and poured into a prepared mold.

Tissue extracts were drawn onto 4 by 6 mm filter paper inserts and placed in a cut in the gel 3 cm from the cathodal end. Current was applied for 10 minutes, after which the filter paper inserts were removed, a glass cooling plate placed on top of the gel and the appropriate voltage applied. At the end of two hours the gels were sliced horizontally by drawing monofilament thread through the gel at three 2 mm intervals. Gel portions were placed into polyethylene containers with the appropriate staining solution (Table 6). After staining, gels were preserved in a 5:5:1 methanol-water-acetic acid solution. Liver was analyzed for lactate dehydrogenase, sorbitol dehydrogenase, tetrazolium oxidase, esterase and isocitrate dehydrogenase (Table 6). Muscle was analyzed for alphaglycerophosphate dehydrogenase, tetrazolium oxidase, isocitrate dehydrogenase, lactate dehydrogenase, malate dehydrogenase, aspartate aminotransferase and phosphoglucomutase (Table 6). Eye was analyzed for lactate dehydrogenase (Table 6).

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[4]Electrostarch Company, Madison, Wisconsin.

Table 6. Specific staining solutions, buffer systems and tissues used for electrophoretic analysis of protein systems.

Protein	Abbreviation	Tissue	Buffer System[a]	Staining Solution
Alphaglycerophosphate dehydrogenase	AGPDH	Muscle	2	100 mg DL-alphaglycerophosphate 5 mg each NAD, NBT, PMS[b] 100 ml Ridgway gel buffer
Sorbitol dehydrogenase	SDH	Liver	1	100 mg D-sorbitol 5 mg each NAD, NBT, PMS 100 ml Ridgway gel buffer
Tetrazolium Oxidase	TO	Muscle Liver	1,2	Viewed on AGPDH stain in muscle and SDH stain in liver
Esterase	Est	Liver	1	10 ml 1% napthyl butyrate in acetone. 10 mg fast blue BB salt. 100 ml Ridgway gel buffer
Isocitrate dehydrogenase	IDH	Muscle Liver	3	30 mg DL-sodium isocitrate 5 mg each NADP, NBT, PMS 50 mg $MgCL_2$. 100 ml Ridgway gel buffer
Lactate dehydrogenase	LDH	Muscle Liver Eye	1	5 ml 60% sodium lactate 5 mg each NAD, NBT, PMS 100 ml Ridgway gel buffer
Malate dehydrogenase	MDH	Muscle	1	100 mg DL-sodium malate 5 mg each NAD, NBT, PMS 100 ml Ridgway gel buffer

Table 6. Continued

Protein	Abbreviation	Tissue	Buffer System[a]	Staining Solution
Aspartate amino-transferase	AAT	Muscle	1	0.08 g alphaketoglutaric acid 0.27 g aspartic acid 0.56 g NaH_2PO_4 (anhydrous) 1.0 g polyvinylpyrrolidinone 0.1 g NaEDTA 0.5 g Fast garnet GBC salt 100 ml H_2O
Phosphoglucomutase	PGM	Muscle	1	500 mg Glucose-1-phosphate (with traces of G-1, 6-diphosphate) 5 mg each NADP, NBT, PMS 20 units Glucose-6-phosphate dehydrogenase, 50 mg $MgCl_2$ 100 ml Ridgway gel buffer

[a]See text for description of buffer system

[b]Abbreviations
NBT - Nitro blue tetrazolium
PMS - Phenazine methosulfate
NAD - Nicotinamide adenine dinucleotide
NADP - Nicotinamide adenine dinucleotide phosphate

RESULTS

Physiological Studies of LDH Phenotype

Swimming experiment one, oxygen at saturation and temperature at 8 C

Data were analyzed statistically with a completely randomized single classification analysis of variance. No significant differences were observed in mean time to fatigue among the three LDH B^2 phenotypes (Table 7). Two phenotype I fish fatigued in three minutes at one body length per second and two phenotype II fish fatigued in one minute at one body length per second. No phenotype III fish fatigued at one body length per second. One phenotype I fish swam continuously at two body lengths per second for 8 hours and was not fatigued when removed from the

Table 7. Mean time to fatigue for Beity rainbow trout LDH B^2 phenotypes at two body lengths per second water velocity, 8 C and oxygen saturation. Times include 5 minutes acclimation at one body length per second, one minute increase to two body lengths per second and the time to fatigue at two body lengths per second.

Parameter	Phenotype I	II	III
Mean time (min)[a]	14.9 ± 9.2[b]	13.3 ± 7.1	18.4 ± 7.7
Range (min)	3-38	1-28	9-35
Number of fish	22	23	18

[a]Means were not significantly different ($p > 0.05$)

[b]± one standard error of the mean

stamina tunnel at the end of that period. The swimming time of that fish was not included in the mean for the group.

Blood pH, pO_2 and pCO_2. No significant differences were observed in blood pH, pO_2 or pCO_2 of 10 randomly selected fatigued fish from each phenotype (Table 8).

Table 8. Mean[a] blood pH, pO_2 and pCO_2 of 10 randomly selected Beity rainbow trout in each LDH phenotype fatigued at two body lengths per second, 8 C and saturated oxygen.

Parameter	Phenotype I	II	III
Blood pH[b]	7.325 ± 0.099[c]	7.341 ± 0.099	7.385 ± 0.111
Blood pO_2(mmHg)	8.9 ± 3.9	10.0 ± 2.6	10.9 ± 2.3
Blood pCO_2(mmHg)	12.3 ± 2.2	11.8 ± 1.9	11.1 ± 4.0

[a]Means were not significantly different ($p > 0.05$)

[b]True mean hydrogen ion concentration

[c]± one standard error of the mean

The mean blood pH (Table 8) was the true mean hydrogen ion concentration and was calculated by:

$$Y = \text{Sum } 10^{-X}/n$$

$$\text{Mean pH} = -\text{Log } Y$$

where: X = observed individual blood pH

n = number of observations

The phenotype I fish that swam continuously for 8 hours had a blood pH of 7.569, pCO_2 of 9.5 mmHg, and pO_2 of 27 mmHg at the end of the 8 hour period.

Swimming experiment two, declining oxygen and temperature at 10 C

Time to fatigue. A relationship between fish weight and fatigue time was observed. Heavier fish reduced the oxygen concentration in the sealed respirometer faster than lighter fish and reached a limiting oxygen concentration at an earlier time. Therefore, swimming time was analyzed with a 3 by 2 factorial analysis of covariance (3 phenotypes and 2 sexes) with weight as a covariant of time. A significant ($p < 0.05$) sex by phenotype interaction was observed with fatigue time. Males showed a slight upward trend in mean time to fatigue adjusted for weight as a covariant from phenotype I through phenotype III, while females showed a downward trend from phenotype I through phenotype III (Table 9). Mean adjusted time to fatigue for phenotype I females was significantly higher ($p < 0.05$) than all other groups (Table 9).

Oxygen concentration at fatigue. The concentration of dissolved oxygen inside the sealed respirometer at the fatigue point was recorded for each fish and compared in a 3 by 2 factorial analysis of variance. A significant difference among phenotypes ($p < 0.05$), a significant difference between sexes ($p < 0.001$) and a significant sex by phenotype interaction ($p < 0.05$) was observed. Mean oxygen concentration at fatigue for phenotype III females was significantly higher ($p < 0.05$) than for all other groups (Table 9). Means for all three groups of males were extremely close (Table 9). The trend in the interaction was upward from phenotype I females through phenotype III females (Table 9).

Blood pH and PO_2. Mean values of blood pH and pO_2 were analyzed statistically with a 3 by 2 factorial analysis of variance. No significant differences were observed in blood pH at fatigue among

Table 9. Performance and oxygen consumption of Beity rainbow trout with different LDH phenotypes. All fish swam at two body lengths per second swimming speed with a declining oxygen level. Values are means plus or minus standard error. Time is in minutes, oxygen concentration in mg/l, pO_2 in mmHg and oxygen consumption in mg O_2/kg/hr.

		Phenotype					
		I		II	III		
Parameter		Males	Females	Males	Females	Males	Females
Fatigue time[a]	b	118 ± 16	165 ± 14[c]	130 ± 19	129 ± 16	133 ± 15	136 ± 16
O_2 conc. at fatigue	b	1.96 ± 2.9	2.22 ± 0.36	1.99 ± 0.43	2.5 ± 0.48	1.95 ± 0.49	3.5 ± 1.6
Blood pH		7.524 ± 0.090	7.498 ± 0.107	7.620 ± 0.107	7.308 ± 0.326	7.479 ± 0.089	7.500 ± 0.086
Blood pO_2	d	4.6 ± 2.6	6.3 ± 2.5	3.3 ± 1.6	6.0 ± 1.6	2.9 ± 2.2	6.3 ± 2.6
O_2 consump. at fatigue	b	140 ± 22 e	102 ± 21	138 ± 47	136 ± 32	123 ± 22	154 ± 68[f]
O_2 consump. at 8 mg/l	d	520 ± 47 e	372 ± 64	528 ± 53 e	371 ± 38	489 ± 69 e	350 ± 42
O_2 consump. at 7 mg/l	d	432 ± 42 e	314 ± 45	408 ± 53 e	337 ± 44	397 ± 41 e	302 ± 32
O_2 consump. at 6 mg/l	d	361 ± 54 e	285 ± 53	347 ± 57 e	291 ± 32	339 ± 35 e	266 ± 22
O_2 consump. at 5 mg/l	d	321 ± 43 e	243 ± 33	308 ± 79 e	250 ± 31	295 ± 21 e	218 ± 18
O_2 consump. at 4 mg/l	d	253 ± 30 e	188 ± 30	251 ± 23 e	200 ± 23	242 ± 27 e	169 ± 18
O_2 consump. at 3 mg/l	d	194 ± 22 e	138 ± 33	189 ± 37 e	149 ± 27	186 ± 25 e	124 ± 18

[a] Means adjusted for weight as a covariant

[b] Sex by phenotype interaction significant ($p < 0.05$).

[c] Means underlined are significantly different ($p < 0.05$) from all others in that row.

[d] Significant difference between sexes ($p < 0.05$).

[e] Mean values by sex are significantly different ($p < 0.05$).

[f] Phenotype III females significantly higher ($p < 0.05$) than phenotype I females.

phenotypes or between sexes (Table 9). Mean pH values (Table 9) are the true mean hydrogen ion concentrations calculated as described earlier. No significant difference was observed in blood pO_2 among phenotypes but females had a significantly higher ($p < 0.001$) mean blood pO_2 at fatigue (6.2 ± 2.1 mmHg for all females) than males (3.6 ± 2.2 mmHg for all males) (Table 9).

Oxygen consumption. Rates of oxygen uptake by each fish were calculated at oxygen concentrations of 8 mg/l (the beginning point), 7, 6, 5, 4, 3, mg/l and finally at the point of fatigue. In cases where fish fatigued prior to 3 mg/l oxygen concentration, consumption rates were determined to the last whole number concentration and at the fatigue point. Consumption rates were taken by expanding the recorder line at the desired point, calculating the rate of oxygen decline per minute in mg/l and using the following formula:

$$\text{Oxygen uptake in } mgO_2/kg/hr = 60 \times (Y - Z)\ 1/Z$$

where: $X = O_2$ decline per minute in mg/l

Y = Total volume of respirometer in liters

Z = Fish weight in kilograms.

The recorder line indicating oxygen decline was linear for at least five minutes before and after each desired point of oxygen consumption calculation.

Since fatigue time was a variable point, oxygen consumption rate at fatigue was treated as a variable separate from the other consumption rates and analyzed statistically with a 3 by 2 factorial analysis of variance. A significant interaction ($p < 0.05$) was observed between phenotype and sex of oxygen consumption rate at fatigue. There was a slight trend downward in mean oxygen consumption rate at fatigue from

phenotype I males to phenotype III males and a definite trend upward from phenotype I females to phenotype III females (Table 9). Mean oxygen consumption rate at fatigue for phenotype I females was significantly lower ($p < 0.05$) than phenotype I males and phenotype III females (Table 9).

Oxygen consumption rates at 8 mg/l through 3 mg/l dissolved oxygen were treated as a regression and analyzed statistically with analysis of covariance. No signficant differences were observed among phenotypes but males were significantly higher ($p < 0.05$) than females at each oxygen concentration (Table 9). Since there were no significant differences among phenotypes, all males and all females were pooled and analyzed with regression statistics. There was a significant regression ($p < 0.01$) of oxygen concentration (X) on oxygen consumption rate (Y). Males (61.0 ± 4.6) had a significantly higher slope ($p < 0.001$) than females (44.7 ± 1.4) (Figure 1). Mean oxygen consumption rates for males were significantly higher ($p < 0.001$) than females at each oxygen concentration (Table 10).

Swimming experiment three, oxygen at 2 mg/l and temperature at 10 C

Time to fatigue. Swimming times to fatigue at a constant 2 mg/l dissolved oxygen and 1.5 body lengths per second were highly variable with no weight-time relationship evident (Table 11). Total swimming time (including 10 minutes at one body length per second) ranged from 11 to 133 minutes for phenotype I, 15 to 182 minutes for phenotype II and 12 to 232 minutes for phenotype III. No significant differences were found in swimming times when analyzed with a 3 by 2 factorial analysis of variance. The frequency distribution of numbers of fish

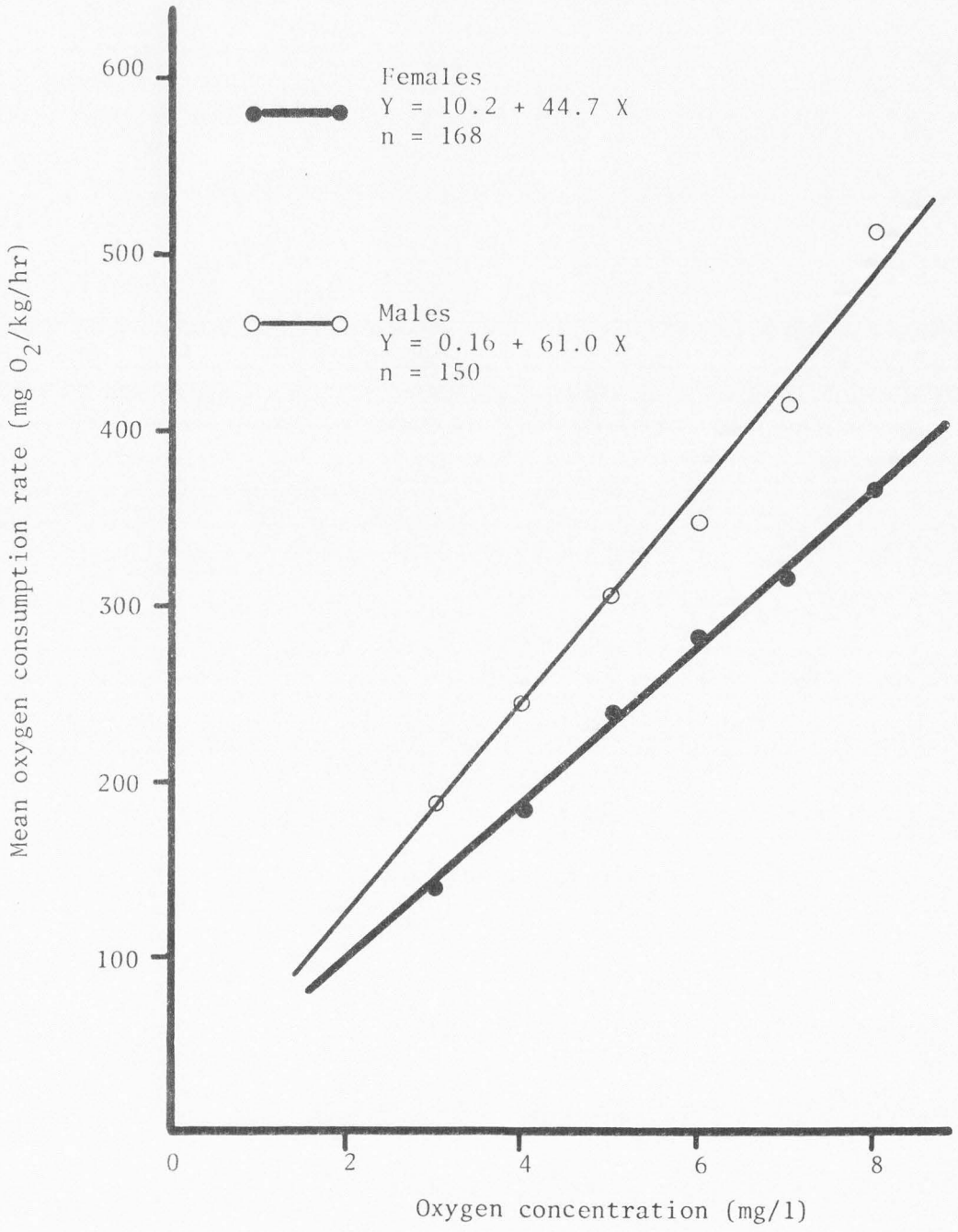


Figure 1. Relation of oxygen consumption rates to oxygen concentration for pooled LDH phenotypes of male and female Beity strain of rainbow trout exercised at two body lengths per second. Slope for males (61.0 ± 4.6) was significantly higher ($p < 0.001$) than for females (44.7 ± 1.4). Mean oxygen consumption for males was significantly higher ($p < 0.001$) at each oxygen concentration than for females.

based on 10 minute intervals of swimming time was highly skewed to the right (Figure 2). The means for the first few intervals fell very near the midpoint of the interval.

The data from the frequency distribution were subjected to chi-square analysis for data arranged in two classes (2 by t contingency tables). A significant ($p < 0.01$) time by phenotype interaction in the distribution was observed (Table 12). The data were broken into 2 by 2 contingency tables and analyzed for differences between phenotypes at various time intervals. In the 11-20 minute interval the

Table 10. Mean oxygen consumption rates ($mgO_2/kg/hr$) for male and female Beity rainbow trout exercised at two body lengths per second.

	O_2 Concentration (mg/l)					
	8	7	6	5	4	3
Males[a]	510 ± 59[b]	412 ± 46	348 ± 47	307 ± 49	248 ± 39	189 ± 27
Number of fish	25	25	25	25	25	25
Females	366 ± 49	319 ± 42	282 ± 39	240 ± 31	188 ± 27	140 ± 28
Number of fish	29	29	29	27	27	27

[a]Males were significantly higher ($p < 0.001$) than females at each oxygen concentration.

[b]± one standard error of the mean.

frequency of phenotype III fish was significantly higher than phenotype II ($p < 0.01$) and phenotype I ($p < 0.10$). In the 21-30 minute interval and 41-50 minute interval the frequency of phenotype III fish was lower ($p < 0.10$) than both phenotypes I and II. No other individual comparisons were significant.

Table 11. Performance of Beity rainbow trout of different LDH phenotypes at 1.5 body lengths per second swimming speed and constant 2 mg/l oxygen (swimming experiment three).

	Phenotype					
	I	II		III		
	Males	Females	Males	Females	Males	Females
Mean time to fatigue (min)	36 ± 23[a]	39 ± 38	28 ± 12	51 ± 50	38 ± 37	52 ± 78
Blood pH	7.633 ± 0.169	7.530 ± 0.175	7.680 ± 0.043	7.591 ± 0.089	7.701 ± 0.144	7.668 ± 0.099
Blood pO_2 (mmHg)[b]	5.89 ± 1.54	7.33 ± 0.87	6.0 ± 0.82	5.69 ± 0.75[c]	6.2 ± 1.23	7.63 ± 1.06
Blood lactate (mg/100 ml)						
At fatigue	25 ± 13	51 ± 38	27 ± 9	29 ± 17	27 ± 11	35 ± 16
8 hrs. post fatigue	23 ± 12	39 ± 46	50 ± 30[d]	21 ± 11	23 ± 9	36 ± 30
21 hrs. post fatigue	11 ± 9	9 ± 7	8 ± 0[d]	9 ± 5	10 ± 3	14 ± 9

[a] ± one standard error of the mean.

[b] Significant difference ($p < 0.05$) between sexes and among phenotypes (phenotype II lower than I and III).

[c] Blood pO_2 for phenotype II females significantly lower ($p < 0.05$) than both phenotypes I and III females.

[d] Two observations

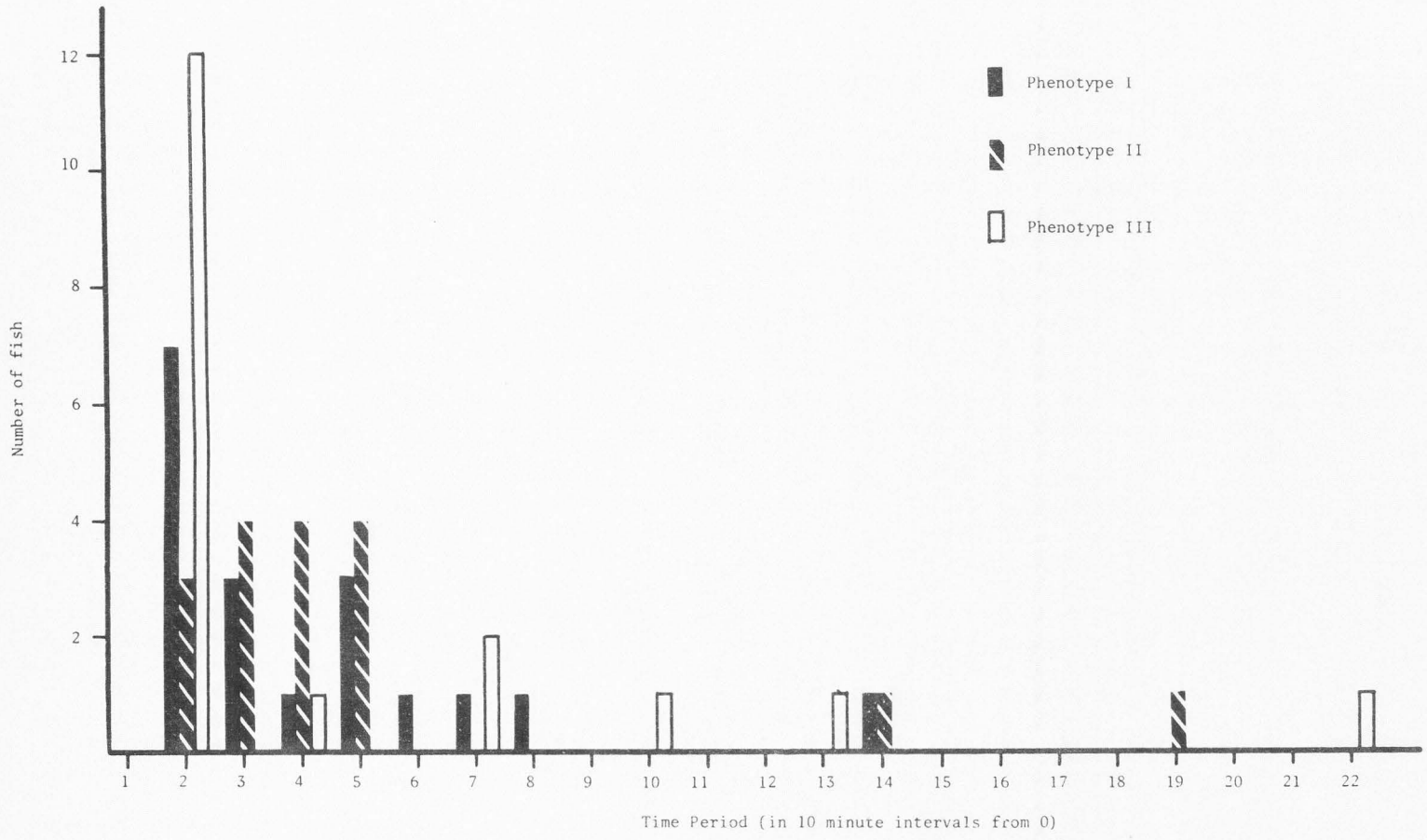


Figure 2. Distribution of Beity rainbow trout with different LDH phenotypes by 10 minute intervals of swimming time until fatigue. The fish swam at 1.5 body lengths per second with a constant 2 mg/l dissolved oxygen (swimming experiment three). There was a significant difference ($p < 0.01$) in distribution among phenotypes.

Table 12. Chi-square analysis (2 by t contingency table) of the distribution of numbers of fish in 10 minute intervals of swimming time to fatigue for Beity rainbow trout of different LDH phenotypes exercised at 1.5 body lengths per second and 2 mg/l dissolved oxygen.

Source of variation	df[a]	Calculated chi-square	Chi-square, p = 0.01
Total	17	71.395	33.409
Time	5	41.740	15.086
Phenotype	2	0.03306	9.210
Time by phenotype	10	29.622[b]	23.209

[a]Degrees of freedom

[b]Significant ($p < 0.01$) time by phenotype interaction in the frequency distribution.

However, the interaction between swimming time and phenotype distribution was clearly significant. There was an initial sharp peak in numbers in the 11-20 minute range for phenotype III fish with the remaining fish scattered from 40 to 232 minutes. There was a lower initial peak in the 11-20 minute interval for phenotype I fish with a gradual decline in frequency as time increased. The distribution for phenotype II fish closely approached a normal type with no sharp peaks in numbers evident (Figure 2).

Blood pH and pO_2. Blood pH and pO_2 were analyzed statistically with a 3 by 2 factorial analysis of variance. No significant differences were observed in blood pH (Table 11). A significant difference ($p < 0.05$) was observed between males and females and among phenotypes in blood pO_2 at fatigue (Table 11). No significant interaction was observed between sex and phenotype. As in swimming experiment two,

females had a significantly higher ($p < 0.05$) mean pO_2 (6.70 ± 1.24 mmHg for all females) than males (6.04 ± 1.26 mmHg for all males) at fatigue. The overall mean pO_2 for phenotype II fish (5.76 ± 0.75 mmHg) was significantly lower ($p < 0.05$) than phenotype I (6.61 ± 1.42 mmHg) and phenotype III fish (6.83 ± 1.34 mmHg). Mean pO_2 for phenotype II females was significantly lower ($p < 0.05$) than both phenotypes I and III females (Table 11).

Blood lactate. Blood lactate was determined at the point of fatigue, 8 and 21 hours post fatigue for a variable number of fish in each phenotype (Table 4). No significant differences were observed with a 3 by 2 analysis of variance of blood lactate (Table 11). Correlations between blood lactate and swimming time were noted in some cases. Regression of blood lactate on swimming time was handled statistically as a Model II regression (i.e. both blood lactate and swimming time were free to vary). All regression slopes presented in this section were calculated by Bartlett's three group method for Model II regression (Sokal and Rohlf 1969, p. 483). Correlation coefficients presented are the product-moment correlation coefficients (Sokal and Rohlf 1969, p. 509).

Blood lactate at fatigue was correlated with swimming time for phenotype I fish (Table 13). There was a definite trend towards higher blood lactate with increased swimming time. There was a trend towards separation of phenotype I male and female fish in blood lactate concentration at fatigue, with females displaced upwards (Figure 3). No correlation of blood lactate at fatigue with swimming time was observed for phenotype II fish (Table 13). Blood lactate at fatigue was correlated with swimming time for phenotype III fish (Table 13). There was no clear distinction between males and females (Figure 4).

Table 13. Correlation between blood lactate concentration and swimming time to fatigue, 8 hours post fatigue and 21 hours post fatigue for Beity rainbow trout of different LDH phenotypes. The fish swam at 1.5 body lengths per second at a constant 2 mg/l dissolved oxygen (swimming experiment three). Regression slopes were calculated by Bartlett's three group method for Model II regression.

Time	Phenotype I	II	III
Fatigue point			
Correlation coefficient	0.87[a]	0.005[c]	0.663[b]
Slope	0.578	-----	0.189
8 hours post fatigue			
Correlation coefficient	0.87[a]	0.216[c]	0.76[b]
Slope	0.551	-----	0.197
21 hours post fatigue			
Correlation coefficient	0.005	0.157	0.617[b]

[a]Correlation coefficient significant ($p < 0.01$).

[b]Correlation coefficient significant ($p < 0.05$).

[c]No slope calculated.

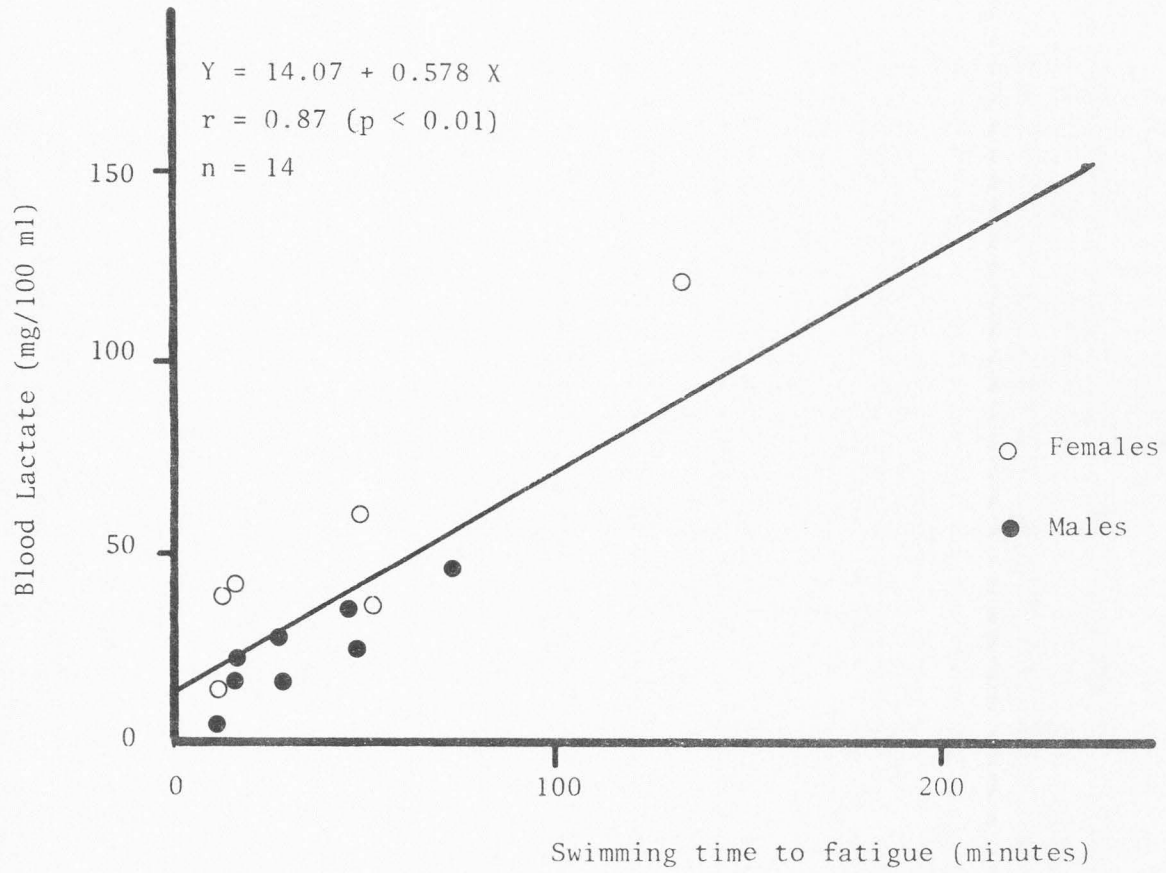


Figure 3. Relation of blood lactate at fatigue to total swimming time at 1.5 body lengths per second and 2 mg/l oxygen for Beity rainbow trout LDH phenotype I. Slope of the regression line was calculated by Bartlett's three group method for Model II regression.

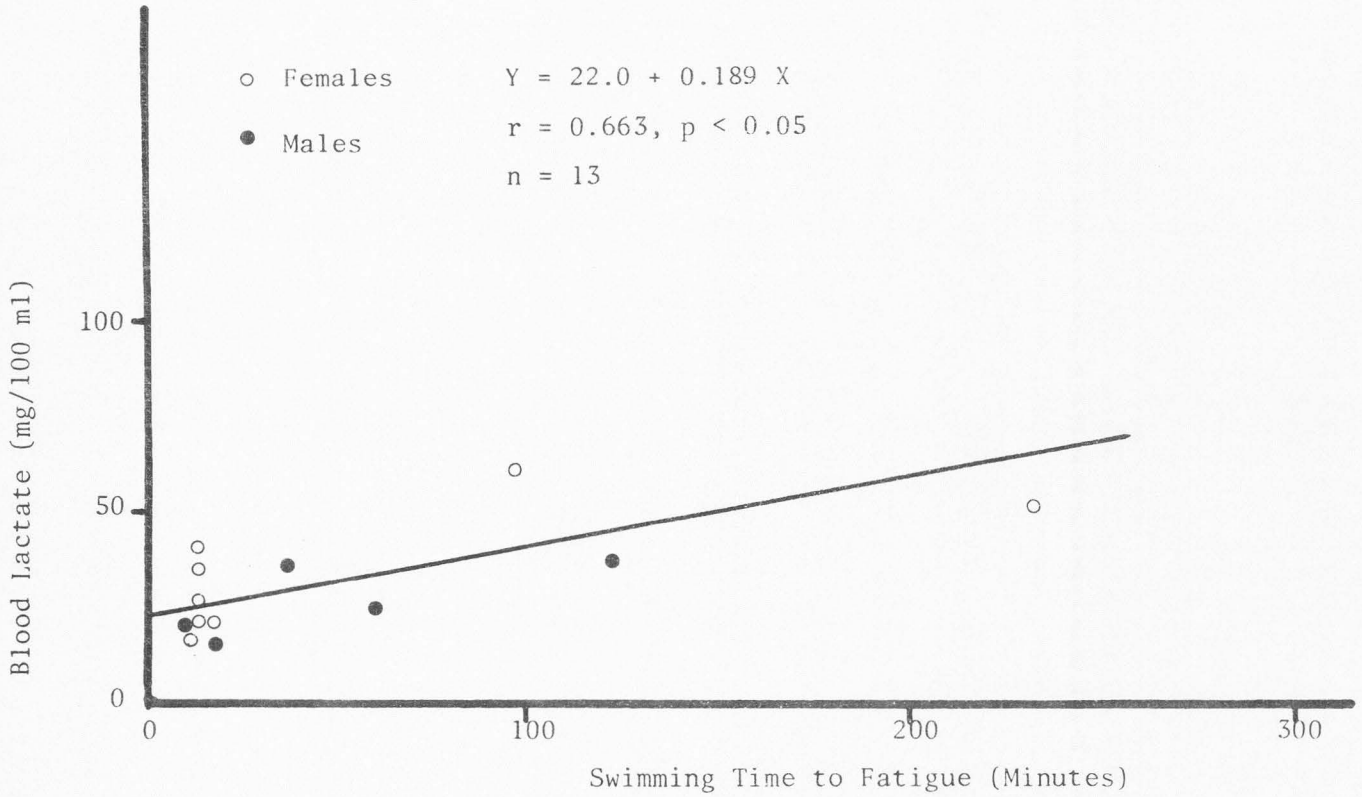


Figure 4. Relation of blood lactate at fatigue to total swimming time at 1.5 body lengths per second and 2 mg/l oxygen for Beity rainbow trout LDH phenotype III. Slope of the regression line was calculated by Bartlett's three group method for Model II regression.

Blood lactate 8 hours post fatigue for phenotype I fish was correlated with total swimming time (Table 13). No male-female separation in blood lactate was evident at 8 hours post fatigue (Figure 5). Blood lactate at 8 hours post fatigue for phenotype II fish was not correlated with total swimming time (Tables 11 and 13). Blood lactate for phenotype III fish (Figure 6) at 8 hours post fatigue was correlated with swimming time (Table 13).

Blood lactate at 21 hours post fatigue was not correlated with swimming time (Table 13) for phenotype I fish (Table 11). Blood lactate for all fish decreased from the 8 hour level. Blood lactate at 21 hours post fatigue was not correlated with swimming time (Table 13) for phenotype II fish (Table 11). Blood lactate for all fish decreased from the 8 hour level. Blood lactate at 21 hours post fatigue was correlated with swimming time (Table 13) for phenotype III fish (Figure 7). Ten fish decreased in blood lactate from the 8 hour level and one remained the same.

In summary, blood lactate for both phenotypes I and III was correlated with swimming time at fatigue and 8 hours post fatigue. No correlation was observed for phenotype II fish. Phenotype I fish had a higher slope (Table 13) than phenotype III fish at both the fatigue point and 8 hours post fatigue. No pattern was evident in blood lactate concentration between the fatigue point and 8 hours post fatigue. All but one fish decreased between the 8 and 21 hour sample.

Static low oxygen experiment one

Blood lactate. It appeared from the blood lactate data of swimming experiment three that the three phenotypes were responding differently

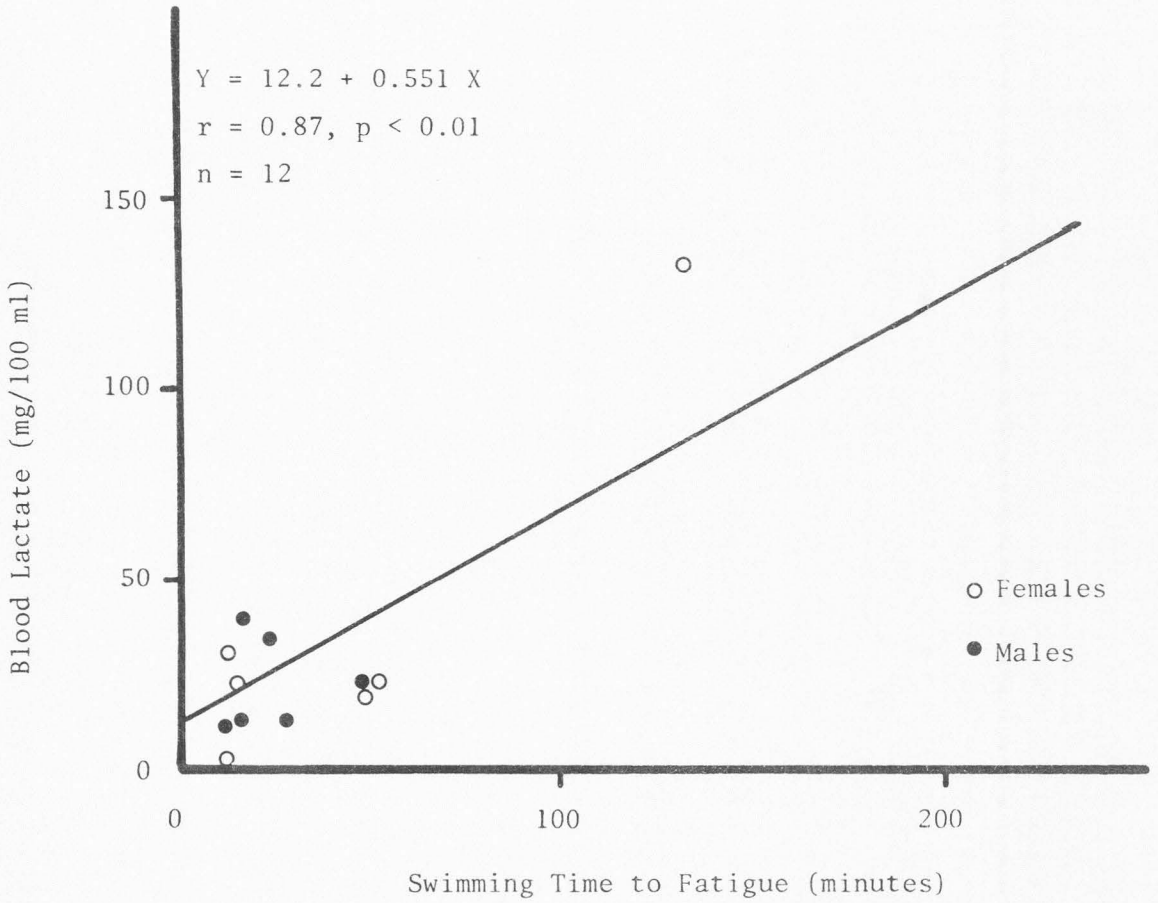


Figure 5. Relation of blood lactate at 8 hours post fatigue to total swimming time at 1.5 body lengths per second and 2 mg/l oxygen for Beity rainbow trout LDH phenotype I. Slope of the regression line was calculated by Bartlett's three group method for Model II regression.

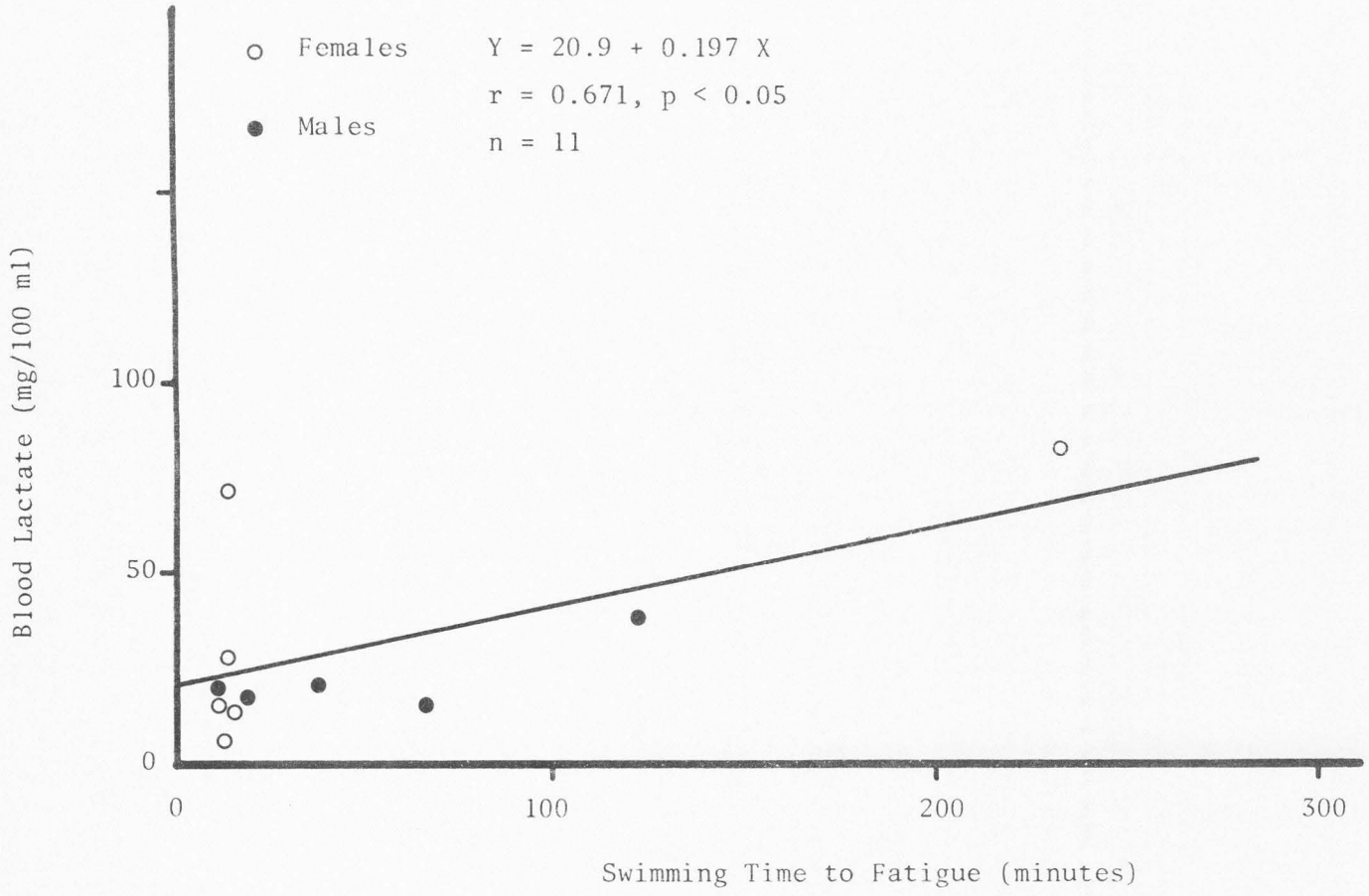


Figure 6. Relation of blood lactate at 8 hours post fatigue to total swimming time at 1.5 body lengths per second and 2 mg/l oxygen for Beity rainbow trout LDH phenotype III. Slope of the regression line was calculated by Bartlett's three group method for Model II regression.

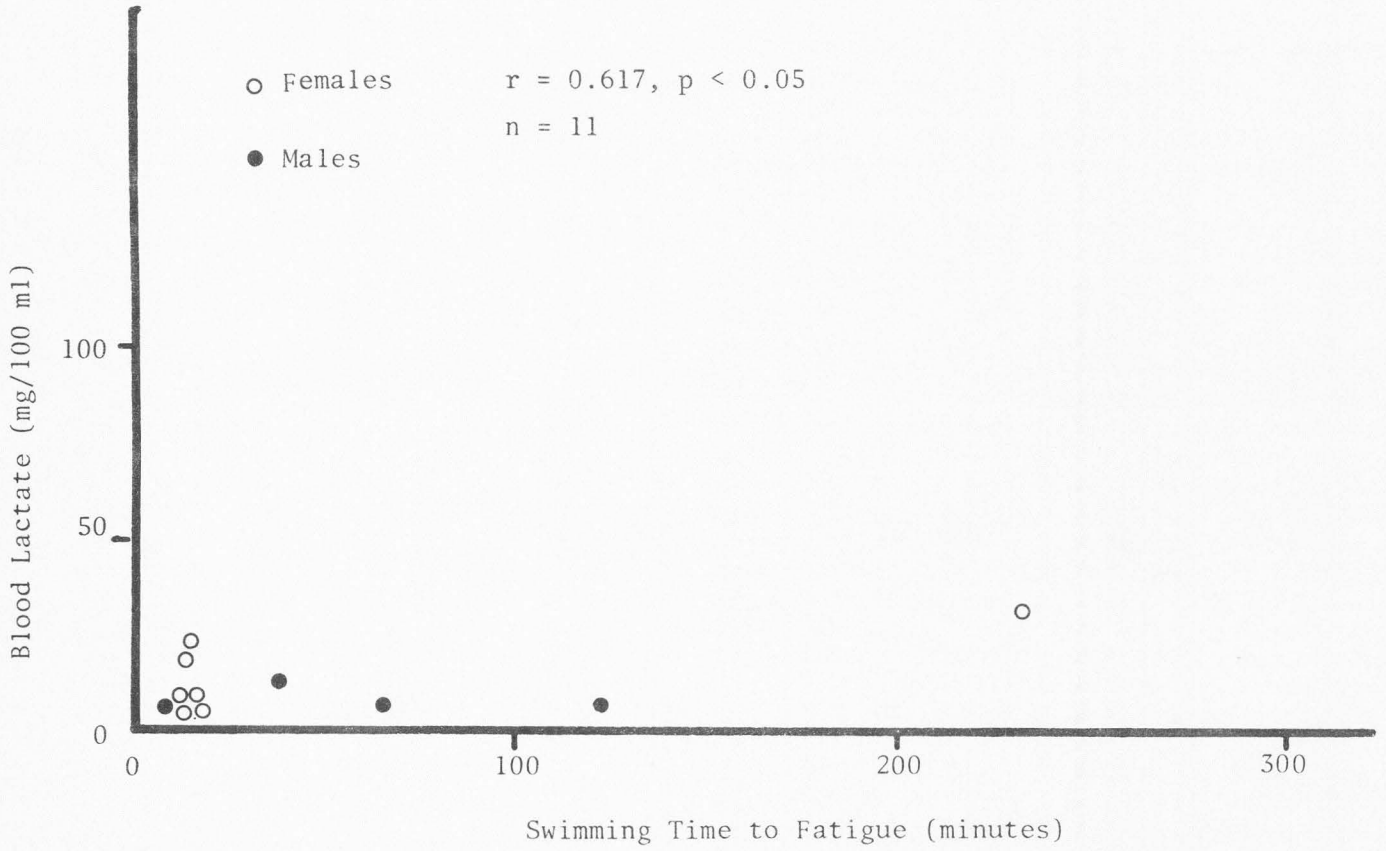


Figure 7. Relation of blood lactate at 21 hours post fatigue to total swimming time at 1.5 body lengths per second and 2 mg/l oxygen for Beity rainbow trout LDH phenotype III.

in blood lactate concentration. Experiments exposing fish to 2 mg/l dissolved oxygen for fixed periods of time were used to test the response difference.

Data from this experiment were analyzed with regression statistics and covariance analysis. Slope of the regression of blood lactate on time for phenotype I was not significantly different from zero ($p > 0.10$, Table 14). A significant regression ($p < 0.025$) was observed for phenotypes II and III (Table 14). Slope increased from phenotype I to phenotype III, with phenotype II between phenotypes I and III and the regression lines intersecting at the same point (Figure 8). Slopes for all three phenotypes were significantly different ($p < 0.001$, Table 14).

Mean blood lactate at 30 minutes exposure for phenotype III was significantly lower ($p < 0.05$) than both phenotypes I and II (Table 14). Mean blood lactate at 15 minutes exposure for phenotype I was significantly higher ($p < 0.05$) than both phenotypes II and III (Table 14).

There were not enough fish available to run a baseline level at the beginning of this experiment. Approximately 9 weeks after the fish were tested, a random sample of 24 fish (8/phenotype) was analyzed for blood lactate. The data were analyzed in a single classification analysis of variance. Mean blood lactate concentrations in mg/100 ml were 5.0 ± 3.5 for phenotype I, 8.5 ± 2.6 for phenotype II and 13.9 ± 2.9 for phenotype III. The three means were significantly different ($p < 0.05$). Assuming those means were reasonable estimates of the zero exposure levels they were plotted as zero time points along with the 15, 30, 45 and 60 minute exposure means (Figure 9). At the zero

point actually phenotype I was the lowest, phenotype III the highest and phenotype II fell between I and III. At the 15 minute exposure time the pattern was reversed and phenotype II again fell between I and III. Straight lines connecting the points intersect at nearly the same place between the zero and 15 minute points and again at a common point between 45 and 60 minutes (Figure 9).

Table 14. Mean blood lactate concentration (mg/100 ml) and regression slope for Beity rainbow trout LDH phenotypes I, II and III exposed to 2 mg/l oxygen for 15, 30, 45 and 60 minutes.

	Phenotype		
Exposure time	I	II	III
60 minutes	36 ± 7[a]	38 ± 8	42 ± 10
45 minutes	38 ± 8	35 ± 5	31 ± 8
30 minutes	36 ± 10	32 ± 5	21 ± 6[b]
15 minutes	38 ± 4[c]	25 ± 8	16 ± 5
Slope[d]	-0.013 ± 0.035[e]	0.277 ± 0.042	0.586 ± 0.066

[a] ± one standard error of the mean

[b] Phenotype III significantly lower ($p < 0.05$) than both phenotypes I and II at 30 minutes.

[c] Phenotype I significantly higher ($p < 0.05$) than both phenotypes II and III.

[d] Slopes for all three phenotypes significantly different ($p < 0.001$).

[e] ± one standard error of the slope.

Static low oxygen experiment two

Blood lactate. In this experiment three time periods (15, 30 and 60 minutes) were used and the fish were tested previously in swimming experiment two. Data were analyzed with regression and covariance statistics.

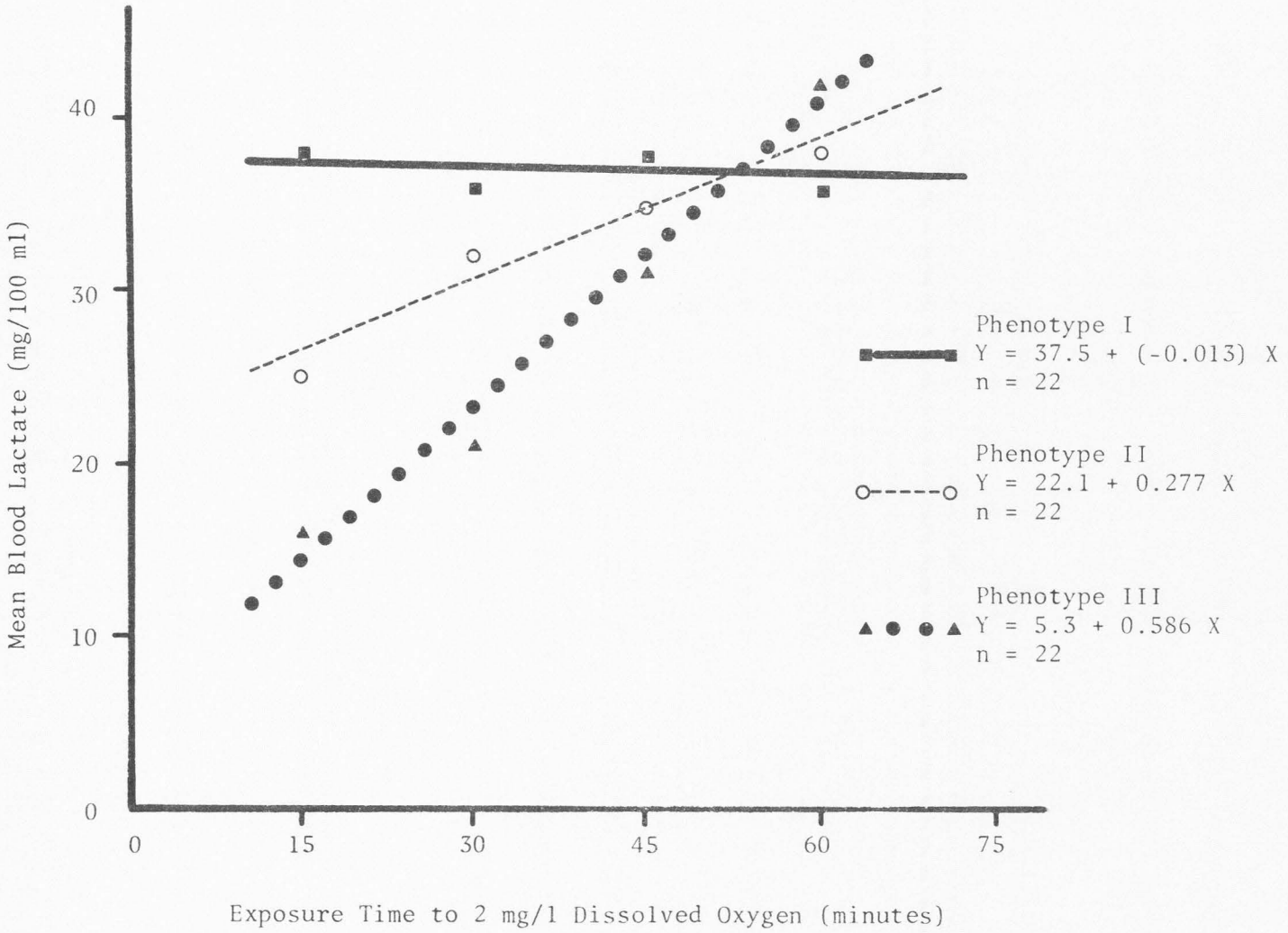


Figure 8. Relation of mean blood lactate to exposure time to 2 mg/l dissolved oxygen for different LDH phenotypes of Beity rainbow trout. All three slopes were significantly different ($p < 0.001$).

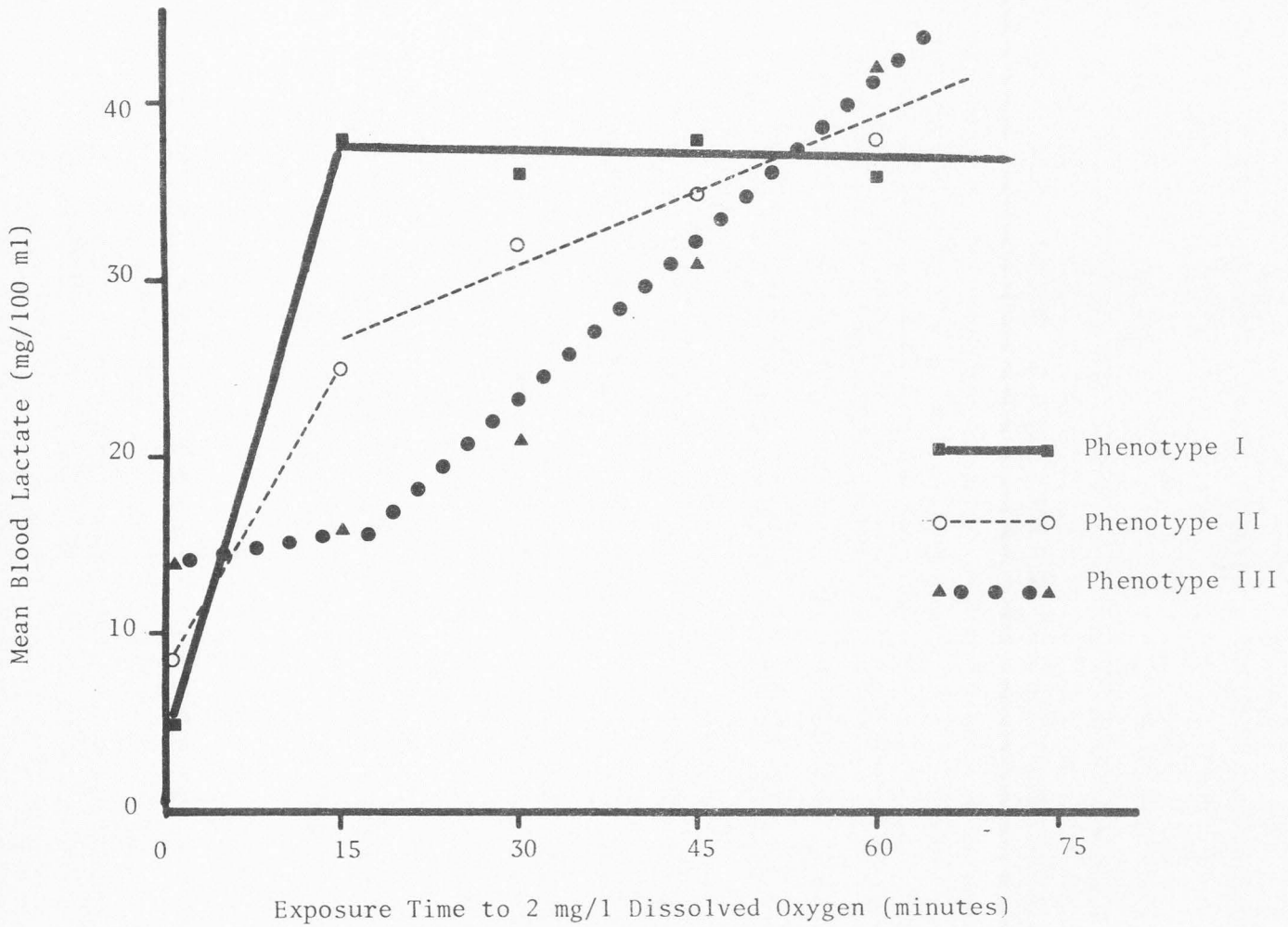


Figure 9. Comparison of mean blood lactate levels with time of exposure to 2 mg/l dissolved oxygen. Straight lines were drawn between the 0 and 15 minute points. See text for determination of values for zero exposure time.

The slope for phenotype I was significantly lower ($p < 0.05$) than the slope for phenotype III (Table 15). The slopes followed the same pattern as those in Low Oxygen Experiment One with phenotype I lower than III and II between I and III (Figure 10). However, the regression lines were somewhat different in that they did not intersect at a common point (Figure 10). The distribution of means was also slightly different. Phenotypes I and III appeared in the same relationship as before but phenotype II was lower at each time period (Figure 10).

Mean blood lactate at 60 minutes for phenotype II fish was significantly lower ($p < 0.05$) than both phenotypes I and III (Table 15). Mean blood lactate at 60 minutes for phenotype I was significantly lower ($p < 0.05$) than for phenotype III (Table 15).

Mortality. In the two low oxygen experiments, four mortalities of phenotype III fish were observed within 24 hours for the 60 minute exposure time and no mortalities occurred in either phenotypes I or II. Those frequencies were tested in a 2 by 2 chi-square contingency table and found to be significantly different ($p < 0.05$). No mortalities were observed at the other exposure periods.

Electrophoretic Analysis of Cutthroat Trout

Aspartate aminotransferase

Several electrophoretic phenotypes of muscle aspartate aminotransferase were observed in cutthroat trout (Figure 11). Allendorf and Utter (1976) reported similar variations for AAT in anadromous cutthroat trout. They concluded that a duplicated locus with three electrophoretically distinct alleles was coding for AAT in cutthroat trout. They further demonstrated that the mode of inheritance was disomic. The

phenotypes observed in the present study would fit Allendorf and Utter's (1976) model of inheritance.

Table 15. Mean blood lactate concentration (mg/100 ml) and regression slope for Beity rainbow trout LDH phenotypes I, II and III exposed to 2 mg/l dissolved oxygen for 15, 30 and 60 minutes.

	Phenotype		
Exposure Time	I	II	III
60 minutes[a]	20.4 ± 7.6[b]	13.3 ± 4.0	25.9 ± 5.3
30 minutes	16.8 ± 4.3	11.5 ± 2.5	13.0 ± 3.4
15 minutes	13.8 ± 5.6	6.8 ± 2.2	10.5 ± 4.7
Slope	0.143 ± 0.017[c]	0.176 ± 0.041	0.360 ± 0.057[d]

[a]Mean values among all phenotypes were significantly different ($p < 0.05$).

[b]± one standard error of the mean

[c]± one standard error of the regression slope.

[d]Phenotype III significantly different from phenotype I ($p < 0.05$).

Lactate dehydrogenase

Electrophoretic variation was observed in white muscle A group LDH of cutthroat trout from Goshute Creek (Figures 12 and 13), Pine Creek (Figure 14), Trout Creek (Figure 15) and Birch Creek, Juab County (Figure 16).

In the genus Salmo, two codominant fixed loci designated A^1 and A^2 produce a slow migrating five isozyme pattern in skeletal muscle (Utter et al. 1973). With the exception of fish from Goshute Creek, Pine Creek, Trout Creek and Birch Creek, Juab County, all cutthroat and rainbow trout examined produced the typical A^1-A^2 muscle pattern. The typical A^1-A^2 pattern was present in Goshute Creek, Pine Creek, Trout

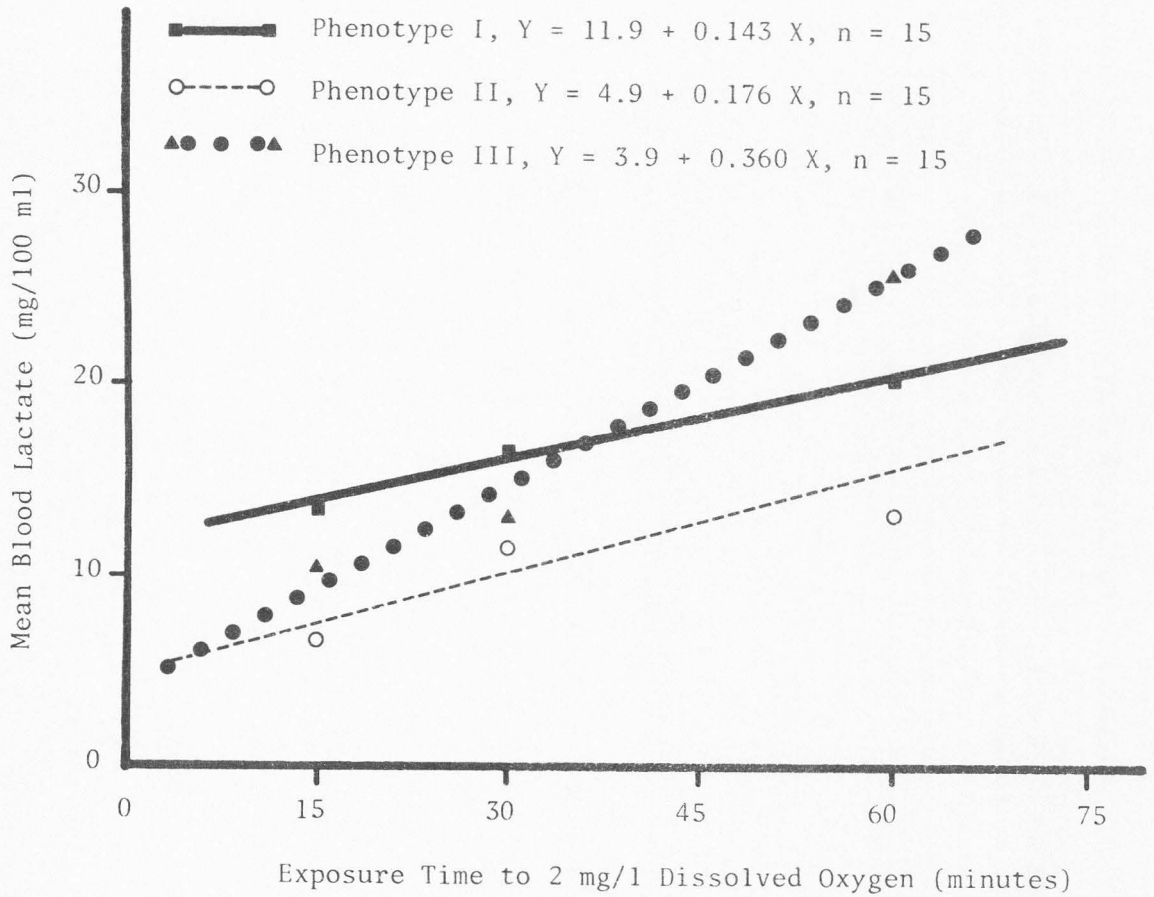
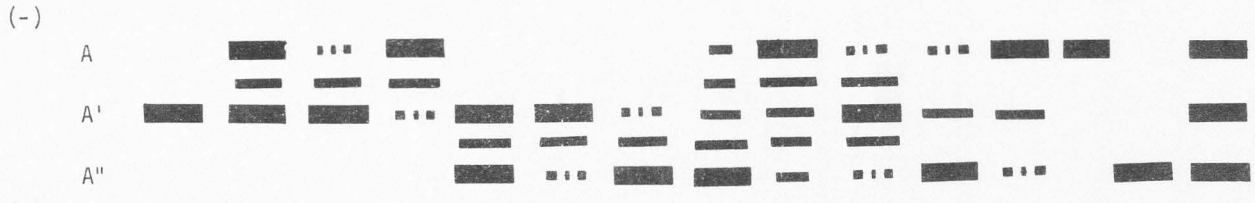


Figure 10. Mean blood lactate values for Beity rainbow trout of different LDH phenotypes exposed to 2 mg/l dissolved oxygen for 15, 30 and 60 minutes. Slopes for phenotypes I and III were significantly different ($p < 0.05$).



Population	A'A' A'A'	A A A'A'	A A' A'A'	AA AA'	A'A' A"A"	A'A' A'A"	A'A" A"A"	A A' A"A"	A A A'A"	A'A' A A"	A A" A"A"	AA AA"	AA AA	A"A" A"A"	A A A"A"
Goshute Creek (1973)	0	0	0	0	10	10	8	0	0	0	0	0	0	0	0
Trout Creek	0	0	0	6	0	0	3	1	2	5	0	0	0	0	0
Birch Creek, Beaver Co., Utah	0	1	0	0	10	0	2	1	2	7	1	1	0	0	4
Water Canyon Creek	0	0	0	0	0	13	0	0	0	0	0	0	0	0	0
Asay Creek	0	0	0	6	0	0	5	1	1	4	1	1	0	0	1
Auburn hatchery	5	2	3	0	0	19	0	0	0	0	0	0	0	0	0
One Mile Creek	0	0	0	0	0	29	0	0	0	0	0	0	0	0	0
Little West Fork Creek	10	0	0	0	0	25	0	0	0	0	0	0	0	0	0
Forest Canyon Creek	0	0	0	0	0	0	10	0	0	0	0	0	0	0	0
Bear Lake (1974)	17	2	7	0	9	6	0	1	0	5	0	0	0	1	0
Bear Lake (1975)	10	3	6	0	0	6	2	0	1	1	0	0	0	0	0
Bear Lake (1976)	0	3	0	0	1	8	0	0	0	4	0	0	0	0	0
Yellowstone Lake	0	4	4	0	8	19	1	3	2	10	0	0	0	0	0
Birch Creek, Juab Co., Utah	0	0	0	0	0	27	0	0	0	4	0	0	0	0	0
Goshute Creek (1976)	0	0	0	0	6	12	10	0	0	0	0	0	0	2	0
Pine Creek	0	0	0	0	3	1	7	0	0	0	0	0	0	2	0

Figure 11. Occurrence of cutthroat trout muscle aspartate aminotransferase phenotypes in 13 Intermountain populations. Allelic designations are from Allendorf and Utter (1976).

RBT

Figure 12. Skeletal muscle LDH electropherogram of cutthroat trout from Goshute Creek (1973) compared with rainbow trout (RBT).

RBT

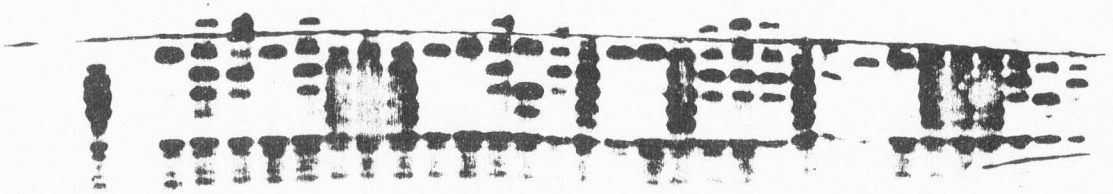


Figure 13. Skeletal muscle LDH electropherogram of cutthroat trout from Goshute Creek (1976) compared with rainbow trout (RBT).

Figure 14. Skeletal muscle LDH electropherogram of cutthroat trout from Pine Creek compared with rainbow trout (RBT).

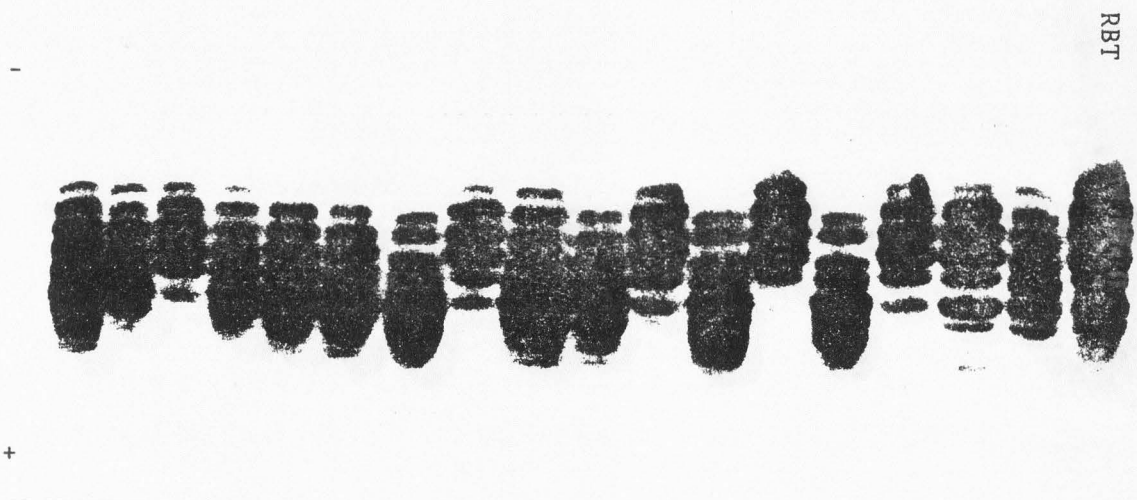


Figure 15. Skeletal muscle LDH electropherogram of cutthroat trout from Trout Creek compared with rainbow trout (RBT).

Figure 16. Skeletal muscle LDH electropherogram of cutthroat trout from Birch Creek, Juab County, Utah compared with rainbow trout (RBT).

Creek and Birch Creek, Juab County cutthroat trout (Figures 17 and 18, pattern I). A variant of A^1 ($A^{1'}$) appears to have been present in Goshute and Pine Creek cutthroat trout (Figure 17, Pattern II). Two additional patterns were present in Goshute Creek and Pine Creek trout that made interpretation difficult. Pattern IV, (Figure 17) suggests the presence of an A^2 null allele or a variant of A^2 that cannot be distinguished from A^1 or B^1. No band was present in the position of A^2 in the electropherograms for those patterns (Figures 12, 13 and 14). The faint band present in pattern IV (Figure 17) may be a recombinant of A^1 and B^1. The slow migrating band in pattern III (Figure 17) is much wider than the slow band in pattern IV (see also Figure 12). Also the middle band in pattern II (Figure 17) is very dark as is the middle band in pattern III, although the two are not in the same position. This suggests a relationship between the two patterns in the presence of similar subunits. The A^2 band appears to be in pattern III. It is possible that pattern III includes the A^1-$A^{1'}$ heterozygote in combination with the A^2-A^2 null heterozygote. It is also possible that there are additional variants of A^2 that migrate slower than A^2 and are present within the patterns, or cannot be distinguished from A^1 or B^1.

Muscle LDH A group variation observed in Birch Creek (Juab County) and Trout Creek cutthroat trout was different from Goshute Creek variation. The former variant pattern was that of two slow bands separated from two fast bands by a distinct gap (Figures 15 and 16). The fastest band of the four is in the position of A^2 and the slowest band is not in the position of A^1 but faster than A^1. In addition there

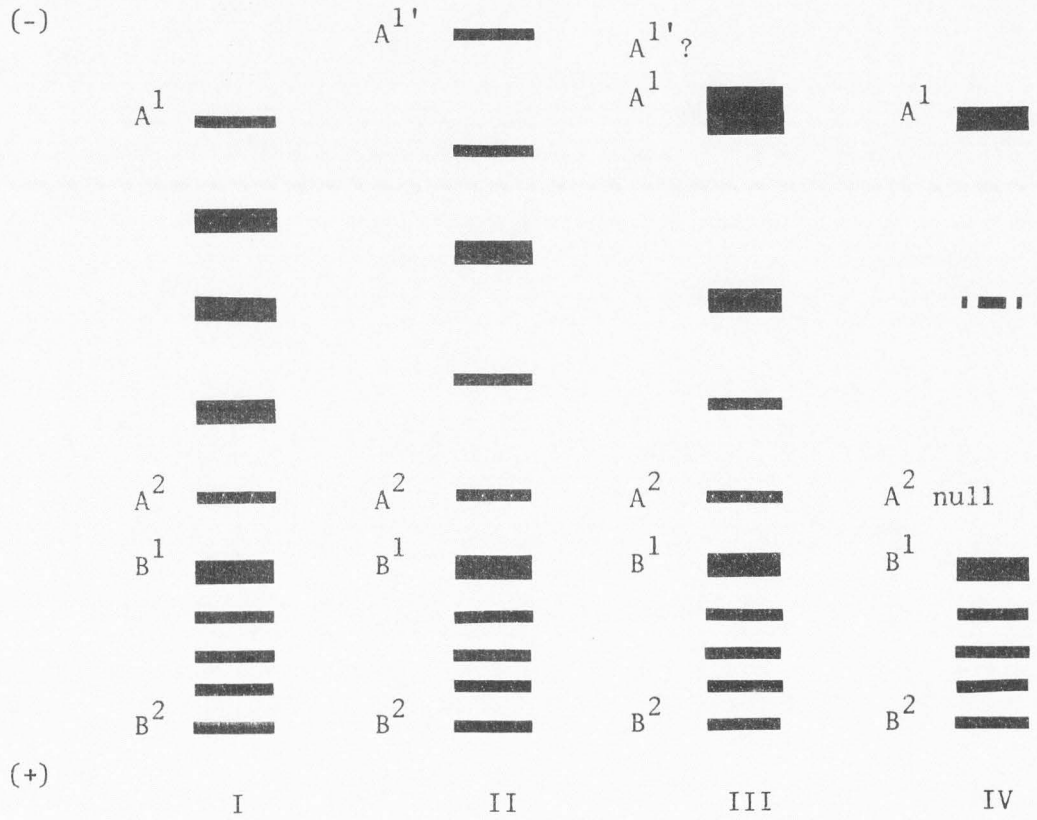


Figure 17. Diagrammatic representation of muscle A group LDH phenotypes in Goshute and Pine Creek cutthroat trout, with possible variant alleles. Phenotype I is the common phenotype present in Salmo.

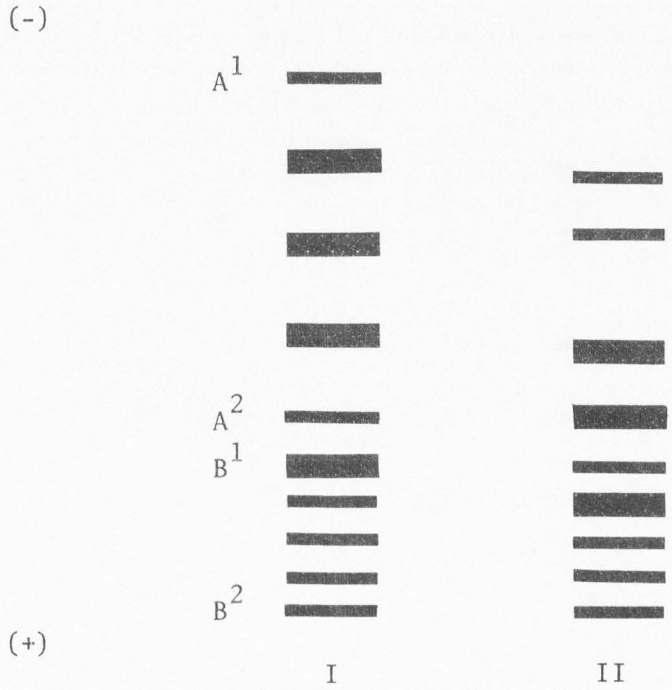


Figure 18. Diagrammatic representation of muscle A group LDH phenotypes in Trout Creek and Birch Creek (Juab County) cutthroat trout. Phenotype I is the common phenotype present in Salmo.

is a heavy dark band migrating within the B group in each variant pattern (Figure 18 pattern II, see also Figures 15 and 16). Analysis of the B group in the eye of those fish indicated a normal pattern with no difference between A group variant and non-A group variant fish. This suggests that a variant of the A group is migrating within the B group in those fish.

Variation in liver LDH was observed in Bear Lake (1974) cutthroat trout. Twenty-five $B^{2'}$ $B^{2'}$, three $B^{2'}$ $B^{2''}$ and no $B^{2''}$ $B^{2''}$ phenotypes were observed. The frequency of $B^{2'}$ was 0.946 and of $B^{2''}$ was 0.054. Hardy-Weinberg expected frequencies were not significantly different from the observed frequencies (Table 16). All other cutthroat trout sampled were $B^{2'}$ $B^{2'}$ (Table 16).

No variation was observed in eye LDH. All cutthroat trout sampled were the CC phenotype.

Phosphoglucomutase, alphaglycerophosphate dehydrogenase and tetrazolium oxidase

Variation was observed in muscle PGM in Bear Lake (1974) cutthroat trout. Phenotypes present were CC (49 fish) and BC (1 fish). Gene frequencies were 0.99 and 0.01 for C and B respectively and expected frequencies were not significantly different from observed frequencies (Table 17). All other cutthroat trout sampled were the CC phenotype for muscle PGM (Table 17).

No variation was observed in muscle AGPDH in the cutthroat trout sampled. All fish were the BB phenotype.

Variation was observed in liver tetrazolium oxidase in Trout Creek and Bear Lake (1974, 1975 and 1976) cutthroat trout. In fish from Trout Creek, 15 BB and 2 AB phenotypes were observed. Allele frequencies were 0.941 and 0.059 for B and A respectively. Expected phenotype

Table 16. Liver lactate dehydrogenase phenotype and allele frequencies in 13 cutthroat trout populations.

Population	Sample Size	Phenotype[a]			Allele frequency	
		$B^{2'}$ $B^{2'}$	$B^{2'}$ $B^{2''}$	$B^{2''}$ $B^{2''}$	$B^{2'}$	$B^{2''}$
Goshute Creek (1973)	28	28	0	0	1.00	0.00
Goshute Creek (1976)	30	30	0	0	1.00	0.00
Pine Creek	13	13	0	0	1.00	0.00
Trout Creek	17	17	0	0	1.00	0.00
Birch Creek (Juab Co.)	31	31	0	0	1.00	0.00
Water Canyon Creek	13	13	0	0	1.00	0.00
Asay Creek	20	20	0	0	1.00	0.00
Birch Creek (Beaver Co.)	29	29	0	0	1.00	0.00
One Mile Creek	29	29	0	0	1.00	0.00
Little West Fork Creek	35	35	0	0	1.00	0.00
Forest Canyon Creek	10	10	0	0	1.00	0.00
Yellowstone Lake	51	51	0	0	1.00	0.00
Bear Lake (1974)	28	25 (25.1)[b]	3 (2.9)	0 (0.08)	0.946	0.054
Bear Lake (1975)	30	30	0	0	1.00	0.00
Bear Lake (1976)	28	28	0	0	1.00	0.00
Auburn Hatchery	31	31	0	0	1.00	0.00

[a]Subunit designations from Utter and Hodgins (1972).

[b]Expected frequencies from Hardy-Weinberg equation

Table 17. Muscle phosphoglucomutase phenotype and allele frequencies in 13 cutthroat trout populations.

Population	Sample Size	Phenotype[a]			Allele frequency	
		CC	BC	BB	C	B
Goshute Creek (1973)	28	28	0	0	1.00	0.00
Goshute Creek (1976)	30	30	0	0	1.00	0.00
Pine Creek	13	13	0	0	1.00	0.00
Trout Creek	17	17	0	0	1.00	0.00
Birch Creek (Juab Co.)	31	31	0	0	1.00	0.00
Water Canyon Creek	13	13	0	0	1.00	0.00
Asay Creek	20	20	0	0	1.00	0.00
Birch Creek (Beaver Co.)	29	29	0	0	1.00	0.00
One Mile Creek	29	29	0	0	1.00	0.00
Little West Fork Creek	35	35	0	0	1.00	0.00
Forest Canyon Creek	10	10	0	0	1.00	0.00
Yellowstone Lake	51	51	0	0	1.00	0.00
Bear Lake (1974)	50	49 (49)[b]	1 (0.99)	0 (0.005)	0.99	0.01
Bear Lake (1975)	30	30	0	0	1.00	0.00
Bear Lake (1976)	28	28	0	0	1.00	0.00
Auburn Hatchery	31	31	0	0	1.00	0.00

[a]Subunit designations from Utter and Hodgins (1972)

[b]Expected frequencies from Hardy-Weinberg equation.

frequencies were not significantly different from observed frequencies (Table 18). In Bear Lake (1974) cutthroat trout 20 BB, 6 AB and 2 AA phenotypes were observed. Allele frequencies were 0.821 ± 0.100 and 0.179 for B and A respectively. Expected phenotype frequencies were not significantly different from observed frequencies (Table 18). In Bear Lake (1975) cutthroat trout 28 BB, and 2 AB tetrazolium oxidase phenotypes were observed. Allele frequencies were 0.967 ± 0.045 and 0.033 for B and A respectively. Expected phenotype frequencies were not significantly different from observed frequencies (Table 18). In Bear Lake (1976) cutthroat trout 8 BB, 18 AB and 2 AA phenotypes were observed. Allele frequencies were 0.607 ± 0.128 and 0.393 for B and A respectively. Expected phenotype frequencies were not significantly different from observed frequencies (Table 18). The 95 percent confidence interval for the B allele frequency in Bear Lake (1974 to 1976) cutthroat trout was calculated by:

$$95\%\ C.I. = p \pm 1.96 \sqrt{P(1-p)/2n}$$

where:

p = frequency of the most common allele

n = sample size

The frequency of the B allele in 1974 was significantly lower than in 1975 and the frequency in 1975 was significantly higher than in 1976. All other cutthroat trout sampled were the BB phenotype (Table 18).

Isocitrate dehydrogenase, malate dehydrogenase, sorbitol dehydrogenase and esterase

No variation was observed in muscle IDH in cutthroat trout sampled. An invariant two banded pattern was observed in all samples.

Table 18. Liver tetrazolium oxidase phenotype and allele frequencies in 13 populations of cutthroat trout.

Population	Sample Size	Phenotype[a]			Allele frequency	
		AA	AB	BB	A	B
Goshute Creek (1973)	28	0	0	28	0.00	1.00
Goshute Creek (1976)	30	0	0	30	0.00	1.00
Pine Creek	13	0	0	13	0.00	1.00
Trout Creek	17	0 (0.05)[b]	2 (1.9)	15 (15.1)	0.059	0.941
Birch Creek (Juab Co.)	31	0	0	31	0.00	1.00
Water Canyon Creek	13	0	0	13	0.00	1.00
Asay Creek	20	0	0	20	0.00	1.00
Birch Creek (Beaver Co.)	29	0	0	29	0.00	1.00
One Mile Creek	29	0	0	29	0.00	1.00
Little West Fork Creek	35	0	0	35	0.00	1.00
Forest Canyon Creek	10	0	0	10	0.00	1.00
Yellowstone Lake	51	0	0	51	0.00	1.00
Bear Lake (1974)	28	2 (0.9)	6 (8.2)	20 (18.9)	0.179	0.821 ± 0.100[c]
Bear Lake (1975)	30	0 (0.06)	2 (1.8)	28 (26.2)	0.033	0.967 ± 0.045
Bear Lake (1976)	28	2 (4.3)	18 (13.4)	8 (10.3)	0.393	0.607 ± 0.128
Auburn Hatchery	31	0	0	31	0.00	1.00

[a]Subunit designations from Utter and Hodgins (1972)

[b]Expected frequency from Hardy-Weinberg equation

[c]± 95% confidence interval

An attempt to analyze for the liver form of IDH was unsuccessful. Reasonable results were obtained with fresh rainbow trout liver but not with the frozen cutthroat trout livers available for this study. The enzyme appears to be unstable in frozen liver tissue.

No variation was observed in muscle MDH in the cutthroat trout analyzed. A single dark invariant band was observed, which was interpreted as the B form of MDH. A single lighter invariant band, presumably the A form, was also observed.

Allendorf (1973) reported that esterase phenotypes were difficult to classify because of non-genetic based differences. In this study, esterase did not give reproducible results from frozen samples and thus was not considered.

An unsuccessful attempt was made to isolate liver SDH in cutthroat trout. This enzyme appears to be very unstable in frozen livers. Livers frozen for more than a few days yielded almost no SDH activity. However, tetrazolium oxidase was found to stain exceptionally well when using SDH stains.

DISCUSSION

Physiological Studies of LDH Phenotype

Physical and physiological performance of Beity rainbow trout

Possession of the LDH $B^{2''}$ allele offered no significant advantage or disadvantage in swimming ability to the fish under conditions of saturating oxygen. Swimming times to fatigue at two body lengths per second were similar for all three LDH B^{2} phenotypes.

Swimming time to fatigue at two body lengths per second and declining oxygen depended on sex as well as LDH phenotype. Phenotype I females had significantly longer swimming times than phenotype II and III females. In addition, phenotype I females fatigued at an oxygen concentration only slightly higher than 2 mg/l. Mean oxygen concentration at fatigue for phenotype III females was considerably higher at 3.5 mg/l. Phenotype II females fell between phenotypes I and III. Oxygen consumption rates at fatigue were influenced by phenotype as well as sex. Phenotype I females had lower oxygen consumption rates at fatigue than did phenotype I males or phenotype III females. The differences noted in oxygen consumption rate at fatigue may be the result of differences in oxygen concentration at fatigue. Ambient oxygen concentration itself influences the rate of oxygen consumption (Brett 1962). As the oxygen concentration is lowered, the rate of oxygen consumption is depressed. Phenotype I females were able to swim to lower oxygen concentrations than phenotype III females and thus had lower rates of oxygen consumption at the fatigue point. However, phenotype

III females were fatiguing with mean rates of oxygen consumption 52 mg O_2/kg/hr higher than phenotype I females.

Phenotype III fish exhibited the poorest performance at 1.5 body lengths per second and a constant 2 mg/l dissolved oxygen with 12 of 18 fish fatigued in the 11 to 20 minute period. Performance of phenotype I fish was better than phenotype III, with 7 of 18 fish fatigued in the 11 to 20 minute period. Phenotype II fish had the best and most consistent swimming performance. Only 3 of 17 fish fatigued in the 11 to 20 minute period. In the 20 to 50 minute period the fatigue frequency of phenotype II fish was higher than both phenotypes I and III.

When the LDH phenotypes were exposed to constant low oxygen (2 mg/l) for fixed periods of time with no forced activity the blood lactate response depended on LDH phenotype. Phenotype I fish reached a blood lactate level (36-38 mg/100 ml) at 15 minutes exposure similar to values reported (Black et al. 1962, Black et al. 1966) for fish subjected to 5 minutes of exercise. That value did not change through 60 minutes of exposure to 2 mg/l dissolved oxygen. Zero time or resting blood lactate for phenotype I fish determined 9 weeks after the exposure was 5 mg/100 ml, in agreement with values reported by Black et al. (1962) and Black et al. (1966). Apparently the phenotype I fish increased blood lactate rapidly from 0 to 15 minutes exposure then leveled off through 60 minutes exposure. This may reflect an initial rapid metabolic adjustment to the low oxygen stress, establishment of a new metabolic equilibrium and maintenance of that equilibrium through 60 minutes exposure. Phenotype III fish had significantly lower blood lactate concentrations than phenotype I fish at 15 minutes and increased gradually through 60 minutes exposure. Phenotype III fish exhibited no rapid metabolic

adjustment to the oxygen stress but exhibited a constantly declining situation with no ability to establish and maintain an equilibrium. Phenotype II fish were intermediate in performance.

There was an observable difference in behavior during static exposure to 2 mg/l dissolved oxygen between phenotypes I and II and phenotype III. Phenotypes I and II fish, when placed in the low oxygen water, would swim about for a few minutes then sink to the bottom and remain there with strong opercle movements. Several fish lost orientation during the longer exposure times (45 and 60 minutes). When a phenotype I or II fish lost orientation it would roll onto its back and remain on the bottom with regular opercle movements. It would occasionally propel itself with one or two movements of its tail. Phenotype III fish, when placed in the test tank, would swim slowly for a few minutes, rest on the bottom for a few minutes, then continue swimming. That swimming was not rapid but effected by only one or two tail movements and drifting. When a phenotype III fish lost orientation it would assume a vertical position in the water with its head up. It would then go through a period of violent convulsive thrashing where the tail would flex maximally from side to side and the opercles would flare outward. These convulsions became more sporadic as time increased and opercle movements became more sporadic and irregular.

All phenotype I and II fish recovered after exposure to 2 mg/l dissolved oxygen when returned to fresh water, including those that lost orientation during testing. Four phenotype III fish failed to recover after the test. Each phenotype III fish that failed to recover demonstrated the behavior described above for fish that lost

orientation. The convulsive type behavior in phenotype III fish suggests nervous system involvement.

In summary, phenotype III females had lower swimming performance at 2 body lengths per second and declining oxygen than phenotype I females and phenotype I females were able to swim at 2 body lengths per second to a lower oxygen concentration than phenotype III females. Phenotype III fish had lower swimming performance at 1.5 body lengths per second and constant 2 mg/l dissolved oxygen than phenotype I fish. Phenotype III fish exhibited a declining blood lactate pattern on exposure to 2 mg/l dissolved oxygen and had higher mortality than phenotype I fish when exposed to 2 mg/l dissolved oxygen.

Adaptive significance of
LDH B^2 alleles

The hypothesis that the LDH $B^{2''}$ $B^{2''}$ (phenotype III) homozygote has a greater metabolic flexibility conferring superior performance at low oxygen must be rejected. The data supports the alternate hypothesis that the $B^{2''}$ $B^{2''}$ phenotype has inferior performance at low oxygen concentrations.

The kinetic characteristics of the $B_4^{2''}$ isozyme reported by Kao (1977) (random kinetic mechanism, pH regulation, product induced modulation) were thought to give the $B^{2''}$ $B^{2''}$ phenotype a greater metabolic flexibility under anaerobic conditions. LDH's of the A or muscle type are generally found in anaerobic tissues such as skeletal muscle. These enzymes are geared kinetically to convert pyruvate to lactate under conditions of high pyruvate and lactate concentrations. Thus the muscle, which obtains most of its ATP from glycolysis, can maintain glycolytic activity with high pyruvate and lactate

concentrations. LDH's of the B type are found in more aerobic tissues such as liver, heart and brain. These LDH's are inhibited by low levels of lactate and pyruvate which prevents a buildup of lactate. Thus pyruvate is diverted towards the Krebs cycle which results in a high yield of ATP (Cahn et al. 1962). Kao and Farley (1977) reported that the $B_4^{2''}$ isozyme has diverged from the $B_4^{2'}$ isozyme towards an A type in its kinetic parameters.

The observation of the convulsive behavior of phenotype III fish under low oxygen stress suggests an explanation for the poor performance of phenotype III fish. Electrophoresis of phenotype III brain tissue revealed the presence of a very high activity of the $B_4^{2''}$ isozyme. The B_4^{1} isozyme also appears in the brain but in much lower activity. Stillings (1974) reported the same finding of $B_4^{2''}$ in the brain. It would be a definite disadvantage for a rainbow trout to have an LDH with the characteristics of $B_4^{2''}$ isozyme (towards an A type LDH) in the brain. As oxygen becomes limiting the $B_4^{2''}$ isozyme would rapidly shift to lactate production, cause a buildup of lactate in the brain cells, loss of energy to those cells and subsequent brain death.

It is not clear how the differential response in blood lactate is related to the functional characteristics of the LDH isozymes. Since muscular activity was not required during the static tests the blood lactate increases may be coming from heart, liver and brain rather than from muscle. The initial response of phenotype I may have been to rapidly produce lactate in these tissues until the $B_4^{2'}$ isozyme was inhibited by lactate, then divert pyruvate to the Krebs cycle using all available oxygen to maintain the brain cells. Phenotype III may have been gradually building up lactate in liver, heart and brain in a manner that would continue until brain death occurred.

Tsuyuki and Williscroft (1977) reported that fingerling Loon Lake rainbow trout $H\alpha^{A}H\alpha^{A}$ ($B^{2''}$ $B^{2''}$, phenotype III) LDH phenotypes had 2.3 times greater stamina than $H\alpha^{B}H\alpha^{B}$ ($B^{2'}$ $B^{2'}$, phenotype I) phenotypes and suggested the use of the superior performance of $B^{2''}$ $B^{2''}$ phenotypes in stocking programs. However, Tsuyuki and Williscroft (1977) also reported a reduction in the stamina of Loon Lake rainbow trout $B^{2''}$ $B^{2''}$ phenotypes when the fish were 27 to 30 months old. During these latter tests the $B^{2'}$ $B^{2'}$ phenotype performed as before but the $B^{2''}$ $B^{2''}$ phenotypes had FT 50 values only 0.79 and 0.28 that of $B^{2'}$ $B^{2'}$ phenotypes. Also they found no stamina difference among $B^{2'}$ $B^{2'}$, $B^{2'}$ $B^{2''}$ and $B^{2''}$ $B^{2''}$ LDH phenotypes in two populations of steelhead trout.

Tsuyuki and Williscroft (1977) suggested that under conditions of lactate buildup during swimming, which would decrease blood pH and thus the oxygen carrying capacity of the blood, the $B_4^{2''}$ isozyme in the liver would remove lactate at a higher rate than $B_4^{2'}$ and postpone the onset of fatigue. The kinetic results of Kao (1977) suggested the opposite, in that when oxygen becomes limiting the $B_4^{2''}$ isozyme would convert to lactate production. Those kinetic results also suggested that the $B^{2''}$ $B^{2''}$ phenotype would be able to remove lactate at a higher rate than $B^{2'}$ $B^{2'}$ only when adequate oxygen is available to maintain low levels of pyruvate. When oxygen becomes limiting and pyruvate levels increase the $B_4^{2''}$ isozyme will convert to lactate production.

Swimming times for phenotype III fish tested in this study under saturated oxygen were not significantly different from phenotype I. When oxygen in the water was reduced the swimming performance of phenotype III was lower than phenotype I. The fish tested in these experiments were sexually mature. This, along with the reduction in stamina

noted by Tsuyuki and Williscroft (1977) in older Loon Lake rainbow trout $B^{2''}$ $B^{2''}$ phenotypes suggests a change in the physiological performance of the $B^{2''}$ $B^{2''}$ phenotypes with the onset of maturity. Ontogenetic changes in hemoglobin fractions have been reported in salmonids (Giles and Vanstone 1976; Hasimoto and Matsura 1960; Iuchi and Yamagami 1969; Iuchi 1973). Iuchi (1973) compared the kinetic characteristics of larval and adult hemoglobins from rainbow trout and found that larval hemoglobin had a higher oxygen affinity than adult hemoglobin. In addition, the adult hemoglobin lost oxygen affinity rapidly below pH 7.6 while the larval hemoglobin was not affected by a decrease in pH. Possession of a hemoglobin by phenotype III fish with the characteristics of the larval hemoglobin may enable the fish to supply sufficient oxygen under swimming stress to maintain low tissue pyruvate levels. Under those conditions the LDH $B_4^{2''}$ isozyme would remove lactate at a higher rate than the $B_4^{2'}$ isozyme which would enable the phenotype III fish to swim for longer periods than phenotype I. Hemoglobins with the characteristics of the rainbow trout adult hemoglobin may not supply sufficient oxygen to maintain low tissue pyruvate levels. An increase in tissue pyruvate level would cause the $B_4^{2''}$ isozyme to convert to lactate production, resulting in increases in lactate in the brain of phenotype III fish and lower swimming performance. The reduction in performance of phenotype III fish with maturity should be investigated from the standpoint of ontogenetic changes in hemoglobin fractions. Also sexual maturation in $B^{2''}$ $B^{2''}$ phenotypes may require sufficient energy, such that in a situation of severe stress, oxygen to the brain becomes limiting.

In the stamina tunnel experiment at saturated oxygen the fish were removed from the water current immediately upon fatigue. Fish that fatigue in natural waters would still be subjected to the water current and would drift downstream. Metabolic activity would continue to build up lactate and may eventually cause a decrease in blood pH. With a decrease in blood pH the oxygen carrying capacity of the blood would be reduced because of the Bohr effect on hemoglobin and a condition of limited oxygen supply to the brain would result. Under those conditions the phenotype III fish would have lower recovery and survival than phenotype I.

From the reports of the distribution of the LDH $B^{2'}$ and $B^{2''}$ alleles in rainbow trout populations (Northcote et al. 1970; Utter and Hodgins 1972; Allendorf 1975; Huzyk and Tsuyuki 1974) it appears that, generally, a high frequency of the $B^{2''}$ allele is associated with a high frequency of heterozygotes. Also when the $B^{2''}$ allele is observed in a population in lower frequency than the $B^{2'}$ allele, the frequency of $B^{2'}$ $B^{2''}$ heterozygotes is higher than $B^{2''}$ $B^{2''}$ homozygotes and in some cases higher than $B^{2'}$ $B^{2'}$ homozygotes. In this study there was no significant difference in swimming ability at saturated oxygen between the heterozygotes (phenotype II) and either homozygote. Phenotype II females were not significantly different from phenotype III females in swimming time to fatigue with declining oxygen and were significantly lower than phenotype I females. Phenotype II fish had better swimming performance than both phenotype I and III at a constant 2 mg/l dissolved oxygen. The increase in blood lactate during exposure to 2 mg/l dissolved oxygen for phenotype II fish was intermediate between phenotypes I and III but mortality from exposure for phenotype II was significantly lower

than phenotype III. Tsuyuki and Williscroft (1977) did not test the stamina of fingerling Loon Lake rainbow trout $B^{2'}$ $B^{2''}$ heterozygotes but reported no difference in stamina among Thompson River steelhead $B^{2'}$ $B^{2'}$, $B^{2'}$ $B^{2''}$ and $B^{2''}$ $B^{2''}$ phenotypes. The heterozygote has both the $B_4^{2'}$ and $B_4^{2''}$ isozymes in its tissues. Possession of both isozymes may, under some conditions, give the heterozygote the advantage of the higher lactate removal of the $B_4^{2''}$ isozyme without the disadvantage of the rapid buildup of lactate in the brain under oxygen stress. Also, the heterozygote may have the advantage of superior performance when they are small without the disadvantage of a drastic reduction in performance as adults. Research should continue to characterize the performance of the heterozygotes under a variety of oxygen and swimming levels at various stages of maturity.

Steelhead habitat and migration have changed drastically in the last few years. Numerous dams and diversions on steelhead streams have created impassable areas, interrupted and irregular flows, nitrogen supersaturation and temperature blocks to migration. Biochemical characteristics that were adaptive or neutral under natural conditions may no longer be so under present conditions. Numerous hatcheries have been built along the steelhead migration routes to artifically spawn migrants and rear and release fry to compensate for the loss in natural reproduction. These hatchery operations must take into account the genetic constitution of the fry released. Inadvertant crosses yielding large numbers of $B^{2''}$ $B^{2''}$ phenotypes could result in poor returns in certain areas. Movement of fish from one drainage to another could cause similar problems.

Some caution is required in the use of the $B^{2''}$ $B^{2''}$ phenotypes in artificial stocking situations on inland waters. The evidence indicates that, although the $B^{2''}$ $B^{2''}$ phenotypes are superior in stamina as fingerlings, they are inferior as adults. Use of the $B^{2''}$ $B^{2''}$ phenotypes may involve a tradeoff between better survival of young and poorer survival and reproduction of adults. Current evidence indicates that, unless survival of young is a severe problem, the $B^{2''}$ $B^{2''}$ phenotype would not be adaptive to most situations. The hypothesis that the heterozygotes have superior stamina when they are young with no reduction in stamina at maturity should be tested. Stocking operations are costly and any increase in survival of fingerlings without a decrease in survival of adults would be desirable.

Swimming times to fatigue

Klar (1973) reported the following stamina results for rainbow trout at 10 C. Of fish exercised at one-half body length per second continuously for 14 days, 41 percent fatigued in 2 hours or less when forced to swim at two body lengths per second. Of fish exercised at one body length per second continuously for 14 days, no fish fatigued in 2 hours at two body lengths per second. Of control fish, which were not exercised but handled three times during the 14 day experiment, 64 percent fatigued in 2 hours or less at two body lengths per second. In this study 98.4 percent of the fish fatigued in 38 minutes or less at two body lengths per second and saturated oxygen, indicating that the fish were in relatively poor physical condition. The fish were held for their entire lives in relatively small laboratory tanks with negligible water currents. Also, during the course of the experiment at two body lengths per second and saturated oxygen the fish were being

fed a diet that was later determined to be rancid (Ronald Goede, personal communication). The length of time the fish were on this diet is unknown but may have been up to a month preceeding the experiment. The fish appeared healthy and no unusual mortality was noted during that time period. The remainder of the experiments in this study were conducted two years after the food problem occurred and were not affected. These factors may account for the relatively poor performance of the fish at two body lengths per second and saturated oxygen.

Numerous authors (Bainbridge 1958, 1960, and 1962; Katz et al. 1959; Thomas et al. 1964; Brett 1964, 1967; Beamish 1966, 1974; Kutty 1968; Houde 1969; Jones 1971; Kutty and Saunders 1973; Greenland and Thomas 1972; Schiewe 1974) have reported on the swimming performance of fish in relation to various factors such as size, temperature and ambient oxygen concentration. No tests were made in their studies to determine if there was a difference between males and females in swimming performance. Swimming times measured at two body lengths per second and declining oxygen depended on sex as well as LDH phenotype. The data suggests that males and females should be treated separately in swimming performance tests under various experimental conditions. Important responses due to such factors as nutritional state, condition factors, temperature, ambient oxygen, etc. may not be evident if males and females are averaged as a group.

Kutty (1968) reported that the swimming ability of rainbow trout fell off sharply at 2 mg/l dissolved oxygen. Under a situation of declining oxygen males were able to swim at two body lengths per second until the oxygen concentration reached approximately 2 mg/l. Females reacted differently depending on LDH phenotype. Again there

were sex based differences that may not have been evident if males and females were not considered separately.

Water velocities of 1.5 body lengths per second and 2 mg/l dissolved oxygen represent a severe test of the fishes ability to perform. Under those conditions most of the fish fatigued in 30 minutes or less. The remaining fish had swimming times spread over a long period. In any group of fish it is not unusual to observe fish with swimming times much longer than the majority of the group. This was noted in the stamina tunnel experiment of this study where one phenotype I fish was able to swim for 8 hours compared to a mean of 15 minutes for the remainder of the group. The performance index of Thomas et al. (1964) takes into account the variation in swimming times. Klar (1973) noted the same kind of variation in testing stamina and used percentages of fish fatigued in a given time period to compare treatment groups. Therefore, it is probably best to handle swimming times as a frequency distribution or percentage to compare treatment groups.

Blood pO_2 and pH

Stevens and Randall (1967) reported the following changes in blood gas concentrations during swimming in rainbow trout. Maximum swimming speed was approximately two body lengths per second and acclimation temperature was 4 to 8 C. Mean preexercise ventral aortic pO_2 was 19 ± 1.4 mmHg and pCO_2 was 5.7 ± 1.5 mmHg. At the end of 5 minutes at the maximum swimming level ventral aortic pO_2 was 16 ± 1.2 mmHg and pCO_2 was 8.0 ± 1.2 mmHg. At the end of 90 minutes post exercise mean ventral aortic pO_2 was 19 ± 1.2 mmHg and pCO_2 was 9.6 ± 3.3 mmHg. Mean dorsal aortic pCO_2 before exercise was 2.3 mmHg and did not change more than 1 mmHg during exercise.

Holeton and Randall (1967) reported the following effects of hypoxia upon the blood gases of rainbow trout. Experiments were carried out at 15 C. Blood pO_2 in the ventral aorta was 30 to 35 mmHg in resting fish and fell to 6 mmHg when the fish were exposed to hypoxia. Ventral aortic pCO_2 was 2.5 mmHg when the fish was in aerated water and increased to 4.5 to 5 mmHg in fish exposed to hypoxia. The blood pH was 7.7 in fish in aerated water and fell to 7.4 when fish were exposed to hypoxia.

Blood circulation in trout proceeds from the heart to the ventral aorta, through the gills to the body and back to the heart. Blood samples taken from fish in this study were from the heart and should be comparable to samples from the ventral aorta. Mean blood pO_2 and pCO_2 at fatigue in fish forced to swim at two body lengths per second at saturated oxygen were comparable to those reported by Stevens and Randall (1967). Mean blood pO_2 at fatigue in fish forced to swim at two body lengths per second with declining oxygen was comparable to that reported by Holeton and Randall (1967) for fish exposed to hypoxia. However, in this experiment blood pO_2 for females (6.2 mmHg) was significantly higher than for males (3.6 mmHg). Blood within the heart is venous blood after it has passed through nearly the entire circulatory system from the gills. Measurement at this point should reflect the relative ability of tissues to extract oxygen from the blood under conditions of limited availability. The difference in pO_2 between males and females may indicate that males were extracting more of the available oxygen from the blood, assuming equal saturation at the gills. When the fish were forced to swim at 1.5 body lengths per second at a constant 2 mg/l dissolved oxygen, females again had a significantly higher blood pO_2 at fatigue than males. Mean blood pO_2 for females at

two body lengths per second and declining oxygen (6.2 mmHg) was very close to that for females at 1.5 body lengths per second and 2 mg/l dissolved oxygen (6.7 mmHg). However, mean blood pO_2 for males at two body lengths per second and declining oxygen (3.6 mmHg) was much lower than for males at 1.5 body lengths per second and 2 mg/l oxygen (6.04 mmHg). Onset of low oxygen stress at 2 mg/l was abrupt, as opposed to gradual under declining oxygen. Thus the males may not have been able to rapidly adjust to the same level of pO_2. In addition, phenotype II fish (when considered as a group) had a significantly lower blood pO_2 than phenotypes I or III. This is probably the result of the longer and more consistent swimming times in the 11 to 50 minute time range for phenotype II fish.

Blood pH values for fish fatigued at two body lengths per second at saturated oxygen and declining oxygen were comparable to those by Holeton and Randall (1967) for fish exposed to hypoxia. However, data by Holeton and Randall (1967) were taken at 15 C whereas data taken in these experiments were at 8 and 10 C. In general fish blood pH increases as temperature is lowered (Rahn 1967). This would accentuate the lowering of the blood pH recorded here. The lower blood pH may have been the result of the swimming activity of the fish and suggests that the fish were physiologically fatigued when removed from the water current.

Blood pH values for fish fatigued at 1.5 body lengths per second and 2 mg/l oxygen were slightly higher than those for fish fatigued at two body lengths per second and declining oxygen. Onset of oxygen stress was abrupt and swimming times were, in general, short in this experiment. Blood lactate increases measured in this

experiment were not drastic. This indicates that the buffering capacity of the blood was not exceeded by increases in blood lactate and blood pH remained within tolerable limits.

Oxygen consumption

Dickson and Kramer (1971) reported active rates of oxygen uptake (no sex separation) of 468 mg O_2/kg/hr for hatchery rainbow trout and 492 mg O_2/kg/hr for wild rainbow trout at 10 C. They further reported that the active rate for males (462 mg O_2/kg/hr) was significantly higher than the active rate for females (408 mg O_2/kg/hr). The active rate is defined as the maximum rate of oxygen uptake. Oxygen consumption rates recorded in this study at 8 mg/l dissolved oxygen can be considered the active rate of oxygen uptake since swimming speed was high enough to induce the active rate. Although no differences were observed among phenotypes in oxygen consumption at 8 through 3 mg/l dissolved oxygen, some important differences were observed between sexes. Active rates of oxygen consumption for males (510 ± 59 mg O_2/kg/hr) and females (366 ± 49 mg O_2/kg/hr) were similar to those reported by Dickson and Kramer (1971).

Brett (1962) reported that oxygen availability influences the rate of oxygen uptake. A lowered oxygen availability depresses the rate of oxygen uptake, probably due to a reduced hemoglobin saturation at lower ambient oxygen concentrations. The experimental design of this study provided an opportunity to observe that dependence and to compare males and females. Rates of oxygen uptake were calculated for each fish at selected oxygen concentrations as the oxygen was reduced by the fish. Males, as expected, were significantly higher than females in oxygen uptake at 8 mg/l dissolved oxygen (the active rate) and maintained a

significantly higher rate of oxygen uptake to 3 mg/l dissolved oxygen. However, the actual difference between males and females became less as the oxygen concentration declined. The regression lines (Figure 1) are converging at low oxygen concentrations. Both regression lines pass very near the origin. The implications are that one or both sexes are making adjustments in oxygen demand or oxygen consumption rate to maintain swimming activity as dissolved oxygen declines. Females may be able to adjust consumption rate upward by increasing ventilatory activity, blood flow through the gills or by increasing blood hemoglobin content under oxygen stress. Males may decrease oxygen demand by shunting blood from non-vital tissues and thus supply available oxygen to vital tissues for function during swimming under oxygen stress. Blood pO_2 at fatigue for males was significantly lower than for females. Males may be extracting more of the available oxygen from the blood during swimming under oxygen stress. It is clear that males not only differ from females in oxygen consumption rates but the response in consumption rate to lowered oxygen concentration differs between the sexes.

Blood lactate

Numerous reports (Black et al. 1966; Stevens and Black 1966; Black 1957; Hammond and Hickman 1966; Black et al. 1959; Black et al. 1962) have dealt with the effects of various levels of exercise on blood lactate concentrations in rainbow trout. The general pattern reported was for blood lactate to increase immediately with the onset of exercise, continue to increase for 2 to 6 hours post exercise, level off for a short period and gradually return to preexercise levels by 24 hours. Preexercise or resting blood lactate values reported in the above publications

range from 4.0 to 16.0 mg/100 ml and most fell in the 7 to 9 mg/100 ml range. This is remarkable agreement considering the potential for differences in sampling techniques, fish populations and handling. Reported increases in blood lactate concentration after 15 minutes of strenuous swimming were of the order of 6 to 10 fold higher than resting levels. Stevens and Black (1966) reported that muscle lactate levels increased 2.3 fold with 1 minute of exercise, 2.9 fold with 2 minutes of exercise and 3.5 fold with 5 minutes of exercise (all increases over resting levels) and remained at that level after 60 minutes of rest. At the same time blood lactate increased 2 fold at 1 minute of exercise, 3.4 fold at 2 minutes, 7.8 fold at 5 minutes of exercise and 20.6 fold after 60 minutes of rest (all increases over resting levels). Black et al. (1962) reported roughly the same changes as Stevens and Black (1966). Hammond and Hickman (1966) reported that conditioned trout exhibited higher muscle and plasma lactate levels when fatigued and a more rapid removal of lactate from muscle and plasma during recovery when compared to unconditioned trout. Blood samples from the studies cited were venous samples generally taken from the heart. Driedzic and Kiceniuk (1976), using dorsal aortic cannulation, reported no increase in blood lactate during sustained swimming but a 4 to 5 fold increase 1 minute after fatigue. However they used only female fish in the 40 to 53 cm size range and conditioned the fish for 2 weeks before testing.

The dynamics of the blood lactate cycle in rainbow trout are not at all clear. Blood lactate concentration, which is the easiest parameter to measure, and thus the most common measurement, reflects the rate of production of lactate in the tissues, the rate of diffusion into the

bloodstream and the rate of utilization by the tissues. It is commonly held that lactate is produced in high quantities in white muscle, diffuses into the bloodstream and is converted to pyruvate in the liver. White muscle has a rather poor blood supply and few mitochondria and large increases in lactate in white muscle have been reported. As Hammond and Hickman (1966) have reported, the rate of diffusion from muscle can be altered by physical conditioning. The fish may be able to control the diffusion rate to some extent by altering the blood flow through the muscle during periods of high activity and recovery. This could account for the large increases during the first few minutes of recovery. Little is known about the lactate contribution to the blood by other tissues such as heart, liver, kidney, etc. under conditions of severe stress. The relative importance of the sites of lactate utilization is not well understood either. Bilinski and Jonas (1972), using ^{14}C labeled lactate, reported that liver tissue had the highest activity in the decarboxylation of pyruvate (which came from the labeled lactate) to acetyl Co A, followed by gill, red muscle, heart and white muscle in that order. However the gill tissue had the highest activity in the further decarboxylation of acetyl Co A in the Krebs cycle, followed by kidney, red muscle, liver, heart and white muscle. They suggested that lactate may be an important energy source for the gill tissue and that much of the lactate is used by the gill and kidney as an energy source, whereas lactate utilized by the liver to produce acetyl Co A is directed towards biosynthetic pathways. The amount of lactate directed into the glucogenic pathway by the liver was not measured and may be substantial. However, even with all the unanswered questions, it appears from the literature reported that a group of fish in

the same relative condition and handled in the same manner should give the same blood lactate results under the same treatment conditions.

Blood lactate data for phenotype I at 1.5 body lengths per second and 2 mg/l oxygen followed the expected pattern for rainbow trout. Blood lactate increased with swimming time, leveled off at 8 hours post fatigue and returned to normal at 21 hours post fatigue. There was an apparent difference between phenotype I males and females at the fatigue point in that females had higher concentrations (Figure 3). Black et al. (1962) found that females had a higher blood lactate concentration than males during swimming and recovery. However, Stevens and Black (1966) did not observe any difference between males and females. Perhaps season or condition influences the response of males and females in blood lactate.

Electrophoretic Analysis of Cutthroat Trout

Cutthroat trout sampled

With the exception of One Mile Creek, Bear Lake and Auburn Hatchery, the cutthroat trout populations sampled were reasonably pure native strains. Goshute Creek, Pine Creek, Trout Creek and Birch Creek (Juab County, Utah) fish are referred to as Snake Valley or Mt. Wheeler cutthroat trout (*Salmo clarki* subsp.) and are located in Western Utah and Eastern Nevada in the Deep Creek Mountains. Water Canyon Creek, Asay Creek and Birch Creek (Beaver County, Utah) fish are the Utah or Bonneville cutthroat trout (*Salmo clarki utah*), located in Southern Utah. Little West Fork Creek cutthroat trout are the Colorado River cutthroat trout (*Salmo clarki pleuriticus*) located in the Uintah Mountains, Utah. Forest Canyon Creek fish are the Greenback cutthroat

trout (Salmo clarki stomias) found in Rocky Mountain National Park, Colorado. Yellowstone Lake fish are the Yellowstone cutthroat trout (Salmo clarki lewisi) located in Yellowstone National Park, Wyoming. Auburn Hatchery fish were reported to be the Snake River strain of cutthroat trout. Many of the populations were found in very small headwater streams that contained no rainbow trout or possessed natural barriers to rainbow trout access. Although rainbow trout influence cannot be ruled out, such influence was probably minimal in most cases.

Utter and Hodgins (1972) and Allendorf (1975) have reported extensive variation in rainbow trout PGM, AGPDH, TO and liver LDH. With the exception of Bear Lake, the only variation observed in the cutthroat trout sampled in those four systems was the presence of two TO heterozygotes in Trout Creek cutthroat trout. This lack of variation in those systems could be due to natural selection, inbreeding or genetic drift. Many of the cutthroat populations sampled were restricted to a small area of the stream where the potential for inbreeding was increased.

One Mile Creek was suspected of containing the Snake River strain of cutthroat trout. Rainbow trout and rainbow-cutthroat hybrids were encountered during sampling. Rainbow-cutthroat hybrids were eliminated and cutthroat trout were selected for analysis on the basis of external appearance. In the cutthroat trout selected from One Mile Creek, no variation was observed in PGM, AGPDH, TO or liver LDH.

Bear Lake cutthroat trout

Bear Lake, on the Utah-Idaho border, is approximately 30 kilometers in length and 6-12 kilometers in width with an average depth of 30.5 meters. At various times in geologic history Bear Lake was connected to Lake Bonneville and the Snake River. Bear Lake was isolated from Lake Bonneville and the Snake River, perhaps as long as 20,000 years

ago. Consequently cutthroat trout in the Bonneville and Snake River basins had access to the lake in the past.

Historically, there was a population of cutthroat trout in Bear Lake. Fishing pressure caused a decline in the original cutthroat trout populations and rainbow trout and Yellowstone cutthroat trout were introduced into the lake. Since there are only three tributary streams of any size to Bear Lake, spawning habitat for trout is limited and cutthroat trout have not recovered. In 1973 the Utah Division of Wildlife Resources began a project to restore cutthroat trout populations in Bear Lake. A trap was constructed on Swan Creek, one of the major tributaries, and ripe fish were collected, spawned artificially, and the progeny reared in a hatchery. Bear Lake samples analyzed in this study were from the progeny of the spawning operations.

Rainbow trout and rainbow-cutthroat hybrids were found in the trap along with cutthroat trout (Bryce Nielson, personal communication). Fish that were trapped were visually classified and only those that had typical cutthroat trout markings were selected for spawning. However, the first years spawning included some fish that were classified as hybrids the following year (Bear Lake 1974 sample of this study) and eliminated from spawning at that time (Bear Lake 1975 sample) (Bryce Nielson, personal communication). Approximately 400 to 500 fish were used as brood stock each year. The fish were marked and used in subsequent years spawning. Eggs were maintained as separate lots until the fry stage was reached. At that time all fry were mixed in a hatchery raceway. As the fish approached fingerling stage they were graded and separated by size (Bryce Nielson, personal communication). Samples were collected for electrophoretic analysis from the larger size lots in March 1974 and 1975 and July 1976.

In the Bear Lake (1974) sample, variation was observed in PGM, TO and liver LDH. The variant PGM allele was very infrequent with one BC heterozygote observed in 50 samples. No PGM variants were observed in the 1975 or 1976 samples. Variants in liver TO were observed in all three sampling years. Allelic frequencies for TO changed significantly between 1974 and 1975 and between 1975 and 1976. However, the observed phenotype frequencies were not significantly different from the expected frequencies for a population in the Hardy-Weinberg equilibrium in any one year. This indicates that a random mix of available spawners was obtained but a different spawning population (in TO allele frequency) was used each year. The elimination of many suspected hybrids between 1974 and 1975 could account for the change in TO allele frequency between those two years. As mentioned, the primary fish trap was on Swan Creek. A second fish trap was operated occasionally on St. Charles Creek a few miles north of Swan Creek. Fish trapped on St. Charles Creek were transported to the trap on Swan Creek and included in the spawning operation. The cutthroat trout migrating up St. Charles Creek may have a different TO allele frequency than those at Swan Creek. Irregular inclusion of St. Charles Creek fish may account for the frequency changes between 1975 and 1976. The progeny from the spawning operations were returned to the lake and will eventually return to the trap to be spawned. With three lots of progeny in the lake at the present time, with known differences in TO allele frequency it will be difficult to stabilize the frequencies of TO alleles in Bear Lake cutthroat trout.

Variants were observed in four out of nine protein systems in Bear Lake cutthroat trout compared to one to three out of nine systems in other cutthroat trout sampled. This includes variation at the liver

LDH B^2 locus in Bear Lake fish which is usually only found in rainbow trout. The additional variation in Bear Lake cutthroat trout could be due to rainbow trout influence on the population.

Snake River cutthroat trout

A sample of cutthroat trout reported to be the Snake River strain was taken from the Auburn Hatchery, Wyoming. This stock of fish had been held in the hatchery for several generations prior to sampling. The degree of artificial selection in that stock was unknown. No variation was observed in PGM, AGPDH, TO or liver LDH in the Auburn population.

Aspartate aminotransferase

Allendorf and Utter (1976) reported that the AAT A allele was the most common allele in anadromous cutthroat trout. It appears that the A' allele was the most common allele in the inland cutthroat trout sampled in this study. The A allele was not observed in Goshute Creek, Water Canyon Creek, Pine Creek, One Mile Creek, Little West Fork Creek or Forest Canyon Creek cutthroat trout (Figure 11).

Snake Valley cutthroat trout

Behnke (1976) reported the current status of the Goshute Creek, Pine Creek and Trout Creek cutthroat trout. The Snake Valley is a part of the Bonneville Basin located in Western Utah and Eastern Nevada. A pure cutthroat trout population discovered in Pine Creek, Mt. Wheeler, Nevada in 1953 was thought to be a native cutthroat trout of the Snake Valley region. The Pine Creek cutthroat trout was differentiated from the cutthroat trout of the main Bonneville Basin (*Salmo clarki utah*)

and all other cutthroat trout in spotting pattern, the number of basibranchial teeth and gillrakers. In 1960, 54 cutthroat trout were transplanted from Pine Creek into Goshute Creek, and a population was established in Goshute Creek. In 1974 and 1975 two virtually pure populations were discovered in Trout Creek and neighboring Birch Creek in Juab County, Utah (Behnke 1976).

Goshute Creek was sampled in 1973 for electrophoretic analysis. Sampling was extended to Trout Creek in 1974 and Birch Creek in 1976. Goshute Creek was sampled again in 1976 along with the parent stock in Pine Creek.

Variation in muscle A group LDH was observed in every population of Snake Valley cutthroat trout sampled to date. In rainbow trout, two codominant fixed loci designated A^1-A^2 produce a slow migrating five isozyme LDH pattern in skeletal muscle. All other cutthroat trout skeletal muscle examined produced the typical A group pattern with isozyme migration rate identical to rainbow trout isozymes. This typical rainbow trout A^1-A^2 pattern was present in low frequency in Goshute Creek, Pine Creek and Birch Creek cutthroat trout and in high frequency in Trout Creek cutthroat trout. Variant LDH A group patterns are similar in Trout Creek and Birch Creek cutthroat trout. Likewise, variant LDH patterns are similar in Goshute Creek and Pine Creek cutthroat trout, but different from Trout Creek and Birch Creek patterns. In Goshute Creek and Pine Creek cutthroat trout there appears to be a slower migrating variant of A^1 ($A^{1'}$) which gives a five isozyme pattern. The remaining variation could be explained by a null allele at A^2, with only A^1 producing an active homotetramer in the A group and the other bands present being heterotetramers between A^1 and B^1 and/or $B^{2'}$. As an

alternative there could be a variant of A^2 which cannot be distinguished from A^1 or B^1.

A rather unusual variation for trout muscle LDH was present in Trout and Birch Creek cutthroat trout. In the muscle group, two slow bands are separated from two fast bands by a distinct gap. The slowest band in this group of four is faster than A^1, while the fastest band is in the position of A^2. Also, the B group appears different in fish with variants in the A group. In the variant fish, a heavy dark band appears in the center of the muscle LDH B group. Electrophoretic analysis of liver and eye B group indicated that the variant fish possessed typical B group subunits (i.e. B^1 and $B^{2'}$). Consequently, there could be a variant of the A group that migrates within the muscle B group in the Trout Creek and Birch Creek cutthroat trout. The distinct gap could be the result of non-random recombination of subunits into heterotetramers. Also the lack of an A^1 in the normal position seems to indicate an additional variant of A^1.

In summary, an unusually complex variation in Snake Valley cutthroat trout muscle LDH was observed, which indicates the presence of several variant alleles.

Trout in the Snake Valley region have been positively isolated from the Bonneville Basin for about 8,000 years. But, as Behnke (1976) suggested, reproductive isolation may have occurred long before that time. Glacial Lake Bonneville was noted for large fluctuations in lake levels during its history. Perhaps during an early inter-glacial dessication, cutthroat trout were isolated in the Snake Valley region for a few thousand years. When lake levels rose again the cutthroat trout may have maintained reproductive isolation due to their parent

stream homing behavior. This would have allowed sufficient time for considerable genetic divergence. In any regard, it appears that a unique evolutionary event, or series of events occurred in the Snake Valley cutthroat trout muscle LDH. Two hypotheses may account for the presence of variants in the A group LDH in the Snake Valley cutthroat trout. The first is that mutations occurred in the A group LDH that were selectively neutral and because of the isolation increased in frequency by chance alone. The second is that mutants in A group LDH occurred that were adaptive to the fish in their isolated habitat and increased in frequency by natural selection. The latter hypothesis could be tested in a manner similar to the test of the B^2 variant in rainbow trout.

SUMMARY

Rainbow trout LDH $B^{2'}$ $B^{2'}$, $B^{2'}$ $B^{2''}$ and $B^{2''}$ $B^{2''}$ phenotypes were tested under a variety of conditions for swimming endurance, blood pH, pO_2 and lactate and oxygen consumption rates. The $B^{2''}$ $B^{2''}$ phenotype females had lower swimming endurance than $B^{2'}$ $B^{2'}$ phenotype females at two body lengths per second and declining oxygen. The $B^{2''}$ $B^{2''}$ phenotype fish had lower swimming endurance at 1.5 body lengths per second and 2 mg/l oxygen than $B^{2'}$ $B^{2''}$ or $B^{2'}$ $B^{2'}$ phenotypes. Blood lactate increased rapidly during the first 15 minutes of exposure to 2 mg/l oxygen for the $B^{2'}$ $B^{2'}$ phenotypes, then leveled off through 60 minutes exposure. Blood lactate for $B^{2''}$ $B^{2''}$ phenotypes continued to increase during 60 minutes exposure to 2 mg/l oxygen. The $B^{2''}$ $B^{2''}$ phenotypes had higher mortality on exposure to 2 mg/l oxygen than $B^{2'}$ $B^{2'}$ phenotypes.

Blood pO_2 was higher for female than male fish fatigued at two body lengths per second and declining oxygen and 1.5 body lengths per second and 2 mg/l oxygen. Oxygen consumption rates were higher for males than females at oxygen concentrations of 8 mg/l through 3 mg/l. The depression in oxygen consumption at low oxygen concentration was greater for males than for females.

Unique muscle LDH A group electrophoretic variation was observed in Snake Valley cutthroat trout which indicated the presence of several variant alleles. Variation was observed in cutthroat trout muscle AAT which would fit a genetic model of two loci and three alleles. No variation was observed in AGPDH, IDH, eye LDH or MDH in cutthroat trout.

Further investigations are needed on the characteristics of the LDH $B^{2''}$ allele in rainbow trout with respect to the influence of maturity on the $B_4^{2''}$ isozyme function. Also the characteristics of the $B^{2'}$ $B^{2''}$ heterozygotes need to be further defined since they may possess the advantages of both homozygotes. The genetic model for the LDH A group variation in Snake Valley cutthroat trout should be determined by test crosses. Hypotheses on the adaptive significance of the LDH A group variation can then be formulated and tested in a manner similar to the tests of the LDH $B^{2''}$ allele in rainbow trout.

CONCLUSIONS

1. Adult rainbow trout of the LDH $B^{2''}$ $B^{2''}$ phenotype had lower swimming endurance under limited oxygen availability than adult trout with the $B^{2'}$ $B^{2''}$ or $B^{2'}$ $B^{2'}$ phenotypes.

2. Male rainbow trout had higher rates of oxygen consumption than female rainbow trout with dissolved oxygen from 3 mg/l to saturation.

3. The depression in the rate of oxygen consumption for rainbow trout at low dissolved oxygen concentrations was greater for males than for females.

4. Mature female rainbow trout had higher blood pO_2 at fatigue under low oxygen concentrations than mature male rainbow trout.

5. The blood lactate concentration in Beity strain of rainbow trout under low dissolved oxygen was dependent on LDH B^2 phenotype.

6. Variation in aspartate aminotransferase from cutthroat trout could be explained by two loci and three alleles.

7. Unique muscle A group lactate dehydrogenase variants occurred in cutthroat trout from the Snake Valley region of Utah and Nevada.

8. Tetrazolium oxidase allele frequencies of the progeny from the Bear Lake cutthroat trout changed in three consecutive years.

9. Very little genetic variation occurred in phosphoglucomutase, alphaglycerophosphate dehydrogenase and liver and eye lactate dehydrogenase in cutthroat trout.

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Doctor of Philosophy

Dissertation: Adaptive Significance of Lactate Dehydrogenase B^2 Isozymes in Rainbow Trout, Salmo gairdneri and A Biochemical Genetic Comparison of Cutthroat Trout (Salmo clarki) Populations

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