AN ABSTRACT OF THE THESIS OF

Joseph Michael Redding for the degree of Master of Science

in Fisheries and Wildlife presented on July 22, 1977

Title: THE ADAPTIVE SIGNIFICANCE OF CERTAIN ENZYME POLYMORPHISMS IN STEELHEAD TROUT (SALMO GAIRDNERI)

Abstract approved: Redacted for Privacy

Carl B. Schreck

Mean weights, lengths, and condition factors of three isozyme phenotypes of lactate dehydrogenase (LDH) enzyme differed significantly for experimentally reared winter steelhead trout (Salmo gairdneri) fry. Time of emergence from the gravel was unrelated to LDH phenotype. Relative mortality of the phenotypes between eyed-egg stage and emergence was unaffected by different sub-gravel conditions of temperature and dissolved oxygen. Differential tolerance to acute challenges of high temperature and low dissolved oxygen was observed between phenotypes of isocitrate dehydrogenase (IDH) enzyme and LDH in juvenile trout. Parental effects may have biased the results for LDH. Differences between IDH phenotypes may be related to intrinsic properties of variant isozymes.

The Adaptive Significance of Certain Enzyme Polymorphisms in Steelhead Trout (*Salmo gairdneri*)

by

Joseph Michael Redding

A THESIS

submitted to

Oregon State University

in partial fulfillment of
the requirements for the
degree of

Master of Science

Completed July 1977

Commencement June 1978

APPROVED:

Redacted for Privacy

Assistant Professor of Fisheries and Wildlife
in charge of major

Redacted for Privacy

Head of Department of Fisheries and Wildlife

Redacted for Privacy

Dean of Graduate School

Date thesis is presented July 22, 1977

Typed by Opal Grossnicklaus for Joseph Michael Redding

ACKNOWLEDGEMENTS

I thank my major professor, Dr. Carl Schreck, for his guidance and encouragement throughout this study. I sincerely appreciate the critical contributions of Drs. Charles Warren, Dick Ewing, Jack McIntyre, and Norbert Hartman.

Special thanks are due to Mr. Ed Labiske of the Marion Forks Salmon Hatchery for providing fish used in this study. The cooperation of the Oregon Department of Fish and Wildlife personnel is also appreciated.

Finally, and most importantly, I thank my parents and family for their inestimably valuable and unflagging support of my endeavors.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

TO PERCEIVE THE RELATIVITY OF TRUTH ITSELF

OUR EYES WOULD HAVE TO POSSESS AS MANY FACETS

AS THOSE OF AN INSECT

LOREN EISELEY

THE ADAPTIVE SIGNIFICANCE OF CERTAIN ENZYME POLYMORPHISMS IN STEELHEAD TROUT (SALMO GAIRDNERI)

INTRODUCTION

The description and quantification of enzyme polymorphisms (isozymes) in fish populations has been the goal of much research during the last decade. The accumulation of such information has been facilitated greatly by advances in electrophoretic techniques (Utter et al. 1974). Having acquired some knowledge about the extent of enzyme polymorphisms and their genetic bases, it is necessary to question their functional and adaptive significance.

Stocks of steelhead trout (Salmo gairdneri) in the Pacific Northwest region exhibit a conspicuous dichotomy in the frequencies of alleles that code for polymorphisms of lactate dehydrogenase (LDH) and isocitrate dehydrogenase (IDH) (Allendorf 1975). Stocks inhabiting coastal areas differ from stocks that originate in interior regions of the Columbia and Snake River systems. This study attempts to evaluate the significance of these and other enzyme polymorphisms in the context of enviornmental adaptation.

LDH catalyzes the interconversion of pyruvate and lactate and serves a critical regulatory function in the balance between aerobic respiration and anaerobic glycolysis. Many species of fish possess LDH polymorphisms (Markert and Faulhaber 1965). The molecular

and genetic bases of LDH isozymes in fish are well understood (Markert 1968). Internal and external factors can modulate the relative activity of LDH isozymes. Studies have demonstrated temperature-dependent induction of LDH isozymes during warm and cold acclimation in a diverse array of vertebrate poikilotherms (Aleksiuk 1971; Hochachka 1965; Kunnemann 1973; Tsugawa 1976; Tsukuda 1975; Hochachka and Somero 1968). Photoperiod may affect LDH isozyme activity in fish (Kent and Hart 1976). Kinetic differences between isozymes of LDH with respect to temperature optima and substrate affinities have been shown to exist in the fathead minnow (Merritt 1972), sockeye salmon (Utter et al. 1974) and rainbow trout (Somero and Hochachka 1969; Hochachka and Somero 1968). Tsuyuki and Williscroft (1973) noted differences in the pH optima of LDH isozymes in rainbow trout and speculated that swimming endurance may be related to LDH phenotype. Geographic variation in the frequencies of alleles that code for LDH polymorphisms have been correlated to environmental temperature (Merritt 1972; Mitton and Koehn 1975). Johnson (1971) demonstrated differential thermal tolerance between LDH phenotypes for a species which exhibits a latitudinal cline in the frequencies of LDH alleles.

Similar observations have been recorded for other polymorphic enzymes in trout: isocitrate dehydrogenase (IDH) (Moon and Hochachka 1972), citrate synthase (Hochachka and Lewis 1970),

acetylcholinesterase (Baldwin and Hochachka 1970), and alkaline phosphatase (Whitmore and Goldberg 1972).

The purpose of my study was to investigate the adaptive significance of various enzyme polymorphisms in steelhead trout, particularly those of LDH, in relation to environmental temperature and dissolved oxygen. Specifically, my objectives were to test the hypotheses that 1) LDH isozyme phenotypes in steelhead trout have differential survival and growth performances during the period between eyed-egg stage and emergence for different regimes of subgravel temperature and dissolved oxygen; and 2) isozyme phenotypes of LDH, IDH, and several other enzyme systems possess different tolerances to acute high temperature and low dissolved oxygen challenges in juvenile trout.

MATERIALS AND METHODS

Experimental Population

The North Santiam River (Oregon) winter steelhead trout stock possesses three electrophoretic variants of LDH. Following the nomenclature of Wright et al. (1975) these variants were designated as the B'B', B'B, and BB phenotypes of the B_1 locus in both liver and eye tissue. These phenotypes are known to exhibit simple Mendelian inheritance patterns (Utter et al. 1973). Because I wanted to test the relative performances of the three phenotypes and they are not present in equal proportions in the wild, the experimental fish had to be selectively bred so that each phenotype would have approximately equal representation in the test groups. Steelhead trout in reproductive condition were captured during their upstream migration in a trap at Minto Pond on the North Santiam River on 7 May 1976. The sperm or eggs from six males and six females were held on ice while the LDH phenotype was determined for each fish by electrophoretic analysis of liver tissue homogenates. Gametes were then selectively crossed so that a single male fertilized the eggs of a single female. The six families resulting from these crosses were mixed randomly to produce a filial population. The fertilized eggs were held in Heath® incubators until they reached the eyed-egg stage at which time they were shocked to identify viable eggs and

divided into two groups. One group was used for the sub-gravel incubation experiment. The other group was kept in the incubators until the fish absorbed their yolk sacs and then placed in circular tanks. Constant conditions in the circular tanks were: temperature, 12° C; hardness ($CaCO_3$), 99 mg/l; pH, 7.3; conductivity, 241 μmho; dissolved oxygen, 8-12 mg/l.

Electrophoresis

Electrophoretic analyses were conducted according to the methods described by Utter et al. (1974). LDH phenotype was determined from eye tissue homogenates for trout fry and from liver tissue homogenates for juveniles. In addition, liver tissue IDH and tetrazolium oxidase (TO) and white muscle tissue malate dehydrogenase (MDH) and alpha-glycerophosphate dehydrogenase (AGPDH) phenotypes were determined for juvenile trout. Phenotype designations for these enzyme systems followed that of Allendorf (1973) and Utter et al. (1973). For IDH I recognized six phenotypes--AA, A^1A, A^1A^1, AA^3, A^1A^3, A^3A^3; for TO three phenotypes--AA, AB, AB; for MDH three phenotypes--B'B', B'B, BB; and for AGPDH three phenotypes--AA, AB, BB. The enzyme systems of malic enzyme, glucose-6-phosphate dehydrogenase, esterase, phosphoglucomutase, and phosphohexoisomerase were unsuitable for analysis due to insufficient variability or inadequate electrophoretic resolution.

EXPERIMENTAL DESIGN AND RESULTS

Sub-gravel Incubation Experiment

Twenty-two days after fertilization 400 viable eyed-eggs from the experimental population were buried beneath 25 cm of gravel ($\geq$ 1.27 cm diameter) in each of 12 flow-through incubation boxes (23 × 23 × 61 cm). The eggs were subjected to one of four different treatment regimes of temperature and dissolved oxygen (D. O.). Roman numerals indicate treatment designations:

		D. O. (mg/l)	
		5	9-10
Temperature (° C)	12	III	IV
	16	I	II

Dissolved oxygen was maintained at a constant concentration by a system in which nitrogen gas was diffused through inflowing water (Eddy 1971). Water velocity through the boxes was constant at 50 cm/h. Emergent fry that had completely absorbed their yolk sac were collected daily. Individual fish were weighed and measured immediately after collection and then frozen for a period of 1-4 wk before electrophoretic analysis.

Treatment effects did not produce any significant deviations in the relative proportion of surviving fish for each LDH phenotype

(Table 1). Confidence intervals ($p < 0.05$) were calculated for the observed proportions ($\hat{p}$) by the formula, $\hat{p} \pm 1.96 \sqrt{\hat{p}(1-\hat{p})/n}$, where n equaled the sample size. Because of variation in the number of eggs contributed by each female the exact proportion of each phenotype was unknown prior to burying the eggs; thus, it was impossible to estimate the relative survival rates between phenotypes within a single treatment. There were no significant differences between the replicates within a treatment group.

Table 1. Proportion of three phenotypes of LDH and the frequency of the B allele for emergent steelhead trout fry and a control sample from a circular tank (± = 95% confidence interval).

Treatment	T (° C)	D.O. (mg/l)	n	phenotype BB	phenotype B'B	phenotype B'B'	B allele
I	16	5.0	660	28	28	44	0.42±0.03
II	16	9.0-10.0	258	31	31	38	0.47±0.04
III	12	5.0	587	30	29	40	0.45±0.03
IV	12	9.0-10.0	627	33	25	42	0.46±0.03
control	12	8.0	174	25	27	48	0.39±0.05

Mean time of emergence, wet weight (W), fork length (L), and condition factor (W/L^3) for each LDH phenotype, replicate, and treatment were analyzed with a three-way analysis of variance (ANOVA) of means (Snedecor and Cochran 1967). There were no significant or consistent differences between the mean times of

emergence for LDH phenotypes within replicates. There were highly significant ($p < 0.01$) differences between treatments and replicates within treatments. I cannot explain the differences between replicates except to attribute them to unknown variations in flow or incident light. The results for time of emergence were pooled for each treatment group:

Temperature (° C)	D. O. (mg/l) 5	D. O. (mg/l) 9-10
12	59.9	55.1
16	49.8	46.6

days to emergence after fertilization

The fry that were reared at 16° C, 9-10 mg/l D. O. had the most rapid development rate while those at 12° C, 5 mg/l D. O. had the slowest rate. Fish reared at 16° C, 5 mg/l D. O. and 12° C, 9-10 mg/l D. O. had intermediate times of emergence. These results are consistent with my expectations based on bioenergetic considerations (Warren 1971).

For all treatment groups the BB phenotype of LDH weighed more than the B'B' phenotype. Both homozygous forms weighed more than the heterozygote, B'B (Fig. 1). The ANOVA for mean weight of

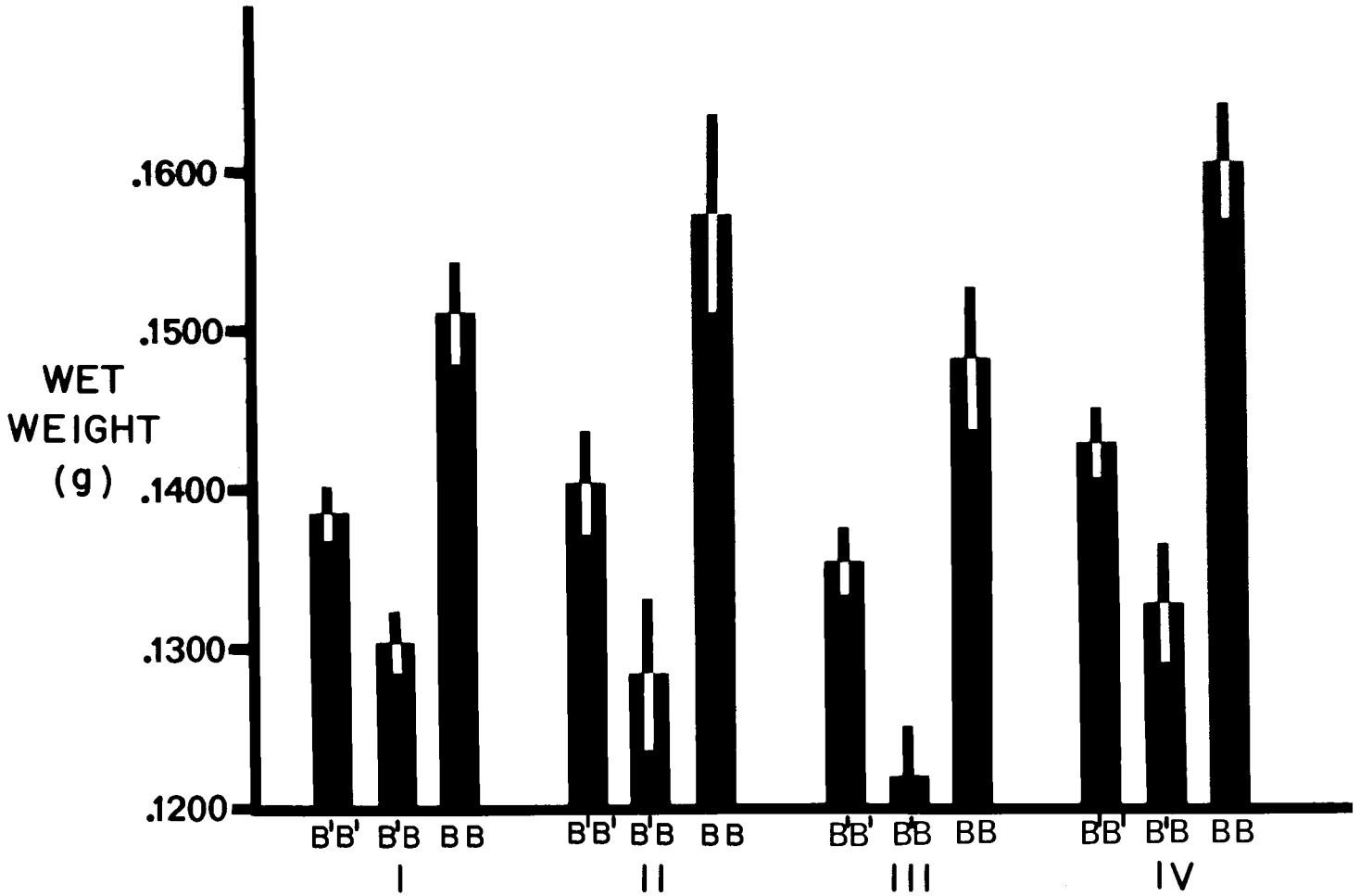

Figure 1. Mean wet weights and confidence intervals ($p<0.05$; vertical bars) of the three phenotypes of LDH in steelhead trout for each treatment after incubation under gravel at various thermal and dissolved oxygen regimes. I = 16° C, 5 mg/l D. O.; II = 16° C, 9-10 mg/l D. O.; III = 12° C, 5 mg/l D. O.; IV = 12° C, 9-10 mg/l D. O.

emergent fry showed highly significant differences for both treatments and LDH phenotypes. Replications within treatments were homogenous. For a given phenotype the fry reared at 12° C, 9-10 mg/l D. O. tended to weigh more at emergence than the fry in either of the 16° C groups. The differences between treatment groups are consistent with the findings of others (Warren 1971).

Differences in length and condition factor between LDH phenotypes were highly significant. Differences between treatments were significant ($p < 0.10$). Replications within treatments were statistically homogenous. The BB phenotype had the greatest mean length and condition factor in all treatments (Fig. 2). B'B was the shortest and had the lowest condition factor except in the 16° C, 5 mg/l D. O. treatment group in which the B'B' form had the lowest condition factor.

Differences in weight, length, and condition factor between LDH phenotypes that were apparent at emergence were not observed in a sample of siblings from the same experimental population hatched in Heath incubators and reared in circular tanks 9 mo after fertilization.

A separate sample of 250 hatchery-reared North Santiam winter steelhead trout eyed-eggs was taken from Marion Forks Hatchery, Oregon on 6 June 1977. These fish were held in incubators until they had completedly absorbed their yolk sacs. The

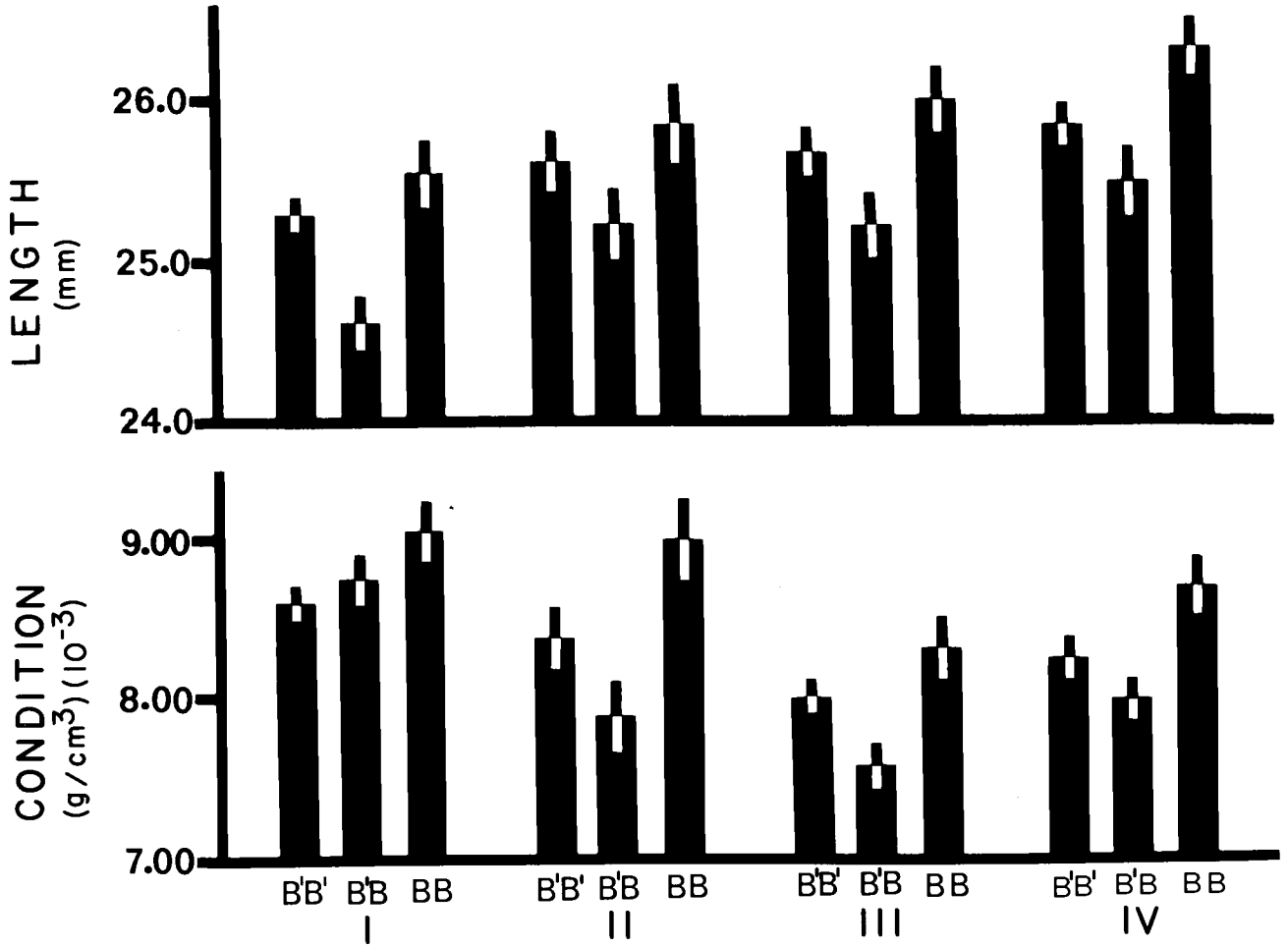

Figure 2. Mean lengths and condition factors and confidence intervals ($p < 0.05$; vertical bars) of the three phenotypes of LDH in steelhead trout for each treatment after incubation under gravel at various thermal and dissolved oxygen regimes. I = 16° C, 5 mg/l D. O.; II = 16° C, 9-10 mg/l D. O.; III = 12° C, 5 mg/l D. O.; IV = 12° C, 9-10 mg/l D. O.

trout fry were then weighed, measured, and analyzed for LDH phenotype. There were no significant differences in the mean weights and condition factors between LDH phenotypes for the hatchery-reared fish. The mean lengths of LDH phenotypes appeared to differ ($p < 0.10$):

	LDH Phenotype		
	B'B'	B'B	B B
n	42	128	80
mean length (cm)	2.81	2.78	2.78

Acute Challenge Experiments

For the acute challenge experiments groups of 100 juvenile trout (5-10 cm in length) were placed in identical 125 liter tanks and allowed to acclimate for 7-9 days before the challenge was imposed. Fish were not fed the day prior to the start of the challenge. After death each fish was immediately weighed, measured, and frozen. Later, the fish were analyzed for their phenotypes of LDH, IDH, AGPDH, MDH, and TO. Time of death for individual fish was calculated from the time at which the ambient acclimation conditions were altered at the beginning of the challenge. I used a probit analysis adapted from Bliss (1938) to calculate the median survival time (MST) and its confidence interval ($p < 0.05$) for each phenotype.

Temperature

In the acute high temperature challenge experiment three replicate groups were acclimated at 12° C and then subjected to a rapid increase in water temperature. After 375 min water temperature had reached 26.5° C. Ambient water temperature was maintained at this level for the duration of the experiment. All fish were dead within 1230 min.

Two groups of 100 trout reared at Marion Forks Hatchery from the same stock and brood year as the experimental population were acclimated at 12° C and then exposed to a challenge of 26.0° C. The maximum temperature was reached in 300 min. All fish were dead within 1860 min. Only the liver tissue enzymes, LDH, IDH, and TO, were assayed for the hatchery-reared groups.

For the experimental population the B'B phenotype of LDH exhibited the highest MST while BB had the lowest (Fig. 3). In the pooled sample confidence intervals for the MSTs of the LDH phenotypes do not overlap. The MST data from the hatchery-reared fish showed no consistent differences between LDH phenotypes (Fig. 4).

There was differential tolerance to acute high temperature challenge by the phenotypes of IDH for both the experimental population and the hatchery-reared fish. In the experimental population the A^3A^3 phenotype tended to have the highest MST. Similarly, in

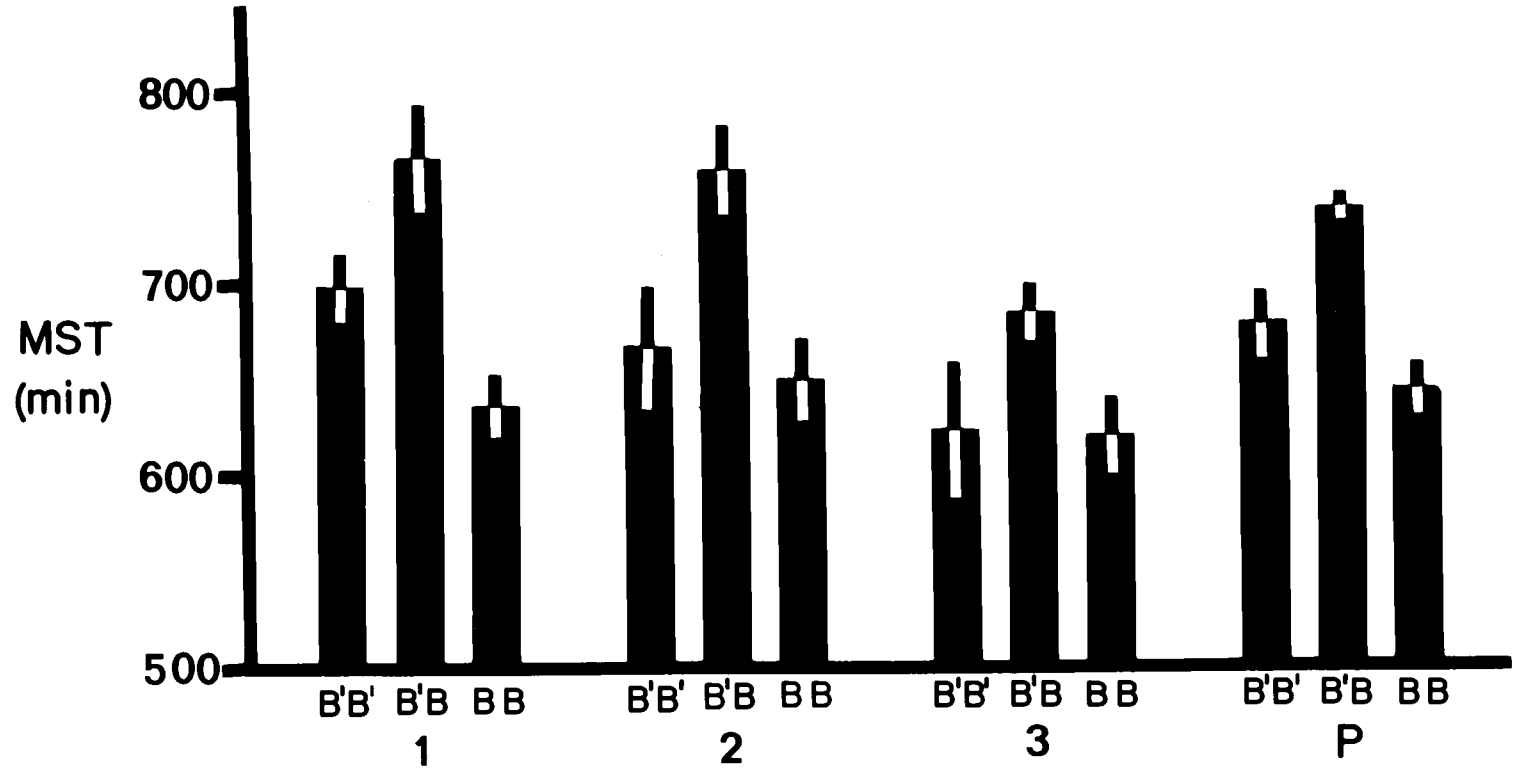

Figure 3. Median survival times (MST) and confidence intervals ($p < 0.05$; vertical bars) of the three phenotypes of LDH for each of three replicate samples and a pooled sample (P) from the acute high temperature challenge experiment with juvenile trout from the experimental population. Fish acclimated at 12° C.

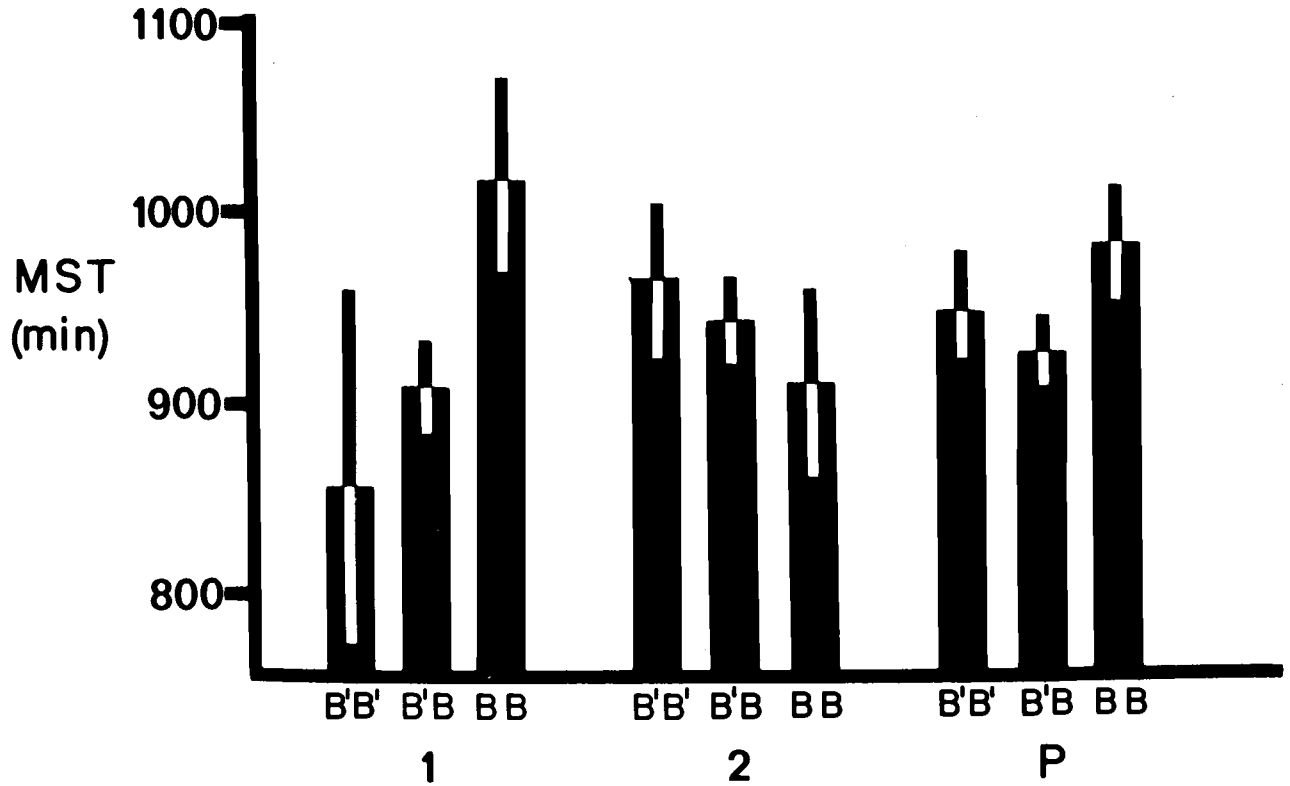

Figure 4. Median survival times (MST) and confidence intervals ($p < 0.05$; vertical bars) of the three phenotypes of LDH for each of two replicate samples and a pooled sample (P) from the acute high temperature challenge experiment with hatchery-reared juvenile trout. Fish acclimated at 12° C.

the hatchery fish A^3A^3 was significantly more tolerant than either AA or AA^3 (Fig. 5).

Dissolved Oxygen

Two replicate groups each from the experimental population were acclimated to 7.5 mg/l and 4.0 mg/l D.O. at 12° C. The challenge condition of 1.5 mg/l D.O. was reached within 270 min and sustained for the duration of the experiment. All fish that were acclimated at 7.5 mg/l D.O. were dead within 675 min. Approximately 10% of the fish that were acclimated at 4.0 mg/l D.O. survived until the experiment was terminated after 2880 min. The relationships of probits versus log time were curvilinear for the groups having incomplete mortality; therefore, I used data only from the linear portion of the curves in the probit analyses for these groups.

For both acclimation groups the BB phenotype of LDH was more tolerant than the B'B' phenotype, significantly so in the 7.5 mg/l D.O. acclimation group (Fig. 6). The B'B phenotype was intermediate in tolerance for the 7.5 mg/l D.O. group but equivalent to the B'B' in the 4.0 mg/l D.O. acclimation group.

The IDH phenotypes had differential tolerance to low dissolved oxygen challenge. In general, the A^1A^1 phenotype had a greater MST than any of the other five phenotypes. Of the 24 fish that survived for the duration of the experiment, 17 were A^1A^1, a far greater

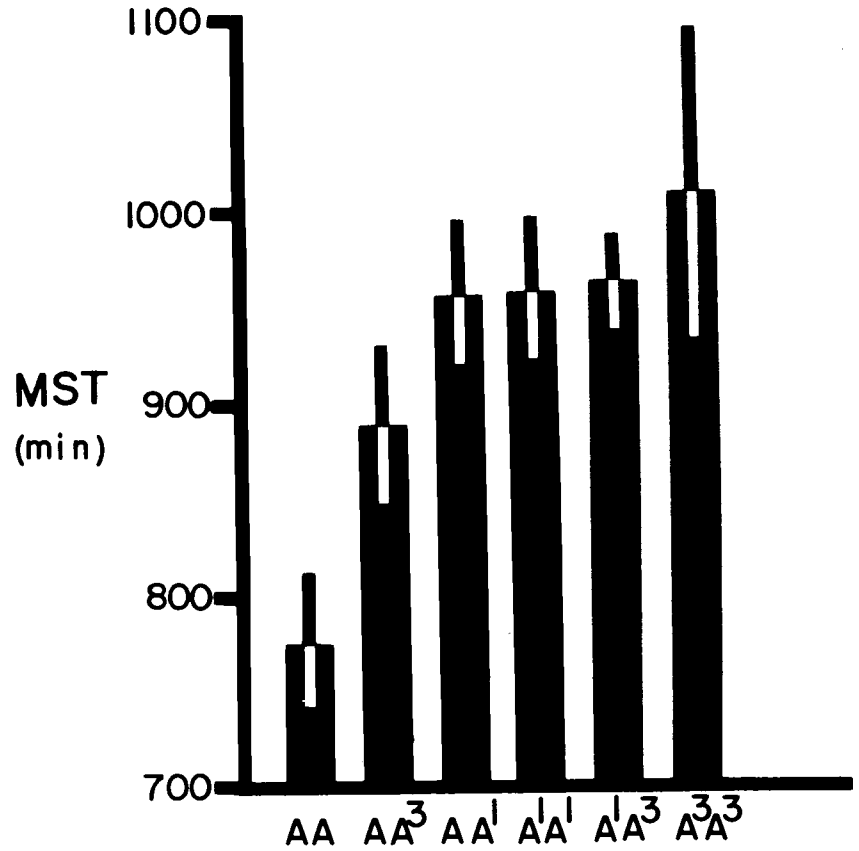

Figure 5. Median survival times (MST) and confidence intervals ($p < 0.05$; vertical bars) of the six phenotypes of IDH from the acute high temperature challenge experiment with hatchery-reared juvenile trout. Data are pooled from two replicate samples. Fish acclimated at 12° C.

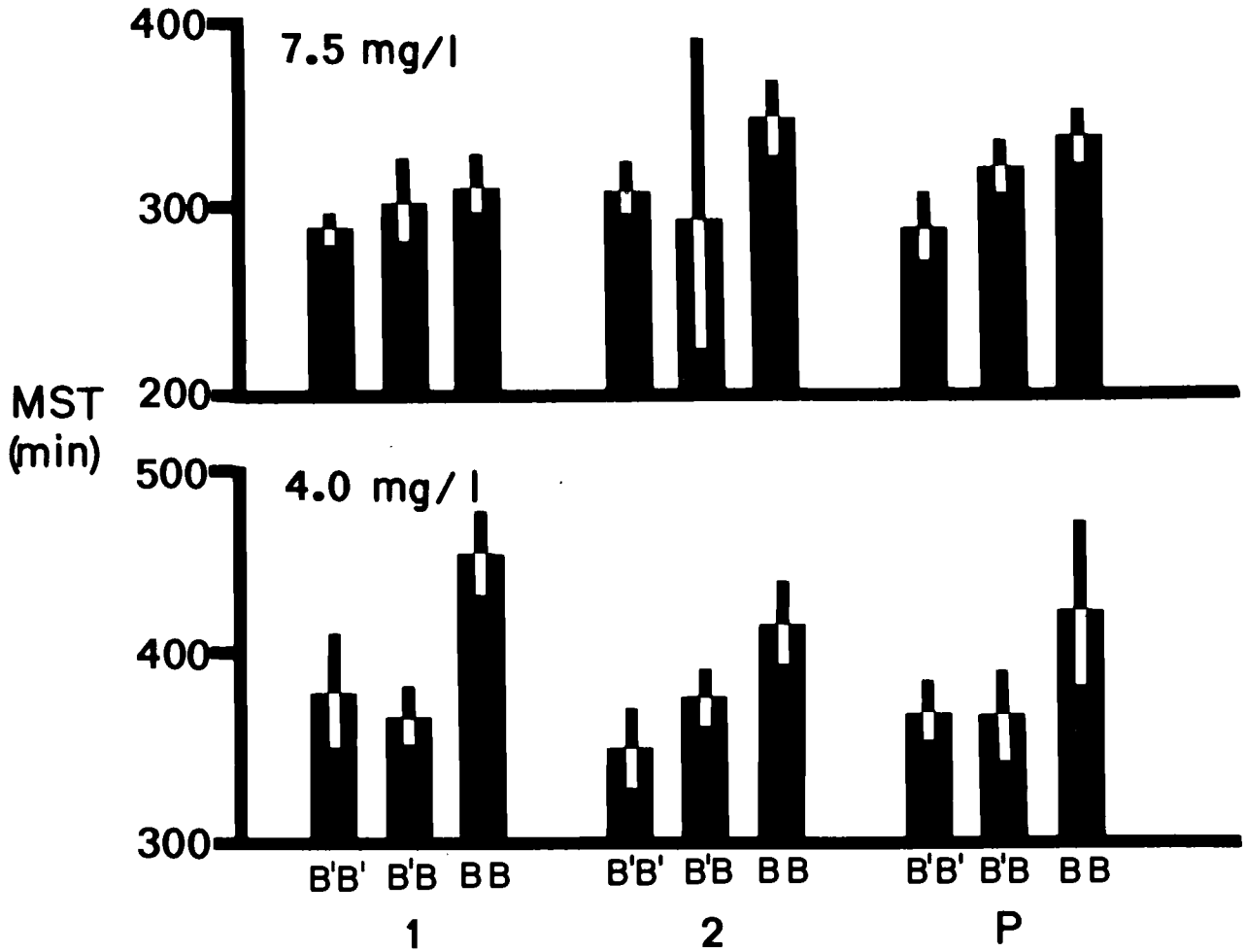

Figure 6. Median survival times (MST) and confidence intervals ($p < 0.05$; vertical bars) of the three phenotypes of LDH for the acute low dissolved oxygen challenge experiment with juvenile trout from the experimental population. Two replicate samples and a pooled sample (P) are shown for each of two acclimation regimes, 7.5 mg/l and 4.0 mg/l dissolved oxygen.

number than one would expect if differential tolerance did not exist.

Temperature and Dissolved Oxygen

Acute challenges of high temperature and low dissolved oxygen were administered simultaneously. Two replicate groups from the experimental population were tested for each of two acclimation regimes. Fish were acclimated at either 12° C, 8.0 mg/l D.O. or 18° C, 4.0 mg/l D.O. The fish were subjected to rapidly increasing temperature (2-3° C/h) and decreasing dissolved oxygen (2.5-3.5 mg/l/h). All fish were dead within 225 min and before the target conditions of 26.5° C, 1.5 mg/l D.O. were reached.

For those fish acclimated at 18° C, 4.0 mg/l D.O. the BB phenotype of LDH was significantly more tolerant than B'B'. B'B had an intermediate value for MST (Fig. 7). There were no significant differences between the MSTs of the three LDH phenotypes for the groups acclimated at 12° C, 8.0 mg/l D.O.

Differential tolerance was apparent between the IDH phenotypes for those fish acclimated at 18° C, 4.0 mg/l D.O. As in the acute low dissolved oxygen experiment the A^1A^1 phenotype tended to have a higher MST than other IDH phenotypes. There were no obvious differences between phenotypes of IDH for those groups acclimated at 12° C, 8.0 mg/l D.O.

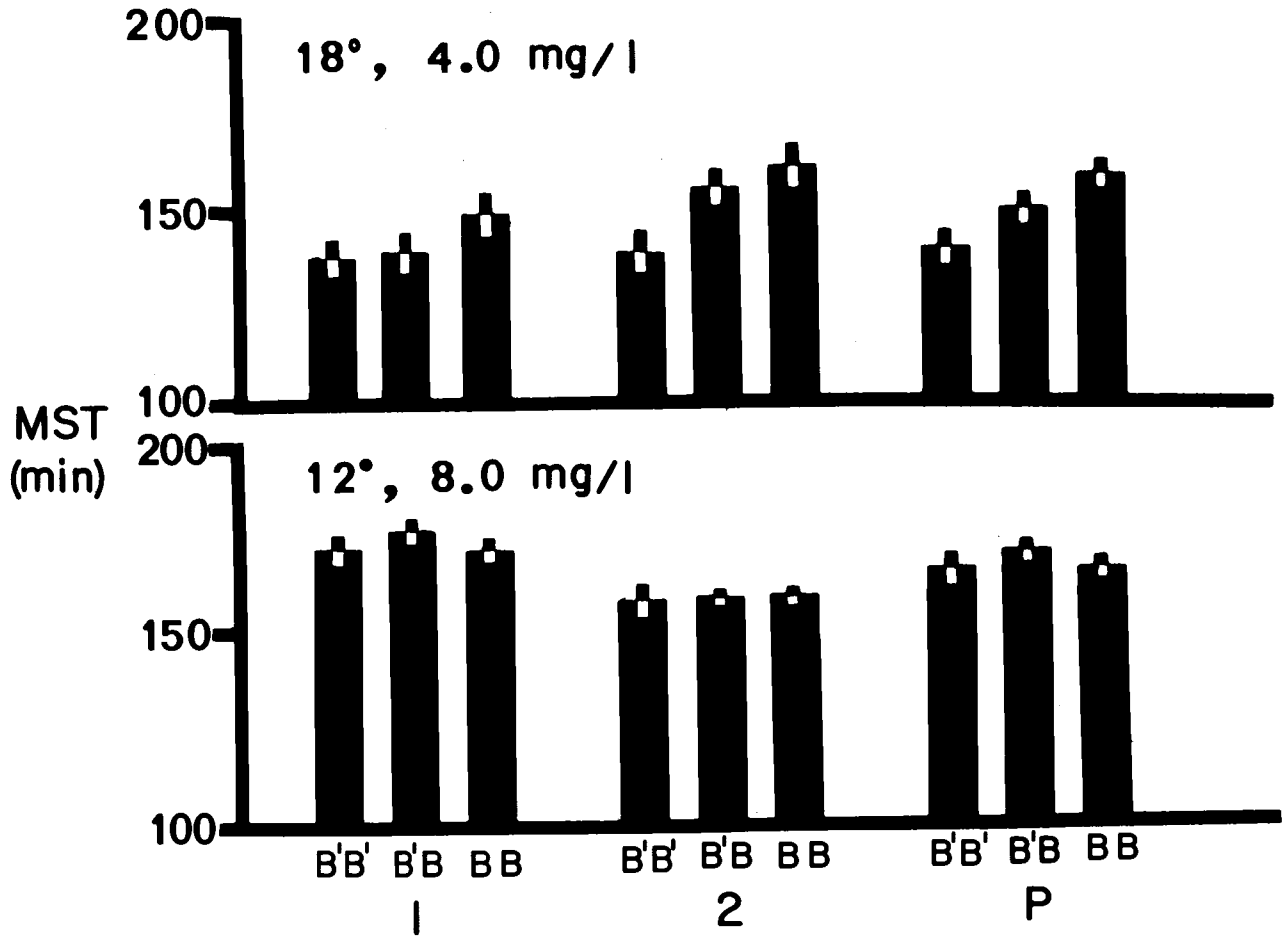

Figure 7. Median survival times (MST) and confidence intervals ($p < 0.05$; vertical bars) of the three phenotypes of LDH for the acute simultaneous high temperature and low dissolved oxygen challenge experiment with juvenile trout from the experimental population. Two replicate samples and a pooled sample (P) are shown for each of two acclimation regimes, 18° C, 4.0 mg/l and 12° C, 8.0 mg/l dissolved oxygen.

Other Observations

There were no apparent differences in tolerance between the phenotypes of TO, MDH, or AGPDH for any of the acute challenge experiments. No significant correlation existed between the weight or condition factor of individual fish and their time of death for any of the acute challenge experiments.

Separate groups of 100 fish from the experimental population were held for 9 days in 125 liter tanks under conditions which approximated each acclimation regime of each acute challenge experiment. No mortality occurred in any of these control groups.

DISCUSSION

Steelhead trout in the experimental population displayed differential performances between isozyme phenotypes with respect to the weight, length, and condition factor of emergent fry (LDH only) and the tolerance of juvenile trout to acute challenges of high temperature and low dissolved oxygen (LDH and IDH). Time of emergence from the gravel was not related to LDH phenotype. Differences in subgravel temperature and dissolved oxygen had no significant effect on the proportionate survival of LDH phenotypes.

I assume that the isozyme phenotypes observed in this study are controlled exclusively by the genome of the fish and are not affected qualitatively by environmental factors. That these isozyme systems exhibit predictable Mendelian inheritance patterns is taken as evidence of the validity of this assumption. Furthermore, the environmental induction of enzymes is thought to require a period of one to two weeks, making it an unlikely event during a 48 h bioassay (Hazel and Prosser 1974).

Granting the above assumption two factors, singly or in combination, may be responsible for the differences I observed. First, they may be related intrinsically to the enzyme polymorphisms. In other words, the functional properties of variant isozymes may result in differential performances by the fish. The performance

traits I observed (i. e., growth and tolerance) are undoubtedly influenced by many gene-enzyme systems; thus, it seems unlikely that allelic combinations at a single locus could be entirely responsible for the observed effects. At best, my results provide correlations which may suggest causal relationships between isozyme variants and performance. I do not exclude the possibility of epistatic interaction with other genes. Secondly, the results may reflect the bias of parental effects. The experimental population was derived from only six families; therefore, the probability that parental effects influenced the parametric means is high. Because I am unable to identify individual fish according to their parentage comparisons between family groups are impossible.

Comparable results from fish that were the progeny of a large number of random matings, such as one would expect to find in a hatchery, would support the hypothesis of intrinsic enzyme related differences. Presumably, under such conditions the probability that a single mating would significantly influence the parametric means of the filial population is minimal.

Steelhead trout fry of the 1977 year class that were spawned and reared at Marion Forks Hatchery until the eyed-egg stage appeared to differ ($p < 0.10$) in length, but not weight or condition factor, between LDH phenotypes. For the hatchery-reared fry B'B' was longer than the other phenotypes while in the experimental group BB was

longest. This implicates parental effects as the most likely cause of the size differences between LDH phenotypes in the experimental population. However, the fact that the hatchery-reared fish showed a LDH-length correlation independently favors the interpretation of intrinsic LDH related differences. It is also possible that environmental factors (e. g., considerably colder water temperature) at Marion Forks Hatchery during early embryonic development of the fish effectively altered or masked differences that might be manifest under other conditions.

Independent results on the growth of different LDH phenotypes for Deschutes River steelhead trout provide supportive evidence for intrinsic LDH related size differentiation. Reisenbichler and McIntyre (1976) used LDH as a genetic marker to distinguish the progeny of multiple matings of hatchery and wild trout. Their experimental crosses were designed specifically to maximize non-LDH genetic variability (i. e., minimize parental effects). In all groups the BB phenotype was largest at the eyed-egg stage (Fig. 8) (Reisenbichler, personal communication). B'B' had the smallest eggs while B'B had eggs of intermediate size. As in the eyed-egg stage, swim-up fry of the BB phenotype had the largest volume (Fig. 8). Note, however, that B'B had a smaller volume than B'B' contrary to what one might expect from the relative sizes of the eggs. This observation (i. e., BB > B'B' > B'B) parallels my findings for the experimental population

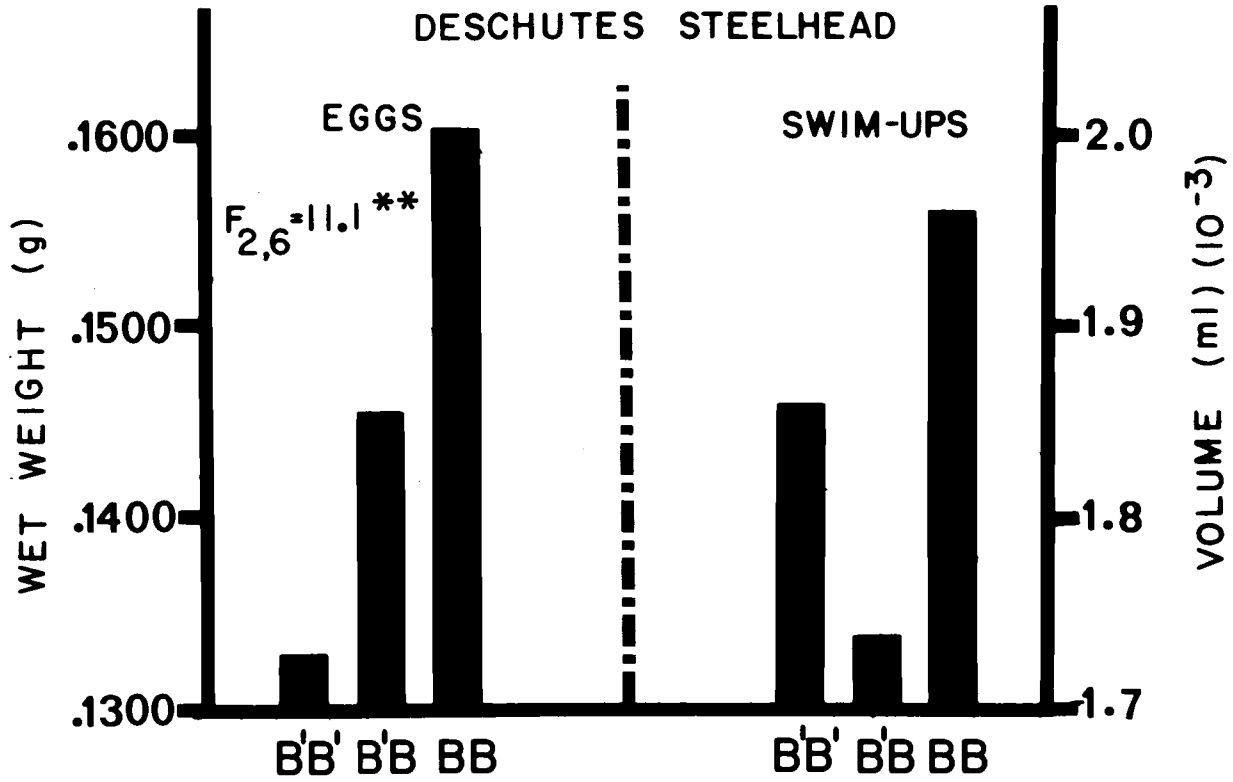

Figure 8. Wet weights for eyed-eggs and volumes for swim-up fry of an experimentally bred population of Deschutes River steelhead trout. ** indicates significant differences ($p < 0.01$) between phenotypes at the eyed-egg stage (Reisenbichler, personal communication).

of North Santiam fry (Fig. 1). Furthermore, Reisenbichler's data show that in the wild environment there were no significant differences or consistent trends between the mean lengths of the LDH phenotypes 4 mo after fertilization, a condition that is analogous to the lack of LDH related size differentiation in juveniles of the experimental population.

Because of the pivotal nature of LDH with respect to anaerobic glycolysis in the white muscle and gluconeogenesis in the liver it is conceivable that functional differences between isozyme variants of LDH could translate into differences in growth performance. Functional differences between LDH isozymes are known to occur in rainbow trout (Tsuyuki and Williscroft 1973; Hochachka and Somero 1968). My results generally support the contention that LDH polymorphism in trout has some adaptive significance with respect to body size at the time of emergence from the gravel under some conditions.

The geographic distribution of alleles coding for particular isozymes can sometimes give an indication of the adaptive significance of those isozymes (Johnson 1971; Koehn 1970). Stocks of steelhead trout in the Pacific Northwest region exhibit a conspicuous dichotomy in the frequencies of the two alleles that code for the LDH polymorphisms described in this study. Coastal stocks have a predominance of the B allele while interior stocks of the Columbia and Snake River systems possess higher frequencies of the alternate allele, B' (Allendorf 1975). Perhaps the size differentiation that I

have observed between LDH phenotypes in trout fry is related to this phenomenon.

In the experimental population the B'B phenotype of LDH exhibited a significantly greater tolerance to acute high temperature challenge than either of the homozygous forms (Fig. 3). Oddly, it was the BB phenotype, not B'B, that was most tolerant when subjected to low dissolved oxygen or simultaneous low oxygen and high temperature (Figs. 6, 7). In nature increased water temperature is usually concomitant with decreased dissolved oxygen concentration. One would expect those fish that are more tolerant to high temperature also to be more tolerant to low dissolved oxygen. Because this was not apparent in my experiments it suggests that certain allelic combinations are better suited for either high temperature or low dissolved oxygen but not both.

Again, these results could signify intrinsic differences between variant forms of LDH or they could simply indicate parental bias. Because hatchery-reared steelhead trout did not exhibit any consistent differential tolerance to high temperature with respect to LDH (Fig. 4), I believe that simple parental effects are the most parsimonious explanation for the thermal tolerance differences observed in the experimental population.

While these results do not support the notion that variants of LDH are associated with differential tolerance, they do suggest that

tolerance to high temperature or low dissolved oxygen is a heritable trait. This fact could be useful to fisheries managers who wish to manipulate stocks of steelhead trout for use in warmer or less aerobic systems. It also implies that alterations in the temperature or dissolved oxygen regimes of an aquatic system can affect the genetic consitution of steelhead trout populations.

Unlike the LDH system, differential tolerance to high temperature and low dissolved oxygen between isozyme phenotypes of IDH cannot be explained solely on the basis of parental effects. For the experimental population A^3A^3 tended to be most tolerant to high temperature relative to other IDH phenotypes. A^3A^3 fish were produced in approximately equal numbers by five of the six experimental families. Thus, if parental effects were the only influence on performance one would expect a more random distribution than that which I observed. Analogously, within the hatchery population the A^3A^3 phenotype possessed the greatest tolerance to thermal challenge, significantly greater than that of the AA or AA^3 forms (Fig. 5). Of the 24 fish that survived the low dissolved oxygen challenge 71% were A^1A^1. Differential tolerance of IDH phenotypes must be attributed, at least partially, to some intrinsic differences correlated to IDH polymorphisms.

IDH catalyzes the reversible oxidation-decarboxylation of isocitrate and is the rate limiting enzyme in the Krebs cycle. Moon

and Hochachka (1971, 1972) demonstrated differential temperature dependency for the reaction kinetics of IDH isozymes in rainbow trout. My results corroborate their interpretation that IDH isozymes are of adaptive significance to this species.

Interior stocks from the Snake and Deschutes River systems tend to have higher frequencies of the A^1 and A^3 alleles of IDH than coastal stocks of steelhead trout (Oregon Cooperative Fishery Research Unit, unpublished data). My experiments suggest that the isozyme phenotypes of the A^1 and A^3 alleles may possess a greater tolerance to high temperature than those of the A allele (Fig. 5). Water temperature in the Snake River can reach as high as 25° C in the summer (Beiningen and Ebel 1971), a value which approaches the upper thermal tolerance limit of steelhead trout. Given the desert climate of the Snake River drainage it is reasonable to suppose that tributaries of the Snake River achieve thermal maximums greater than 25° C. In contrast, a typical coastal stream in Oregon may have summer maximums of only 15° C (Moring 1975). If water temperature in the interior regions is warmer during the summer than that in coastal areas, a possible consequence might be the natural selection of isozyme phenotypes with greater tolerance to high temperature in interior stocks. The relatively higher frequencies of A^1 and A^3 alleles in interior stocks is consistent with this explanation.

My results support the notion that enzyme polymorphisms within

fish species can have adaptive significance to the organism. This contrasts to the belief held by some that most protein variation is effectively neutral and that protein evolution occurs via random fixation processes rather than natural selection (e. g., King and Jukes 1969). Polymorphisms of LDH within steelhead trout populations appear to be correlated in some cases to size differences between individual fry. Polymorphisms of IDH, but not LDH, seem to be associated with differential tolerance to acute challenges of high temperature and low dissolved oxygen.

LITERATURE CITED

Aleksiuk, M. 1971. An isoenzyme basis for instantaneous cold compensation in reptiles: Lactate dehydrogenase kinetics in Thamnophis sirtalis. Comp. Biochem. Physiol. 40B:671-681.

Allendorf, F. W. 1973. Genetic variation, inheritance and preliminary population distribution of some proteins of Salmo gairdneri. M. S. Thesis, Univ. of Washington, Seattle. 61 pp.

Allendorf, F. W. 1975. Genetic variability in a species possessing extensive gene duplication: Genetic interpretation of duplicated loci and examination of genetic variation in populations of rainbow trout. Ph. D. Thesis, Univ. of Washington, Seattle. 98 pp.

Baldwin, J., and P. W. Hochachka. 1970. Functional significance of isoenzymes in thermal acclimatization: Acetylcholinesterase from trout brain. Biochem. J. 116:883-887.

Beiningen, K., and W. Ebel. 1971. Dissolved nitrogen, dissolved oxygen, and related water temperatures in the Columbia and lower Snake Rivers, 1965-1969. Data Report 56, National Marine Fisheries Service, U. S. Dept. of Commerce, Seattle. 60 pp.

Bliss, C. I. 1938. The determination of dosage mortality curves from small numbers. Quart. J. Pharmacy Pharmacol. 11: 192-216.

Eddy, R. M. 1971. The influence of dissolved oxygen concentration and temperature on the survival and growth of chinook salmon embryos and fry. M. S. Thesis, Oregon State Univ., Corvallis. 45 pp.

Hazel, J. R., and C. L. Prosser. 1974. Molecular mechanisms of temperature compensation in poikilotherms. Physiol. Rev. 54:620-677.

Hochachka, P. W. 1965. Isoenzymes in metabolic adaptation of a poikilotherm: Subunit relationships in lactate dehydrogenases of goldfish. Arch. Biochem. Biophys. 111:96-103.

Hochachka, P. W., and J. K. Lewis. 1970. Enzyme variants in thermal acclimation: Trout liver citrate synthases. J. Biol. Chem. 245:6567-6573.

Hochachka, P. W., and G. N. Somero. 1968. The adaptation of enzymes to temperature. Comp. Biochem. Physiol. 27:659-668.

Johnson, M. S. 1971. Adaptive lactate dehydrogenase variation in the crested blenny, Anoplarcus. Heredity 27:205-226.

Kent, J. D., and R. G. Hart. 1976. The effect of temperature and photoperiod on isozyme induction in selected tissues of the creek chub, Semotilus atromaculatus. Comp. Biochem. Physiol. 54B:77-80.

King, J. L., and T. H. Jukes. 1969. Non-Darwinian evolution. Science 164:788-798.

Koehn, R. K. 1970. Functional and evolutionary dynamics of polymorphic esterases in catostomid fishes. Trans. Am. Fish. Soc. 99:219-223.

Kunnemann, H. 1973. The influence of temperature on thermostability, isoenzyme pattern and reaction kinetics of lactate dehydrogenase from fishes. Marine Biol. 18:37-45.

Markert, C. L. 1968. The molecular basis for isozymes. Ann. N. Y. Acad. Sci. 151:14-40.

Markert, C. L., and I. Faulhaber. 1965. Lactate dehydrogenase isozyme patterns of fish. J. Exp. Zool. 159:319-332.

Merritt, R. B. 1972. Geographic distribution and enzymatic properties of lactate dehydrogenase allozymes in the fathead minnow, Pimephales promelas. Am. Nat. 106:173-184.

Mitton, J. B., and R. K. Koehn. 1975. Genetic organization and adaptive response of allozymes to ecological variables in Fundulus heteroclitus. Genetics 79:97-111.

Moon, T. W., and P. W. Hochachka. 1971. Temperature and enzyme activity in poikilotherms: Isocitrate dehydrogenases in rainbow trout liver. Biochem. J. 123:695-705.

Moon, T. W., and P. W. Hochachka. 1972. Temperature and the kinetic analysis of trout isocitrate dehydrogenases. Comp. Biochem. Physiol. 42B:725-730.

Moring, J. R. 1975. The Alsea watershed study: Effects of logging on the aquatic resources of three headwater streams of the Alsea River, Oregon. Part II - Changes in environmental conditions. Fishery Research Report No. 9, Oregon Dept. Fish and Wildlife, Dec. 1975. 39 pp.

Reisenbichler, R. R., and J. D. McIntyre. 1977. Genetic differences in growth and survival of juvenile hatchery and wild steelhead trout, Salmo gairdneri. J. Fish. Res. Board Can. 34:123-128.

Snedecor, G. W., and W. G. Cochran. 1967. Statistical methods. Iowa State Univ. Press, Ames, Iowa. 593 pp.

Somero, G. N., and P. W. Hochachka. 1969. Isoenzymes and short-term temperature compensation in poikilotherms: Activation of lactate dehydrogenase isoenzymes by temperature decreases. Nature 223:194-195.

Tsugawa, K. 1976. Direct adaptation of cells to temperature: Similar changes of lactate dehydrogenase isozyme patterns by in vitro and in situ adaptations in Xenopus laevis. Comp. Biochem. Physiol. 55B:259-263.

Tsukuda, H. 1975. Temperature dependency of the relative activities of liver lactate dehydrogenase isozymes in goldfish acclimated to different temperatures. Comp. Biochem. Physiol. 52B:343-345.

Tsuyuki, H., and S. N. Williscroft. 1973. The pH activity relations of two LDH homotetramers from trout liver and their physiological significance. J. Fish. Res. Board Can. 30:1023-1026.

Utter, F. M., H. O. Hodgins, and F. W. Allendorf. 1974. Biochemical genetic studies of fishes: Potentialities and limitations, p. 213-238. In D. C. Malins and J. R. Sargent (ed.) Biochemical and biophysical perspectives in marine biology. Vol. I. Academic Press, New York.

Utter, F. M., H. O. Hodgins, F. W. Allendorf, A. G. Johnson, and J. L. Mighell. 1973. Biochemical variants in Pacific salmon and rainbow trout: Their inheritance and application in population studies, p. 329-339. In J. H. Schroder (ed.) Genetics and mutagenesis of fish. Springer-Verlag, Berlin.

Warren, C. E. 1971. Biology and water pollution control. W. B. Saunders, Philadelphia. 434 pp.

Whitmore, D. H., and E. Goldberg. 1972. Trout intestinal alkaline phosphatases II. The effect of temperature on enzymatic activity in vitro and in vivo. J. Exp. Zool. 182:59-68.

Wright, J. E., J. R. Heckman, and L. M. Atherton. 1975. Genetic and developmental analyses of LDH isozymes in trout, p. 375-403. In C. L. Markert (ed.) Isozymes. Vol. III. Academic Press, New York.