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Possible Adaptive Significance of Certain Enzyme Polymorphisms in Steelhead Trout (*Salmo gairdneri*)[1]

J. M. REDDING AND C. B. SCHRECK

Oregon Cooperative Fishery Research Unit,[2] Oregon State University, Corvallis, OR 97331, USA

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In experimentally reared winter steelhead trout (*Salmo gairdneri*) fry, mean weights, lengths, and condition factors of three isozyme phenotypes of lactate dehydrogenase (LDH) enzyme differed significantly. 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 subgravel 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 result for LDH. Differences between IDH phenotypes may be related to intrinsic properties of variant isozymes.

Key words: isozymes, lactate dehydrogenase, isocitrate dehydrogenase, temperature, dissolved oxygen, adaptive significance, *Salmo gairdneri*

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Chez des alevins de truite steelhead d'hiver (*Salmo gairdneri*) élevés expérimentalement, on observe des différences significatives dans le poids, la longueur et les facteurs de condition moyens de trois phénotypes d'isozymes de l'enzyme lactate déshydrogénase (LDH). Le moment de l'émergence des alevins du gravier n'a pas de rapport avec le phénotype de LDH. La mortalité relative des phénotypes entre le stage d'oeuf oculé et l'émergence n'est pas affectée par des conditions différentes de température et d'oxygène dissous sous la surface du gravier. Nous observons des différences dans la tolérance à des expositions intenses à une température élevée et un bas niveau d'oxygène dissous entre phénotypes de l'enzyme isocitrate déshydrogénase (IDH) et LDH chez de jeunes truites. Les résultats obtenus avec LDH peuvent être biaisés par des effets parentaux. Il se peut que les différences entre les phénotypes d'IDH soient reliés à des propriétés intrinsèques d'isozymes différentes.

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STOCKS of steelhead trout (*Salmo gairdneri*) in the Pacific Northwest 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. In this study we attempt to relate these and other enzyme polymorphisms (isozymes) to their possible functional and adaptive significance.

LDH catalyzes the interconversion of pyruvate and lactate and regulates the balance between aerobic respiration and anaerobic glycolysis. Many species of fish possess LDH polymorphisms (Markert and Faulhaber 1965) and the molecular and genetic bases of these isozymes are well understood (Markert 1968). Internal and external factors can modulate the relative activity of LDH isozymes. Temperature-dependent induction of LDH isozymes occurs during thermal 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 LDH isozymes in temperature optima and substrate affinities exist in the fathead minnow, *Pimephales promelas* (Merritt 1972), sockeye salmon, *Oncorhynchus nerka* (Utter et al. 1974), and rainbow trout, *Salmo gairdneri* (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 later (1977) demonstrated that swimming endurance was related to LDH phenotype. Geographic variation in the frequencies

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of alleles that code for LDH polymorphisms has been correlated with environmental temperature (Merritt 1972; Mitton and Koehn 1975). Johnson (1971) demonstrated differential thermal tolerance between LDH phenotypes for a species that exhibits a latitudinal cline in the frequencies of LDH alleles.

Similar observations have been recorded for other polymorphic enzymes in trout: 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 this study was to investigate the adaptive significance of various enzyme polymorphisms, particularly those of LDH, in steelhead trout in relation to environmental temperature and dissolved oxygen. Specifically, we tested the hypotheses that (1) isozyme phenotypes of LDH in steelhead trout have differential survival and growth performances during the period between the eyed-egg stage and emergence, when exposed to different combinations of subgravel temperature and dissolved oxygen; and (2) in juvenile steelhead trout, isozyme phenotypes of LDH, IDH, and several other enzyme systems possess different tolerances to acute high temperature and low dissolved oxygen challenges.

Materials and Methods

The North Santiam River (Oregon) winter steelhead trout stock possesses three electrophoretic variants of LDH. Following the nomenclature of Wright et al. (1975) we designated these variants 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 we wanted to test the relative performances of the three phenotypes, it was necessary that they have approximately equal representation in the test groups. Since the phenotypes are not present in equal proportions in the wild, the experimental fish were selectively bred.

Steelhead trout in reproductive condition were captured during their upstream migration in a trap at Minto Pond on the North Santiam River on May 7, 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 an experimental population. The fertilized eggs were held in Heath® incubators until they reached the eyed-egg stage; they were then shocked to identify viable eggs, and divided into two groups. One group was used for the subgravel incubation experiment. The other group was kept in the incubators until the fish absorbed their yolk sacs; then they were reared in circular tanks until used in the acute challenge experiments. Various physiochemical conditions in the circular tanks were held constant: temperature, 12°C; hardness ($CaCO_3$), 99 mg/L; pH, 7.3; conductivity, 241 μmho; dissolved oxygen, 8–12 mg/L.

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, phenotype were determined for four enzyme systems in juvenile trout: IDH and tetrazolium oxidase (TO) in liver tissue and malate dehydrogenase (MDH) and alpha-glycerophosphate dehydrogenase (AGPDH) in white muscle tissue. Phenotype designations for these enzyme systems followed that of Allendorf (1973) and Utter et al. (1973). We recognized six phenotypes for IDH (AA, A^1A, A^1A^1, AA^3, A^1A^3, A^3A^3) and three each for TO (AA, AB, BB), MDH (B′B′, B′B, BB), and AGPDH (AA, AB, BB).

Experimental Design and Results

Subgravel 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 diam) in each of 12 flow-through incubation boxes (23 × 23 × 61 cm). The eggs were subjected to one of four treatment regimes of temperature and dissolved oxygen (DO). Each treatment had three replicates. Roman numerals indicate treatment designations:

Temp (°C)	Dissolved oxygen (mg/L) 5	9–10
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 sacs were collected daily. Individual fish were weighed and measured immediately after collection and then frozen for 1–4 wk before electrophoretic analysis.

Treatment effects produced no significant differences in the relative proportion of surviving fish for each LDH phenotype (Table 1). Confidence intervals ($P < 0.05$) were calculated for the observed proportions (p) by the formula $p\sqrt{1.96\,p(1-p)/n}$, where n equaled the sample

TABLE 1. Percentage 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	Temp. (°C)	Dissolved oxygen (mg/L)	No. of fry	Percentage of phenotype BB	B′B	B′B′	Frequency of *B* allele
I	16	5	660	28	28	44	0.42±0.03
II	16	9–10	258	31	31	38	0.47±0.04
III	12	5	587	30	30	40	0.45±0.03
IV	12	9–10	627	33	25	42	0.46±0.03
Control	12	8	174	25	27	48	0.39±0.05

size. Because the number of eggs contributed by each female varied, the exact proportion of each phenotype was unknown before the eggs were buried; thus, we could not estimate the relative survival rates between phenotypes within a single treatment. There were no significant differences between the replicates within a treatment group.

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 any treatment group. Significant differences in time of emergence were observed between treatment groups as one would expect on the basis of bioenergetic considerations (Warren 1971).

For all treatment groups, fish of the BB phenotype of LDH weighed more than the B'B' phenotype. Both homozygous forms weighed more than the heterozygous form, B'B (Fig. 1). The ANOVA for mean weight of 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 DO tended to weigh more at emergence than did 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. 1). B'B was the shortest and had the lowest condition factor, except in the 16°C, 5 mg/L DO treatment group in which the B'B' form had the lowest condition factor.

Differences in weight, length, and condition factors between LDH phenotypes that were apparent at emergence were not observed 9 mo after fertilization in a sample of siblings from the same experimental population that were reared in incubation trays and circular tanks at 12°C, 9–10 mg/L DO.

A separate sample of 250 eyed eggs was taken on June 6, 1977 from North Santiam winter steelhead trout reared at Marion Forks Hatchery, Oregon. These fish were held in incubators until they had completely absorbed their yolk sacs. The fry were then weighed, measured, and analyzed for LDH phenotype. There were no significant differences in the mean weights, lengths, or condition factors between LDH phenotypes in the hatchery-reared fish.

Acute Challenge Experiments

For the acute challenge experiments, groups of 100 juvenile trout (5–10 cm long) were placed in identical 125-L tanks and allowed to acclimate for 7–9 d before challenged. Fish were not fed the day before the start of the challenge. Immediately after death, each fish was weighed, measured, and frozen. Later, the fish were analyzed for LDH, IDH, AGPDH, MDH, and TO phenotypes. Time of death for individual fish was calculated from the time at which the ambient acclimation conditions were altered. We 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.

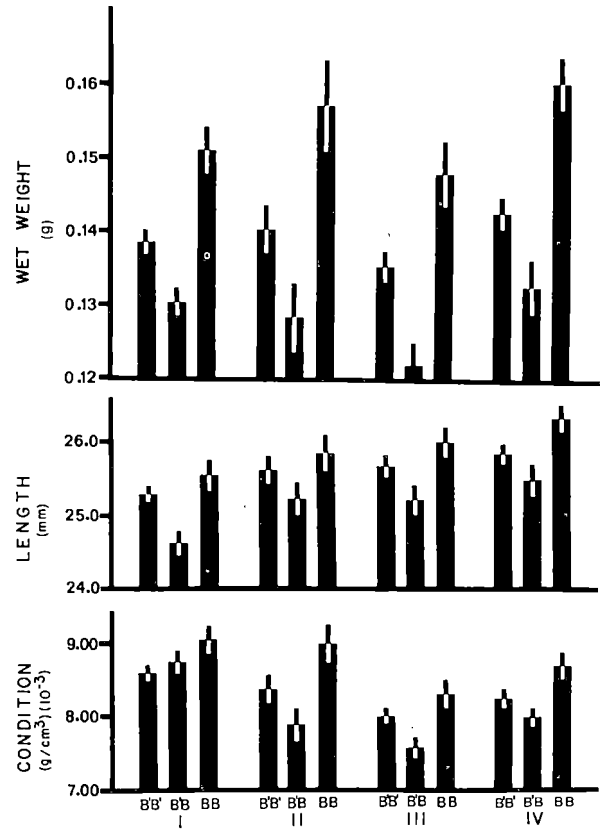

FIG. 1. Mean wet weights, length, and condition factors and confidence intervals ($P < 0.05$; vertical bars) of the three phenotypes of LDH in steelhead trout for each treatment, after incubation under gravel at various thermal and dissolved oxygen regimes: I = 16°C, 5 mg/L DO; II = 16°C, 9–10 mg/L DO; III = 12°C, 5 mg/L DO; IV = 12°C, 9–10 mg/L DO.

Temperature

In the acute high temperature challenge experiment three replicate groups from the experimental population 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; it was main-

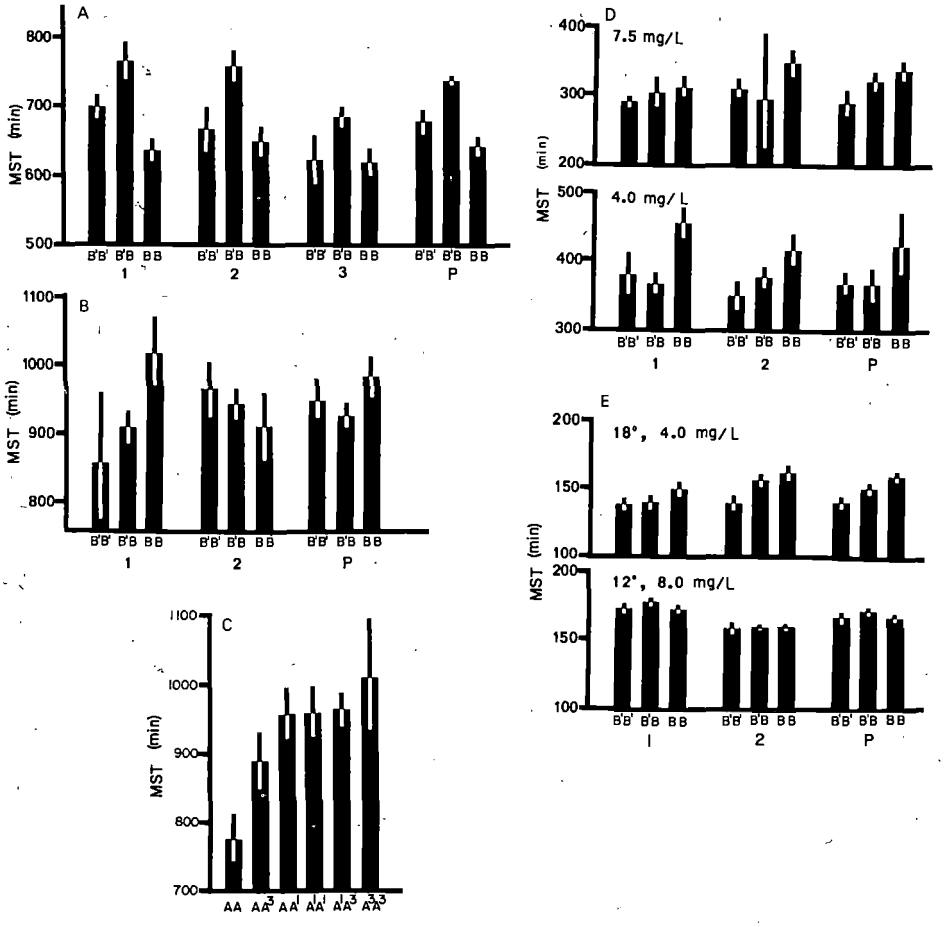

FIG. 2. Median survival times (MST) and confidence intervals ($P < 0.05$; vertical bars) of (A) the three phenotypes of LDH for the acute high temperature challenge experiment with juvenile trout from the experimental population (three replicate samples and a pooled sample (P) are shown, fish acclimated at 12°C); (B) the three phenotypes of LDH for the acute high temperature challenge experiment with hatchery-reared juvenile trout (two replicate samples and a pooled sample are shown, fish acclimated at 12°C); (C) 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); (D) 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 are shown for each of two acclimation regimes, 7.5 mg/L and 4.0 mg/L dissolved oxygen); and (E) 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 are shown for each of two acclimation regimes, 18°C, 4.0 mg/L and 12°C, 8.0 mg/L dissolved oxygen).

tained at this level for the duration of the experiment. All fish died within 1230 min.

Two groups of 100 juvenile trout reared at Marion Forks Hatchery and randomly bred 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 died within 1860 min. Only the liver tissue enzymes, LDH, IDH, and TO, were assayed for the hatchery-reared groups.

In the experimental population the B'B phenotype of LDH exhibited the highest MST, and BB had the lowest (Fig. 2A). In the pooled sample, confidence intervals for the MST's of LDH phenotypes do not overlap. The MST data from the hatchery-reared fish showed no consistent differences between LDH phenotypes (Fig. 2B).

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 the hatchery fish, A^3A^3 was significantly more tolerant than either AA or AA^3 (Fig. 2C).

Dissolved Oxygen

Two replicate groups each from the experimental population were acclimated to 7.5 and 4.0 mg/L DO at 12°C. The challenge condition of 1.5 mg/L DO was reached within 270 min and sustained for the duration of the experiment. All fish that were acclimated at 7.5 mg/L DO died within 675 min. About 10% of the fish that were acclimated at 4.0 mg/L DO survived until the experiment was terminated after 2880 min. The relationships of probits vs. log time were curvilinear for the groups in which mortality was incomplete; therefore, we 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 (Fig. 2D). In the pooled samples the B′B phenotype was intermediate in tolerance for the 7.5 mg/L DO group but equivalent to the B′B′ in the 4.0 mg/L DO 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 the duration of the experiment, 17 were A^1A^1, a far greater 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 DO or 18°C, 4.0 mg/L DO. 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 died within 225 min, and before the target conditions of 26.5°C, 1.5 mg/L DO were reached.

For fish acclimated at 18°C, 4.0 mg/L DO, the BB phenotype of LDH was significantly more tolerant than B′B′. The MST value for B′B was intermediate (Fig. 2E). There were no significant differences between the MST's of the three LDH phenotypes for the groups acclimated at 12°C, 8.0 mg/L DO.

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

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 to death in any of the acute challenge experiments.

Separate groups of 100 fish from the experimental population were held for 9 d in 125-L tanks under conditions that approximated each acclimation regime of each acute challenge experiment. None of the control fish died.

Discussion

Isozyme phenotypes in the experimental population of steelhead trout differed in weight, length, and condition factor of emergent fry (LDH only) and in 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.

We 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 believed to require a period of 1–2 wk (Hazel and Prosser 1974), making it an unlikely event during a 48-h bioassay.

Granting the above assumption, two factors, singly or in combination, may be responsible for the differences we 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 characteristics we 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, our results provide correlations that may suggest causal relationships between isozyme variants and performance. We do not exclude the possibility of epistatic interaction with other genes. Secondly, the results may be biased by uncontrolled genetic variability between family groups (parental effects). The experimental population was derived from only six families; therefore, the probability that parental effects influenced the parametric means is high. Because we could not identify the parentage of individual fish, comparisons between family groups were 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. Pre-

sumably, under such conditions the probability that a single mating would significantly influence the parametric means of the filial population is small.

Steelhead trout fry of the 1977 year-class that were spawned and reared at Marion Forks Hatchery until the eyed-egg stage did not differ in length, weight, or condition factor between LDH phenotypes. This fact implicates parental effects as the most likely cause of the size differences observed between LDH phenotypes in the experimental population. However, it is also possible that environmental factors (e.g. considerably lower 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 ($P < 0.01$) (R. R. Reisenbichler, U.S. Fish and Wildlife Service, Red Bluff, Calif., personal communication). The B′B′ phenotype 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. However, 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 our findings for the experimental population of North Santiam fry (Fig. 1). Furthermore, R. R. Reisenbichler's unpublished 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). Tsuyuki and Williscroft (1977) correlated variation in swimming stamina in rainbow trout to LDH phenotype. Our results generally support the contention that LDH polymorphism in trout, under some conditions, has some adaptive significance with respect to body size at the time of emergence from the gravel.

The geographic distribution of alleles coding for particular isozymes sometimes provides an indication of the adaptive significance of those isozymes (Johnson 1971; Koehn 1970). Stocks of steelhead trout in the Pacific Northwest exhibit a conspicuous dichotomy in the frequencies of the two alleles that code for the LDH polymorphisms described here. 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 *B′* allele (Allendorf 1975). Perhaps the size differentiation that we 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 did either of the homozygous forms (Fig. 2A). Oddly, it was the BB phenotype, not B′B, that was most tolerant when subjected to low dissolved oxygen or simultaneous low dissolved oxygen and high temperature (Fig. 2D, E). 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. The absence of this relation in our experiments 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. 2B), we believe that simple parental effects are the most parsimonious explanation for the differences in thermal tolerance observed in the experimental population.

Although 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 at least partially heritable. Significant differences in a trait between families are expected in this case only if the trait is heritable. The fact that tolerance to high temperature and low dissolved oxygen seems to be heritable could be useful to fishery 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 constitution 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. The 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 we

observed. Analogously, within the hatchery population the A^3A^3 phenotype showed the greatest tolerance to thermal challenge, significantly greater than that of the AA or AA^3 forms (Fig. 2C). 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.

The IDH enzyme 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. Our results corroborate their interpretation that IDH isozymes are of adaptive significance in 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 do coastal stocks of steelhead trout (Oregon Cooperative Fishery Research Unit unpublished data). Our 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. 2C). Water temperature in the Snake River may rise as high as 25°C in the summer (Beiningen and Ebel 1971), a value that approaches the upper thermal tolerance limit of steelhead trout. In contrast, a typical coastal stream in Oregon may have summer thermal maximums of only 15°C (Moring 1975). If water temperature during the summer is higher in the interior regions than 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.

Our results support the notion that enzyme polymorphisms within fish species can have adaptive significance to the organism. This contrasts to the opposing view (e.g. King and Jukes 1969) that most protein variation is effectively neutral and that protein evolution occurs via random fixation processes rather than by natural selection. 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.

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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, Wash. 61 p.

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, Wash. 98 p.

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. U.S. Nat. Mar. Fish. Serv. Data Rep. 56: 60 p.

BLISS, C. I. 1938. The determination of dosage mortality curves from small numbers. Q. J. Pharm. 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, Oreg. 45 p.

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. Mar. 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 dehydro-

genases in rainbow trout liver. Biochem. J. 123: 695–705.

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. Oreg. Dep. Fish Wildl. Fish. Res. Rep. 9: 39 p.

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 p.

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.

1977. Swimming stamina differences between genotypically distinct forms of rainbow (*Salmo gairdneri*) and steelhead trout. J. Fish. Res. Board Can. 34: 996–1003.

UTTER, F. M., H. O. HODGINS, AND F. W. ALLENDORF. 1974. Biochemical genetic studies of fishes: Potentialities and limitations, p. 213–238. *In* C. C. Malins and J. R. Sargent [ed.] Biochemical and biophysical perspectives in marine biology. Vol. I. Academic Press, New York, N.Y.

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, Pa. 434 p.

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., F. 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, N.Y.