Liver Enzyme Activity as a Growth Index in Wild Rainbow Trout

Jan C. Dean,[1] *Department of Fisheries and Wildlife Sciences, Virginia Polytechnic Institute and State University, Blacksburg, VA 24061*

Gino L. Luchetti, *Department of Fisheries and Wildlife Sciences, Virginia Polytechnic Institute and State University, Blacksburg, VA 24061*

Abstract: The relationship between fish size and the activity of certain liver enzymes was assessed in age I rainbow trout (*Salmo gairdneri*) from 3 Virginia streams. Total length and body weight of the trout differed significantly among streams. Activity of glutamate dehydrogenase and aspartate aminotransferase also differed significantly among fish in the streams and both were negatively correlated with fish size. Differences in size and enzyme activity probably relate to differential caloric intake of fish in the 3 streams. Condition factor and alanine aminotransferase activity of the trout did not differ among streams.

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An index of growth rate in wild fish would allow quick evaluation of various management strategies. This would benefit the fishery manager since conventional means of evaluation may require years of growth and production data, and variation among or between years can complicate interpretation of results.

Indices of growth rate should be biochemically based since biochemical mechanisms control growth and respond rapidly to changes in food intake or energy expenditure. Research concerning biochemical indices of growth in fish is sparse but short-term growth in fish has been correlated with cellular RNA content, amino acid uptake into scales, and the alkaline phosphatase activity of dermal tissue connected to fish scales (Bulow 1970, Shul'man 1974, Kayes 1978, Adelman 1980).

[1] Present address: Oklahoma Department of Wildlife Conservation, 1801 N Lincoln Boulevard, Oklahoma City, OK 73105.

The purpose of this study was to determine if the activities of certain liver enzymes are related to growth in wild rainbow trout (*Salmo gairdneri*). The enzymes tested included glutamate dehydrogenase (GDH; E.C.1.4.1.3.),[2] aspartate aminotransferase (GOT; E.C.2.6.1.1.) and alanine aminotransferase (GPT; E.C.2.6.1.2.); they are involved in the metabolism of nitrogen, carbohydrates, and energy. Laboratory experiments with channel catfish have demonstrated that the activity of the enzymes tested here do vary with dietary proteins and with growth rate (Dean 1982). Therefore, the inference is that these enzyme activities may be related to growth rate in wild fish as well; this study was an initial attempt at evaluating that inference.

Methods

Fish

The fish used in this study were age I rainbow trout from 3 streams in southwestern Virginia. The exact genetic history of the fish is unknown but they are thought to be several generations removed from stocked trout. Age I trout were selected since they were plentiful, could be easily identified, and were growing at a fast rate; differences in growth rate and enzyme activity should be easier to detect in young fish since they are more metabolically active than older fish.

Study Area

The 3 streams sampled were Spring Branch, the South Fork of the Holston River, and Lewis Fork; they were selected for this study based on differences in water quality and inferred differences in trout growth and productivity (Brayton 1981; G. Sandone, pers. commun.).

Total hardness, conductivity and pH are highest in Spring Branch and lowest in Lewis Fork (Table 1). Spring Branch is a small spring fed, second-

Table 1. Average Annual Physical-Chemical Characteristics of Study Streams

Stream	Conductivity (μmhos)	pH	Total Hardness (mg/l $CaCO_3$)	Discharge (m^3/s)
Spring Branch[a]	500	8.2	370	0.15
South Fork Holston River[b]	131	7.6	90	0.80
Lewis Fork[a]	10	6.8	6	0.12

[a] G. Sandone, pers. commun.
[b] Brayton 1981

[2] Letters immediately following the enzyme names are their abbreviations used in this paper. The second set of letters and numbers are the systematic names recognized by the International Union of Biochemistry.

order stream which flows primarily through pastures. The South Fork of the Holston River is a larger, spring fed, third-order stream which flows alternately through forests and pastures. Lewis Fork is a typical steep-gradient, second-order trout stream which flows through a heavily wooded area.

Collection, Handling and Measurements

Samples were taken in early July when water temperature was approximately 14 C in each stream. Approximately a dozen small trout were collected from each stream with a backpack electroshocker (Model BP-1C, Coffelt Electronics, Inc., Denver, CO). The stunned fish were quickly pithed and placed on ice for transport back to the lab.

Once at the lab, all fish were numbered, weighed to the nearest 0.1 g and measured to the nearest mm; some scales were removed for age determination; and livers were removed, weighed to the nearest mg and frozen for subsequent determination of enzyme activity and soluble protein concentration.

Enzyme Assays

Within 2 weeks of collection, the trout livers were thawed and homogenized in 19 volumes of 0.1 M potassium phosphate buffer (pH 7.6; 0–4 C) with a motor-driven Potter Elvehjem-type tissue homogenizer. The homogenate was centrifuged at 15,000 × g for 30 minutes at 0–4 C. The supernatant was then divided as necessary for enzyme assays and frozen at −20 C for later determination of enzyme activity and soluble protein concentration; all enzyme assays were performed within 48 hours of homogenization. Wilson (1973) reported no noticeable loss in the activity of these enzymes during a 6-month period of tissue storage. The supernatant was thawed as needed, kept on ice, and assayed within 2-3 hours for enzyme activity. The reaction mixture, to which supernatant was added, was preheated in a water bath to the reaction temperature.

Enzyme activity was quantified kinetically using a narrow band-width spectrophotometer coupled to a strip-chart recorder. Enzyme assays were conducted at 30 C and the reactions were monitored at 340 mm during the first 1-2 minutes after addition of supernatant to the reagent mix. Initial absorbance values were set at 0.6 OD and all assays were conducted in the linear range of response in measured activity to enzyme concentration. Initial rates were calculated as tissue activity (units/g liver) and specific activity (units/g soluble liver protein); a unit was defined as 1 μmole of NADH oxidized per minute at 30 C. Tissue activity corresponds to enzyme concentration in a particular tissue, e.g., the liver; it probably is most meaningful when using crude homogenates, as in this study.

Specific activity defines enzyme activity in terms of soluble tissue protein concentration. Since enzymes are proteins, specific activity reflects the relative concentration of a particular enzyme to soluble proteins, including enzymes; it becomes a more important measure when dealing with purified tissue extracts.

The supernatant was analyzed for soluble protein content by the method of Lowry et al. (1951) using bovine serum albumin as the standard. Details of enzyme assays are given by Dean (1982). All assays were performed in duplicate.

Statistical Methods

All data were subjected to 1-way analyses of variance and Duncan's new multiple range procedure (Steel and Torrie 1960). Relationships between fish length and enzyme activity were assessed with Pearsons's correlation coefficient (R). Probabilities of Type I errors are given for each correlation and analysis of variance; probabilities of 0.05 or less were considered statistically significant. Pooled SE values were computed based on the formula for pooled n with unequal sample sizes presented by Sokal and Rohlf (1969: 207).

Results and Discussion

Fish Size

A few trout collected from Lewis Fork and the South Fork of the Holston River were determined by scale analysis to be age II and, therefore, were not included in the results. The final sample sizes were 7, 11 and 11 age I rainbow trout from Lewis Fork, the South Fork of the Holston River and Spring Branch, respectively.

Table 2. Average Size and Condition Factor of Age I Rainbow Trout from Study Streams

Stream	n	Total Length (mm)	Body Weight (g)	Condition Factor (g/dm^3)
Spring Branch	11	173A[a]	55.5A	10.44
		(4)[b]	(4.5)	(0.31)
South Fork Holston River	11	156B	36.1B	9.35
		(3)	(1.9)	(0.14)
Lewis Fork	7	115C	14.9C	9.84
		(1)	(0.9)	(0.60)
P-value		<0.001	<0.001	ns[c]

[a] Means within a column not sharing a common letter are statistically different ($P < 0.01$).
[b] Values in parentheses are SE.
[c] $P > 0.05$

Average total length and body weight values differed significantly among trout in the three streams, as expected ($P < 0.001$, Table 2). The observed lengths and weights covered the range expected for age I trout (Carlander 1969); wide differences in growth were needed for evaluating the growth indices. Trout from Spring Branch were 50% longer and 272% heavier than trout in Lewis Fork; the South Fork of the Holston River trout were intermediate in both parameters.

Condition factor of the trout did not differ significantly among streams (Table 2). The fish were apparently in good health and none appeared to be starving.

Enzyme Activity

GOT tissue and specific activity, and GDH tissue activity, differed significantly among trout in the 3 streams (Table 3, $P < 0.01$ and $P < 0.05$ respectively). GOT and GDH activities were both higher in trout from Lewis Fork than trout from Spring Branch. The most reasonable inference is that differences in enzyme activities relate to differences in food intake and nitrogen and energy metabolism. Both enzymes are important in the production of glucose and energy from amino acids, an active process in fish metabolism (Nagai and Ikeda 1973, Wilson 1973).

The implication, then, is that the fish with the lower food supply had to use proportionately more of their available amino acids to meet their energy needs. The enzymes necessary for the conversion of amino acids to glucose or energy (GOT and GDH) become more concentrated, and growth was lower since amino acid intake was reduced and proportionately more of the amino acids were being used for energy, not tissue synthesis. The strong,

Table 3. Average Liver Enzyme Activity in Age I rainbow Trout from Study Streams

		Enzyme					
		GDH		*GOT*		*GPT*	
Stream	n	(U/g)[a]	(U/P)[b]	(U/g)	(U/P)	(U/g)	(U/P)
Spring Branch	11	55.2A[c]	690A	35.1A	439	7.9	99
South Fork Holston River	11	64.7B	691A	41.5AB	444	7.0	75
Lewis Fork	7	75.9C	829B	48.7B	532	10.0	109
Pooled SE		3.0	37	3.2	38	0.9	10
P-value		<0.01	<0.05	<0.05	ns[d]	ns	ns

[a] Enzyme activity in units per g of liver, 1 unit = 1 μmole of NADH oxidized per minute at 30 C.
[b] Units per g of soluble liver protein.
[c] Means within a column not sharing a common letter are statistically different ($P < 0.05$).
[d] $P > 0.05$.

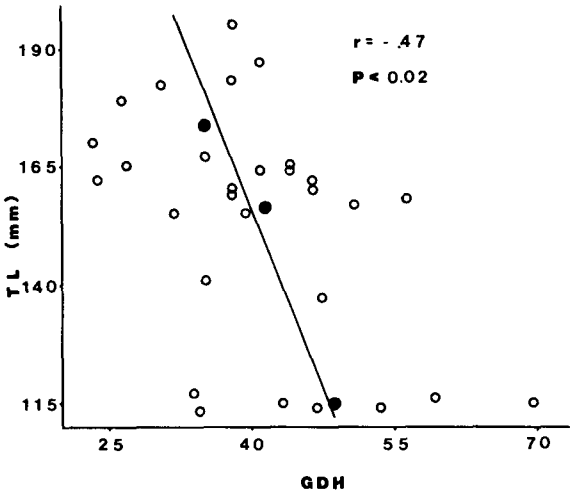

Figure 1. Relationship between hepatic glutamate dehydrogenase activity and total length in age I rainbow trout from 3 streams. Open circles represent individual fish whereas closed circles represent stream means.

positive correlation between tissue GOT and GDH activity ($P < 0.0001$) supports the hypothesis that both enzymes are involved in the same metabolic process.

In contrast, GPT activity did not differ among trout in the 3 streams ($P > 0.05$, Table 3). Apparently, GPT is not actively involved in the same metabolic pathways as GOT and GDH. GPT is, however, known to function in the transport of alanine, an amino acid, from skeletal muscle to the liver (Cornish et al. 1978). The alanine transport pathway, actually an alanine-glucose cycle, does not necessarily involve GOT or GDH.

Enzyme activity values presented in Table 3 must be adjusted for the difference in temperature between the streams (14 C) and the assay procedure (30 C) to be physiologically meaningful. Assuming a Q_{10} coefficient of 2, the given values are about 3 times higher than they would be if the assay temperature had been 14 C; i.e., $2^{1.6} = 3.0$. Lack of proper equipment prevented us from performing the assays at 14 C, as desired, but the effect was the same for all samples.

Similar adjustments were made to published values so that results could be compared. The GOT values reported here are in the range found by Schlisio and Nicolai (1978), 10 to 50% higher than given by Gaudet et al. (1975), and up to double that presented by D'Apollonia and Anderson (1980) and Jurss (1978). Comparisons among studies are complicated by

differences in assay procedures and conditions. Gaudet et al. (1975) reported GDH values for rainbow trout as low as ½ of our values, but their assay conditions were different.

In contrast, GPT values reported in the other studies were 4- to 9-fold higher than those in the present study (Gaudet et al. 1975, Jurss 1978, Schlisio and Nicolai 1978, D'Apollonia and Anderson 1980). The reason for our relatively low GPT values is not known. All 4 of the published studies, however, involved cultured trout fed artificial feeds on an *ad libitum* basis whereas the present study involved wild trout which had to compete for natural food items and were presumably more active. Differences in GPT values may simply reflect differences in metabolic needs between fish in wild versus cultured conditions. Wilson (1973) found that cultured channel catfish had significantly higher ($P < 0.05$) liver tissue GPT activity than did wild channel catfish.

Growth and Enzyme Activity

To be useful as an index of growth, enzyme activity must be correlated with some measure of growth. For development of a growth index, the best parameter to compare with enzyme activity data is short-term growth rate data based on changes in either length or weight. In this preliminary study, however, we chose total length of same age trout as our measure of growth rate.

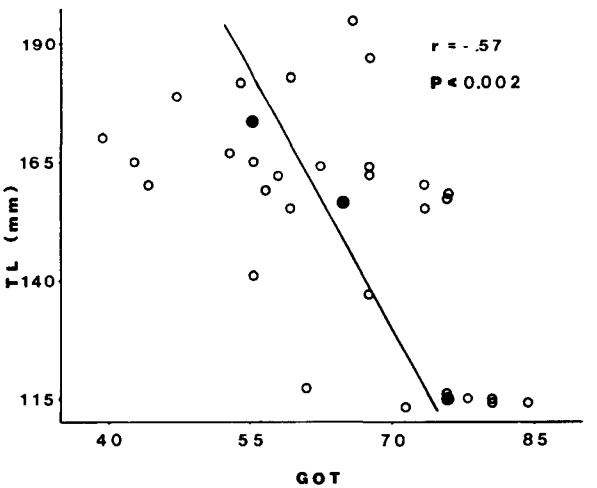

Figure 2. Relationship between hepatic aspartate aminotransferase (glutamate oxaloacetate transaminase) activity and total length in age I rainbow trout from 3 streams. Open circles represent individual fish whereas closed circles represent stream means.

Total length of age I trout from the 3 streams was negatively correlated with liver tissue GOT and GDH activity ($P < 0.002$ and $P < 0.02$ respectively; Figs. 1 and 2). Although the correlations were statistically significant, the correlation coefficients were not high and, thus, predictions based on individual fish would probably not be accurate; $r = -0.47$ and -0.57 for total length versus GDH and GOT activity, respectively (Figs. 1 and 2). We suggest basing any inferences about growth rate on the enzyme activity data from the mean of at least 10 fish. Also, the relationships presented here are really based on 2 different time periods—growth since hatching, and enzyme activity for a particular instant in July.

We feel that liver enzyme activity is a potential index of growth rate in wild fish and may prove useful in rapidly evaluating various management strategies. Future investigations should be based on growth rate data collected over small time intervals (1 month) throughout the year with concomitant determinations of enzyme activity and other factors including water temperature and food consumption.

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