Electrophoretic patterns of blood serum proteins from rainbow trout (Salmo gairdneri)
by Robert Vance Thurston

A thesis submitted to the Graduate Faculty in partial fulfillment of the requirements for the degree of DOCTOR OF PHILOSOPHY in Zoology
Montana State University
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Abstract:
Electrophoretic patterns on acrylamide gels of blood serum proteins from over 400 hatchery rainbow trout (Salmo gairdneri) subjected to different environmental stress have been examined. Stresses to which fish were subjected were: reduction of oxygen, addition of copper sulfate, addition of sodium sulfite, and different methods of capture. Comparisons of patterns were made on the basis of sex, degree of maturity, and location of blood extraction. Comparisons were also made with blood protein patterns of wild rainbow trout taken from a local stream.

Patterns showed marked intraspecific differences, and revealed a characteristic band for sexually ripe females. Significant variations were found which were attributed to degree of maturity, method of sampling, repeated sampling of the same individual, and method of capture. No significance was attributed to variations related to reduction of environmental oxygen or the addition of sub-lethal levels of sodium sulfite. Significant pattern variations were noted with the addition of copper sulfate. Comparisons with wild rainbow trout showed significant pattern differences.

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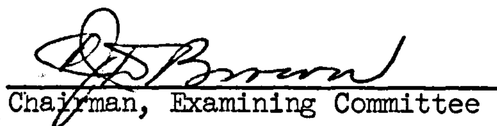
Approved:

Head, Major Department


Chairman, Examining Committee

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ABSTRACT

Electrophoretic patterns on acrylamide gels of blood serum proteins from over 400 hatchery rainbow trout (Salmo gairdneri) subjected to different environmental stress have been examined. Stresses to which fish were subjected were: reduction of oxygen, addition of copper sulfate, addition of sodium sulfite, and different methods of capture. Comparisons of patterns were made on the basis of sex, degree of maturity, and location of blood extraction. Comparisons were also made with blood protein patterns of wild rainbow trout taken from a local stream.

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INTRODUCTION

Intraspecific differences in the electrophoretic patterns of fish blood serum proteins have been reported by several researchers. Many of these are described in a review by Booke (1964). Changes in the serum protein patterns of fish subjected to lethal concentrations of sodium fluoride were observed by Neuhold and Sigler (1960). Pattern changes related to pollution stress from Kraft pulp mill wastes were reported by Fujiya (1961), and changes as a result of low dissolved oxygen tension and method of capture were studied by Bouck and Ball (1965, 1966). Other electrophoretic studies concerning variations in fish serum proteins were related to: changes in diet (Drilhon, et al., 1956, Lysak and Wojik, 1960, and Thomas and McCrimmon, 1964); sexual differences and degree of sexual maturity (Rall, et al., 1961, Vanstone and Chung-Wai Ho, 1961, Drilhon and Fine, 1963, Kirsipuu, 1964a, and Thomas and McCrimmon, 1964); temperature differences (Meisner and Hickman, 1962); seasonal variations (Saito, 1957, Kirsipuu, 1964b); disease (Sindermann and Mairs, 1958, Fine et al., 1963, and Thomas and McCrimmon, 1964). No study was found which included a variety of tests on fish from the same stock.

In the present study electrophoretic patterns of blood serum proteins were determined for 402 hatchery and 12 wild rainbow trout (Salmo gairdneri). The objectives were to determine: if a characteristic serum protein pattern is present among fish of the same species and from the same stock; what variations exist among serum patterns of individuals subjected to physical or chemical stress prior to blood extraction; what variations may

exist among serum patterns of hatchery and wild fish of the same species. The study was done between July, 1964, and July, 1966.

MATERIALS AND METHODS

Test Fish and Holding Facilities

Approximately 450 blood samples were taken from 414 rainbow trout. Of these fish, 402 were from the Bozeman National Fish Hatchery, Montana. These ranged in total length from 15.2-34.3 cm and in age from 15-25 months. Twelve wild fish of unknown ages from a local stream were used. These varied in total length from 15.2-21.6 cm. A total of 340 fish from the same stock was used in tests to compare age and sex differences and effects of physical stress. The remaining 62 fish were from a different stock and were used for chemical stress tests.

The total length, sex, and degree of maturity of each fish were determined after blood sampling. Fish were classified as "immature" if the gonads were poorly developed and sex was difficult to determine without microscopic examination. Fish were classified as "mature" if the gonads were developed so that sex was apparent without magnification. Mature fish were classified as "ripe" if the gonads were in spawning condition to the degree that eggs or sperm were readily released when the fish was handled.

Outside raceways at the hatchery and laboratory tanks at Montana State University were used for holding test and control fish. The raceways were of concrete (1.8 x 18.5 m), and supplied with spring water having an annual temperature range of 5-15 C. The laboratory tanks were of fiberglass (1.2 m diameter), and supplied with dechlorinated city water with a 10-17 C annual temperature range. The tanks were maintained at 700 l capacity

during all tests. Fish transported to the University from the Hatchery were allowed an adjustment period of 24-48 hours before experiments began. One thousand fish were planted in a local pond which had a surface area of approximately 2500 m^2 and a maximum depth of 7 m. Of these, seven were recovered after 5 days and sampled.

Blood Collection and Preparation

Immediately after removal from their test environments, fish were anaesthetized for a period of 90-120 sec in a solution (0.25 grams per liter) of tricaine methane sulfonate (Sandoz MS-222). Blood samples were taken by means of a hypodermic syringe. Blood was extracted from the heart by inserting a needle into the center of the isthmus on a line drawn between the origins of the pectoral fins. A number 18 needle was used which allowed blood to flow freely and with minimum damage to the blood cells. A total of 370 fish was sampled in this manner. In an experiment requiring repeated sampling of the same individual, blood was extracted from the dorsal aorta (Schiffman, 1959) using a number 22 needle to minimize hemorrhaging and damage to the muscle tissue. A total of 19 fish was sampled by this means. Blood was taken from 25 fish by a variety of other methods, including transversely slitting the isthmus, severing the caudal peduncle, and opening the pericardial cavity to pierce the heart directly. Some satisfactory serum samples were obtained by each of these methods, but none produced clear samples with the consistency of the first two methods described.

Each sample, after being drawn into a syringe, was slowly ejected into a centrifuge tube and stored at 3-5 C for 2-18 hr to allow clotting. Serum was centrifuged at 15,500 rcf x g for 3-5 min, and stored at -15 C for periods up to 18 months before electrophoresis.

Electrophoresis

The gel electrophoresis method used for serum separation was modified from Davis (1964). An eight-position plexiglass electrophoresis cell was built using platinum electrodes in place of carbon electrodes to eliminate polarization. Double O-rings were used in place of grommets to ensure constant alignment of gel tubes with relation to the poles of the electric field, thereby reducing inconsistencies among tubes and between runs. A separate cell which would accommodate tubes of larger diameter was employed for destaining. The tube diameter difference permitted insertion of stained gels into the destaining tubes without damage. The amount of serum used was usually 1.5 µliter, although comparative samples were run in amounts varying from 0.5-5.0 µliter. The reagent concentrations used for the small pore gel were those recommended by Davis (ibid.). The large pore gel and spacer gel concentrations were increased by one-third to ensure rapid and firm setting. Freshly purchased acrylamide gave more consistant results than that which had been on hand for over a year without refrigeration. The aniline blue black fixative stain was re-used no more than once. Both electrophoresis and destaining were performed under refrigeration at 3-5 C to minimize protein denaturation. The electrophoresis was done at 2.5 ma/tube and at approximately 30 v/cm for 1.5 hr.

The destaining was accomplished at 5.0 ma/tube and at approximately 20 v/cm for 3-4 hr. Each electrophoresis run included seven test samples and one control sample. The control was horse serum which maintained a constant protein pattern throughout the study.

Data Analyses

Comparisons among gels were made on the basis of protein distribution as reflected in the number of bands, the relative distance migrated, and the concentration. As many as 16 bands were discernable in some gels, although 10 was the maximum number that appeared with consistency. In order to make meaningful comparisons among gels it was necessary to have some common method of comparison. A densitometer tracing or "profile" was obtained for each gel using a National Instruments Laboratory "Chromoscan." A special cam was used in the Chromoscan to approximate a direct relationship between light absorption and protein-bound dye. The height of the profile curve describing any point along the gel was assumed to correspond to the amount of protein-bound dye in the gel at that point. All gel profiles were divided into 10 fractions, corresponding to the 10 most commonly occurring bands.

A representative gel and its corresponding densitometer profile are illustrated in Figure 1. Although this profile is divided into 10 standard fractions, two bands can be seen grouped in fraction 10, and three bands in fraction 9. The profile indicates the possibility of additional bands in fractions 8 and 6 although these are not clearly discernible in the gel.

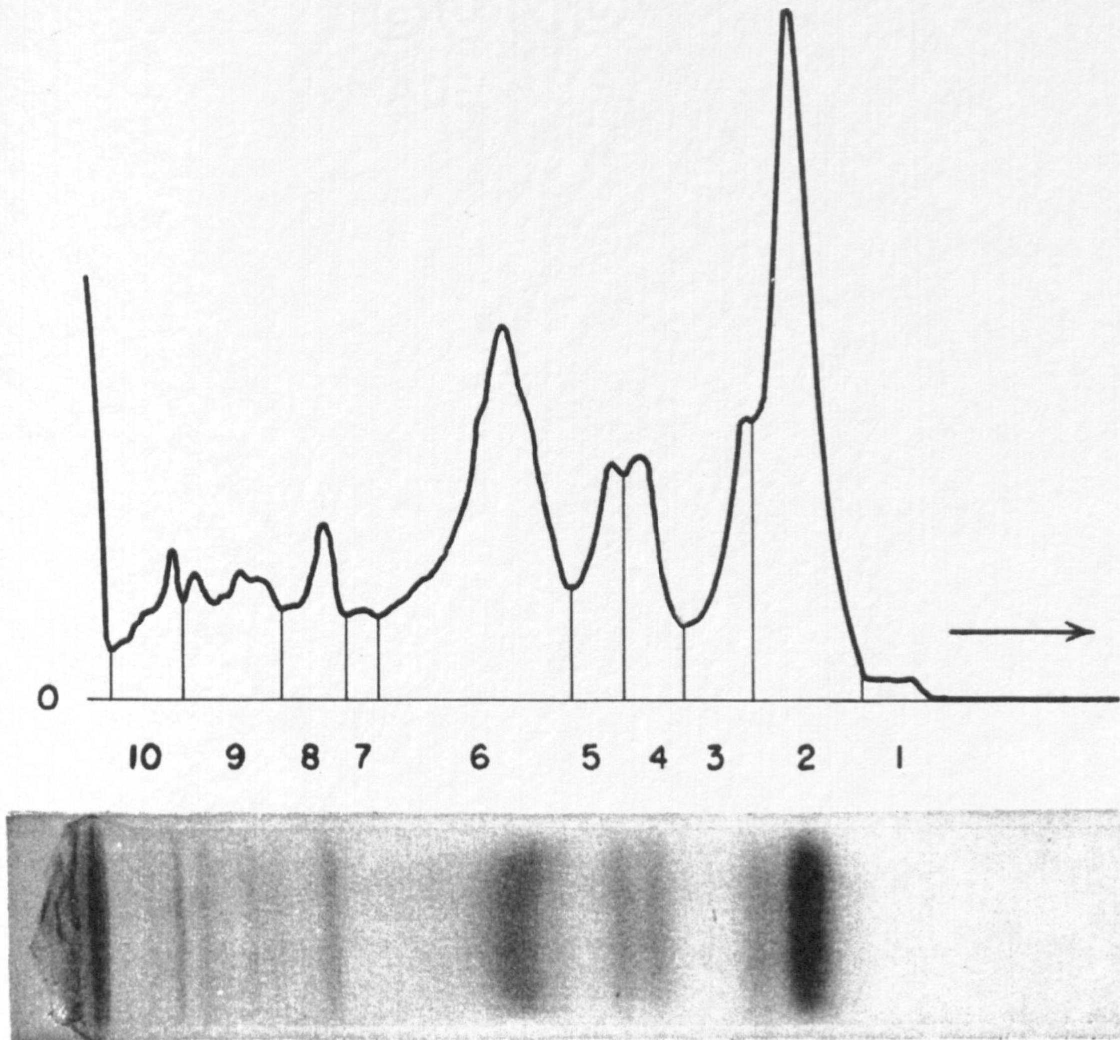

Figure 1. Disc gel electrophoresis pattern of serum from wild rainbow trout (Figure 5C) and corresponding densitometer profile. Origin for protein migration is at "O" and direction of migration toward positive electrode is shown by arrow. Numbers indicate profile fractions.

Fraction 1 contains the most rapidly migrating band and fraction 10 contains the bands with the least electrophoretic mobility. Fraction 10 is directly adjacent to the starting plane for all protein, i. e. the surface between the spacer gel and the small pore gel.

The relative distances these 10 groups of bands migrated from the starting plane were consistent, although there were wide variations in their intensities. These intensity variations occurred among different bands within the same gel and between corresponding bands of different gels. Some of the wide intraspecific variations among rainbow trout serum patterns are illustrated in Figures 2-5. The patterns obtained from the same serum sample in different gels were consistent, even when run at different times (Fig. 2).

Each pattern profile was measured with a polar planimeter. The area of each profile fraction was computed as a percentage of the whole. Comparisons were then made among profiles on the basis of these percentages for correspondingly numbered fractions. In a test to determine the replicability of this method, 2 runs were made each containing 2 gels at four different concentrations of the same serum sample (0.6, 0.9, 1.2, 1.5 µliter). At any concentration level the deviation among corresponding protein fractions did not exceed 3% of the total protein.

Statistical comparisons among gel profiles of fish used in a test to determine maturity and sex related differences were made by a two-way analysis of variance. A one-way analysis of variance and Duncan's New Multiple Range test were used for all other test comparisons. Significance was assigned whenever the probability of random occurrence was less than 5%.