ELECTROPHORETIC PATTERNS OF BLOOD SERUM PROTEINS FROM RAINBOW TROUT (SALMO GAIRDNERI)

by

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of

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in

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VITA

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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.

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 an imaginary 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.

which 25 fish ; in what tests

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 separations were affected by centrifugation at 15,500 rcf for 3-5 min. Serum was then stored at -15 C for periods up to 18 months before electrophoresis.

Electrophoresis

The gel electrophoresis method used for serum separations 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. 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.

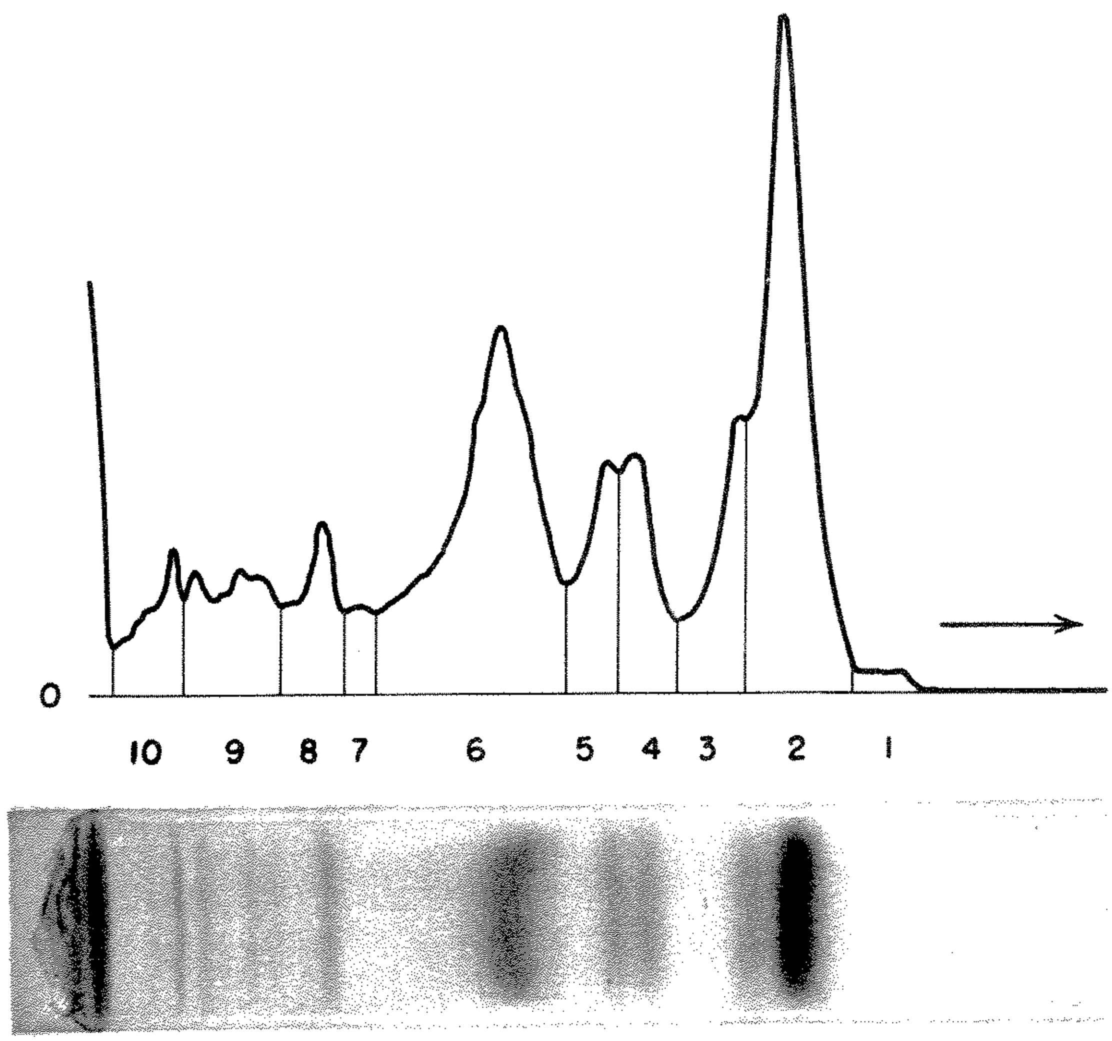


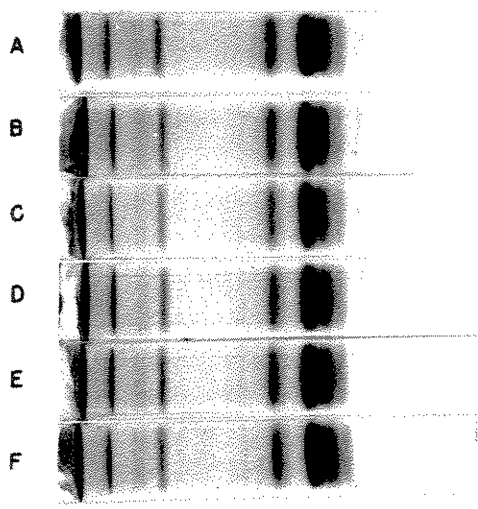
Figure 1. Disc gel electrophoresis pattern of serum from wild rainbow trout (Figure 5C) and corresponding densitometer profile. Origin for protein migration is at "0" and direction of migration toward positive electrode is shown by arrow. Numbers indicate profile fractions.

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%.



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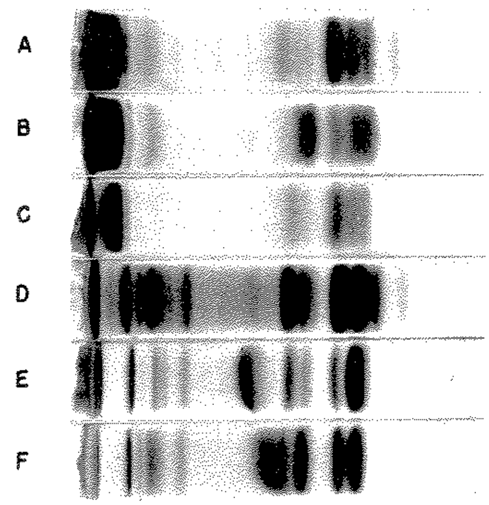

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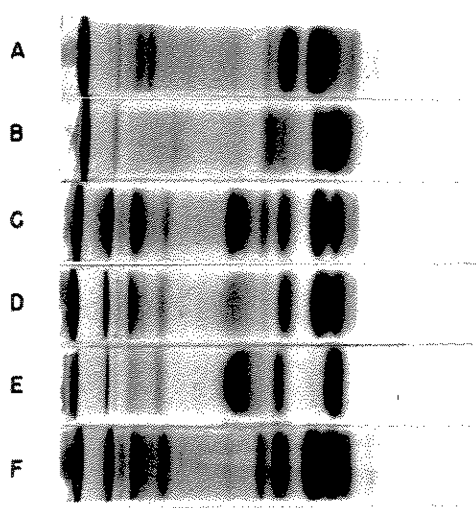

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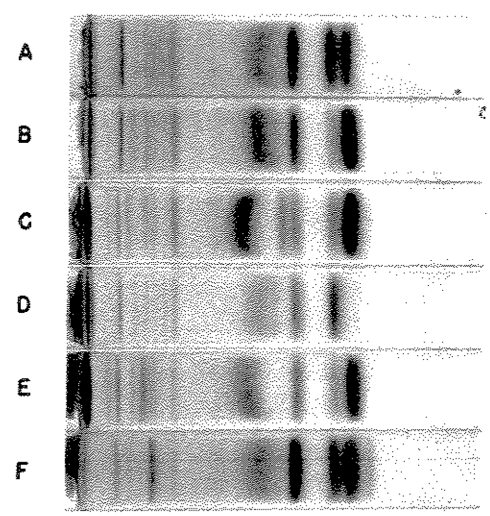

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Figure 2. Patterns from one rainbow trout serum sample. A, B, C run simultaneously, D, E, F run simultaneously.

Figure 3. Serum patterns of female rainbow trout. A, B ripe, C, D mature, E, F immature.

Figure 4. Serum patterns of male rainbow trout. A, B ripe, C, D mature, E, F immature.

Figure 5. Serum patterns of wild rainbow trout. A, B, D, E mature females, C mature male, E ripe male.

RESULTS

Sex and Maturity

To determine possible pattern variations attributable to sex and maturity differences, an analysis was made of serum samples from 20 male and 28 female rainbow trout which were collected by dip net directly from the hatchery raceway. Two of the males were immature, 14 were mature, and four were ripe. Fourteen of the females were immature, and 14 were mature. The mean values and range for each fraction in each of these sex-maturity categories are presented in Table 1.

Table 1. Serum protein fractions of blood from rainbow trout in relation to sex and maturity. Fractions expressed as per cent of total protein. Number of individuals in parentheses.

Sex and Maturity	Fraction Number									
	1	2	3	4	5	6	7	8	9	10
Immature males (2)										
Mean	1.3	28.5	9.8	15.3	5.4	12.5	2.8	4.7	11.5	8.3
Range	0.6- 2.0	23.7-33.3	8.8-10.7	11.6-18.9	4.8- 5.9	12.0-13.0	2.4- 3.2	4.0- 5.3	11.2-11.8	7.7- 8.8
Mature males (14)										
Mean	1.3	25.2	17.2	13.3	7.5	11.2	2.8	4.5	9.4	7.5
Range	0.8- 2.3	15.2-38.2	7.8-32.7	5.7-22.9	3.4-11.6	8.5-16.1	1.3- 4.7	2.9- 6.3	7.5-11.4	4.9-11.7
Ripe males (4)										
Mean	1.2	21.4	23.1	15.7	4.4	17.2	2.6	3.3	6.6	4.5
Range	0.7- 2.0	13.6-31.6	16.2-35.9	11.2-19.5	2.8- 5.3	9.0-26.8	2.0- 3.0	2.3- 4.2	5.5- 8.9	4.2- 4.9
Immature females (14)										
Mean	1.4	25.6	17.5	12.7	5.1	14.6	3.2	4.3	9.4	6.3
Range	0.5- 1.9	13.5-36.8	9.2-33.5	8.3-19.3	2.4- 8.8	10.7-19.3	1.8- 4.7	2.8- 5.8	7.0-11.2	4.3- 8.8
Mature females (14)										
Mean	1.6	22.2	20.3	14.3	5.8	10.7	2.5	3.8	8.6	10.1
Range	0.3- 2.8	5.0-42.4	6.3-35.4	10.1-18.5	2.2-13.8	4.3-14.6	0.6- 6.4	2.6- 5.2	4.4-11.8	5.8-33.1

Significant differences were observed in fractions 6 and 9 which were related to maturity, but none in any fraction which could be attributed to sex. The F values of all fractions are reported in Table 2.

Table 2. Profile fraction F values and corresponding significant F values at 95% confidence level.

Experiment	Profile Fraction F Values 1	2	3	4	5	6	7	8	9	10	Significant F Values
Sex	1.09	0.15	0.37	0.23	2.93	0.03	0.06	0.85	0.00	0.99	4.08
Maturity	0.39	0.70	1.36	1.04	2.98	9.96*	1.16	2.09	6.09*	3.04	3.23
Method of capture	7.00*	1.86	0.72	3.25*	0.96	0.06	0.22	5.12*	1.16	3.80*	2.68
Repeated sampling (males)	0.21	0.06	0.17	0.12	0.55	0.88	0.69	2.83	2.19	11.03*	6.94
Repeated sampling (females)	1.08	0.28	0.29	0.38	0.16	4.53*	0.73	0.45	4.03*	2.74	3.47
Location of blood extraction	0.83	2.13	0.52	5.69*	0.71	0.67	2.36	6.85*	1.49	4.88*	4.49
Low dissolved oxygen	0.40	4.32	2.71	0.34	2.01	0.13	1.85	1.21	0.02	0.52	4.35
Copper sulfate and sodium sulfite	1.25	0.53	0.02	2.93*	0.88	1.43	5.53*	1.89	0.72	5.03*	2.84
Wild and hatchery stocks	16.64*	2.43	0.14	1.26	2.55	69.84*	2.24	13.35*	0.53	2.70	4.20

* Significant differences

Only two ripe females were found in all the hatchery fish tested. Both of these showed amounts of protein in fraction 10 which were too large to measure by the Chromoscan. These bands were estimated to contain more than 70% of the total protein (Fig. 3). Samples from eight mature females observed in other tests also evidenced this exceptional fraction — the gel from one of these is shown in Figure 3. Fraction 10 did not exceed 13% in samples from males or immature females. An intense band also appeared in serum samples from two ripe rainbow trout and two ripe cutthroat trout (Salmo clarki) taken from nearby streams and lakes.

Because of the statistical differences related to maturity, and the observed differences related to sex, certain data were discarded from subsequent experiments so that comparisons were made between test and control groups only for fish of like sex or maturity.

Method of Capture

Comparisons were made among 126 fish to determine the effects of different methods of capture on blood serum patterns. The methods used were: hook and line, gill net, electroshocking, dip net. Twenty-one fish were caught with artificial bait by hook and line after they were placed in an isolated section of the hatchery raceway. Each fish was played for approximately 45 sec, although it was noted that active resistance to capture diminished markedly after 15-25 sec. Eighteen fish were "caught" by a gill net placed in the hatchery raceway. Each fish was placed into the net by hand, ensuring that the threads did not inhibit the opercular apparatus. Fish were left in the net for 4-21 hr before removal for blood extraction. Blood was taken from 39 fish after electroshocking. Twenty fish were electroshocked simultaneously in an isolated raceway section. Five of these were removed, placed in a holding tank, and blood was taken from them within 5 min. The remaining 15 fish were then re-shocked, four of these were removed and placed in a holding tank, and these were also sampled within 5 min. This procedure was continued, 4-5 fish being removed after each electroshocking, until the last fish taken from the raceway had been electroshocked five times in a period of 20 min. Another group containing 19 fish was treated in an identical manner. A total of 48 fish was captured by dip net. This was the same group of fish used in the sex and maturity variation study.

Comparisons were made among the serum protein patterns of fish caught by these four methods, and the mean profile values and ranges are presented in Table 3. The sex and maturity of these fish are listed in Table 4.

Table 3. Serum protein fractions of blood from rainbow trout captured by different methods. Fractions expressed as per cent of total protein. Number of individuals in parentheses.

Method of Capture	Fraction Number									
	1	2	3	4	5	6	7	8	9	10
Dip net (48)										
Mean	1.4	24.3	18.4	13.7	6.0	12.6	2.8	4.1	9.0	7.7
Range	0.3- 2.8	5.0-38.2	6.3-35.9	5.7-22.9	2.2-13.8	4.3-26.8	0.6- 6.4	2.4- 6.3	4.4-11.8	4.2-33.1
Hook and line (21)										
Mean	1.0	28.2	19.4	11.5	6.8	12.3	3.1	4.0	8.2	5.5
Range	0.3- 3.0	3.7-49.6	7.9-47.6	7.8-16.1	3.5-16.5	9.8-16.5	1.1- 6.1	2.0- 6.2	5.9-11.4	2.2- 8.5
Gill net (18)										
Mean	0.9	23.3	21.7	13.4	5.7	12.7	3.0	4.5	8.8	6.2
Range	0.3- 2.6	4.1-37.9	8.3-50.6	7.8-20.4	4.1- 9.5	9.6-18.0	1.8- 4.3	2.7- 6.9	6.3-11.9	3.6- 7.8
Electroshocking (39)										
Mean	0.9	22.5	20.2	14.0	6.2	12.6	3.0	5.0	8.8	6.8
Range	0.3- 2.3	4.0-39.6	9.5-35.8	4.8-19.7	2.1-10.6	8.0-23.9	0.8-14.0	3.1- 8.2	4.6-11.6	3.4-11.9

Table 4. Sex-maturity categories of rainbow trout used in analyses of different capture methods.

Sex-Maturity	Dip net	Hook and line	Gill net	Electro-shocking
Immature males	2	6	3	6
Mature males	14	0	0	14
Ripe males	4	4	9	0
Immature females	14	10	6	18
Mature females	14	1	0	1
Ripe females	0	0	0	0
Totals	48	21	18	39

It was not possible to select any one sex-maturity category which contained enough individuals to represent adequately all four capture methods. Accordingly, any interpretation of the data from these experiments must be in the light of possible sex or maturity bias.

The dip net method of capture showed significant differences from all other methods in fraction 1, between hook and line in fractions 4 and 10, and between electroshocking in fraction 8. The electroshocking and hook and line methods showed significant differences between each other in fractions 4 and 8. The two fractions which showed significant differences in sex and maturity experiments showed no significant differences in these experiments.

Bouck and Ball (1966) have reported significant differences in plasma protein patterns of rainbow trout caught by seining, hook and line, and electroshocking. They used paper electrophoresis and obtained a profile of five plasma fractions. The differences found by these researchers and those observed in the present study cannot be further compared because the methods of analysis were different.

Repeated Sampling

Four mature males and eight immature females were sampled three times to determine the effect of repeated sampling on blood serum patterns. Each sample contained 0.4-0.6 ml whole blood drawn from the dorsal aorta through the roof of the mouth. During the experiment the fish were held in tanks with a maximum of 15 in each. The second blood sample from each individual was taken eight days after the first, and the third sample seven weeks after the second. Blood was taken from additional fish but these sustained considerable tissue damage during one of the first two blood extractions and were removed from the test lot. Some samples were unusable because of excessive hemolysis.

In order to avoid bias in analysis which might result from sex or maturity, the mature males were treated in one group and the immature females in another (Table 5). A separate one-way analysis of variance was made on the data from each of these groups. The males showed a significant increase in fraction 10 between the second and third samples, and the females showed a significant increase in fractions 6 and 9 between the first and second samples.

Table 5. Serum protein fractions of blood from rainbow trout subjected to repeated blood extraction. Fractions expressed as per cent of total protein. Number of individuals in parentheses.

Time of Sample	Fraction Number									
	1	2	3	4	5	6	7	8	9	10
Males (4)										
First										
Mean	1.0	21.0	18.0	17.1	5.1	11.5	2.8	6.0	9.5	8.2
Range	0.5- 1.9	6.3-40.2	5.4-26.7	16.9-18.8	4.4- 6.3	9.8-12.7	2.0- 3.8	4.4- 8.3	8.3-10.6	6.4- 9.1
Second										
Mean	1.3	22.4	17.2	16.7	4.8	13.1	2.6	4.4	10.6	7.1
Range	0.6- 2.4	14.7-39.2	7.4-26.5	15.3-18.0	2.8- 7.0	9.8-15.9	1.2- 3.8	3.4- 5.3	10.2-11.4	6.1- 8.0
Third										
Mean	1.7	24.1	21.3	17.3	4.3	10.9	2.0	4.1	9.2	5.3
Range	0.4- 5.4	13.4-42.2	6.0-34.4	15.3-20.1	4.0- 4.8	7.5-13.0	1.0- 3.2	3.3- 5.2	7.7-10.1	5.0- 5.7
Females (8)										
First										
Mean	1.3	18.3	26.7	15.1	4.9	11.4	2.0	5.3	8.0	7.1
Range	0.5- 2.1	6.6-38.5	7.2-38.5	13.5-18.0	3.3-6.4	9.5-11.8	1.3- 2.9	4.5- 7.1	6.4- 9.9	5.4- 8.0
Second										
Mean	1.4	15.6	24.2	15.2	4.8	14.0	2.3	5.0	10.0	7.5
Range	0.5- 2.9	4.3-29.5	12.1-33.7	13.2-16.9	3.6- 6.3	11.2-18.6	1.7- 3.3	3.3- 6.3	7.5-11.8	4.1- 9.3
Third										
Mean	1.0	15.2	28.0	15.7	4.6	12.7	2.4	5.5	8.9	6.0
Range	0.2- 1.7	6.0-26.8	15.6-41.2	13.6-18.7	2.8- 5.8	10.6-15.4	1.6- 4.0	3.9- 7.3	7.7-12.8	4.7- 8.9

Location of Blood Extraction

An analysis was made comparing the profiles of eight immature females sampled from the dorsal aorta with 10 immature females sampled from the heart to determine possible pattern variations resulting from the location of blood extraction (Table 6). All fish were held in tanks at the University. Data were not analysed for other fish which were bled after severing the caudal peduncle or opening the pericardial cavity because only a few satisfactory samples were obtained from immature females.

Table 6. Serum protein fractions of blood taken from different locations in rainbow trout. Fractions expressed as per cent of total protein. Number of individuals in parentheses.

Location	Fraction Number									
	1	2	3	4	5	6	7	8	9	10
Heart (10)										
Mean	0.9	25.3	22.8	12.6	5.4	12.3	2.4	3.7	8.8	5.7
Range	0.2- 3.3	5.4-35.2	13.5-49.4	9.0-17.5	3.0- 7.2	8.6-16.5	1.6- 3.0	1.9- 7.1	6.3-11.5	3.9- 7.8
Dorsal aorta (8)										
Mean	1.3	18.3	26.7	15.1	4.9	11.4	2.0	5.3	8.0	7.1
Range	0.5- 2.1	6.6-38.5	7.2-38.5	13.5-18.0	3.3- 6.4	9.5-15.3	1.3- 2.9	4.5- 7.1	6.4- 9.9	5.4- 8.0

Significant differences in profiles of blood taken from the dorsal aorta and blood taken from the heart were found in fractions 4, 8, and 10.

Low Dissolved Oxygen

An analysis was made of blood serum profiles of 10 fish subjected to low dissolved oxygen for periods up to 48 hr. Duplicate experiments were run in tanks, each involving 10 test and 10 control fish. At the start of each experimental period the incoming water was shut off in both test and control tanks. Dissolved oxygen was maintained in the control tanks by bubbling air through the water, and during the experiments the levels declined from 10.0 ppm to 7.0 ppm. In both test tanks the dissolved oxygen levels dropped from 10.0 ppm to 5.5 ppm during the first 12 hr, to 3.0 ppm after 24 hr, and to 1.3 ppm by the end of 48 hr. In the test tanks the carbon dioxide levels rose from 4.0 ppm to 11.0 ppm, while in the control tanks it remained at 4.0 ppm. Ammonia levels in the test tanks rose from 0.5 ppm to 1.8 ppm, and in the control tanks from 0.5 ppm to 1.3 ppm.

Only profiles of immature fish were subjected to data analysis because these comprised the largest maturity group tested. The pooled data (Table 7)

Table 7. Serum protein fractions of blood from rainbow trout subjected to low dissolved oxygen, copper sulfate, and sodium sulfite. Fractions expressed as per cent of total protein. Number of individuals in parentheses.

Test	Fraction Number									
	1	2	3	4	5	6	7	8	9	10
Low oxygen (10)										
Mean	1.0	19.1	28.4	11.8	6.4	11.8	2.0	4.6	9.0	6.0
Range	0.3- 1.5	4.4-33.6	9.2-41.0	6.4-16.4	3.8-11.2	9.1-19.5	1.2- 3.4	2.3- 8.1	6.7-12.0	3.7- 9.2
Control - Low oxygen (12)										
Mean	1.0	27.4	20.8	12.5	5.2	12.2	2.5	3.9	9.1	5.5
Range	0.2- 3.3	5.4-42.0	8.8-49.4	9.0-17.5	3.0- 7.2	8.6-16.5	1.5- 4.8	1.9- 7.1	6.3-12.7	3.6- 7.8
Copper sulfate (10)										
Mean	0.8	20.9	14.3	10.7	6.0	21.8	3.1	5.6	9.2	7.6
Range	0.2- 1.4	9.9-29.5	5.8-26.4	8.4-12.9	3.5- 9.5	16.1-28.2	1.8- 4.6	4.0- 9.9	5.8-13.0	5.3-12.2
Sodium sulfite-1 (9)										
Mean	0.7	22.3	13.8	10.8	7.6	24.4	2.1	4.2	8.3	5.7
Range	0.3- 1.3	15.4-33.6	6.2-20.2	4.3-15.0	2.9-17.3	20.4-28.5	1.1- 3.3	2.7- 4.9	7.4-11.1	4.8- 7.2
Sodium sulfite-2 (12)										
Mean	0.6	18.8	14.2	10.7	7.8	24.4	2.5	4.5	9.4	7.2
Range	0.3- 1.2	9.1-31.3	4.4-23.4	6.9-14.5	3.3-13.5	19.6-28.9	1.9- 3.2	2.8- 6.7	6.5-12.3	5.2-11.8
Control - Copper sulfate, Sodium sulfite (20)										
Mean	0.6	20.3	13.8	13.1	6.6	24.1	2.1	5.0	8.6	5.9
Range	0.2- 1.2	8.3-33.6	4.8-25.4	6.5-19.9	3.5-15.5	13.4-28.7	1.0- 3.4	2.5- 8.3	5.0-15.7	3.5- 8.0

include four test and five control fish from the first experiment, and six test and seven control fish from the second experiment. No significant differences occurred in the serum fractions between test and control fish.

Copper sulfate and Sodium sulfite

The effects of copper sulfate and sodium sulfite on serum protein patterns of 51 fish were studied in a series of four experiments conducted in tanks at the University. Fifteen test and 15 control fish were used in each experiment, although in the final analysis only data from immature fish were used, this being the largest maturity category tested. Incoming water was shut off in both test and control tanks and air was bubbled through the tank water in an attempt to maintain dissolved oxygen levels. Water temperatures rose from 6 C to 8 C during the experiments.

Sufficient copper sulfate was added to bring the copper ion level to 5.0 ppm in the first copper sulfate experiment. By the end of a 12 hr period all but four fish had died. Blood was then taken from the surviving fish and the experiment was terminated. In the second experiment the copper ion level was also started at 5.0 ppm but it decreased to 1.5 ppm by the end of the test period. Thirteen of the test fish were alive after 9 hr, but experiencing difficulty in maintaining equilibrium. Blood was taken from these fish and the experiment was terminated.

Fish were initially subjected to 400 ppm sulfite ion in the first sodium sulfite experiment. During the first 6 hr they evidenced some difficulty in breathing but after 24 hr their breathing and movement appeared normal. None of the fish died and blood samples were taken from each. The change in dissolved oxygen was from 7.0 ppm to 4.5 ppm during the test period. Starting conditions were identical to the first in the second sodium sulfite experiment, but the experiment was terminated after 2.5 hr. This was the time that fish showed the greatest loss of equilibrium in the first sulfite experiment. Dissolved oxygen dropped from 7.0 ppm to 0.1 ppm in the first 15 min and remained near this low concentration for the rest of the test period.

Because the first copper experiment and its control included only two immature fish, data from this experiment were omitted in the final analysis. The remaining data included 5 males and 5 females from the second copper experiment, 4 males and 5 females from the first sulfite experiment, 5 males and 7 females from the second sulfite experiment, and 9 males and 11 females from the control groups (Table 7). A one-way analysis of variance was made on these three experimental groups and on the combined control groups. The control fish showed significant pattern differences from the copper experimental fish in fractions 7 and 10. The first sulfite experimental fish (those tested for 24 hr) also showed differences from the copper experimental fish in these same fractions. The two sulfite experimental groups showed differences from each other in fraction 10. The analysis of variance also showed significant differences in fraction 4. Differences among groups in this fraction were not revealed by Duncan's New Multiple Range test because the confidence level of 95% significance in the analysis of variance was actually lower than the Range test application level (98% or more).

Large concentrations of sulfite dissolved in water caused a marked decrease in dissolved oxygen. Even so, there were no significant differences between the control fish and either of the groups of fish subjected to sulfite salts. These results are similar to those obtained earlier in the experiment on low oxygen concentrations in which no significant differences were obtained between test and control groups.

Wild and Hatchery Stocks

Nine wild rainbow trout were sampled to see if variations existed between their serum protein patterns (Figure 5) and patterns of hatchery fish. The wild fish selected were all mature females captured by electroshocking and transplanted to the University where they were held for an adjustment period of 48 hr before blood sampling. A comparable number of immature females from hatchery stock captured by electroshocking were sampled immediately after capture. Mature hatchery females available for comparison were taken by dip net.

A one-way analysis of variance was made to compare data from serum profiles of wild fish and of both hatchery groups (Table 8). Significant

Table 8. Serum protein fractions of blood from wild and hatchery rainbow trout. Fractions expressed as per cent of total protein. Number of individuals in parentheses.

Origin of Stock	Fraction Number									
	1	2	3	4	5	6	7	8	9	10
Wild mature females (9) (Electroshocked)										
Mean	0.6	13.8	20.0	13.2	3.8	25.6	2.7	6.3	7.9	6.1
Range	0.3- 1.1	3.3-30.5	7.8-34.2	8.7-18.1	2.1- 5.3	21.9-28.9	2.1- 3.4	3.7- 9.0	3.4-13.0	4.1-10.2
Hatchery immature females (8) (Electroshocked)										
Mean	0.5	19.8	22.0	12.5	5.9	14.0	4.5	6.1	8.9	6.0
Range	0.3- 0.7	8.4-28.1	14.1-34.4	7.9-15.6	4.7- 8.8	9.1-18.8	1.3-14.0	5.4- 8.1	6.5-11.2	4.4- 7.7
Hatchery mature females (14) (Dip net)										
Mean	1.6	22.2	20.3	14.3	5.8	10.7	2.5	3.8	8.6	10.1
Range	0.3- 2.8	5.0-42.4	6.3-35.4	10.1-18.5	2.2-13.8	4.3-14.6	0.6- 6.4	2.5- 5.2	4.4-11.8	5.4-33.1

differences were found between the profiles of wild fish and both hatchery groups in fractions 1 and 6. The hatchery fish which were not electroshocked showed differences from both other groups in fractions 6 and 8.

In the experiment on different capture methods a significant difference in fraction 8 was also shown, although none was shown for fraction 6. Differences in fraction 6 between mature and immature fish have also been shown in the experiment on sex and maturity, although none was shown for fraction 8.

DISCUSSION

Most electrophoretic studies of fish blood proteins reported to date have used paper as the separation support medium. The present study on rainbow trout used acrylamide gel as the support medium and achieved a greater degree of serum protein separation than has been possible with paper. Because of the many pattern differences obtained in this study it has not been possible to characterize a constant electrophoretic pattern for this species.

No reports of intraspecific differences among fish related to repeated sampling of the same individual or to location of blood extraction are known to this investigator, although studies have been made on variations attributable to sex and maturity, method of capture, and chemical stress. VanStone and Chung-Wai Ho (1961) reported a slowly migrating fraction in the serum of maturing 2.5 year-old female coho salmon (*Oncorhynchus kisutch*) which they believe may be lipovitellin. It was absent from the serum of spawning fish and those that had completed spawning. Rall, et al. (1961) described the appearance of a rapidly migrating plasma fraction from mature lampreys (*Petromyzon marinus*), and Thomas and McCrimmon (1964) studied the same species and found serum pattern differences between sexes in the pre-spawning phase which did not appear in the mature phase. Kirispuu (1964) studied eight Estonian fish and found no intraspecific pattern differences among males and immature females, but albumins and rapidly migrating globulins in mature females increased during the spawning season. Bouck and Ball (1965) used only male fish in their study because of observed sex-related differences. Tsuyuki and Roberts (1966) have noted a fraction separable on acrylamide gel which appears to be similar to the slowly migrating fraction characteristic of ripe females reported in this study (Figure 3). This fraction has not been identified.

Fujiya (1961) described a protein pattern shift in the blood of *Sparus macrocephalus* exposed to varying concentrations of Kraft pulp mill wastes. He suggested that electrophoretic analysis of fish blood serum might prove to be a useful tool as a pollutant detector. Significant differences have been reported by Bouck and Ball (1965) in the blood plasma profiles of two out of three species of fish studied under conditions of low oxygen. Changes were noted in two of the six fractions obtained in the paper electrophoresis profiles of bluegills (*Lepomis macrochirus*) and in one of the five profile fractions obtained for largemouth bass (*Micropterus salmoides*). Yellow bullheads (*Ictalurus natalis*) evidenced no profile change, and these authors have suggested that the dissolved oxygen levels (2.7 ppm) in the test conditions may have presented little or no stress for this species. In the present study, no significant changes were noted in the serum profile fractions of rainbow trout subjected to dissolved oxygen levels as low as 1.3 ppm for short periods of time. In addition, no significant changes were noted in the serum patterns of rainbow trout subjected to sodium sulfite salt causing a dissolved oxygen decrease to 0.1 ppm for up to 2 hr.

Significant changes were noted in the patterns of rainbow trout subjected to lethal levels of copper sulfate. The full nature of the reactions of copper to fish is not understood. Heavy metals, including copper, are known to form insoluble compounds when combined with proteins in the mucus secreted by external epithelial tissues of fishes. This condition is known to interfere with respiration (Doudoroff and Katz, 1953, Fromm and Schiffman, 1958). Holland, et al. (1964) concluded that blockage by outer epithelial tissue is sufficient to keep heavy metals, including copper, from penetrating to internal tissues. Some recent works show definite histopathologic changes under prolonged conditions of low concentrations of heavy metals (Crandall and Goodnight, 1963) as well as actual tissue accumulation of certain heavy metals (Fromm and Stokes, 1962). Tissue destruction, releasing cellular proteins into the blood stream might be a cause of altered serum patterns. This has been referred to as cellular leakage by Bouck and Ball (1965).

It is more difficult to account for serum protein changes not related to sex differences or tissue alterations as a result of chemical stress. The degree of variability in serum protein patterns encountered in this investigation of one stock of hatchery rainbow trout, and the further variability between this stock and a group of wild fish would suggest that variations within species may be even greater. Caution should be exercised in the interpretation placed on electrophoretic patterns in pollution investigations, pathological examinations, and taxonomic studies.

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