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DETERMINATION OF ELECTROPHORETIC VARIATIONS IN
DIFFERENT POPULATIONS OF CHANNEL CATFISH
(*ICTALURUS PUNCTATUS*) BASED ON SERUM
PROTEIN POLYMORPHISM *mu*

by

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INTRODUCTION

INTRODUCTION

The importance of genetics and selective breeding studies in animal husbandry and agriculture have long been recognized. Genetic studies have previously limited contributions in the development of aquaculture stocks. In this nascent field of husbandry, where much of the work is focused on culture techniques, genetics is often viewed as difficult, long term, low priority research.

There is presently a surge of interests in determining and understanding the genetics of cultured and wild fishes. The growing interests are triggered by both biological and economic reasons (Hedgecock et al., 1976). First, the very process of domestication alters the genotype of a population by forcing the fish to adapt to artificial environments used in aquaculture systems. Some genetic changes result from conscious efforts to improve stocks; but most changes probably pass unrecognized as a result of unconscious husbandry practices and natural selection. Second, genetic improvements may be required in many cases to reduce intensive aquaculture costs to competitive levels by improving the growth and reproductive characteristics of various stocks. Many of the problems associated with the culture of fishes have been solved. The major remaining way to improve fish production is through improved genetic stocks (Stickney, 1979).

Intensification of commercial fishing for some species of fish has produced a dramatic decline in harvestable weights due to overfishing (Gwahaba, 1973; Silliman, 1975). These intense harvest rates, if remain unchecked may result in size changes, age of sexual maturation and altered behavioral patterns thereby posing a threat to the ecological system. All these various processes create a strong incentive for genetic "correction" of the damage done by overfishing (Moav et al., 1978).

Meager information exists relating to fish genetics because a great deal of the research conducted by aquaculturists has been related to environmental and nutritional requirements, methods of disease control, and ways to design and manage culture systems for optimum production (Stickney, 1979). A myriad of techniques have been recently used to improve stocks of fish such as mass selection, individual selection (Kirpichnikov, 1970), system of crossing and utilizing heterosis (Kirpichnikov, 1970; Stickney, 1979) and gynogenesis (Stanley and Sneed, 1974).

Genetic markers greatly aid in the accomplishment of aforementioned techniques. Two methods that are generally employed are: the use of morphological markers such as body coloration and scale pattern for progeny identification (Kirpichnikov, 1970; Katasonov, 1973, 1974; Moav and Wohlforth, 1973), and the use of electrophoretic techniques (biochemical markers). The obvious major advantage of morphological markers over biochemical markers is direct visual identification. However, these markers do suffer the overwhelming disadvantage of a complete dominance of the wild type allele not permitting the identification of heterozygotes. Furthermore, most morphological markers are likely to have adverse effects on performance characteristics as well as market desirability (Moav et al., 1976). Electrophoretic markers have the following advantages over morphological markers: (a) they are invisible and, therefore, do not impose marketing problems; (b) they are more or less "neutral" as far as performance is concerned; (c) they segregate within the populations; and (4) most importantly, heterozygotes can be identified.

The inception of electrophoretic techniques has provided a new insight in the world of fish genetics. It has assured fish geneticists of methods for identifying genotypic variants. This technique is used in two generally accepted methods: zone electrophoresis and free boundary electrophoresis. Zone electrophoresis is widely used because of its theoretical advantages

over the free boundary electrophoresis (Smithies, 1955). This electrophoresis method is expensive but effective as a method for separating mixtures of proteins in extracts from biological tissues (Smithies, 1959; Davis, 1964). The use of electrophoresis with improved histochemical staining techniques increases the ability to study polymorphisms in soluble protein and enzymes particularly in blood among various groups of fishes (Rogers, 1972).

Electrophoretically identifiable protein polymorphisms have been used widely for investigations of variations of wild and domesticated fish populations and for marking fish populations (Moav et al., 1976). It has become an effective tool in the identification of hybrids (Aspinwall and Tsuyuki, 1968), population identification (DeLigny, 1969; Skow, 1976; Morgan and Koo, 1973), species identification (Mairs and Sindermann, 1960; Bouck and Bail, 1968; Utter and Hodgins, 1972; Morgan and Koo, 1973; Avise et al., 1977; Menzel, 1977; Slinko and Semenova, 1978; Klar, 1978; Herzberg, 1978) and systematics (Tsuyuki et al., 1968).

Much of the works in protein polymorphisms using electrophoresis as the biochemical tool were focused on commercial fishes like salmon, sunfish, trout, and bass. However, very limited research has been done on catfish (*Ictalurus punctatus*), a commonly cultured species of warmwater fish in the United States (Brown, 1977). The lack of information in this facet of genetics poses a problem for warmwater culturists in selecting good brood stock. Generally, culturists base their selection of brood fish on growth rate which results in little or no improvement of the fish stock (Stickney, 1979). Studies on protein variation in channel catfish will yield information about the genetic background, which will eventually aid in the selective breeding of channel catfish as a commercial aquaculture species.

This report is a synthesis of several works on protein variations among populations of fishes with particular emphasis on channel catfish. Actual investigations of blood protein variants in three populations of channel catfish was undertaken using horizontal zone starch gel electrophoresis to determine electrophoretic variations in these fish groups based on esterase, lactate dehydrogenase and serum protein activity. The study was confined to blood proteins since they can be readily obtained without sacrificing the animal, thereby enhancing their usefulness as genetic markers.

Numerous investigations of blood groups in fishes during the past few years indicate the following: (a) variability of blood proteins in fish populations is widespread; (b) heterogeneity of blood proteins in fish populations is large; (c) the hereditary basis of antigenic differences is formed by allelic variation. Many of the systems include so called "zero" allel-genes, the products of which are still undetected (Kirpichnikov, 1973). Population within a species differ from others in blood group genotype. Examples are found in works conducted on herring (Sindermann, 1959;), anchovy (Limansky, 1964), sardine (Sprague and Vrooman, 1962; Vrooman, 1964) and on tuna (Suzuki, 1967; Fujino, 1969). A differentiation of populations by the frequencies of individual genes enables the investigators to analyze the species structure and detect true intraspecific groups. A good example is in the pacific tunas that make up the three main reproductively isolated populations (southern, northern and western ones). They are closely related morphologically, but can nevertheless be easily recognized by their blood protein patterns (Fujino, 1970). The adaptive significance of variability in blood groups is obvious. By analogy with higher animals the diversity of erythrocyte antigens in fish can be regarded as a good means of protection from parasite infestations and infectious diseases.

Since there is significant evidence that proteins vary among members of a population of fishes and other animals, a knowledge of the manner in which they may vary is needed. Bailey (1957) found differences in the concentration of serum total proteins after injection of estroïdol. Chum salmon exhibited sexual differences in serum protein patterns (Hara, 1978).

Pacific salmon (Robertson et al., 1961) subjected to starvation for a long period of time during migration had decreased concentrations of plasma total proteins, globulins and albumin. Phillips et al. (1957) found that the A/G (Albumin/Globulin ratio) value in brown trout affected with kidney disease was the reverse of that obtained from brown trout not affected by the disease. They suggested that an examination of serum proteins in hatchery reared fish may be an important aid for predicting disease outbreaks in these fish. Sindermann and Mairs (1958) showed that sea herring suffering from acute fungus disease had a sharp reduction in concentration of the albumin fraction of their blood serum. Booke (1962) found an increase in the two serum globulin components in a population of white fish (*Corengus clupeaformis*) with increasing age. Kiryakov (1979) revealed variations of the blood serum in the bream (*Abramis abrama*) that were age related. Klar (1978) showed the effects of temperature, salinity, and feeding on amino transferase activity in the liver and white muscle of rainbow trout (*Salmo gairdneri*). It is expected that workers in this discipline will take note on these variations to have good results.

MATERIALS AND METHODS

MATERIALS AND METHODS

Collection of specimens

Channel catfish for use in this study were collected from three populations. Channel catfish from wild populations were obtained from the Blue River and Tuttle Creek Reservoir (Manhattan, Kansas). The third population of channel catfish was obtained from a commercial catfish fish farm in Slovak, Arkansas, owned by Raymond F. Schroeder.

Most of the specimens taken from the Blue River were collected by setting trotlines downstream. Collection of specimens from the Tuttle Creek Reservoir was assisted by the men of Kansas Fish and Game Commission using gill nets and seines. Trotlines were also set in the reservoir (McIntire Cove) for collecting channel catfish.

Sample collection and preparation

Blood samples were taken by means of hypodermic syringe. Two methods were used in withdrawing the blood from the fish. Cardiac puncture was used for extracting blood from the heart. The other method for collecting blood was by puncture of the caudal artery. A 20 GX1-1/2 needle was used in the collection of blood. This needle size allows for blood to flow freely and with minimum damage to the blood cells.

Each sample, after being drawn into a syringe, was transferred into a glass culture tube fitted with screw cap and then placed in a cold storage (4°C) for one day to allow clotting of the blood. Serum samples were withdrawn by a Pasteur pipette. The serum was then placed in glass culture tubes, sealed and stored frozen. Samples collected were later analyzed for protein variation by electrophoresis.

Electrophoresis

General. A horizontal zone starch gel electrophoresis was used to run the blood samples taken from the three fish populations. This method was used for the following reasons: (a) freedom from quantitatively important boundary anomalies; (b) the possibility of preparing electrophoretically discrete proteins; and (c) adaptability to small quantities of material (Smithies, 1955).

This method comprises zone electrophoresis with a starch gel as a supporting medium. The gel containing a Tris Citrate (discontinuous buffer) was prepared in a gel mold (Skow, 1976). This prepared gel was used to run the blood samples in the electrophoresis chamber (Figure 1). Staining of the gel was performed just after the termination of the electrophoresis depending on the protein to be studied.

Composition and preparation of the gel. The starch gel used in this work was prepared by adding 45 grams of hydrolyzed starch (Sigma #S-4501) to a dry liter pyrex side-arm flask and dissolving in the following:

12 ml of electrolyte buffer NaOH-boric acid (2.4 g sodium hydroxide,
23.6 g boric acid dissolved in distilled water and brought to
2000 ml volume)

23 ml stock gel buffer (Tris Citrate, pH 8.3); 17.3 g Tris Trizma Base
Sigma #T-1503); 9.68 g citric acid dissolved in distilled water
and diluted to 2000 ml volume

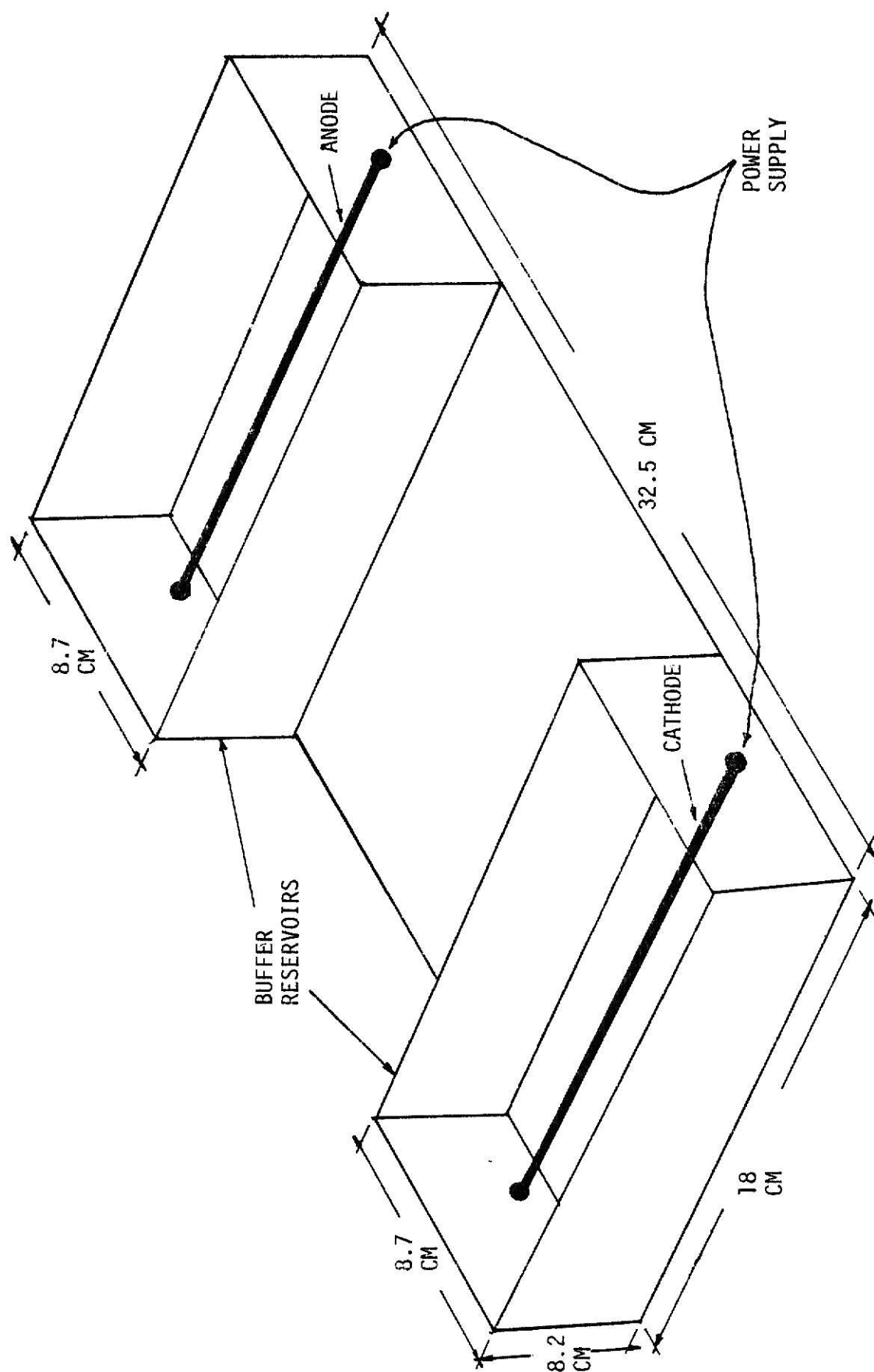
265 ml distilled water

The mixture was heated gradually over a burner with constant swirling until the gel becomes semi-solid. The heating and swirling was continued until a viscous homogeneous solution was obtained. To remove small air bubbles, a negative pressure was applied with a vacuum pressure pump until the whole contents bubbled vigorously. Degassing was terminated when large bubbles

Figure 1. Diagram of electrophoresis chamber.

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started to appear. The hot starch solution was then poured into the previously assembled gel mold (Figure 2) and immediately covered with a glass plate in a manner to prevent the occurrence of trapped air bubbles between the gel and the plate. Gels may be used within 24 hours after they are made without any effect on the electrophoretic behavior of the serum samples.

Introduction of the blood samples into the gel. In preparation for the introduction of blood samples, the glass plate cover was gently removed from the mold by inserting a flat object (like a spatula) between the cover and the mold. Plexiglass strips along the periphery of the gel were removed by making a cut between the gel and the plexiglass strips. The gel was cut at about 1/4 of its length. The smaller portion of the gel was pulled back gently to provide a space for the samples absorbed in filter papers. Blood samples were first introduced on filter papers which were cut into small squares (1 mm^2) by a Pasteur pipette. The papers containing the samples were inserted in a slit created in the gel. After the insertion of these samples, the separated gel parts were pushed together making the gel ready for electrophoresis.

The prepared starch gel with the samples was placed on the electrophoresis chamber (Figure 3) with a buffer reservoir at each end. These two reservoirs contain an electrode of 16 gauge platinum wire. The samples in the gel were run in the chamber for 15 minutes at 375 volts in a cold place (4°C). After running for 15 minutes, the inserted filter papers were removed and the gel was run in the electrophoresis chamber for 4 hours at 325 volts. Electrical contact between the gel and the buffer was achieved by the use of wick papers (Smithies, 1955). A Heatkit Regulated H.V. Power Supply Model IP-17 was used as the current supply.

Figure 2. Diagram of gel mold.

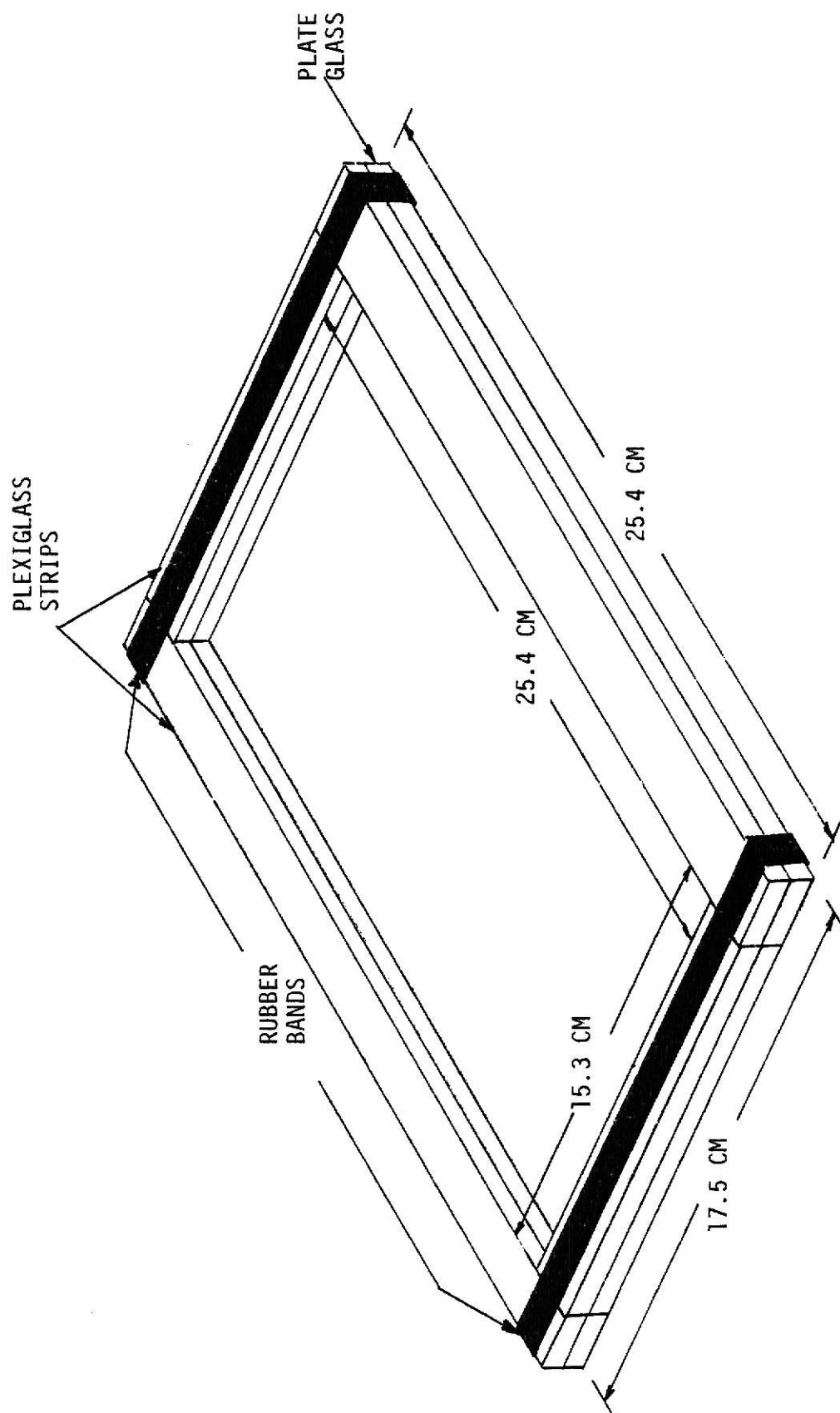
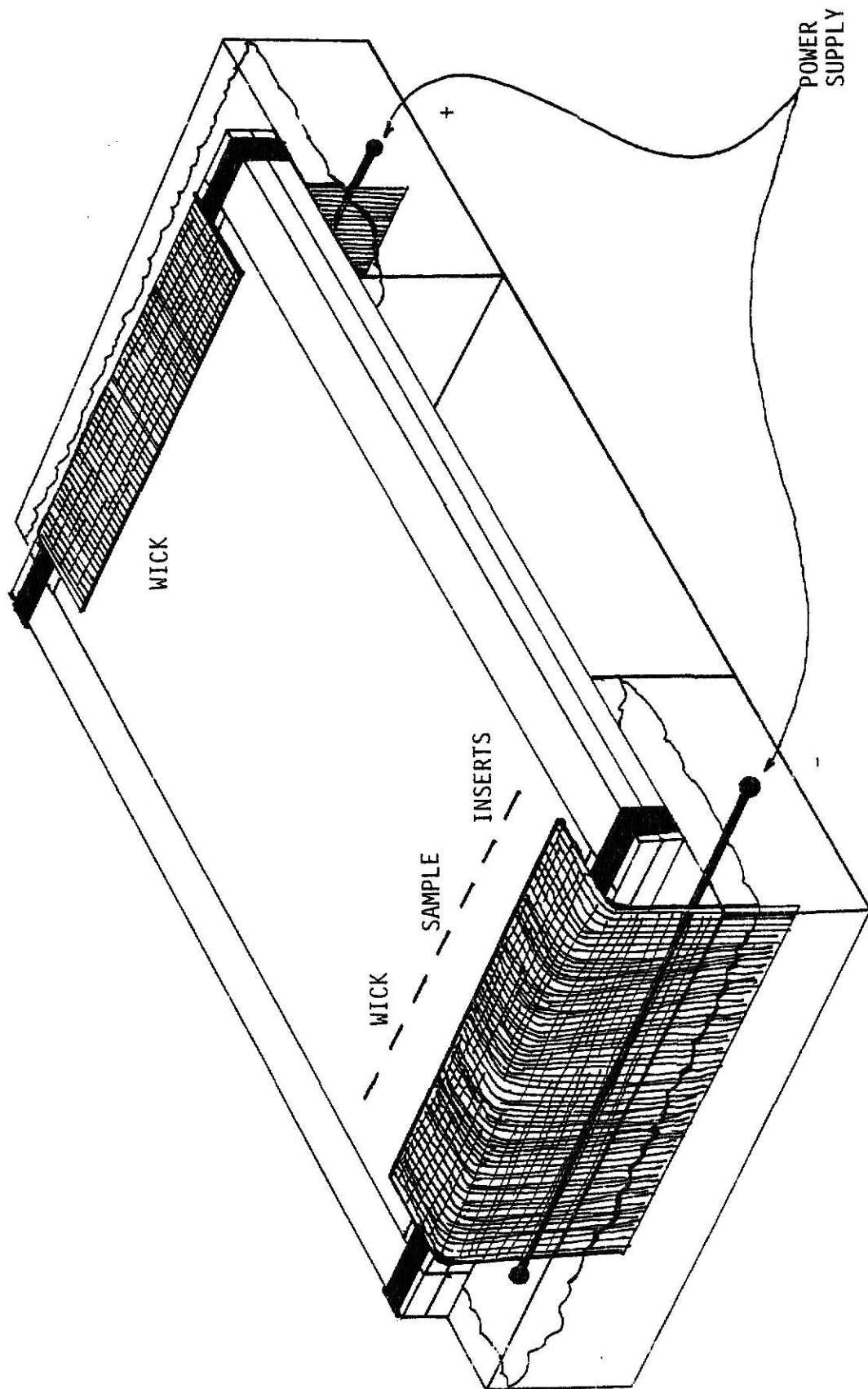


Figure 3. Diagram of electrophoresis chamber and prepared starch gel plate assembled for horizontal starch gel electrophoresis.



After completing the electrophoresis, the gel was removed from the chamber, laid on flat surface and disassembled. Strips of 2.5 mm thick plexiglass replaced the 5 mm thick longitudinal strips used to form the mold; and the cover plate was repositioned on the top of the gel. A gel slicer constructed of 20 gauge nichrome wire mounted in a hack saw frame, was positioned between the cover plate and the base plate at the front of the mold with the wire resting on the 2.5 mm plexiglass strips. Firm pressure was applied uniformly to the cover plate, and the gel slicer was quickly moved lengthwise through the gel. This procedure produced mirror-image gel halves of uniform thickness that could be simultaneously stained for different proteins.

Staining techniques

Staining the gels for different protein studies were carried out following the staining techniques presented in the work of Skow (1976).

Serum proteins (Sp). Serum proteins were visualized by staining for 3 hours in a saturated solution of Naphthol Blue Black dissolved in 4:4:1 solution of water, methanol and glacial acid. Destaining was accomplished in a solution of water-methanol-acetic acid alone on a pan. Destaining was continued for approximately 36 hours.

Esterase (Es). To study esterase, serum samples were stained for esterase activity by the procedure of Ruddie et al. (1969) and Skow (1976). To 150 ml of 0.05 M phosphate buffer (equimolar concentration of Na_2HPO_4 and KH_2PO_4) containing a trace of CaCl_2 , 0.5 ml of alpha naphthyl proprionate solution (1 g/6 ml acetone) and 750 mg Fast Blue BB were added. The staining solution was poured over the gel and the gel incubated in the dark for 2 to 3 hours at room temperature.

Lactate dehydrogenase (LDH). A method following Skow (1976) was used in LDH staining. To a buffer solution containing 25 ml of 0.2 M Tris-HCl (pH 8.0) and 75 ml water was added, in order: 1 ml of 40% sodium-lactate (Sigma), 1 ml of 0.1 M diphosphopyridine dinucleotide, 1 ml of phenazine methosulfate (2.4 mg/ml), 1 ml of Nitro Blue tetrazolium solution (10 mg/ml), and 1 ml of 0.01 M KCN. The stain was immediately poured over the gel and the gel incubated in darkness for 2 hours. Regions of LDH activity appeared as a dark blue on a white background.

Collection and analysis of data

Length of migration of each serum sample on the starch gel after electrophoresis and staining was measured in millimeter by a metric rule. Proteins were compared on the basis of relative number of bands (loci) (Gorman and Renzi, 1979) and the frequency of occurrence of each band. Calculations were made on ITEL AS/5 computer using the Statistical Analysis System (SAS) for analysis of variance, the Duncan's Multiple Range Test and Chi-Square test.

RESULTS

RESULTS

The determination of electrophoretic variations in the three populations (BR, RFS, TCR) of channel catfish showed encouraging results in the biological differentiation of these three fish populations. Of particular significance is the enzyme polymorphisms which are reflected in the occurrence of bands that appeared in the electropherograms of the channel catfish sera. Electrophoretic differences among populations were confined primarily on the occurrence of bands in the electropherograms.

An analysis of variance was run on the number of bands for each fish to determine if the populations differ in number of bands. Electrophoretic pattern variations were distinctly observed in the electropherograms of each of the three stocks of channel catfish (Table 1). Duncan's Multiple Range Test (Table 2) for variable bands shows significant differences in number of bands between populations. The test indicates a significant difference between Blue River and RFS stocks while TCR and BR populations showed no significant difference in this particular enzyme. The analyses also indicate that no significant difference exists between the TCR and the RFS fish stocks. A total of five bands were observed in the electropherograms of channel catfish serum examined for both esterase and serum protein activity (Figures 4 and 5). Sp-1 and Sp-3 bands occurred frequently in the TCR population. Sp-2 band was common in RFS fish population and Sp-4 and Sp-5 bands were observed frequently in BR population (Table 3).

In the electrophoretic esterase activity analysis, the 124 blood samples from the three populations showed electrophoretic esterase polymorphism (Table 4) revealing a heterogeneous population. Statistical analysis using Duncan's Multiple Range Test for variable bands based on esterase enzyme (Table 5)

Table 1. Test of significance of serum protein patterns among three populations of channel catfish using the SAS General Linear Model

Dependent Variable	Bands					
Source	DF	Sum of Squares	Mean Square	F Value	PR > F	
Population	2	7.13198653	3.56599327	8.09	0.0005	
Error	105	46.27542088	0.44071829	STD DEVIATION		BANDS MEAN
Corrected Total	107	53.40740741		0.66386617		2.9259

Table 2. Duncan's Multiple Range test for the number of bands based on serum protein activity.

Grouping	Mean	Number	Population
A*	3.227273	44	Blue River
A B	2.900000	10	Tuttle Creek Reservoir
B	2.685185	54	Raymond F. Schroeder

*Note: Means with the same letter are not significantly different.

Table 3. Differences in the percentage frequency occurrence of serum protein band and chi-square values for three stocks of channel catfish.

Band	BR	RFS	TCR	Chi-square values	Probability
Sp-1	90.91	53.70	100.00	21.32	0.0001
Sp-2	25.00	48.15	40.00	5.54	0.0628
Sp-3	13.89	17.59	6.48	4.82	0.0900
Sp-4	93.17	85.19	50.00	12.04	0.0024
Sp-5	81.82	46.30	30.00	16.58	0.0003
Sample Size	44	54	10		

Table 4. Test of significance of esterase enzyme patterns among three populations of channel catfish using the SAS General Linear Model

Dependent Variable	Bands					
Source	DF	Sum of Squares	Mean Square	F Value	PR	F
Population	2	6.68122698	3.34061349	5.20	0.0068	
Error	121	77.73812785	0.64246387	STD DEVIATION		BANDS MEAN
Corrected Total	123	84.41935484		0.80153844		2.11290323

Table 5. Duncan's Multiple Range test for the number of bands based on esterase enzyme activity.

Grouping	Mean	Number	Population
A*	2.266667	15	Tuttle Creek Reservoir
A	2.260274	73	Raymond F. Schroeder
B	1.750000	36	Blue River

*Note: Means with the same letter are not significantly different.

Figure 4. Graphic presentation of serum protein electrophoretic patterns of channel catfish on the basis of their mobility at 15% starch gel.

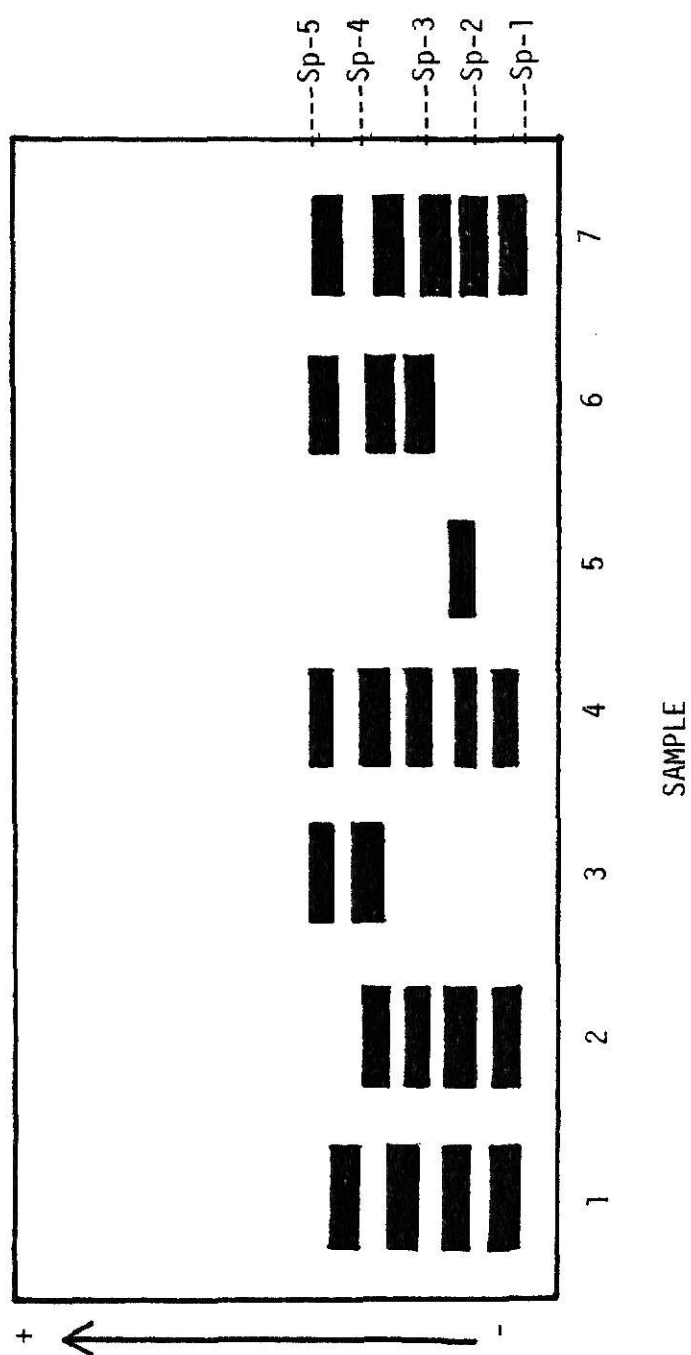
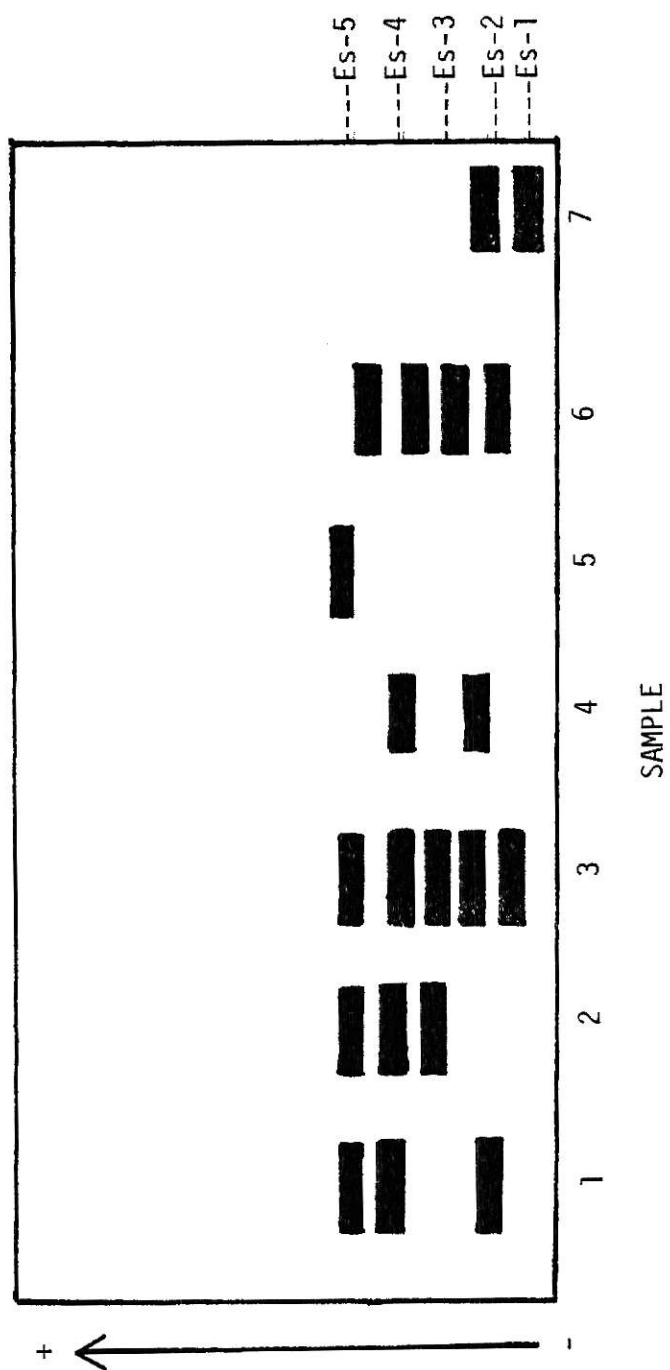


Figure 5. Graphic presentation of esterase electrophoretic patterns of channel catfish on the basis of their mobility at 15% starch gel.



between the means of the three populations shows a remarkable difference between TCR and BR fish populations despite the relationship of these two bodies of water. RFS fish stocks were significantly different than the BR stocks.

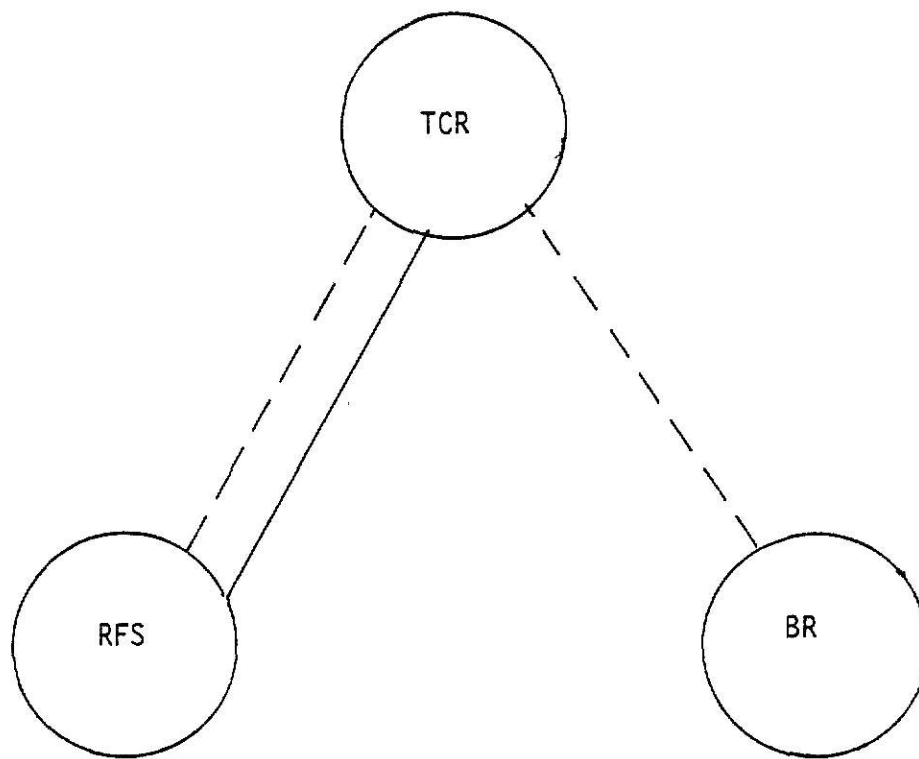
Differences in band occurrence were prominent in the sera of the three fish populations studied. Band combinations varied between serum samples. Bands Es-1, Es-2 and Es-4 predominated in the sera of the fish blood samples from TCR stock. The Es-3 band was observed frequently in the blood samples of the RFS fish population, while Es-5 was common in the BR fish blood samples (Table 6).

Finally, Figure 6 presents the relationships of these three fish populations studied based on serum protein and esterase activity. From Figure 3 we can discern that fish populations from TCR and RFS are related for both esterase and serum protein activities. TCR and Blue River fish populations exhibit kinship in serum protein only; but, BR and RFS fish populations are distinctly unrelated.

Table 6. Differences in the percentage frequency occurrence of esterase enzyme band and chi-square values.

Band	BR	RFS	TCR	Chi-square values	Probability
Es-1	0.00	13.70	20.00	6.47	0.0394
Es-2	2.78	43.84	73.33	28.43	0.0001
Es-3	38.89	72.60	53.33	11.83	0.0027
Es-4	52.78	79.45	80.00	9.04	0.0109
Es-5	83.33	16.44	0.00	56.91	0.0001
Sample Size	36	73	15		

Figure 6. The relationships of the three stocks of channel catfish based on esterase and serum protein activities after electrophoresis. Es = ———, Sp = -----. Inter-connecting lines means no significant differences in the frequency of bands that occurred.



DISCUSSION

DISCUSSION

Serum protein (Sp)

Fish serum proteins have been extensively studied in fish systematics but imperfectly studied from the genetic point of view. Early works on serum proteins were focused on the blood levels of protein, electrolytes, etc. The advent of electrophoresis brought a long series of studies of fish sera beginning with Deutsch and Goodloe (1945) who used the Tiselius (1937) method to report species specificity of the electrophoretic patterns of the blood proteins in fishes. An array of electrophoretic studies immediately followed resulting in various reports on fish phylogeny, systematics, taxonomy and lately on fish genetics. Lush (1970) reported the proteins and enzymes present in serum occurring in genetically variable forms. Species variability was found in albumins and haptoglobulins. Infrequently polymorphic systems of these proteins were found to include "zero" alleles. They are found for example in haptoglobin of the sea bass (*Sebastes marinus*) (Nefedov, 1969).

Results of the electrophoretic study in the sera from 108 fish blood samples of channel catfish obtained from the three populations revealed some variations in the electrophoretic patterns. Serum protein polymorphisms based on the occurrence of Sp-bands were indicative of the significant differences observed among the populations. Variations were observed on every identified band. Skow (1976) found allozymic variation in the Sp-4 region in the serum of fish blood samples of channel catfish. In the evaluation of the differences between populations, RFS stocks showed a wide variation in the serum protein activity indicating that this channel catfish population was significantly different from the BR population. The variation was attributable to the origin of the experimental fish stocks. On the other

hand, no significant difference was observed between BR and TCR based on serum proteins which is in contrast with the result obtained from the esterase enzyme.

The sera of trout was found to have significant differences in fractions 6 and 9 which were related to maturity, but none in any fraction was attributed to the sex (Thurston, 1967). An intense band also appeared in serum samples from two ripe rainbow trout (*Salmo gairdneri*) and two ripe cutthroat (*Salmo clarki*) taken from nearby streams and lakes. The pattern from the disc-gel electrophoresis provided a greater number of apparent serum fractions. As many as 18 fractions were noted in some of the samples studied and in others as few as 10. Thurston (1967) found some gross similarities among the patterns revealed in the gel. The patterns also have similarities to the disc-gel patterns of rainbow trout plasma reported by Tsuyuki and Roberts (1965, 1966). No two patterns of different serum samples could be considered identical, and few were sufficiently alike that some kind or degree of difference could be readily perceived. Because of this variability of individual differences, he concluded that it is not possible to characterize a "normal" disc-gel electrophoresis pattern for rainbow trout serum. He also reported that changes in the serum profile of any one individual may be brought about by the introduction or increase of a given protein by the decrease or elimination or by any change in the structure of a protein which is sufficient to alter its electrophoretic mobility. Increased endocrine flow into the blood stream as a result of stress can affect the total serum protein. Tissue destruction releasing cellular proteins into the blood serum may be one of the causes in serum protein variations. The damage may be from trauma or from physiological degradation. Thurston (1967) indicated that some protein is added to any serum sample as a result of hemolysis during even the most careful blood extraction.

To be sure that individual variation is genetically controlled, breeding experiments, if at all possible, are needed. Less satisfactory is population genetic data which may be explained in several different ways. DeLigny (1969) also suggested that the "components which have been identified with proteins found to be polymorphic in higher vertebrates" might be most selected for study, although she wisely added the warning "it ought to be put in mind that more direct proof of the inheritance of the components in the fish species studied is to be preferred."

Using polyacrylamide gel electrophoresis, Rolan (1968) compared the sera of 13 Ictalurids and 1 Ariid with that of human serum. He reported that the human serum has 13 protein fractions. The fastest moving component fraction, a, is albumin; all the other components precipitate as globulins. Lipoproteins are present in fractions a, e, i and m. Fractions f, i, and j are haptoglobulins since they bind hemoglobin and show a positive benzidine reaction. There is no ambiguity inasmuch as the migration of pure hemoglobin complexes have no region of overlap. Cytochrome oxidase activity was found localized in the albumin. The other components of human serum were tentatively identified only through a comparison with parallel electropherograms of the human Cohn fractions. Fractions c and d apparently are alpha globulins while fractions f are distributed through the region occupied by fractions i through m. All the Ictalurid species studied possess similar serum electrophoretic patterns. There are reports on the electrophoretic patterns of sera of other vertebrates which suggest that the Ictalurids and Galeichids are not alone in possessing different albumin components which may, under certain conditions, migrate together.

Brown (1964) studied the sera of *Bufo americanus* and *B. woodhousei* by paper electrophoresis and found that the fast-moving component, which precipitated as albumin was a lipoprotein. On occasions, however, this

fraction apparently splits into two, each having slightly different mobilities. When this happened, the slower moving albumin was shown to be one containing lipoproteins. Dessauer et al. (1962), by paper electrophoresis, occasionally observed incomplete resolution of the two most rapidly migrating components of *Cnemidophorus tigris* serum. These two components are the most abundant ones in this serum and are probably albumins. Similar observations have been noted for fishes. Mairs and Sindermann (1960) found occasional splitting of the leading fraction of the sera of *Clupea harengus*, especially in immature individuals. Vanstone and Ho (1961) were able to demonstrate that lipoproteins are the second fastest migrating fraction in the sera of coho salmon. The fastest and second fastest migrating fraction appear to be albumins.

One of the biological roles of albumins of catfish serum is their role in maintaining osmotic pressure of the capillary membrane. Compared with human albumin, the catfish albumins seem to be somewhat less capable of generating osmotic pressure. They occur in lesser concentrations and probably have larger molecular sizes since they migrate electrophoretically much more slowly than human albumin. These two factors mean that the osmotic pressure developed by catfish albumin is much less than that for humans. This is a desirable state of affairs since the blood pressure of fishes is lower and consequently there is a lesser tendency toward the formation of tissue fluid. The osmotic concentration of the plasma and the blood pressure must be properly balanced. It is clear that studying the plasma proteins of lower vertebrates by electrophoresis is not adequate to assume that albumins are lacking simply because there are no fractions which migrate to the same extent as human albumin. A corollary to this assumption is that the fastest moving components of fish serum must be alpha globulins since they migrate in the same region as human alpha globulins. This conclusion is no more

justified than the first. On the other hand, it should not be construed as fish albumin in every other regard.

Catfish serum fraction g, in both Ictalurids and Galeichids, is a lipoprotein. Brown (1964) found a lipoprotein in total serum which seems to correspond to the slow-moving lipoproteins of catfishes. In both cases the lipoproteins scarcely move from the point of origin. These proteins may correspond to human beta-lipoproteins, which is either or both of the slow-moving lipoproteins of human serum.

Although proteins analogous to human haptoglobins are lacking in catfish sera, they may yet be present in the sera of other groups of fishes. Blumberg (1960) reported the occurrence of two haptoglobins each in the sera of the three species of marine fishes. Evidently, the complexing of hemoglobin with haptoglobin is not a universal characteristic of vertebrate blood, and species specificity in the formation of such complexes.

Skow (1976) worked on 921 samples of channel catfish sera and the electrophoretic patterns revealed a closely similar pattern as studied by Rolan (1968). The electrophoretic patterns analyzed shared four major components two of fast mobility and two of slow mobility. Differences in electrophoretic patterns were detected in the minor components of intermediate mobilities. The protein fractions revealed by Skow's work confirmed with Rolan's study that channel catfish albumins are not precipitated by the standard ammonium sulfate fractionation procedures. Conflicting results were found in the number of albumins present in the serum of channel catfish. Skow (1976) found one albumin while Rolan (1968) reported two. Because of these conflicting results, Skow suggested the use of a more refined biochemical analysis on the proteins to adequately resolve their identities and physiological roles.

Esterase (Es)

This term is used to designate a few groups of enzymes that vary in their functions. Aryl-, acetyl-, cholin-, and carboxyl- esterases are known (Holmes and Whitt, 1970; Manwell and Baker, 1970). According to Nyman (1971), a serum esterase probably belongs to a type of carboxyl esterases. In the overwhelming majority of cases, no precise determination of enzyme has been conducted. Esterases are inherited codominantly without formation of hybrid rings. As a rule, the esterase molecule is a homopolymer (probably a dimer) and, therefore, the number of isozymes is relatively low. Some fishes do, however, exhibit a large number of esterases (Holmes and Whitt, 1970). Polymorphism with respect to esterase genes has been discovered in representative of clupeoids, salmonids, osmerids, cyprinids, catostominids, eels, gadoids, tunas, flatfishes and other families. By the frequency and range of variability, esterases resemble transferrins. Not infrequently, a population contains three-four or even six-seven alleles of one locus and in the capelin (*Mallotus villosus*) their number reaches 10 (Nyman, 1971). One esterase is sometimes coded by several loci.

Stable polymorphism has been detected in the liver and heart esterases of the Atlantic herring (*Clupea harengus*). It was determined by the three allelic systems with concentrations of three genes in different populations amounting to 0.71-0.77, 0.22-0.28 and 0.005-0.016, respectively. The populations are rather alike, and the distribution of genotypes is in good agreement with Hardy-Weinberg's equation while at the same time, there are small reliable differences in allelic concentration between local groups (Ridgway et al., 1970). It appears that the herring populations are reproductively isolated.

The results from the electrophoretic esterases activity analysis of 124 sera of blood samples indicate that the three populations of channel

catfish varied. The differences between RFS and BR fish populations was due to the place of origin. Between TCR and BR populations, it is suspected that the ecological adaptations might be responsible for the variation. Exposure to certain environmental conditions may alter esterase activity. The sensitivity of some esterases to inhibition by organophosphate pesticides may also represent an important source of quantitative esterase variation in channel catfish, especially in agricultural areas. Temperature, diet, age, and disease may also affect the activity of esterase isozymes (Skow, 1976). The Blue River has a stronger current than Tuttle Creek Reservoir, and this current may be responsible for observed differences. Another reason might be the migration of channel catfish from the TCR to BR. Migration of individuals from one type of habitat to another is also an important way for genes to be added or subtracted from a population. The complete absence of Es-5 bands in the TCR fish population was noticeable, whereas a high frequency of occurrence of this band in BR fish population was observed. We can deduce from this result that Es-5 band may serve as a genetic marker for these two populations. On the contrary, the non-significant result derived from the Sp-band for the two populations is still subject to further research. There is a strong possibility that the channel catfish in BR still retain the Sp-band similar to their TCR counterparts because of some restricted breeding between the two populations.

Skow (1976) reported three variable regions of esterase activity in channel catfish sera. These regions include Es-5, Es-6 and Es-7 and probably occur in serum as products from other tissues. Es-5 and Es-6 comprise the major regions of serum esterase activity. Es-7 was a lightly staining and highly variable region that is visualized only after prolonged staining. Due to the large amount of variation in the Es-7 region, he found it difficult to categorize all samples; and, the variation was not further evaluated.

He also reported that three zones of esterase activity were found in the Es-5 region and designated F (fast), I (intermediate) and S (slow) corresponding to their relative electrophoretic mobilities. The electropherograms also indicated the occurrence of different isozymes, in all three populations of channel catfish checked.

Lactate dehydrogenase (LDH)

Four subunits constituting a LDH tetrameric molecule are usually coded by two genes, the products of which make up a series of 3-5 isozymes. Most of the products of "a" and "b" genes are combined. As a result, isozymes A_4 , A_3B , A_2B_2 , AB_3 and B_4 are formed. In some fishes combinations A_3B , AB_3 and sometimes A_2B_2 are absent. Likewise, other genes, for instance gene "c" which manifest itself in gonads participate in the formation of LDH heteropolymers (Holmes and Markert, 1969). According to the recent data, white fish from the genus *Prosopium* contains 10 LDH genes constituting 5 enzymes (a, a', b, b', d, d', e, e', f, f'). Each of the 3-5 isozymes were found to be related to a definite tissue (Massaro, 1972). Generally, the tissue specificity is typical of the majority of LDH isozymes detected in fishes.

Markert and Faulhaber (1965) found a very negatively charged LDH in eye extracts of 30 species of fish. They also studied the muscle LDH of these species and arranged them in four groups depending on another four major isozymes. A single fraction was found in the fluke (*Paralichthys dentatus*), two in the porgy (*Stenotomus versicolor*), yellow perch (*Perca flavescens*), bream (*Diplodus argenteus*), striped mullet (*Mugil cephalus*) and many other species. Three isozymes were found in some genera including sea trout (*Cynoscion*), bass (*Morone*) and walleye (*Stizostedion*). Five isozymes were observed in herring (*Clupea harengus*).

Whitt (1970) has pointed out that in many vertebrates the LDH polypeptide subunits (A and B) are encoded in at least two codominant loci. Mammals and birds have three such loci, the c locus functioning only in primary spermatocytes. In teleosts a third locus, termed E by Markert and Faulhaber (1965) has been postulated it has marked specificity. The E_4 isozyme normally has a very high negative charge and is localized in the nervous system, especially in the retina. Occasionally it is found in the non-neural tissues of teleosts. Whitt (1969) has proposed the E_4 isozyme may be more closely related to the B_4 than the A_4 isozyme because the kinetic and physical properties are closely related. The genetic origin of the E subunit was investigated in a panmictic population of 245 adult killifish (*Fundulus heteroclitus*) from Wood's Hole, by studying the allelic isozyme variations at the LDH B locus. Three LDH phenotypes were found slow (BBB) and the heterozygote hybrid (BB') with intermediate mobility.

Hodgins et al. (1969) have found LDH variants in the sera of sockeye salmon (*Onchorynchus nerka*). There are three phenotypes probably due to two alleles at the B locus. Ultracentrifugation showed that all variants have molecular weight of about 150,000 and a similar number of subunits.

Using polyacrylamide gel electrophoresis of tissue extracts from brook trout collected in Gaspé Hatchery, Quebec, an isolated selected inbred population, nine bands of LDH were reported in an early paper of Goldberg (1965) who obtained no evidence of tissue specificity but found evidence of a C gene locus in all tissues giving three homopolymers AAAA, BBBB and CCCC and 12 heteropolymers by random combination of the polypeptides. This theory was subsequently withdrawn by Goldberg (1966) and reported the occurrence of mutants at the A locus. He found that nine bands occurred only in hybrids (splake) between brook trout and lake trout and between female splake and male brook trout. Five LDHs with species and tissue specificity occurred in

the parental species from other areas of Canada and the U.S.A. He also found multiple LDHs in the lake trout which had LDH bands with mobilities similar to the closely related brook trout while rainbow trout had a different pattern. He proposed that the synthesis of the three polypeptides LDH subunits was controlled by three non-allelic gene loci and that there should be 15 different LDHs if the three subunits were arranged in all possible subgroups of four. In the splake nine LDHs have been found and in the latter paper (Goldberg, 1966) LDH¹ (BBBB) has a common gene locus in both species which would give the nine forms found. In vitro "hybridization" of LDH from both parental species also gives nine LDHs on electrophoresis because some of the forms have identical mobilities.

Minor isozymes have been found in the eyes and gonads of many species of fish and not elsewhere in their tissues. A separate unit C was proposed for the isoenzyme in the eye of all salmonids (Goldberg, 1966). This C appears to combine predominantly with B because of this preferential association. Morrison and Wright (1966) suggested that the subunit may have evolved from B gene duplication and mutation. The muscle specific subunits D and E found by Hochachka (1966) may have arisen from A in a similar way but some hybridization may occur between all types of subunits. Cytogenetical evidence has been adduced by Ohno et al. (1968) and Klose et al. (1968) to back up the gene duplication theory.

Bouck and Ball (1968) studied the electrophoretic patterns of LDH in brook, lake and rainbow trout. Each species showed an LDH that was unique for that species. However, no variation was observed in the LDH patterns for each of these species. This might be due to the lack of samples used in this study. There were marked differences in the LDH patterns among the three species, hence, the patterns served as a tool for biological identification.

The tissue extracts of brook, lake and rainbow trouts each had a total of exactly 15 isoenzymes as judged by the production of colored bands. These consisted of three systems of isozymes (1, 2 and 3) and each system had five isozymes (a, b, c, d and e).

Working on the electrophoretic LDH pattern of rainbow trout, Utter and Hodgins (1972) found a single banded form in homozygous individuals and a five banded form in the heterozygous phenotype. They also found that the mobility of the LDH phenotypes from the rainbow trout livers belonged to the B₂ subunit.

Skow (1976) reported the presence of A and B subunits in the distribution of the 10 channel catfish LDH electrophoretic patterns which conformed with other findings in other fishes mentioned earlier. However, he revealed that the LDH E gene was non-functional in this species which is in contrast with the findings of Whitt (1969) that this gene is functional. Differences in species may be the reason for this result. Erythrocyte LDH activity was studied in 921 specimens of channel catfish showing no variation in the electrophoretic patterns revealing a single band of LDH activity. My actual investigation on the LDH electrophoretic patterns of channel catfish from the three populations (BR, TCR, and RFS) showed a similar result obtained by Skow (1976). There was a low LDH activity in the serum and this result suggests the unlikelihood of this pattern as a genetic marker.

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APPENDIX 1
OBSERVED BANDS IN BR FISH POPULATION
BASED ON ESTERASE ENZYME ACTIVITY

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OBSERVED BANDS IN BR FISH POPULATION
BASED ON ESTERASE ENZYME ACTIVITY

Sample	Number of Bands	Es-1	Es-2	Es-3	Es-4	Es-5
22	1	0	0	0	0	1
6	2	0	0	1	0	1
2	3	0	0	1	1	1
1	1	0	0	1	0	0
3	1	0	0	1	0	0
26	2	0	0	1	0	1
1	2	0	0	1	0	1
6	2	0	0	1	0	1
4	2	0	0	1	0	1
3	3	0	0	1	1	1
5	3	0	0	1	1	1
2	3	0	0	1	1	1
7	3	0	1	0	1	1
18	3	0	0	1	1	1
30	2	0	0	1	1	1
4	2	0	0	0	1	1
1	1	0	0	0	1	0
23	1	0	0	0	1	0
20	1	0	0	0	0	1
25	1	0	0	0	0	1
34	1	0	0	0	0	1
10	2	0	0	1	0	1
14	2	0	0	0	1	1
7	2	0	0	0	1	1
11	1	0	0	0	0	1
38	1	0	0	0	0	1
36	2	0	0	0	1	1
12	2	0	0	0	1	1
9	2	0	0	0	1	1
39	1	0	0	0	0	1
25	2	0	0	0	1	1
27	1	0	0	0	0	1
23	1	0	0	0	0	1
24	1	0	0	0	1	0
21	1	0	0	0	1	0

APPENDIX 2
OBSERVED BANDS IN RFS FISH POPULATION
BASED ON ESTERASE ENZYME ACTIVITY

APPENDIX 2
OBSERVED BANDS IN RFS FISH POPULATION
BASED ON ESTERASE ENZYME ACTIVITY

Sample	Number of Bands	Es-1	Es-2	Es-3	Es-4	Es-5
50	2	0	0	1	0	1
79	3	1	1	1	0	0
99	4	0	1	1	1	1
11	3	1	0	1	1	0
61	3	1	0	1	1	0
23	4	1	1	1	1	0
71	2	0	0	1	1	0
21	3	1	0	1	1	0
30	4	1	1	1	1	0
27	2	0	1	0	1	0
13	1	0	0	0	1	0
4	1	0	0	0	1	0
28	2	0	0	1	1	0
29	3	1	1	0	1	0
24	1	0	1	0	0	0
22	1	0	0	0	1	0
31	2	0	0	1	1	0
67	2	0	0	1	1	0
12	2	0	0	1	1	0
77	1	0	0	0	1	0
79	1	0	0	0	1	0
98	1	0	0	0	1	0
61	1	0	0	0	1	0
11	1	0	0	1	0	0
78	2	0	0	1	1	0
10	2	0	1	0	1	0
5	4	1	1	0	1	1
6	3	0	1	1	1	0
5	3	0	1	1	1	0
23	3	0	1	1	1	0
21	3	0	1	1	1	0
17	3	0	1	1	1	0
2	3	0	1	1	1	0
60	2	0	1	1	0	0
68	3	1	0	1	1	0
57	2	0	1	0	1	0
62	3	0	1	1	1	0
72	2	0	0	1	1	0
52	3	0	1	1	1	0
55	3	0	1	1	1	0
57	2	0	0	1	1	0
86	2	0	0	1	1	0

Sample	Number of Bands	Es-1	Es-2	Es-3	Es-4	Es-5
59	2	0	0	1	1	0
64	2	0	0	1	1	0
61	2	0	0	1	1	0
74	2	0	0	1	1	0
69	2	0	0	0	1	1
53	2	0	0	1	1	0
80	1	0	0	0	1	0
90	2	0	0	0	1	1
22	2	0	1	1	0	0
4	2	0	1	1	0	0
14	3	0	1	1	1	0
19	3	0	1	1	1	0
20	3	0	1	1	1	0
16	3	0	1	1	1	0
15	3	0	1	1	1	0
13	3	0	1	1	1	0
9	3	0	0	1	1	1
1	3	0	0	1	1	1
7	2	0	0	0	1	1
1	3	0	0	1	1	1
8	1	0	0	0	0	1
2	1	0	0	0	0	1
3	2	0	0	1	0	1
63	2	1	0	1	0	0
65	2	0	0	1	1	0
51	2	0	0	1	1	0
75	2	0	1	1	0	0
54	2	0	0	1	1	0
76	2	0	1	1	0	0
70	1	0	1	0	0	0
80	2	0	1	1	0	0

APPENDIX 3
OBSERVED BANDS IN TCR FISH POPULATION
BASED ON ESTERASE ENZYME ACTIVITY

APPENDIX 3
OBSERVED BANDS IN TCR FISH POPULATION
BASED ON ESTERASE ENZYME ACTIVITY

Sample	Number of Bands	Es-1	Es-2	Es-3	Es-4	Es-5
1	1	0	0	0	1	0
2	3	1	1	1	0	0
3	3	0	1	1	1	0
4	3	1	1	1	0	0
5	3	0	1	1	1	0
6	3	0	1	1	1	0
7	3	0	1	1	1	0
8	3	1	1	1	0	0
9	3	0	1	1	1	0
10	1	0	0	0	1	0
41	2	0	1	0	1	0
2	1	0	0	0	1	0
5	2	0	1	0	1	0
12	2	0	1	0	1	0
11	1	0	0	0	1	0

APPENDIX 4
OBSERVED BANDS IN BR FISH POPULATION
BASED ON SERUM PROTEIN ACTIVITY

APPENDIX 4
OBSERVED BANDS IN BR FISH POPULATION
BASED ON SERUM PROTEIN ACTIVITY

Sample	Number of Bands	Sp-1	Sp-2	Sp-3	Sp-4	Sp-5
21	3	1	0	1	1	0
4	3	0	0	1	1	1
1	2	1	0	0	0	1
2	2	1	0	0	0	1
6	5	1	1	1	1	1
2	5	1	1	1	1	1
1	2	1	0	0	1	0
3	3	1	0	0	1	1
26	4	1	1	0	1	1
1	5	1	1	1	1	1
6	3	1	0	0	1	1
4	4	1	0	1	1	1
3	3	1	0	0	1	1
5	4	1	1	0	1	1
2	3	1	0	0	1	1
7	4	1	1	0	1	1
22	3	1	0	1	1	0
25	3	1	0	0	1	1
27	3	1	0	0	1	1
23	2	1	0	0	0	1
24	2	1	0	0	1	0
23	4	1	1	0	1	1
20	4	1	0	1	1	1
25	4	1	1	1	1	0
21	3	1	0	0	1	1
30	4	1	0	1	1	1
27	2	1	0	0	1	1
13	3	1	0	0	1	1
4	3	1	0	0	1	1
28	3	1	0	0	1	1
29	3	1	0	0	1	1
24	3	1	0	0	1	1
94	3	1	0	0	1	1
31	4	1	1	0	1	1
34	3	0	1	0	1	1
10	3	0	1	0	1	1
14	2	0	0	0	1	1
18	4	1	0	1	1	1
7	3	1	0	0	1	1
36	3	1	0	0	1	1
11	4	1	0	1	1	1
12	3	1	0	1	1	0
39	3	1	0	1	1	0
25	3	1	0	1	1	0

APPENDIX 5
OBSERVED BANDS IN RFS FISH POPULATION
BASED ON SERUM PROTEIN ACTIVITY

APPENDIX 5
OBSERVED BANDS IN RFS FISH POPULATION
BASED ON SERUM PROTEIN ACTIVITY

Sample	Number of Bands	Sp-1	Sp-2	Sp-3	Sp-4	Sp-5
50	3	1	0	0	1	1
78	3	1	0	0	1	1
63	2	1	1	0	0	0
65	2	1	1	0	0	0
51	1	0	1	0	0	0
75	2	1	1	0	0	0
54	2	0	0	1	1	0
76	2	0	0	1	1	0
70	2	0	0	1	1	0
80	2	0	1	1	0	0
60	3	1	0	0	1	1
68	3	1	0	0	1	1
57	3	1	0	0	1	1
62	3	1	0	0	1	1
3	3	1	0	0	1	1
9	3	1	0	0	1	1
10	3	1	0	0	1	1
86	2	0	1	1	0	0
59	3	0	1	1	1	0
64	3	1	1	0	1	0
61	2	0	1	0	1	0
74	3	0	1	1	1	0
69	2	0	0	0	1	1
80	2	0	0	1	0	1
53	2	0	0	1	1	0
90	2	1	0	1	0	0
9	3	1	0	0	1	1
16	3	1	0	0	1	1
6	3	1	0	0	1	1
1	3	1	0	0	1	1
8	3	1	0	0	1	1
2	3	1	0	0	1	1
72	3	1	0	0	1	1
52	3	1	0	0	1	1
55	3	0	1	0	1	1
57	3	1	0	0	1	1
71	3	0	1	1	1	0
79	3	1	0	0	1	1
22	4	1	1	1	1	0
4	3	0	1	1	1	0
14	3	0	1	1	1	0
19	3	1	1	0	1	0

Sample	Number of Bands	Sp-1	Sp-2	Sp-3	Sp-4	Sp-5
20	2	0	1	0	1	0
16	2	0	1	0	1	0
15	2	0	1	0	1	0
13	4	1	1	1	1	0
18	2	0	1	0	1	0
30	3	0	1	1	1	0
10	3	0	1	0	1	1
5	3	0	1	0	1	1
6	3	1	0	1	1	0
5	3	0	1	1	1	0
23	3	0	1	1	1	0
21	3	0	1	0	1	1

APPENDIX 6
OBSERVED BANDS IN TCR FISH POPULATION
BASED ON SERUM PROTEIN ACTIVITY

APPENDIX 6
OBSERVED BANDS IN TCR FISH POPULATION
BASED ON SERUM PROTEIN ACTIVITY

Sample	Number of Bands	Sp-1	Sp-2	Sp-3	Sp-4	Sp-5
1	3	1	1	1	0	0
2	3	1	1	0	1	0
3	3	1	1	1	0	0
4	3	1	1	0	1	0
5	2	1	0	1	0	0
6	3	1	0	1	1	0
7	3	1	0	1	1	0
8	3	1	0	0	1	1
9	3	1	0	1	0	1
10	3	1	0	1	0	1

DETERMINATION OF ELECTROPHORETIC VARIATIONS IN
DIFFERENT POPULATIONS OF CHANNEL CATFISH
(*ICTALURUS PUNCTATUS*) BASED ON SERUM
PROTEIN POLYMORPHISM

by

Tereso A. Abella

B.S., Central Luzon State University, Philippines, 1976

AN ABSTRACT OF A MASTER'S REPORT

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Manhattan, Kansas

1980

ABSTRACT

Serum was collected from three populations of channel catfish (*Ictalurus punctatus*) and analyzed for differences using horizontal zone starch gel electrophoresis. The populations sampled were from Tuttle Creek Reservoir (TCR), the Blue River, Kansas, (BR) and from Raymond F. Schroeder's (RFS) fish farm in Slovak, Arkansas. Significant differences among the three populations were found to exist relative to esterase (Es) and serum protein (Sp) bands observed in the electropherograms of blood samples examined. There was an apparent distinction between the RFS fish stock and BR stock for both Es and Sp activities. No significant difference was found between the TCR and BR stocks in the serum protein activity but differences were found between these two fish populations in the esterase enzyme activity.

Electropherograms of samples for lactate dehydrogenase (LDH) showed a very low reactivity, therefore, no statistical analysis was made.

A review of works on several species revealed differences in the serum protein and esterase enzyme electrophoretic patterns. Serum protein and esterase enzyme polymorphisms were found among Ictalurids, Clupeids, Salmonids, Cichlids, Cyprinids and other families.