ABSTRACT

Robert Allan Smith, BIOCHEMICAL GENETIC MARKERS IN BLUEBACK HERRING SUBPOPULATIONS. (Under the direction of Dr. Charles W. O'Rear, Department of Biology, East Carolina University) 1984.

Blueback herring (Alosa aestivalis) were collected from three streams within the Chowan River complex. The larval stages from 14-22 mm. were examined electrophoretically.

Muscle tissue extracts were separated via electrophoretic techniques and the electromorphs examined. A geographic spatial distribution appeared to be statistically evident, as distances increased so did electrophoretic dissimilarity increase.

The biochemical data in conjunction with the natal stream tendency of the blueback herring lend credence to the isolation concept in subpopulation definition.

BIOCHEMICAL GENETIC MARKERS IN BLUEBACK HERRING SUBPOPULATIONS

A Thesis

Presented to

the Faculty of the Department of Biology

East Carolina University

In Partial Fulfillment

of the Requirements for the Degree

Master of Science in Biology

by

Robert A. Smith

May 1984

BIOCHEMICAL GENETIC MARKERS IN BLUEBACK HERRING SUBPOPULATIONS

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ACKNOWLEDGEMENT

I would like to thank the following persons for their help in accomplishing this research: Dr. Charles O'Rear, for conceptual assistance; Bennett Wynne, for assisting in larval blueback collections; and Linda Hall, for editorial assistance. Partial funding for this project was provided by Anadramous Fish Conservation Act funds through the North Carolina Department of Natural Resources and Community Development, Division of Marine Fisheries.

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INTRODUCTION

The objective of this research was to determine if geographically isolated subpopulations of larval bluebacks were biochemically discernable. Larvae were used in this study because of the ease of collection and coincidence of funding for related studies.

Blueback herring (Alosa aestivalis), Mitchell, are anadramous fish of the family Clupeidae. The blueback along with the alewife (Alosa pseudoharengus) are collectively called river herring due to the difficulty in separation. The herrings are characterized by a saw-toothed edge on the belly, one dorsal fin, no lateral line, deeply forked caudal fin, with the body possessing deciduous cycloid scales.

The blueback differs from the alewife in the peritoneal coloring, with the blueback possessing a black peritoneum and the alewife a whitish-pink peritoneum. The adults and juveniles are a silvery schooling fish which are strongly laterally compressed (Jones, et. al., 1976). Generally, the adults have a distinct longitudinal line with a single dark shoulder spot behind the opercle.

The adult river herring generally adhere to coastal waters but move offshore during the winter months (Jones, et. al., 1976). Hardy (1959) states that populations of river herring serve as a forage fish for pelagic predators.

The blueback reaches sexual maturity in about four years and returns to spawn in freshwater and brackish water. Reed (1964), has found the herring to exhibit a natal stream tendency. Spawning occurs in the spring, from March through May, in North Carolina. Spawning occurs from 14°C through 27°C with 21-25°C being the optimum. Fecundity ranges from 45,000 at 238 mm. to about 350,000 at 310 mm. of size (Jones, et. al., 1976).

The eggs are demersal, somewhat adhesive and pelagic. Spawning will occur in streams and ponds with an access to the ocean. Loesch (1969), reports that the blueback generally spawns closer to the mouth of streams than do the alewife, although numerous other authors have not found this to be true. The eggs hatch at approximately 5 mm. to become yolk-sac larvae.

The larval stage ranges from 6 to 20 mm. in total length. Species identification is generally determined by meristic counts, like pre- or post-anal myomere counts. I used the method of Lipson (1974), to separate bluebacks from alewives by postanal myomere counts, with the bluebacks possessing 5 or less and the alewives more than 5. Total preanal myomere counts of 44 and 40, respectively, were also used as a cross-checking criteria. (See Figure 1: COMPARISON OF BLUEBACK AND ALEWIFE LARVAE). The ventral pigmentation pattern is another way to separate morphologically similar Clupeidae (Leim, 1924).

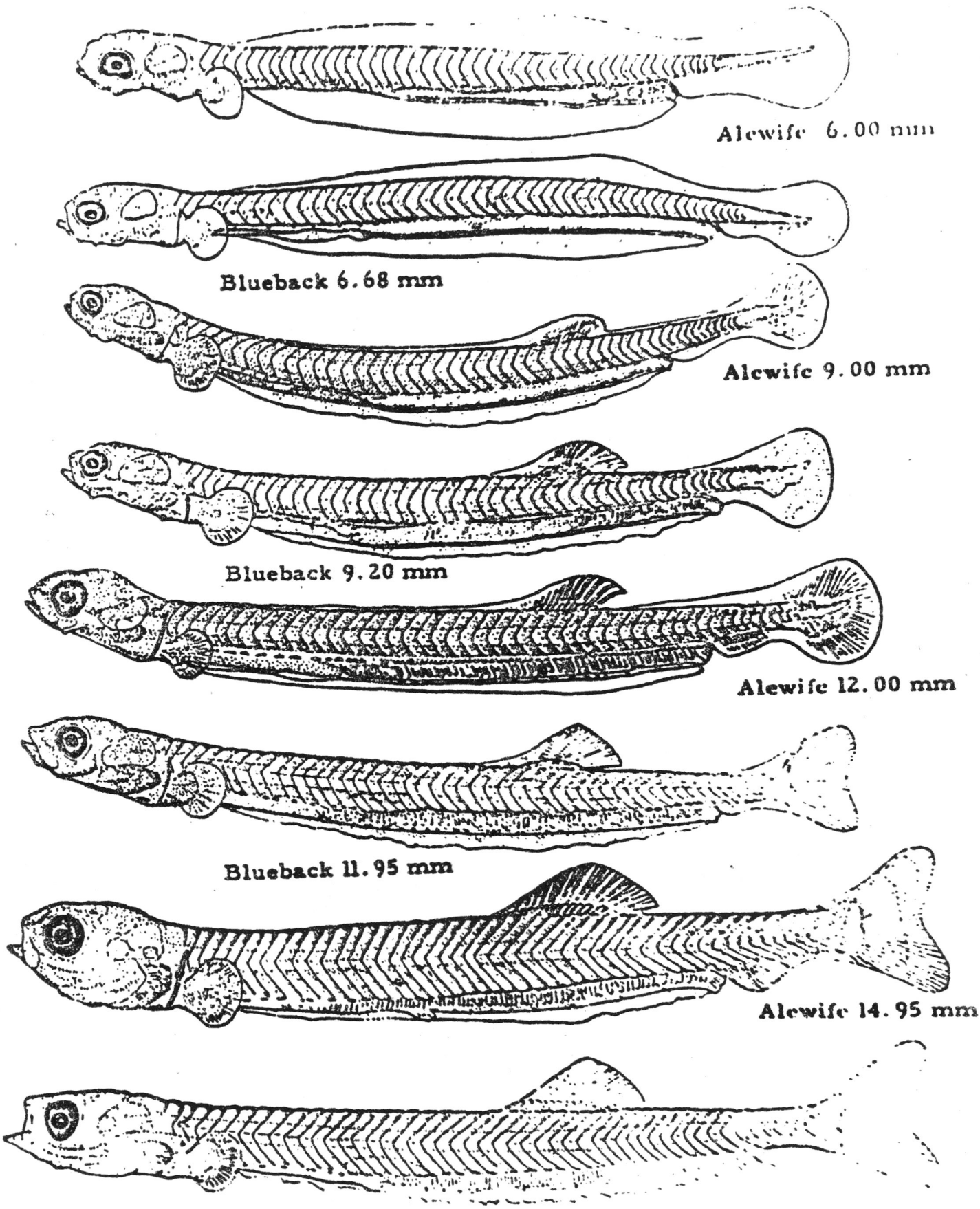

Alewife 6.00 mm
Blueback 6.68 mm
Alewife 9.00 mm
Blueback 9.20 mm
Alewife 12.00 mm
Blueback 11.95 mm
Alewife 14.95 mm
Blueback 14.80 mm

FIGURE 1.

The blueback larvae remain relatively sedentary in small groups while growing. They migrate to the mouths of streams there forming larger and larger groups or subpopulations. The juveniles migrate into estuaries, remaining there until the fall at which time they leave for the ocean (Burbridge, 1974).

River herring form an integral part of the freshwater estuarine food chain. Manooch (1972) and Trent and Hassler (1966), have found that striped bass utilized the juvenile and adult river herring as a food source. Dickson (1955), found that crappie and channel catfish also feed on the herring in its juvenile stage of development.

Virtually all the commercial herring fisheries are in the states of North Carolina and Virginia. In fact, these two states are the only ones which routinely sample for river herring in their fisheries program. Due to the difficulty in separation of the two species, alewife and blueback, most fisheries statistics are for the combined catch of the two species. Loesch and Lund (1977), state the trend in recent years has been toward a dramatic decline in the numbers of adult fish.

Since the overwhelming majority of the commercial catch in North Carolina comes from the Albemarle Sound and its drainage basin, it seems obvious that this nursery ground should be more extensively studied. The decline in abundance from about 19 million pounds in 1969 to a low of 5

million pounds in 1979 has prompted the North Carolina Division of Marine Fisheries to investigate the problem (Leete, 1981).

Some speculation has arisen over water quality problems reducing the catch in North Carolina (Street, 1979). Other significant problems are from the increased fishing pressure applied by offshore foreign catch. As the foreign catch increased, diminishment of inshore catches occurred.

The Fishery Conservation and Management Act of 1976 extended the territorial rights of the United States to 200 miles. This extension would prohibit the catch of prespawned populations in early spring by foreign vessels; hence, theoretically an increase in the spawning population should occur and a significant increase in the major fisheries stocks has been accomplished, except for the herring stocks (Gordon, 1982).

Since the overall abundance of herring stocks has not significantly increased, further investigation of the biology of the river herring is needed. It is hoped that this study should shed some light on a small portion of the stock defintion. Surely, any definitive criteria that can be found to help define subpopulations would be of enormous potential for inherent recruitment and predictive purposes.

Of late, several biochemical techniques have been utilized to distinguish between inter and intraspecific groups or species of fish. Electrophoretic analysis has

been employed in many groups of organisms for various reasons. It is a common immunological technique used today for a variety of reasons. Its uses as a biochemical genetic tool have been well exploited in the field of aquaculture of both vertebrates and invertebrates. Hedgecock et. al. (1976) found that individuals with distinct advantages could be depicted and used for development of increased hybrid success. Utter and Folmar (1978), used electrophoretic techniques to determine genetic variation in the protein of grasscarp stocks. The United States Department of Agriculture has for many years used electrophoretic analysis of muscle for species identification of fish (Lane, et. al., 1966).

Avise (1974) found that in bluegill, specific proteins could be assayed for population defintion. Utter (1974), found that with sockeye salmon, biochemical genetic data could be used for broad defintion of stocks taken at sea. This author (1979) has found with bluegill populations that eyelens protein variability can be used for population definitional purposes.

Environmental as well as physical stress has been shown to affect electrophoretic patterns. The method of capture has been shown to affect the resultant protein electrophoretic patterns in fish by producing differential stress (Schmitten, 1967; Ney and Smith, 1976).

There are basically three types of media which can be used for electrophoretic or biochemical analysis; blood,

muscle, and eyelens. Each system within the body is influenced differently by somewhat different forces, genetic expression and environmental regulation.

The serum component of the blood appears to be the most sensitive to environmental stress and the eyelens the most conservative (Huntsman, 1970; Bloemendal, 1977). Owing to the size of the eyelens in larval fish, eyelens protein analysis was prohibitive. I chose to examine the intermediate form, muscle tissue, for potential electrophoretic protein variability.

MATERIALS AND METHODS

Larval blueback herring were collected from three streams in the Chowan River drainage systems. These were geographically from S. E. to N. W., Rockyhock, Catherine's, and Bennett's creeks. (See Figure 2: MAP OF CHOWAN RIVER). For purposes of clarification, the study sites are divided by approximately 25 km. with Catherine's creek and Bennett's creek being separated by about 2 km. from each other. From a geographically reproductive isolated perspective, one would assume that it would be more probable that if intraspecific variation did occur, that it would be greatest in areas which were more geographically separated. To a certain extent, this variation did occur.

The sizes of fish collected ranged from 6 mm. total length to 25 mm. total length. Although all sizes were examined, only the range of 14-22 mm. of length were used for comparative purposes. The method of capture varied according to the size of the fish. The behavioral biology of the blueback indicated that several methods were necessary in order to effect a large enough catch to be utilized.

The nets used in this study were of three types; dipnet, a modified forward mounted side plankton net, and a channel net. The dipnet was used for yolksac larvae and very small larvae. Herring larvae tend to stay in small groups after hatching in relatively shallow water which is somewhat protected from the main channel of the stream. Since boats do

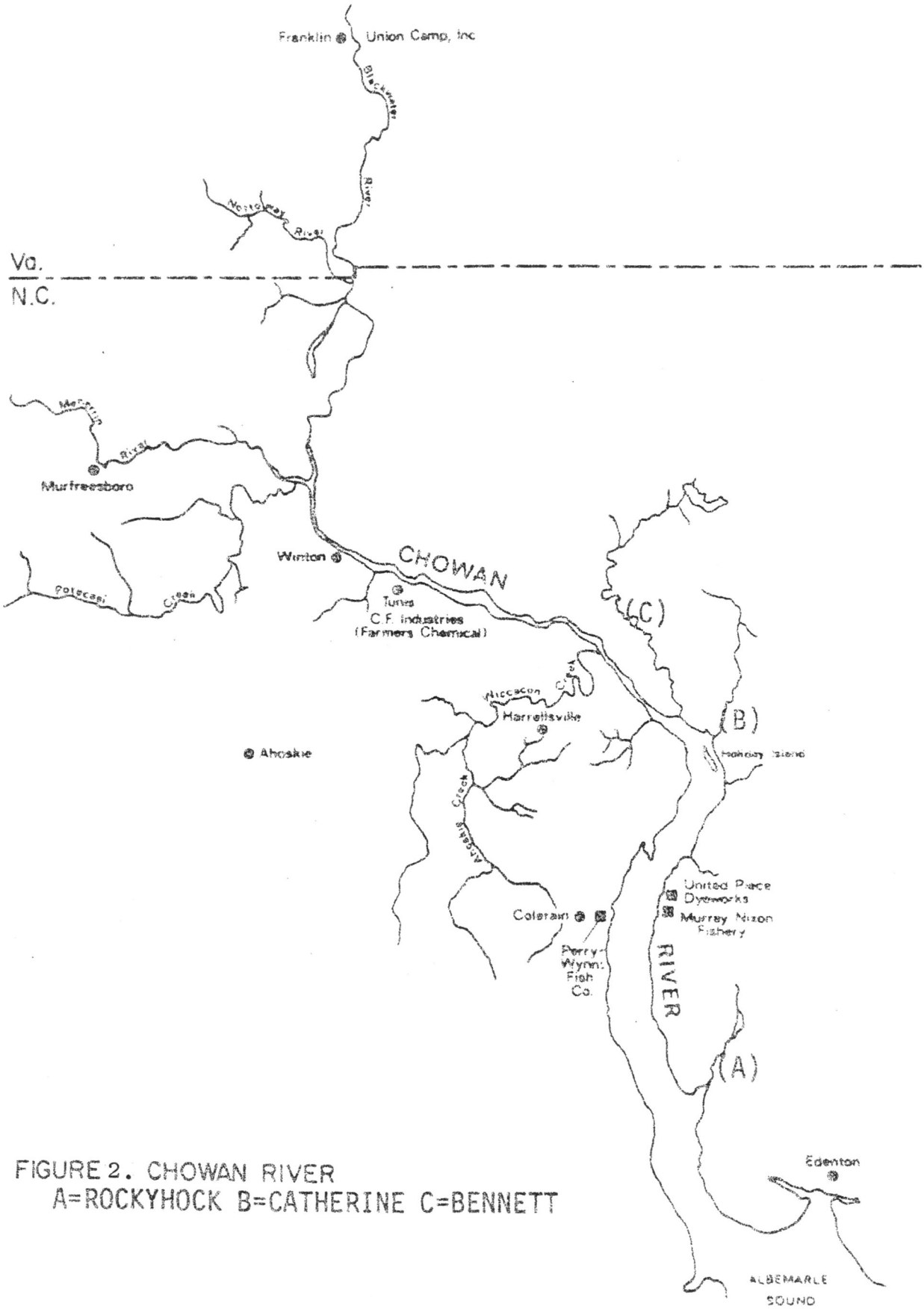


FIGURE 2. CHOWAN RIVER
A=ROCKYHOCK B=CATHERINE C=BENNETT

not have the ability to maneuver very well in the underbrush, wild rose bushes, etc., a long handled dipnet was used with a very fine plankton net sewn into it. With this net, one could reach into the "protected areas" favored by herring larvae. As well, small plankton nets (20 cm.) mounted on frames were pulled in a premeasured fashion, but discarded due to the presence of numerous cypress knees.

As bluebacks grow and develop, they tend to become more mobile; hence, a different type of net needed to be employed in order to catch them. We used a 1/2 meter plankton net which was mounted to a frame. This net was attached to the bow of the boat via an eyebolt and long metal pole. With this setup, the distance from the bow of the boat to the aperture of the net could be controlled as well as the angle of the net relative to the movement of the boat.

Inside the mouth of the plankton net was mounted a general oceanic flowmeter. Using this, one could, if timed, get a relative abundance measurement for statistical purposes (catch per unit volume, etc.). This type of net was favored due not only to the behavioral aspects of the river herrings, but also to the increase in efficiency that it afforded.

Many plankton nets are dragged behind a boat with the results being somewhat erroneous due to several considerations. One sampling consideration is the movement of water off to the bow. Another consideration should be the habits of the fish themselves. Since the larvae are essentially primarily on

the surface of the water, the physical action of the boat itself limits a plankton tow behind the boat. For these reasons, I chose to employ the above mentioned net which could be maneuvered by hand and extended beyond the bow wave.

As bluebacks become more developed and approach the juvenile stage of development, they tend to migrate toward the mouth of streams (Jones, et. al., 1976). These fish are exceedingly more accomplished in their swimming prowess. This prowess necessitated a different approach to sampling. A channel net similar to the channel net used by Lewis, et. al., (1970) was constructed and pulled behind the boat. This net was the only trapping device which was found to have any success catching the more rapidly swimming larvae and juveniles. Large numbers of fish were caught using this net.

Several variations were tried in net location for abundance studies. The favored one by the N. C. Division of Marine Fisheries is simply to sample directly from existent or permanent structures, bridges and the like. We did employ nets constructed as above which were tied to bridges and timed, replete with flowmeter, but found that the more accurate approach should be from boats.

Larval hatching containers were experimented with for two years and found to have moderate to poor results. Adult fish were captured, artificially spawned and placed in the larval container. Muscle tissue extracts were prepared from

the adults to see if electrophoretic protein patterns resembled their progeny. Since the speciality of the offspring were definitely known, the hatched larvae could be used as a comparator for common meristic counts or to verify the speciality of the larvae caught in the immediate proximity for electromorph similarity.

Chemical analysis of each stream was performed for a period of three years. The parameters of conductivity, dissolved oxygen, pH, chlorinity, hardness, and ammonia-nitrogen were examined and found to exhibit no statistical difference. These were for the most part performed in the field with meters and the use of a Hach Digital Titrator. The ammonia-nitrogen analyses were done in the laboratory. Although this sampling was done for grant compliance purposes, it was investigated and included because Ney and Smith (1976) found that intralacustrine differences produced variations in electrophoretic patterns due to differential stress.

The biochemical technique chosen to examine the subpopulations of blueback larvae was an electrophoretic examination of the muscle proteins. Essentially, electrophoresis is the separation of proteins, using an electrical field, according to several characteristics in a supportive media. The theory behind electrophoresis is that proteins will align themselves (stacking) and migrate from an inoculation point (cathode) toward the opposite pole (anode). The action of movement is similar to diffusion but differs in that the movement can be

accelerated in the presence of an electrical field.

In an electrophoretic examination, certain characteristics should be considered. The electrophoretic properties of proteins such as the size and conformation of the protein, the net electrical charge of its amino groups, the solubility of the protein in the electrophoretic buffer, the porosity of the supporting media, the electrical voltage applied, and the time interval of electrophoretic exposure are all taken into consideration.

There are several types of electrophoresis which have been employed of late. I chose to use the 7.5% polyacrylamide gel tube approach (PAGE). The polyacrylamide gel is a cross-linked polymer of acrylamide with an average pore size of 50Å. The gel tubes are placed into a Buchler electrophoretic chamber, sample added, and an electrical current applied. The proteins present will stack up and migrate through the gels from the cathode toward the anode. Those proteins which offer the least resistance to the gel will migrate the furthest and those which create the most friction will migrate the least amount.

For example, serum albumin, which is cigar shaped (150 x 40Å) can orient itself for a minimum amount of resistance or friction and readily pass through the gel. In contrast to this would be a large globular protein like serum lipoprotein (185 x 185Å) which would experience a great deal of friction and have difficulty moving through the pores present in the

gel and consequently stay very close to the cathode or inoculation point (Rendina, 1971).

I chose to use a basic buffer of 0.188M tris-glycine which has a pH of 8.9. A barbitol buffer with a pH of 8.6 was initially tried but discarded because of poor resolution. A basic buffer was employed so that the terminal amino groups would have a net negative charge. Generally, with unknown protein, one uses a basic buffer although techniques exist for acidic, basic, and gradient pH's.

Sample preparation can be altered in a myriad of ways. Two years of experimentation showed that the best resolution was obtained using the following techniques:

(1) Using a binocular dissecting microscope, removal of the head, digestive tract, and fins from fish frozen alive was accomplished leaving solely muscle tissue. Extraneous protein can be associated with these body parts (Bloemendal, 1977).

(2) Rinse the muscle tissue with distilled-deionized water.

(3) Cell destruction was accomplished with a tissue grinder and a Vortex.

(4) 12% Sucrose was added in equal measure to the ground muscle protein. Distilled water and solutions of varying concentrations of $NaCl/H_2O$ showed a poorer resolution and were

abandoned for the sucrose preparation. Hjerten, et. al. (1965) suggests at least a 6% sucrose by weight sample preparation.

(5) The sample was then centrifuged for 5 minutes to remove all stroma.

(6) 100 ul - 200 ul of the sucrose-protein mixture was decanted using a pipette and layered onto the top of the polyacrylamide gels.

(7) A tracer dye of Bromophenol Blue was added so as to visually monitor the electrophoretic process.

(8) A layer of 50% glycerol was applied to the top of each gel so as to limit the flushing out of the protein into the upper reservoir.

(9) A constant current was applied of 90 volts and 18 milliamps for 5 minutes until the sample was introduced into the gel. The current was then increased to 150 volts and 25 milliamps and electrophoresised until the tracer dye migrated to the bottom of the gel. Samples showed much better resolution when a second application of the marker dye was administered and stopped at 1 cm. from the bottom of the gel. This application, of course, would have eliminated the fastest moving protein

from consideration, but nevertheless was chosen because of the increased resolution that it afforded in the remaining protein bands.

(10) Gels were extracted and removed from the glass tubes by rimming with a hypodermic needle and ice water.

(11) The freed gels were then placed into a 12% Trichloroacetic Acid (TCA) solution and the protein fixed for 30 minutes.

(12) The fixed gels were rinsed with distilled water several times to remove any excess TCA and placed in a 0.05% Coomassie Blue 7% acetic acid staining solution for 1 hour. Amido Scharz (black) stains were run on 50% of the gels in the first few trials but discarded because the Coomassie Blue stain was approximately 10 times more sensitive (Biorad, 1979).

(13) The stained gels were then placed in a 10% Acetic Acid 10% isopropanol destaining solution for 1 hour, rinsed with distilled water, and then destained overnight.

(14) Gels were then stored in the buffer solution and analysed for the electromorph variation by measuring with a ruler. Some gels were also measured using a scanning densitometer at 590 nm.

Prior to electrophoresis, the quantity of protein per unit volume was determined. A technique developed by Bradford (1976) and refined commercially by Biorad Laboratories (1979) was employed. When one uses electrophoretic techniques, it is sometimes wise to know the quantity of protein present. Many procedures require a specified amount of protein where in a dilution may be necessary prior to the actual electrophoresis.

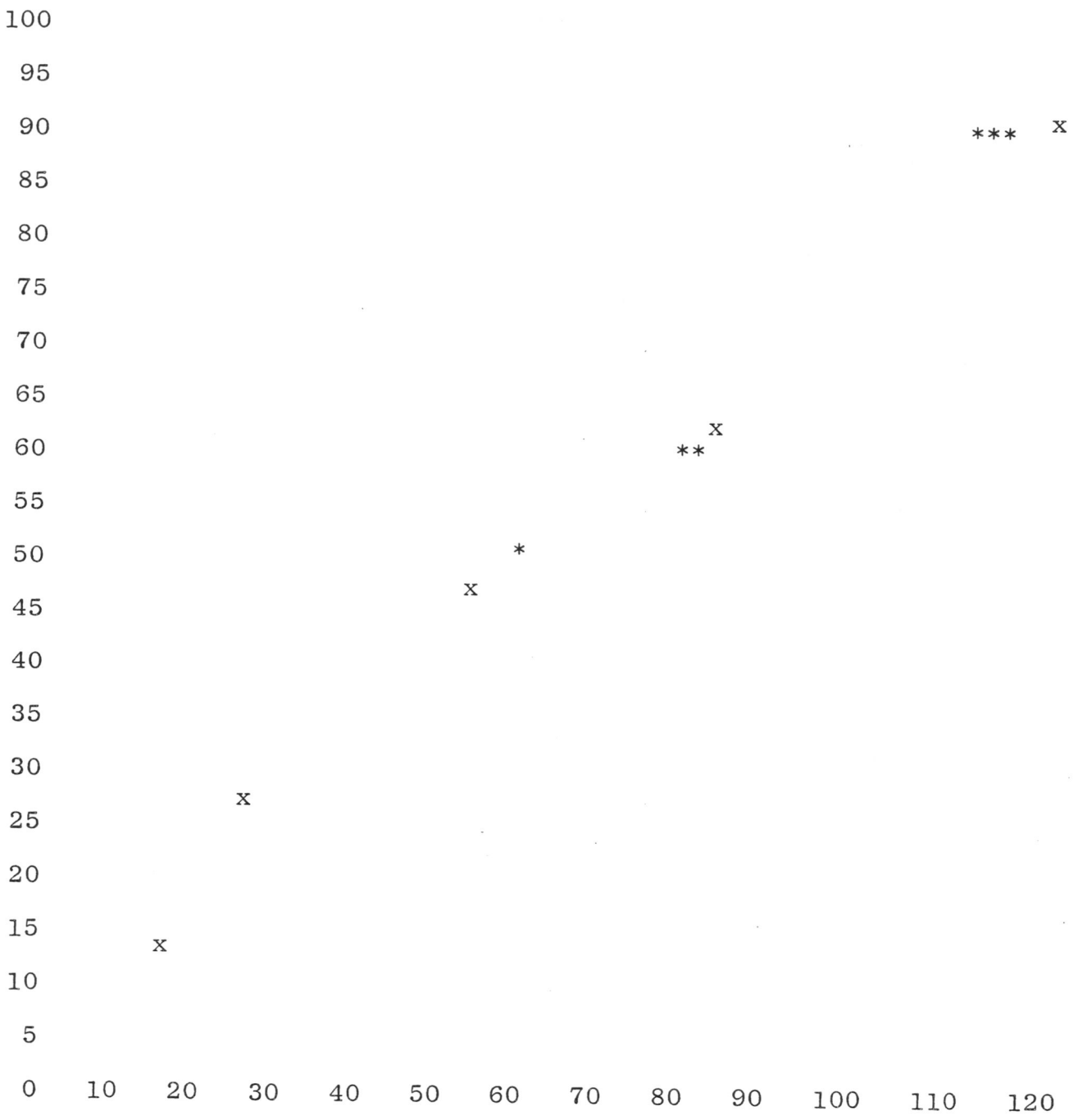
The Biorad (Bradford) technique is based on the maximum absorbance shift from 465 nm. to 595 nm. of protein bound to an acidic Coomassie Brilliant Blue G-250 solution. When using a protein source with unknown proteins, and an unknown quantity of proteins, several techniques could be used. A comparison of the Biuret, Lowry, and Biorad (Bradford) techniques shows very little difference between the three. The Biuret method necessitates a minimum of 100 ug protein; the Lowry method can detect differences as low as 1 ug but has several interferences inherent to unpurified protein. The methodology of Biorad (Bradford) was also enhanced by the simplicity that it afforded.

RESULTS

Prior to an electrophoretic analysis, it is wise to determine the quantity of protein present so as to keep as many factors constant as possible. A protein analysis of three sizes of larval fish indicated that an increase in the size of the fish corresponded to an increase in the concentration of protein present. At a total length of 15 mm., larval blueback muscle tissue exhibits a concentration of 600 ug/ml. As the fish grows to 20 mm., the concentration of protein increases to 790 ug/ml. Finally, at 22 mm. total length, the point at which the blueback changes from the larval to the juvenile stage, the concentration of protein increases to 1400 ug/ml. (See Figure 3: PROTEIN ASSAY LARVAE).

The electrophoretic sample size was regulated in an attempt to keep the quantity of protein constant for each size examined. For example, at size 15 mm., 200 ul. of inoculum was administered to the upper gel surface with a corresponding amount of 60 ug. protein. As the size increased and subsequent protein concentration increased, the quantity of inoculum was decreased. At 20 mm. of size, 150 ul. of inoculum was administered for a total quantity of 60 ug. protein. At size 22 mm., the amount of protein inoculum was further reduced to 100 ul. (See Table 1: PROTEIN ANALYSIS).

PROTEIN ASSAY LARVAE AT 15 mm., 20 mm., & 22 mm.



N = 15
* = Size 15 mm.
** = Size 20 mm.
*** = Size 22 mm.

FIGURE 3.

TABLE 1. PROTEIN ANALYSIS

BIORAD (BRADFORD) TECHNIQUE

Size 22 mm.	x = 1400 ug/ml
Size 20 mm.	x = 790 ug/ml
Size 15 mm.	x = 600 ug/ml

SAMPLE SIZE FOR ELECTROPHORESIS

Size 22 mm.	1400 x 0.100 ml = 140 ug
Size 20 mm.	790 x 0.150 ml = 120 ug
Size 15 mm.	600 x 0.200 ml = 120 ug

N = five per size class

Electropherograms were prepared by staining the gels with Coomassie Blue and Amido Scharz stains. The Amido Scharz stained gels were discarded as owing to the small quantity of protein present in the gels, resolution was poor. The Coomassie Blue stained gels showed much better resolution and subsequently were the only ones analyzed for electrophoretic variation. Eyelens extracts were prepared and electrophoresised, but owing to their small size and quantity of protein which they contained, were discarded. Agarous slab gels were employed to see if the patterns present were comparable to those found by Leete (1981). This technique was discarded as being far less sensitive than the polyacrylamide disc gel technique (Broome, 1963; Clarke, 1965).

A total of seven bands or electromorphs were detected on the polyacrylamide gels. Several approaches were utilized for comparative purposes so as to view the results from different perspectives. The percent occurrence was calculated for each band in each sample. (See Table 2: PERCENT OCCURRENCE OF PROTEIN BANDS BY STREAM). This was determined by a simple presence or absence of a particular band. Of the bands present, the relative mobility (Rf) was determined. In this approach, the amount of migration of each band from the inoculation point (cathode) toward the opposite end of the gel (anode) was measured. The movement of each band was compared to a maximum amount of movement, and a ratio computed. Band 7 (that band which migrated the furthest) was statistically

TABLE 2. PERCENT OCCURRENCE OF PROTEIN BANDS BY STREAM

STREAM	BAND 1	2	3	4	5	6	7	N
Rockyhock	100	69.6	0	95.7	43.5	0	100	37
Catherine	100	75.0	0	100	18.7	100	100	37
Bennett	100	30.4	8.7	100	52.1	60.8	100	34
N =	108	64.0	3.0	106	41.0	58.0	108	

found to be the same in each of the streams. Since this band was consistently present (100% occurrence), and the movement identical for each sample, it was employed as the reference point. All of the other bands were measured for the amount of migration relative to the leading band using the following equation:

$$Rf = \frac{\text{Migration of sample band}}{\text{Migration of the leading (front) band}}$$

A chi-square analysis was performed to see if the three populations of fish were indeed discrete. (See Table 4: CHI-SQUARE ANALYSIS). The percent occurrence of all bands from each sample in each stream were compared both against every other stream and against the overall sample of bluebacks. The results proved that each stream was a distinct subpopulation differing from both the overall population and each of the other two subpopulations.

Certain similarities did exist between each of the subpopulations of bluebacks. The median frequency distribution of each stream was calculated and compared. The median was chosen as a comparator instead of the mean for several reasons. The mean generally gives the "average" amount of migration of each band, but the median gives that point at which exactly half of the measurements are above and half are below a central point (Zar, 1974). The mean will, to a certain extent, exclude outlying values. The median frequency, on the other hand, will present a way to examine the variation exhibited within each band by use of the range of values

associated with an electromorph in deference to a particular central value.

A chi-square analysis of the three streams by percent occurrence indicated that some electromorphs or protein groups are more variable than others. Band No. 1, that band at the inoculation point, was present in all of the samples. This band more than likely is a composite of several protein groups.

All protein groups which are physically very large would of course have greater difficulty migrating through the gel matrix and would tend to stay very close to the inoculation point. As well, since the inoculation point is at the cathode, any protein which could have a cathodic tendency would tend to stay at the inoculation point. Bands 4 and 7 also show little variation in occurrence. These two bands were present in virtually all samples.

The greatest variation in the percent occurrence was exhibited in Bands 2, 3, 5, and 6. Bands 2, 3, and 5 all show significant differences between two of the three streams compared, although not always between the same stream combinations. Bennett's creek showed significant differences when compared to either Rockyhock or Catherine's creeks. In bands 2 and 3, a comparison of Rockyhock and Catherine's creeks for these same two bands showed no significant difference between the two subpopulations.

When comparing Bennett's creek and Rockyhock creek, a difference was not detected in band number 5. Catherine's creek differed from both Rockyhock and also from Bennett's creek in regard to this band. Band number 6 showed the greatest amount of variation in the percent occurrence. All of the streams studied were shown to be significantly different from each other. (See Table 3: CHI-SQUARE ANALYSIS).

When a band was present, the Rf values associated with it were computed and compared statistically to see if variation was present as well. This comparison takes into account the differences in electrophoretic mobility exhibited by each protein group, within each organism, per stream. A comparison of all streams by Rf values showed that each stream was a separate entity differing from each other stream, effectively a subpopulation. There existed a significant subspecific variation by each stream. A chi-square analysis of the Rf values showed a rather distinct trend. When examining each stream geographically, more similarities existed between Catherine's creek and Bennett's creek than dissimilarities.

Catherine's creek and Bennett's creek are geographically much closer to each other than to Rockyhock. Bands 1, 2, 4, 5, and 7 exhibited no significant difference between the two study groups. Rockyhock differed from both streams in all bands except bands 1, 4, and 7. The differences between Rockyhock and Catherine's creeks were equivalent to the differences between Rockyhock and Bennett's creeks.

TABLE 3. CHI-SQUARE ANALYSIS

A. MEDIAN ANALYSIS

1. By % occurrence: ROCKYHOCK ≠ CATHERINE ≠ BENNETT: Therefore, Rockyhock, Catherine, and Bennett are not from the same population. N = 108

2. By Rf values:

	BANDS	
	Same	Differ
a. Rockyhock vs. Catherine N = 74	1,7	2,3,4,5,6
b. Catherine vs. Bennett N = 71	1,2,4,6,7	3,5
c. Bennett vs. Rockyhock N = 71	1,2,3,7	4,5,6

B. MEDIAN FREQUENCY DISTRIBUTION

1. By % occurrence:

a. Rockyhock vs. Catherine N = 74	1,2,3,4,7	5,6
b. Catherine vs. Bennett N = 71	1,4,7	2,3,5,6
c. Bennett vs. Rockyhock N = 71	1,4,5,7	2,3,6

2. By Rf values:

a. Rockyhock vs. Catherine N = 74	1,4,7	2,3,5,6
b. Catherine vs. Bennett N = 71	1,2,4,5,7	3,6
c. Bennett vs. Rockyhock N = 71	1,4,7	2,3,5,6

DISCUSSION

The results of various statistical analyses indicated that differences existed between the three streams studied. Two general approaches were employed to see if a delineation of these differences could be exacted. The first approach was based on the percent occurrence of protein bands exhibited by polyacrylamide disc gel electrophoretic (PAGE) techniques. The second approach was based on the electrophoretic mobility of the protein bands or electromorph diversity. Both approaches should be taken into consideration when examining the apparent biochemical differences between the study groups. One should be able to speculate upon the mechanisms necessary for biochemical variation knowing certain inherent criteria.

The examination of the percent occurrence of protein bands was chosen as a means to evaluate certain, rather broad, abilities of an individual within a group to produce a similar protein. By this is meant that an electromorph, or electrophoretic protein band, is the result of at least one protein reacting to the electrophoretic criteria. It should be noted that differentiation or identification of each electromorph was not accomplished.

It was not my objective to accomplish these tasks but to examine the overall stream constituency. An electromorph is the result of a very precisely controlled phenomena; a soluble protein subjected to an electrical field in a defined

media. Without identification of each band shown on the electropherogram, it would be impossible to say if each band did indeed represent solely one protein or several proteins which reacted similarly under the conditions imposed.

Each individual within each stream exhibited from 5 to 7 protein bands. Each band in every group was considered individually and statistically evaluated against each other stream. Differences were detected in some bands but not others. Certain bands were consistently present both to the group or subpopulation and also to the overall population of the three streams. These bands should be considered as constants or consistent with the overall population of blueback herring.

Variation is exhibited in several of the bands. The mechanism used by some individuals to produce a given type of protein while at the same time others within the same group apparently not producing the same protein is unknown. Biochemical variation is shown here in that some individuals react to a stimulus and produce a certain protein group while others apparently given the same stimulus will not produce the protein. The cause of repression can but be speculated upon. The percent occurrence approach should always be coupled with the Rf value analytical technique when examining the overall biological variation.

The Rf value approach can be employed so as to indicate that if a protein is present exactly how varied it is. A

slightly different trend is evidenced when comparing the cumulative Rf values of each stream. Zar's median analysis indicated that the greatest amount of similarity between two streams was evidenced by the streams closest together, namely Catherine's and Bennett's creeks. Both Catherine's and Bennett's creeks showed a marked divergence from the fish found in Rockyhock. Bennett's creek did indicate, though, a closer similarity to Rockyhock than did Catherine's creek.

An analysis of the intervals associated with the median frequencies of the Rf values yielded similar results to the median analyses. The streams closest together once again proved to be statistically more similar than those further apart. Geographic distance seemed to be equated with electrophoretic similarity. Rockyhock has three bands identical to Catherine's creek and also to Bennett's creek. Catherine's creek and Bennett's creek had five bands identical with only two bands different.

Certain factors of the life history of the blueback lend creedence to the reproductive isolation concept for population defintional purposes other than the results of the above biochemical data. A phenomena known as "homing behavior" must be accountable. Messieh (1977), in his paper on the population structure of the river herring, found that the herring exhibited a much higher degree of separation during the spawning season than before spawning. This supports

a homing hypothesis for river herring. Wright and Hassler (1967) earlier had found using serum proteins that the homing behavior and geographic distance were effective isolating mechanisms for determining populations of white bass. The natal tendency of the river herring in general, and the blueback in particular, has been well documented. (Thunberg, 1971; Loesch and Lund, 1977; Durbin, et al., 1979).

The mechanism for natal tendency has been worked on by Thunberg (1971), and he suggests that the "odor hypothesis" could be a part of the mechanism. When a fish was placed in a simulated stream with different water types at each end, the fish consistently selected one water type over the other. This selection was olfactory; hence, the term "odor hypothesis." Wright and Hassler (1969) contend that the ability to return consistently to a specified spawning ground confers a selective advantage in that it leads to a maximum annual production of young. This natal tendency is utilized extensively in stocking practices today whenever possible. Virgin river spawning grounds are seldom stocked with adult fish, usually with "fry" or juvenile fish. The reason for this stocking technique is as Thunberg (1971) states, that this stage of development is the "imprinting" period.

Knowledge of the population substructure is always a useful tool for predictive purposes. Seldom are all of the reasons known for exactly why one group of individuals react differently to a stimulus than do another group. Avise and

Felley (1979) contend that population structuring can result from inbreeding, selection, or assortive mating due to either environmental or behaviorally imposed restrictions on migration or mating success. These must act upon a set of historical developments. The ability to select a natal stream by olfactory detection may be a possible isolating factor in the migration of blueback populations.

Whenever examining populations of organisms, one must always take great care in their procedures. Electrophoresis is a valuable tool yet to be exploited to its fullest potential in determining population substructure. Certain criteria must always be taken into account in order for the resultant data to be reliable. The number of samples in the electrophoretic examination must be large (Booke, 1964). A total of 108 individual fish were sampled in this research. Gorman and Renzi (1979) have found, at least in regard to electrophoretically examined heterozygosity estimates, that 8-12 individuals will yield estimates within 1% of the total estimates for a larger sample. All study groups exceeded these numbers.

The type of body part used for analysis should also be carefully considered, as the common types, blood serum, muscle tissue, and eyelens differ in their respective origins. All proteins respond to genetic, physiological, and environmental factors although each somewhat differently (Huntsman, 1970). Huntsman found serum protein to be too sensitive to use for taxonomic purposes, but he did note that perhaps muscle tissue

extracts could be utilized for differentiating racial stocks. Manwell (1970) as well states that muscle tissue protein tend to be more conservative than do blood serum protein. Leete (1981) found that eyelens protein electrophoresis could be used to separate groups of adult bluebacks.

The method of preservation always should be taken into consideration. Proteins tend to denature rather quickly when exposed to heat; thus, all samples were kept alive until frozen. Huntsman (1970) found no difference in fish sera frozen for 9 months. All fish were analysed in a shorter time interval than this.

Specific enzyme analysis has also been utilized by many as a method for distinguishing both interspecific and intra-specific variation (McKenzie, 1973; Johnson, 1974; Avise, 1975). The determination of the correct enzyme can sometimes be a rather long arduous procedure, although more specific genetic information can be extracted and expounded upon. Clearly, this analysis would be a preferable examination if time permits. The purpose of this study was simply to see if larval blueback subpopulations were distinct. If, as is my contention, that the larval groups are biochemically distinct, then the exact differences should be investigated as a continuance of this project.

It seems that more than one general conclusion can arise from this study. When comparing solely whether or not a fish has the ability to produce a protein or electromorph, then no

distinct statement can be made about distance and its relationship to electrophoretic similarity or dissimilarity. On the other hand, if a fish produces a given protein, then a comparison of the electromorph variation exhibited can be related to the proximity of the comparators.

Many times, one becomes involved with the specifics of a research topic and loses the significance of the results. I contend that if discernable biochemical markers exist, then this technique can be used to separate subpopulations of larval blueback herring. The next step in this research should be a continuance of the examination of the biology of the subpopulations throughout their life history. Juvenile fishes should be examined in the sounds to see if the biochemical differences exhibited in the larval stage are carried over to the juvenile stage of development.

In addition, the overall population of fish offshore should be likewise examined. If these differences continue to exist in the adult stage, the offshore captured adults can be delineated into their respective spawning groups and reliable importance values assigned to specified nursery grounds. At present, this examination is not being done, but as more of the biology of the herring is known, more precise predictions can be made. As segregation of adults for spawning does occur (Messieh, 1977), the knowledge of the biochemical genetics of river herring could prove to be a valuable asset to fisheries biologists.

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