PROTEIN PATTERNS FROM LENS OF BLUEBACK HERRING (*Alosa aestivalis*) FROM EASTERN NORTH CAROLINA

by

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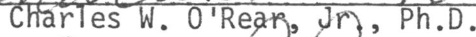

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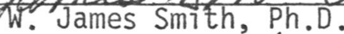

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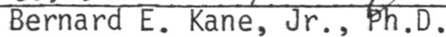

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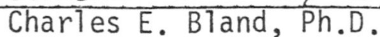

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ABSTRACT

Jerome B. Leete. PROTEIN PATTERNS FROM LENS OF BLUEBACK HERRING (*Alosa aestivalis*) FROM EASTERN NORTH CAROLINA. (Under the direction of Charles W. O'Rear, Jr.) Department of Biology, August 1981.

Blueback herring were obtained from four river systems in eastern North Carolina to determine if the populations were genetically divergent. The proteins of eye lenses were studied using agarose gel electrophoresis.

The protein patterns were subjected to chi-square analysis to determine if blueback stocks could be identified by presence or absence of specific banding patterns. The bands were described for each geographic location and tested for correlation with sex, year, and age.

Sokal's D values for geographic patterns indicated greatest similarity among the closest river systems. The degree of divergence did not allow sub-population identification from lenses proteins.

The banding patterns showed no significant influence when tested for correlation with sex, year, and age. Length-frequency groupings were used for age approximations. Further study was suggested using a less conservative protein source or a combination of sources to gain more insight than one source would allow.

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PROTEIN PATTERNS FROM LENS OF BLUEBACK HERRING (*Alosa aestivalis*) FROM EASTERN NORTH CAROLINA

A Thesis

Presented to

the Faculty of the Department of Biology

East Carolina University

In Partial Fulfillment

of the Requirements of the

Master of Science Degree

in Biology

by

Jerome B. Leete

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INTRODUCTION

The blueback herring, *Alosa aestivalis* (Mitchill), is an anadromous fish that, when reaching sexual maturation, returns from the open sea to spawn in natal streams. Many aspects of its life history are unknown or sparsely studied. The migration from the sea begins after four years of existence for most bluebacks (Marcy, 1969). The spawning migration occurs primarily during the spring in the central Atlantic area. The months of March and April have the highest activity for North Carolina. The peak runs for this study area come in early April with water temperatures between 50° and 60° F.

Most of the spawning activity takes place in freshwater, but brackish water is also utilized. Mortality of the spawned adults can be high, reaching above 50% in some cases (Kissil, 1974). Duration on the spawning grounds varies from several days to several weeks for the adults. After leaving the spawning areas, the adults begin to feed again and return to the sea (Durbin, *et al*, 1979).

The blueback fry are highly salinity tolerant and can utilize either freshwater or saltwater for nursery areas (Chittenden, 1972). This is a significant advantage for the survival and growth of the fish by increasing the potential food supply. The fry migrate downstream, remaining in the estuaries until fall before leaving for the ocean (Burbridge, 1974). The adults range from St. John's River, Florida, to Cape Breton, Nova Scotia (Loesch and Lund, 1977).

The herring fishery is an important source of revenue to commercial fishermen in North Carolina. The fishery consists of the blueback and

the alewife, Alosa pseudoharengus (Wilson), which are not separated in most catch statistics because of the virtually indistinguishable external morphology of the two fish. See Figures 1 and 2. They are commonly referred to as river herring. Commercial uses of the river herring range from human consumption to pet food (McKenzie and Martin, 1975). North Carolina and Virginia annually produce the highest catches for the Atlantic coast and population parameters for these two states reflect the condition of the fishery as a whole. In both states, the fishery has been declining in recent years (Loesch and Lund, 1977).

The largest landings in North Carolina for river herring are from the Albemarle Sound and its tributaties. Other productive waters include the Pamlico and Neuse Rivers. North Carolina catches had a recent peak in 1969 of 19,762,000 pounds with a dollar value of $304,000. The decade of the 1970's proved to be disastrous for the herring industry of North Carolina. The 1970 harvest recorded a 42% drop in poundage and a 36% drop in dollar value from the previous year. The yields dropped steadily and by 1975, the catch was down to 5,952,000 pounds. The only positive aspect for the fishermen was the rise in price brought on by world demand for protein. The price in 1969 was 1.5 cents per pound, which increased to 3.9 cents per pound in 1974. Slight yield increases for 1976-78 were followed by the lowest catch on record in recent times with a poundage of 5,119,000 pounds for 1979. The increasing price, up to 6.1 cents per pound brought $314,000 for the fishermen.

The central problem confronting the industry for the decade of the 1970's was reduction of foreign catch in the Atlantic. The sharp

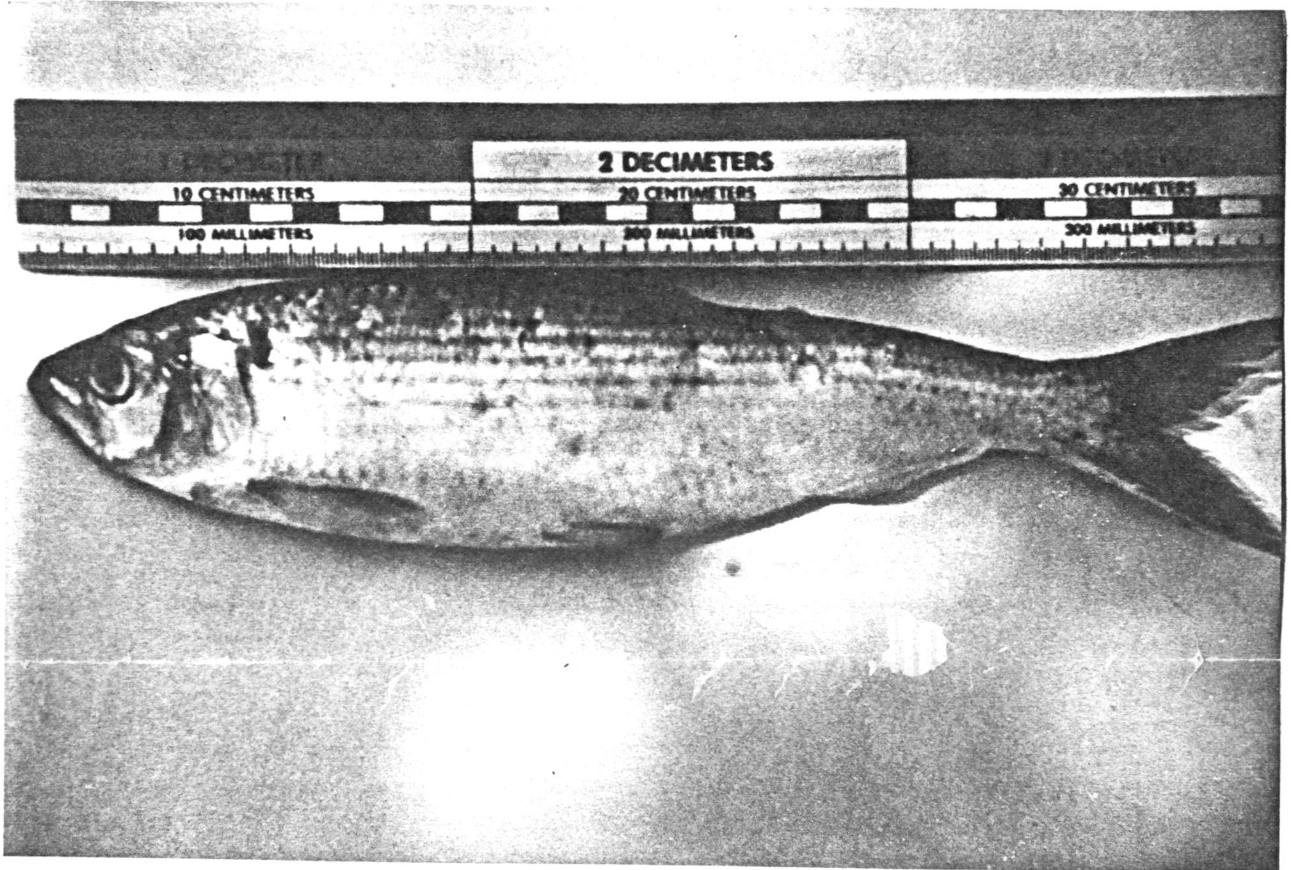


Figure 1. Blueback herring (Alosa aestivalis)

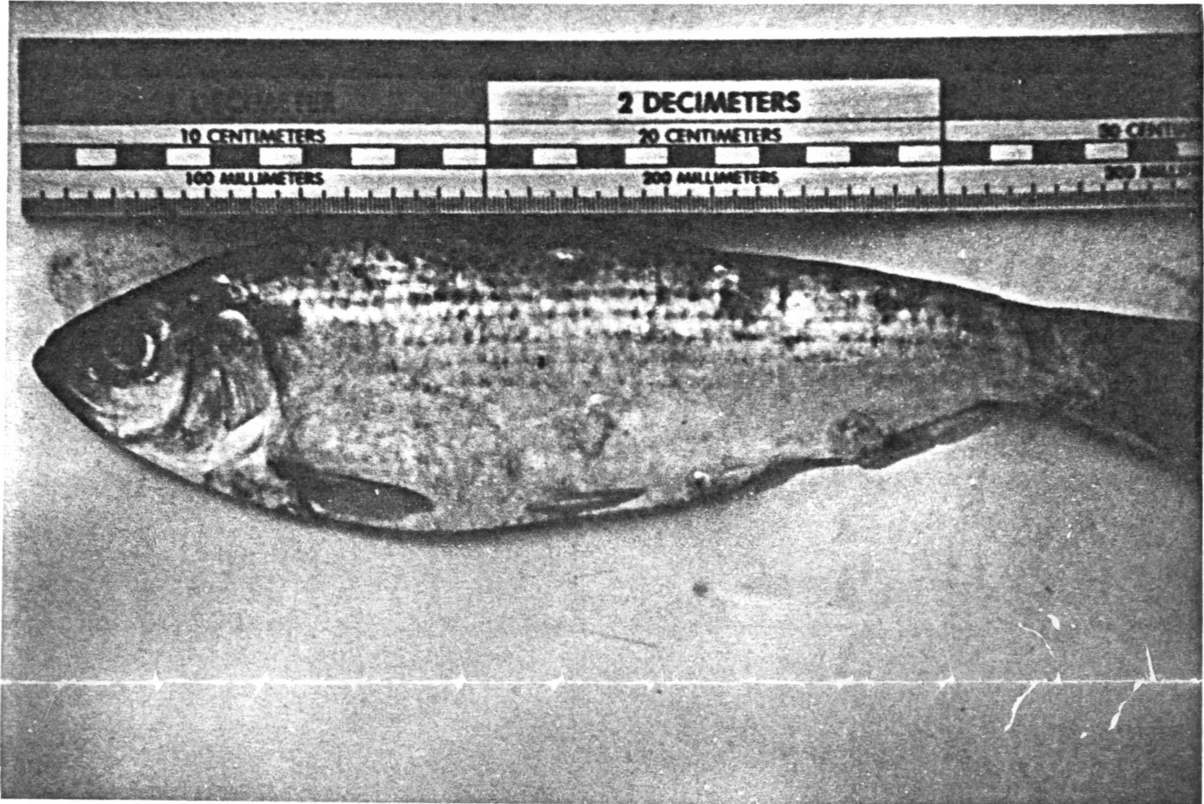


Figure 2. Alewife herring (*Alosa pseudoharengus*)

drop in inshore landings coincided with increased offshore harvest. For relief, the United States negotiated bilateral treaties with Russia, Poland, and Romania during 1975 and 1976. Catch limits were set and certain North Carolina coastal waters were closed to these countries in February and March to protect the herring spawning stock. The Fishery Conservation and Management Act of 1976 extended the United States' fishery jurisdiction out to 200 nautical miles. These actions were successful in reducing the Atlantic catches of river herring by foreign nations. The low catch in 1979 is thought to be related to reduced water quality in the Chowan River and Albemarle Sound. This problem is currently under investigation. These efforts may help raise the yield and dollar value of the herring catch.

The dollar value does not reflect the value of the river herring in their ecological role. During their spawning run, river herring provide nitrogen and phosphorous which increase microbial activity thus releasing energy to the stream stored in leaf litter (Durbin, 1979). The river herring eggs hatch out at the same time that phytoplankton blooms are commonly occurring, leading to an increase in the zooplankton. Burbridge, 1974, working with blueback fry in the James River, Virginia, found that on a monthly average, seven zooplankton species constituted not less than 96% of the plankton and not less than 92% of the stomach contents. The river herring are an important forage fish for predators in fresh water and marine environments (Bigelow and Schroeder, 1953). These ecological roles are important to community stability.

It would be helpful to the understanding and identification of the herring's ecological benefits if the sub-populations spawning in a particular stream could be identified. The impact of the spawning run could be predicted if an estimate of sub-population spawners were known. Also, fishery management scientists could manage the blueback population better if sub-populations could be identified. Fishing regulations could be adjusted to sub-population needs, allowing maximum harvest without devastating the population.

Several methods are currently in use for sub-population identification. Morphological characteristics have been used to identify sub-populations, but problems are encountered in using this method. An often found size gradient among fish occurs in which the more northern member of a species is generally larger than its southern counterpart (Barlow, 1961). This size variation is based on a tempe[illegible]re cline in which lower temperatures produce larger individuals. [illegible] the Southern Hemisphere, the north-south variation in size reverses to south-north. Also, within populations, there is variation in expression of size. For example, similar fish of the same area will vary in many comparative forms such as weight, size of head, or size of eye orbit. There will be a high-low range common to the species in which the measurements taken of the individuals will fall.

Other factors such as sex can influence morphological characteristics of fish. Pollard and Pichot (1971) in trying to classify fishes of the genus <u>Spicara</u> found their work difficult because of the color variations caused by sexual dichromatism and state of sexual maturity. These variations and changes make reliance on morphological

characteristics for sub-population markers insecure.

Another method employed in identifying fish sub-populations is meristic counts of serial elements such as finray or vertebrae. Barlow (1961) found that a slow developing rate in a fish produced higher meristic counts in fish of the same age. Fish in lower temperature waters normally have a slower developing rate than warm water counterparts resulting in meristic differences based upon temperature. Other stress factors in the fish's environment can alter its rate of development. An example would be a stream with high rates of sewage discharge causing depressed values for dissolved oxygen. Stress factors normally act by slowing development and increasing meristic counts.

Messieh (1977), working with breeding stocks of alewives and bluebacks from different areas of the St. John River, studied the groups by comparison of eight meristic characters. He was able to show significant differences between the two species. He was not as successful in differentiating between alewife breeding groups that were in the same river.

A mean and range must be established for meristic counts and for morphological studies. A disadvantage in establishing the mean is that a relatively high sample number is required. This is a problem in situations where only a small sample is available. Meristic counts are a valuable research method, but more accurate methods are available for identification of fish sub-populations.

Use of gene frequencies as a means of identifying populations has been advanced in recent years by discoveries in molecular genetics

involving protein synthesis. One of the methods frequently used in work involving sub-populations is a biochemical technique, electrophoresis. This technique is separation of the proteins in a supporting media while subjected to an electrical field. The rate of movement depends primarily on the net electrical charge of the protein. Also, size and shape of the protein alter its movement. The genetic mechanisms for the use of electrophoresis in identifying fish populations centers around the "one gene-one protein" concept or more accepted as "one gene-one polypeptide." Proteins can be studied for variability reflecting the variance of the gene and how many forms it may have, and the frequency of expression of each form.

Lane, et al (1966) reported that the United States Department of Agriculture was an early user of electrophoresis to identify species of commercially prepared fish fillets. Ground up muscle tissue was their protein source. Each species produced a unique, reproducible pattern. Huntsman (1970) was successful in separating three species of Carpiodes using electrophoretic techniques. Muscle tissue extracts were used for the protein source. Morgan (1975) was able to distinguish larval forms of white perch (Morone americana) from striped bass (Morone saxatilis). He had not been able to do so using morphological or meristic characters. McKenzie (1973) working with muscle tissue protein established separate patterns for the blueback and the alewife but was not able to determine any differences within each species, even though the collection sites were geographically separated. He concluded that muscle protein was not a suitable source of protein to study variation within a species.

Blood serum is frequently used in electrophoretic work. Morgan, *et al* (1973) was able to detect intraspecific differences in serum protein patterns of striped bass from several of the rivers sampled in the Upper Chesapeake Bay. He felt this method was more accurate than using meristic or morphological characters. McKenzie and Martin (1975) worked with serum transferrins from blueback herring collected from four areas of the St. John River, New Brunswick. The fish samples were separated by time from geographically different collection points. They concluded that the serum transferrins were of limited use in identifying sub-populations of blueback herring in the St. John River.

A problem in using serum proteins is the close relationship between gene expression and the physiological condition of the fish. This varies the quantity of an individual protein present in the blood stream. The concentrations of protein present may be altered by various activities as feeding or spawning. Environmental factors may stimulate quantitative changes in serum proteins. Booke (1964) found eleven factors, physiological and environmental that effected the serum proteins of fish. Dorfman (1973) reported that stressful methods of capture could alter serum proteins. These possible influences make precise interpretation of serum protein patterns difficult. Large samples are needed because of these variables. Tissues containing proteins that are less variable are desirable to use as sub-population markers.

The eye lens of a fish provides such a tissue for use in sub-population identification. The lens has no contact with the blood

circulatory system and is not as subject to alteration from physiological or environmental influences. The lens is thirty-five percent protein and ninety percent of these are water soluble (Bloemendal, 1977). The conservative trend of the lens is especially emphasized in the lens' nucleus. Proteins found in the nucleus are present from the time the fetus was formed (Bloemendal, 1977).

The nucleus is frequently isolated and used alone in searching for genetic intraspecific variation in protein patterns. The cells of the nucleus do not undergo mitotic activity after formation and are buffered from contact with surrounding media, such as aqueous or vitreous humors, by the lens cortex. This buffering from other protein systems and lack of mitotic activity produce a source of proteins that is not readily subject to qualitative or quantitative change. These proteins are resistant to denaturation (Smith and Goldstein, 1967). Saunders and McKenzie (1971) used eye lens nuclei in electrophoretic work with Arctic char. They found inter-population variation in protein patterns and suggested that these variations could identify sub-populations of char. Smith (1971) obtained best results with eye lens nucleus while working with the rock fish family, Scorpaenidae. He considered the nucleus to be of value in further study of this family.

In contrast to isolating the nucleus, many researchers prefer to use the entire lens. The outermost layer of the lens is the only site of mitotic activity. This activity decreases as the fish ages, except when the lens is injured. The outer section of the lens, or cortex, does not contain proteins from blood or muscle systems. The proteins

of the cortex are more subject to environmental influences than the nucleus but are more conservative than either blood or muscle protein systems.

Cobb, *et al* (1968) used the entire eye lens and worked with thirteen different species of saltwater fish ranging from Atlantic croaker (*Micropogon undulatus*) to bonnethead shark (*Sphyrna tiburo*). The analyzed pattern of their eye lens proteins showed each species identifiable from the results. Pollard and Pichot (1971) selected the eye lens for its protein constancy to identify three forms of the genus *Spiraca*. Eckroat (1971) separated brook trout (*Salvelinus fontinalis*) that came from different sample sites. He employed the eye lens as a protein source. Brassington and Fergusson (1975) identified hybrids of roach, rudd, and bream on the basis of their protein from the entire eye lens.

A statistical method usable to analyze presence or absence frequencies in protein banding patterns was developed by Sokal (1961). Sokal developed a distance coefficient computation for measurement of similarities between pairs of taxa. Equally weighted characters of observed values must be used in his formula to compare pairs of taxa. Weinstein and Yerger (1976) employed Sokal's taxonomic distance measure to compare protein pattern results among four sub-populations of seatrout. Values for the calculations were obtained from the percentage occurrence of the observed protein bands. As taxonomic distance between compared pairs increases, the calculated values increase.

The whole lens provides more protein bands for study than the

isolated nucleus. It is relatively constant and less variable than protein systems from either the blood or muscle systems. The lens is easy to remove from fish and requires little more equipment than a scalpel and containers. Its compact size makes possible storage of samples in a small area.

The objective to be accomplished is the identification of sub-populations of blueback stocks. The specific objectives are to: identify stocks by presence or absence of specific banding patterns using eye lens as the protein source; describe banding patterns from various sample areas; correlate sex, year, and size with any bands they influenced.

MATERIALS AND METHODS

Collections of spawning blueback herring were made in the spring of 1977, 1978, and 1979. The herring were sampled for these three years from the Roanoke River, Meherrin River, Chowan River, and the Neuse River. Single samples were taken in 1977 from the Albemarle Sound and Blounts Creek in Camden County (see Figure 3). Data derived from samples for these last two collection sites was determined to be of less significance than data from the other sample locations. This was done because of the small sample size from the same year. The Meherrin, Chowan, and Roanoke Rivers are all tributaries of the Albemarle Sound. Blounts Creek is a tributary of the Pasquotank River which also flows into the Albemarle Sound. The Neuse River is a tributary of the Pamlico Sound and is geographically separately from the other sources. Tables 1 through 3 give locations and numbers of fish sampled by sex and year. The herring were purchased from net fishermen who caught the fish in gill nets. Samples were also obtained for me and kept frozen by employees of the North Carolina Division of Marine Fisheries at Elizabeth City.

The non-herring species sampled consisted of gray trout, croaker, and bluefish. Single samples were taken for the study in the summer of 1978. These fish were caught by hook and line in the Pamlico Sound near the vicinity of the Neuse River entrance buoy.

With fresh fish, preparation was done primarily in the field. The fork length of the fish was measured on a board with a built-in metric ruler. The fish were sexed by applying palm pressure to the fish's

Figure 3. Study area in coastal North Carolina
1. Roanoke River
2. Chowan River
3. Neuse River
4. Meherrin River

abdomen and observing the vent for release of milt or eggs.

The bluebacks were identified from the alewives by opening the body cavity and checking the abdominal peritoneum for color, noting that bluebacks have a bluish-black peritoneum, and alewives have a pink peritoneum (see Figures 4 and 5).

The entire eyeball was then removed and dissected for the lens (see Figure 6). Both lenses were placed in a glass vial, then on ice, and transported to the lab. In the lab, the lenses were processed immediately or frozen for later use. Freezing the lenses did not alter their protein patterns which was in agreement with published literature (Brassington and Freguson, 1976), (Utter and Folmar, 1978), Weinstein and Yerger, 1976).

The Marine Fisheries employees froze the fish they collected. The frozen fish were picked up and transported to the lab on ice and kept frozen until processed. When required for use, these samples were thawed at room temperature and immediately processed. There was no refreezing of the lens. Once defrosted, the fish were subjected to the same procedure as the non-frozen samples, except that all the work was done in the lab.

The first step in lens preparation was a wash in distilled water, repeated three times, to remove any protein sources attached to the outside. Next, I added one milliliter of 0.018 percent saline solution to a test tube and placed the lens from one fish in the solution. This saline solution helped extract water soluble proteins and caused no interference in their patterns (Smith and Clemens, 1973). This was

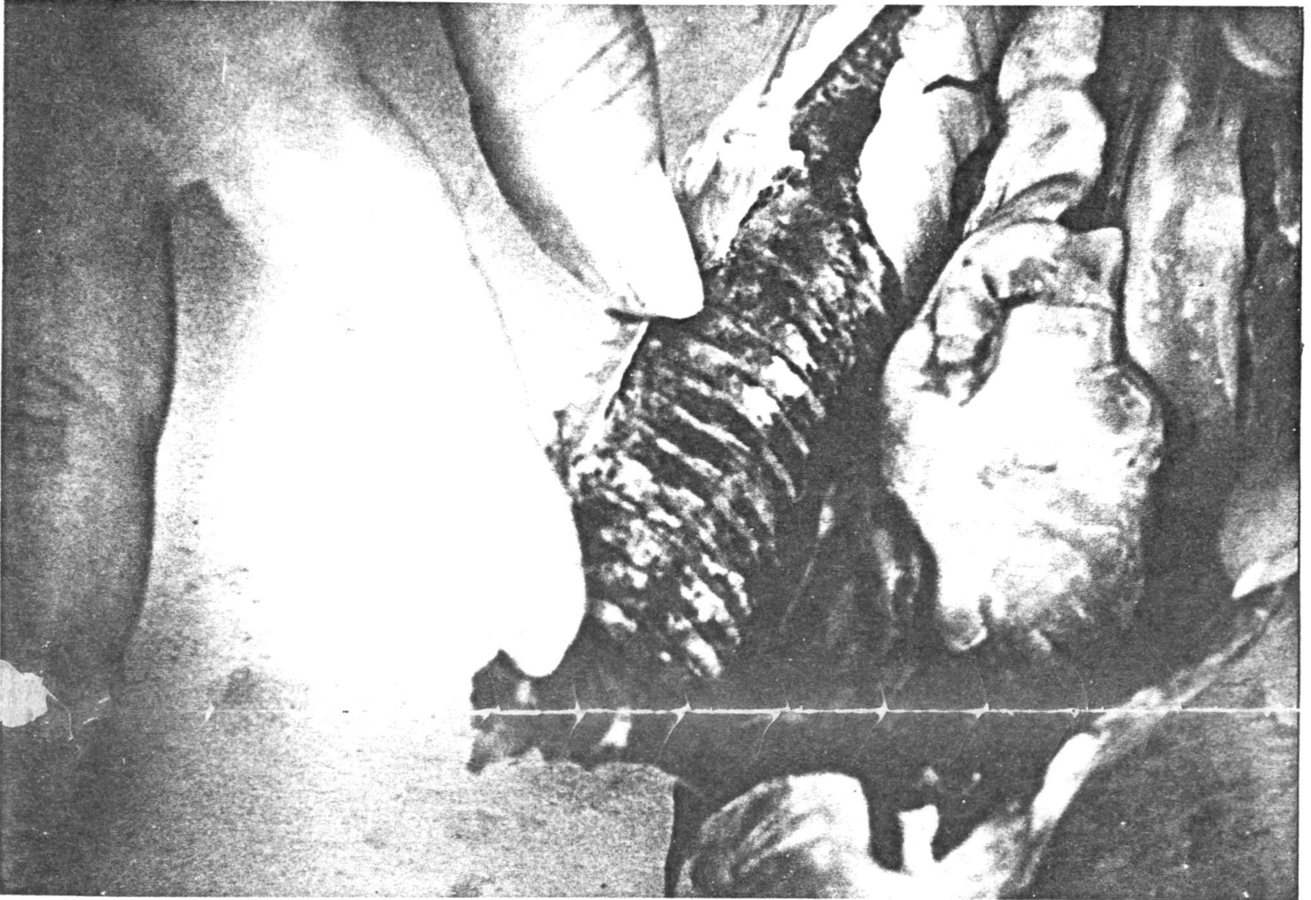


Figure 4. Peritoneum color of Blueback herring

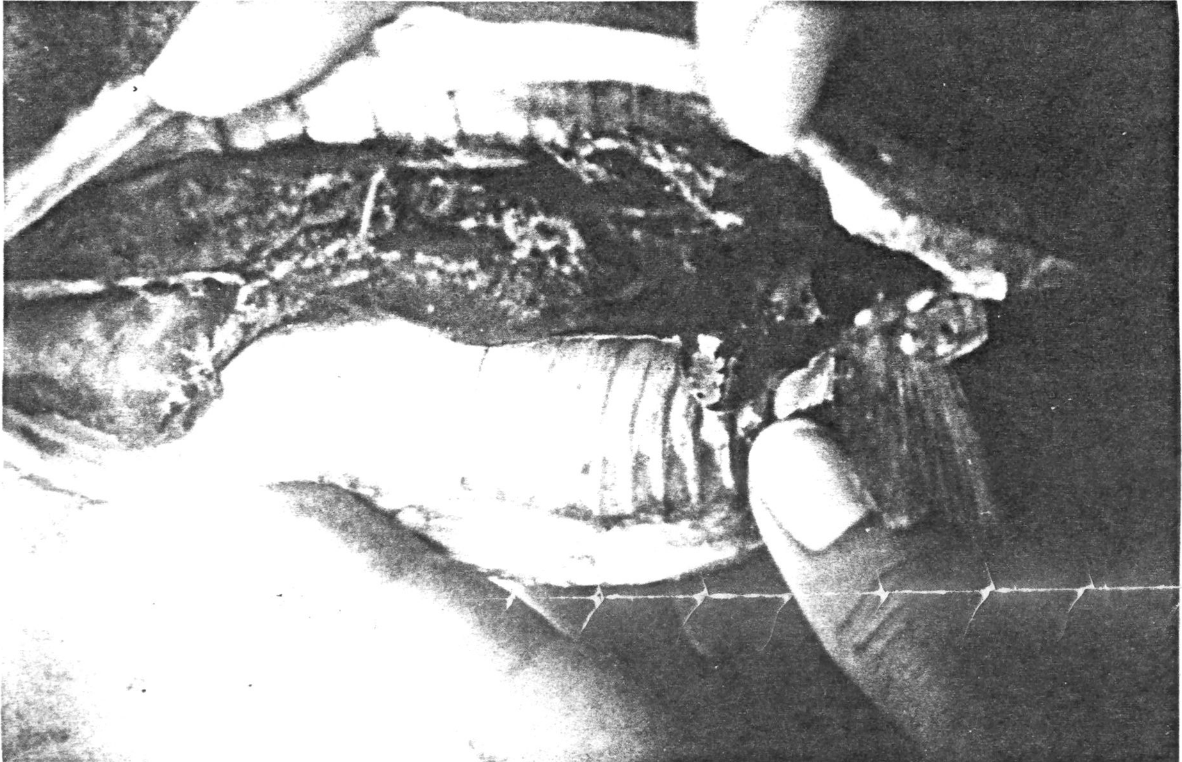


Figure 5. Peritoneum color of Alewife herring

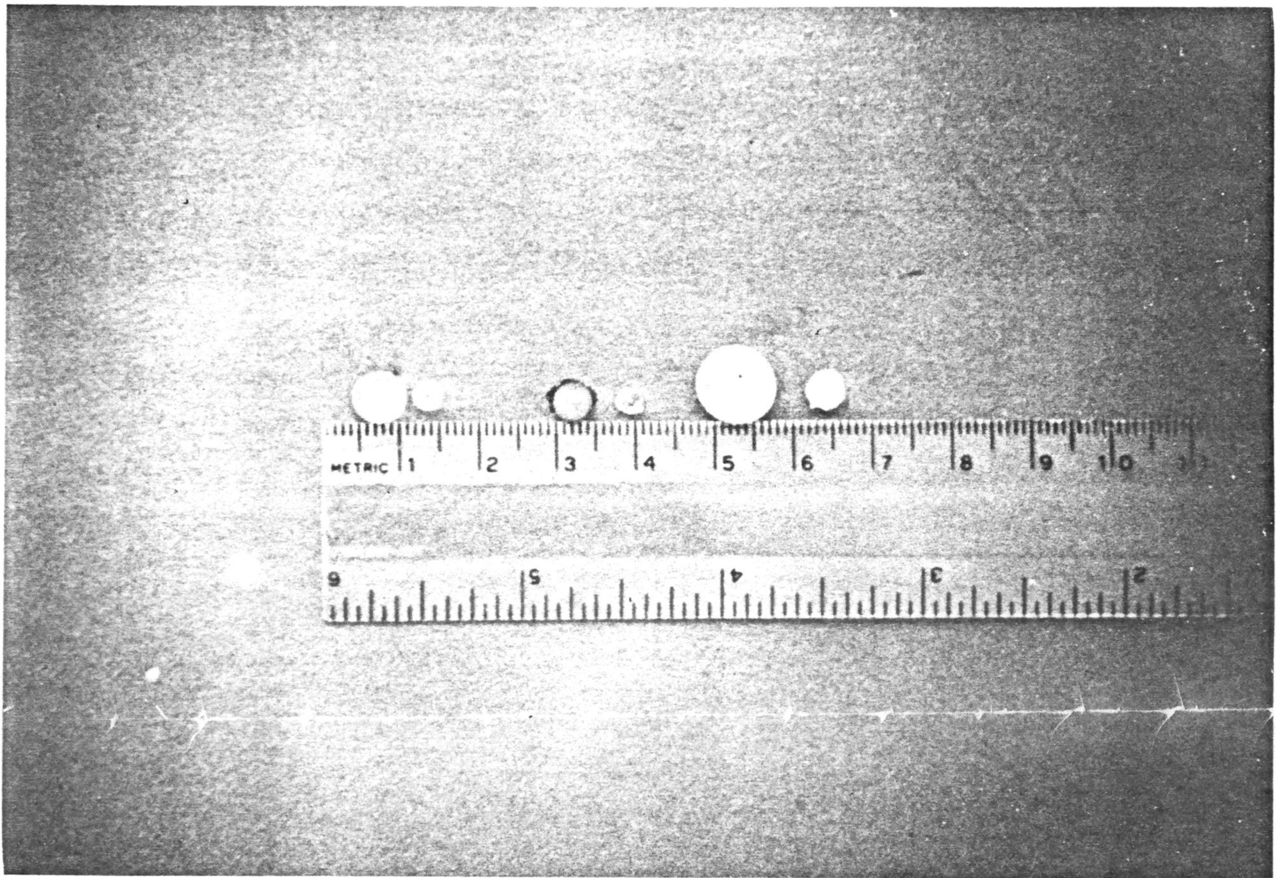


Figure 6. Whole lens and nucleus of (left to right) Alewife herring, Blueback herring, and King mackerel (*Scomberomorus cavalla*)

followed by cell destruction with a manual tissue grinder. After grinding, the mixture was sealed and placed in a cold room on a shaker for twenty-four hours to enhance extraction of water soluble proteins. When removed from the shaker, the solution was centrifuged for five minutes at twelve hundred revolutions per minute and the supernatant was used for the sample.

The system used to run the samples was the Corning-ACI Agarose Film/Cassette System using Universal Electrophoresis Film Agarose. This film utilizes Agarose as the media through which the proteins migrate and is imprinted with eight slots for sample application. One microliter of sample was applied to each slot using the Corning ACI microliter sample dispenser and disposable sample tips. This system was calibrated to deliver one microliter of sample. The power source was designed to produce a constant ninety volts. The samples were run for forty minutes. Longer running times expanded and lightened the bands making them difficult to read. Shorter running times kept the bands close together and caused severe masking of the proteins. The buffer used was Barbitol Buffer with EDTA and a pH of 8.6. Amido Black 10B stain was used to mark the location of the proteins on the film after running, and excess stain was removed with a five percent acetic acid wash. The film was then allowed to dry.

Proteins were studied on the basis of electrophoretic migration. The migration distance of the protein bands were read visually by using the film as an overlay on one millimeter graph paper. Distances were recorded in millimeters. Protein appearance frequencies have been treated statistically to determine degrees of similarity between

populations of a species by the author.

A zone electrophoretic method utilizing an agarose gel media was chosen by me for separating the lens proteins. This method allowed a wide range of application with basic apparatus that could be operated by technicians without long periods of training. There should be less technician error and less technician bias introduced in a method of this sort. Expenses from supplies were lower than for many electrophoretic systems. The speed of this method was high allowing greater sample numbers to be run and less technician time devoted to the mechanics of the system.

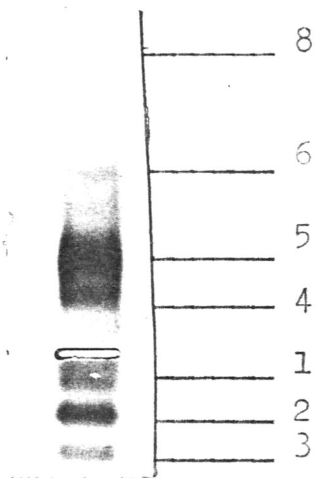
Computer key punch cards were prepared for each fish recording all data for statistical analysis by computer. Statistical Analysis System (SAS 76, 1976), and the Statistical Package for the Social Sciences (SPSS, 1976) were used for analysis. Sokal's method was used to compare taxonomic distance between sample sites.

RESULTS AND DISCUSSION

The lens protein patterns for the blueback herring produced a maximum number of eight bands and a minimum number of seven. All bands were not distinct in each fish. The bands migrating the least stained darkest, and those migrating farthest were the lightest. The bands with highest consistency in staining intensity and distance traveled were three bands migrating toward the negative pole. These proteins possessed a net positive charge. They were present in 100 percent of the samples run and displayed little variation in band characteristics. The other bands migrated toward the positive pole, representing a net negative charge for the protein. Of these five bands, the band closest to the point of sample application showed highest consistency in staining. Bands occurring in nearly 100 percent of the samples for all locations were of least interpretive value in statis[illegible] analys[illegible] See Figures 7 and 8 for typical patterns.

The protein patterns were compared by collection location and sex. Data are presented in Tables 1 through 3. Summaries are given by each sex and for both sexes combined. Frequency of occurrence for bands one through four were consistent for all locations and for both sexes. Bands firve through eight displayed more variation based on presence or absence.

For age analysis of protein patterns, data was grouped by sex and by size. Figures 9 through 12 are the length frequency histograms. The bluebacks were separated by age and by sex, followed by a summary for the three years. Herring were sorted by size to determine



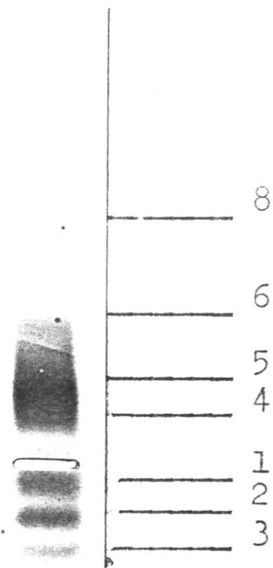


Figure 7. Examples of lens patterns. Anode is to top of page for each. Application point is slot in film. Chowan River, 1977, at top and Neuse River, 1977, at bottom.

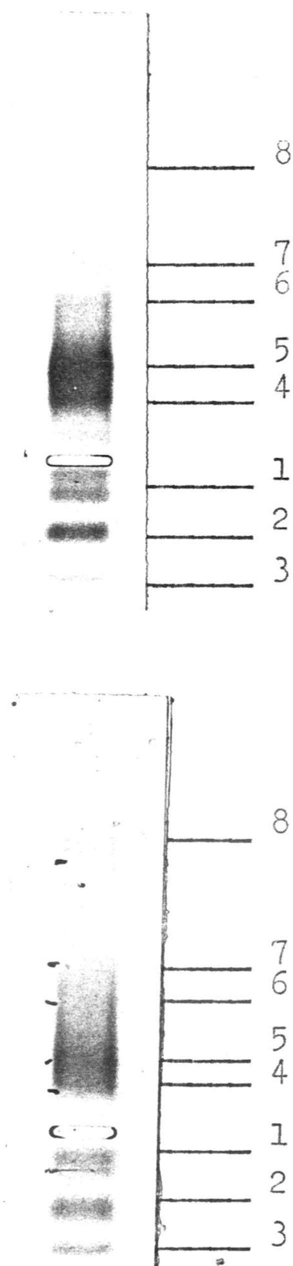


Figure 8. Examples of lens patterns. Anode is to top of page for each. Application point is slot in film. Neuse River, 1978, at top and Roanoke River, 1979, at bottom.

Table 1. Percentage occurrence of bands by location, females.

Location	Band 1	Band 2	Band 3	Band 4	Band 5	Band 6	Band 7	Band 8	N
Roanoke River	100	100	100	98	64	88	69	64	42
Chowan River	100	100	100	100	33	74	67	85	17
Neuse River	100	100	100	100	52	100	83	93	27
Meherrin River	100	100	100	100	91	36	91	36	22
Albemarle Sound	100	100	100	100	100	100	0	88	8
Blounts Creek	100	100	100	100	67	67	17	42	12

Table 2. Percentage occurrence of bands by location, males.

Location	Band 1	Band 2	Band 3	Band 4	Band 5	Band 6	Band 7	Band 8	N
Roanoke River	100	100	100	100	53	90	58	47	19
Chowan River	100	100	100	100	13	47	87	67	15
Neuse River	100	100	100	100	61	83	100	100	18
Meherrin River	100	100	100	100	100	50	93	29	14
Albemarle Sound	100	100	100	100	100	100	0	75	4
Blounts Creek	100	100	100	100	0	100	50	0	4

Table 3. Percentage occurrence of bands by location, both sexes.

Location	Band 1	Band 2	Band 3	Band 4	Band 5	Band 6	Band 7	Band 8	N
Roanoke River	100	100	100	98	61	89	66	59	61
Chowan River	100	100	100	100	26	64	74	79	32
Neuse River	100	100	100	100	55	94	89	96	45
Meherrin River	100	100	100	100	94	42	92	33	36
Albemarle Sound	100	100	100	100	100	100	0	83	12
Blounts Creek	100	100	100	100	50	75	25	31	16

Figure 9. Length-frequency for 1977 sample.

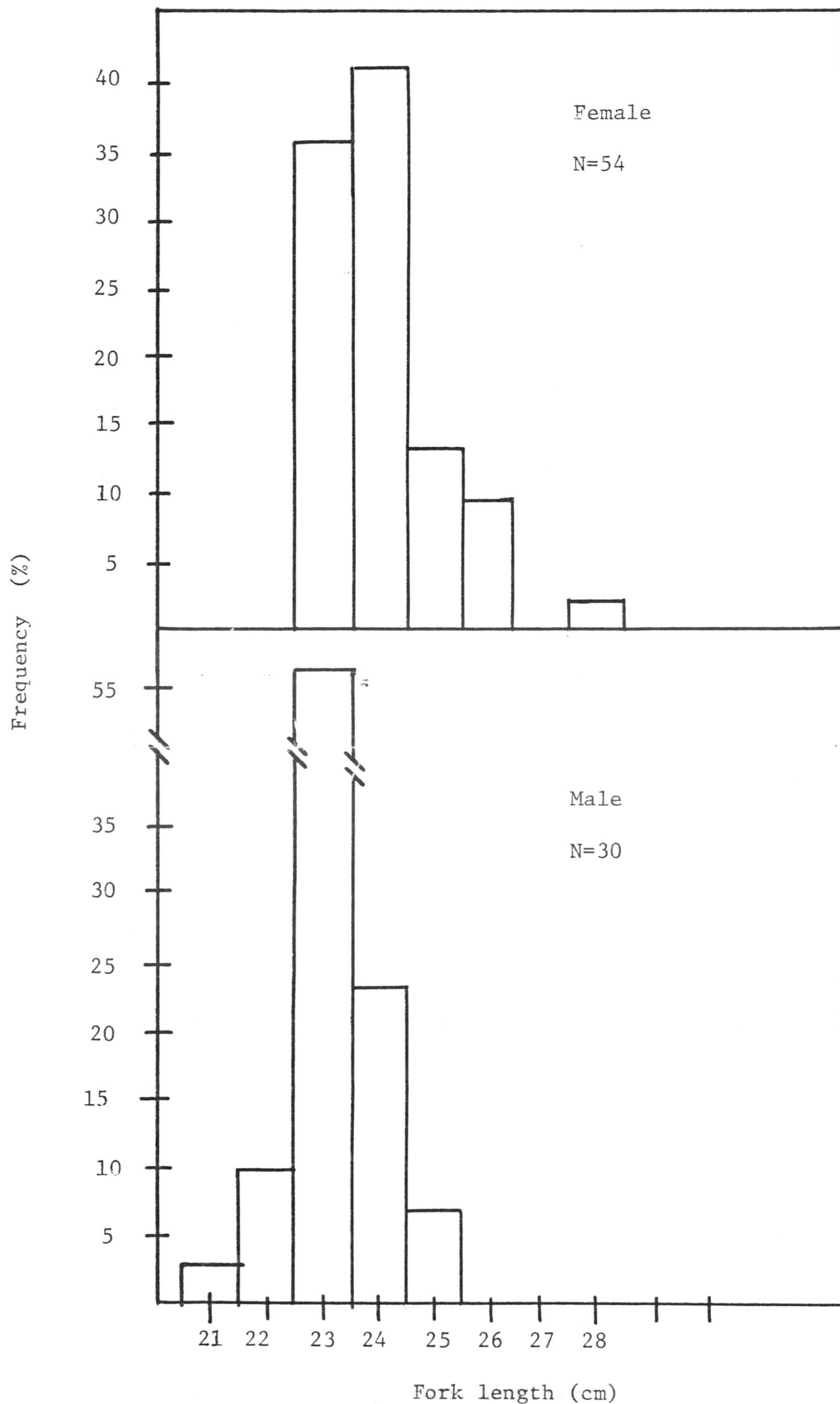


Figure 10. Length-frequency for 1978 sample.

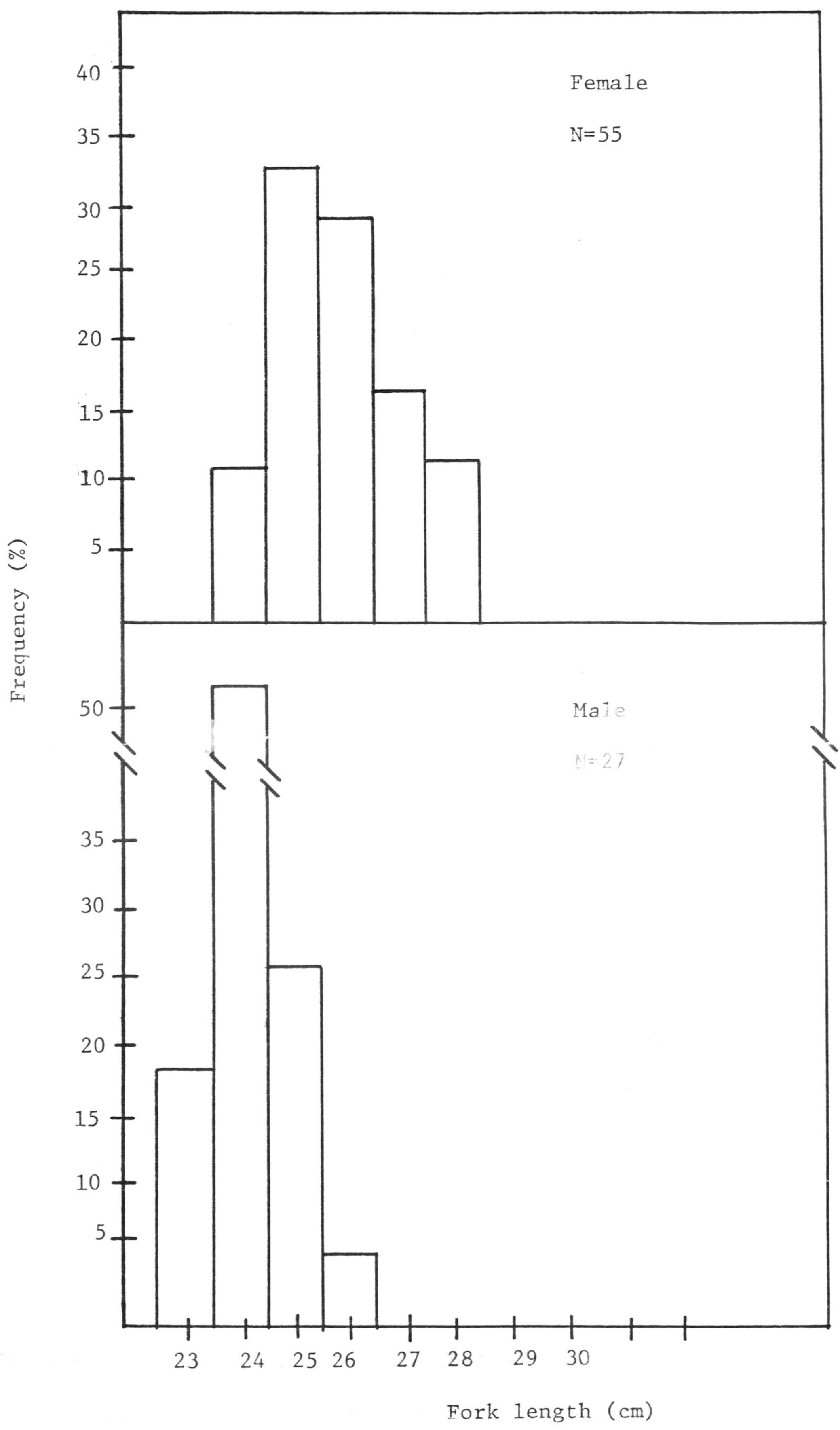


Figure 11. Length-frequency for 1979 sample.

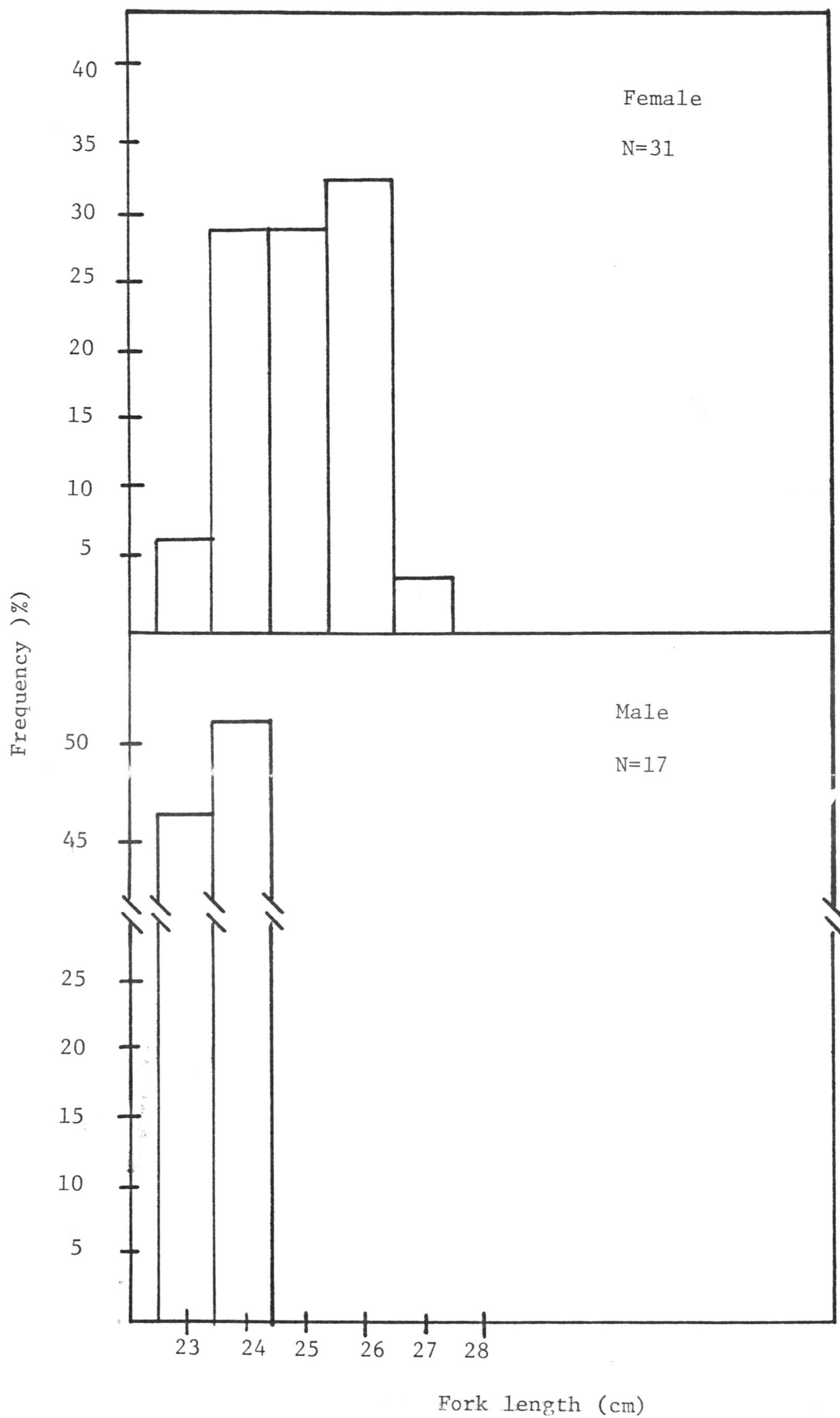
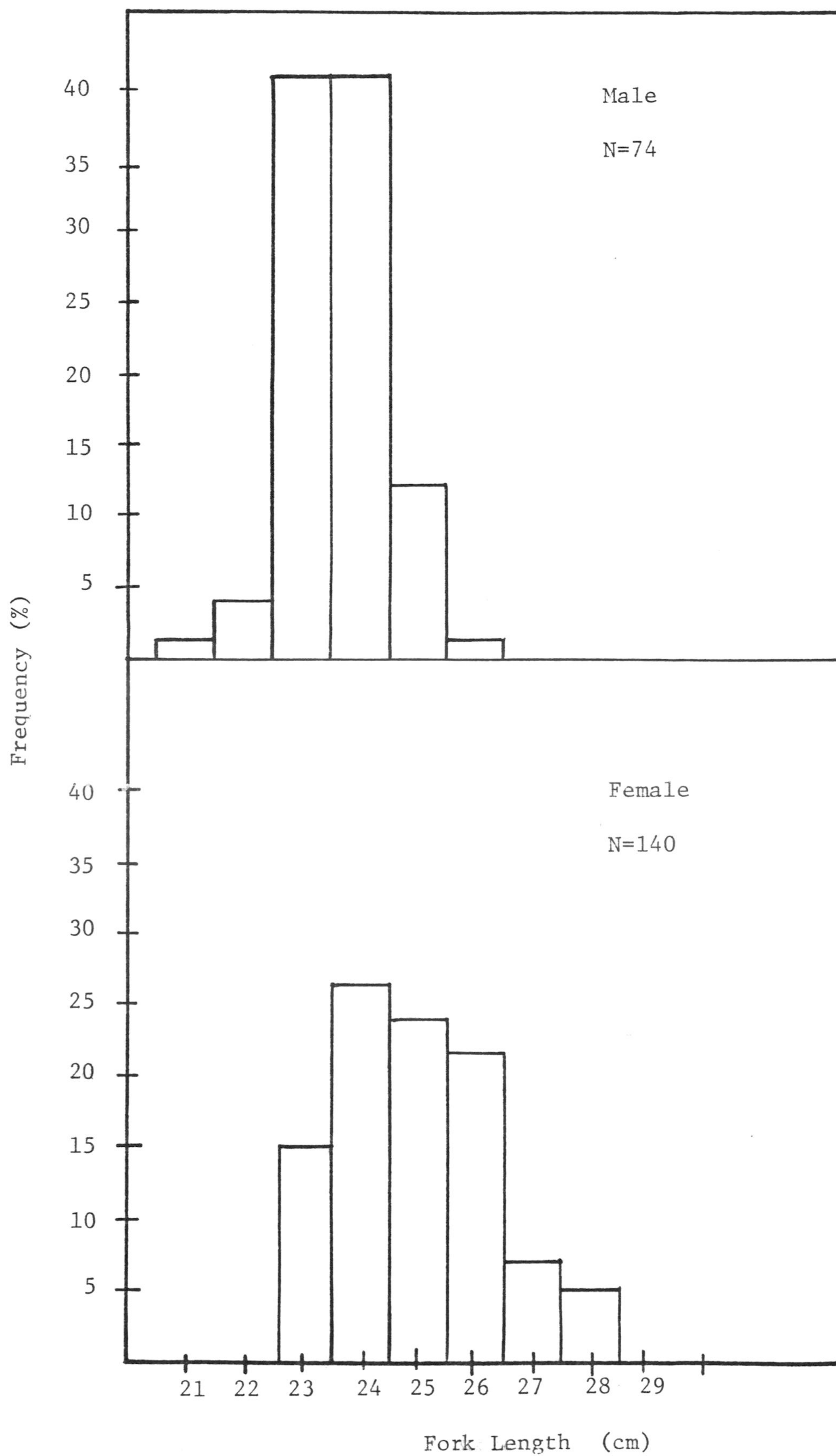


Figure 12. Length-frequency for total sample.



approximate age according to the data of Holland and Yelverton (1973). The major portion of the herring were estimated to be four to six years old.

Tables 4 and 5 present the percentage occurrence of bands by length of fish. The appendix tables separate the fish by sex and by year of capture. Variance in terms of present or absent began with band five. The female values for percent present were not as variable as the male. The males' presence values ranged from 40 to 100 percent. The band showed no significant relationship to age.

The range of frequency of occurrence of band six did not differ greatly by sex. It was present in all samples for males either twenty-two or twenty-six and above centimeters in length. Band six showed a nonsignificant relationship with age, as did bands seven and eight. Band seven was especially consistent for females ranging from a percentage present of 61 to 71 for all size classes.

Protein patterns for non-blueback species in this study produced interspecific variation that permitted species identification for fish utilized. Cobb *et al* (1968) used lens protein patterns to identify thirteen saltwater fish. Croaker and trout were included in their study. The soluble protein distributions for each fish were characteristic for the species. The limited croaker, bluefish, and trout samples in my work were used primarily as a check on technique, procedure, and sensitivity of the method employed.

The literature on the homing ability of the blueback and alewife herring shows that these fish return to their natal streams for spawning.

Table 4. Percentage occurrence of bands by length of the males. N=74

	Length (cm)				
Band No.	22 N=4	23 N=30	24 N=30	25 N=9	26 N=1
1	100	100	100	100	100
2	100	100	100	100	100
3	100	100	100	100	100
4	100	100	100	100	100
5	100	50	43	89	100
6	100	73	67	78	100
7	75	67	93	89	100
8	25	37	77	89	100

Table 5. Percentage occurrence of bands by length of the females. N=140

Band No.	Length (cm)				
	22	23 N=21	24 N=37	25 N=34	26 N=48
1		100	100	100	100
2		100	100	100	100
3		100	100	100	100
4		100	97	100	100
5		76	62	[illegible]7	67
6		81	70	74	88
7		29	49	82	85
8		43	46	88	85

Bigelow & Schroeder (1953) reported that alewife runs could be introduced into suitable unpopulated rivers by allowing gravid adults to spawn upstream. Their work showed that the offspring of the adults returned 3 to 4 years later upon reaching sexual maturity. This would support a home stream capability for the herring. Durbin, Nixon, and Oviatt (1979) in a paper on the impact of the spawning alewives on nutrient cycles found the homing ability of the alewife to be similar to the salmon in returning to natal waters. Thunberg (1971) tested alewives for their ability to select home waters from other waters that may or may not have supported an alewife run. The other waters were selected from streams in the same geographic area as the home stream. In his results, the alewives were able to detect and select water from their natal streams. These results support the concept of homing ability for the alewives. Tyus (1971) did a two year study of alewife spawning movement at Lake Mattamuskeet, North Carolina. The lake is connected to the Pamlico Sound by four separate canals. For the two year study, 97.5% of the fish taken for samples were from the same canal. All four canals were set up in a similar manner for sampling. The canal being almost exclusively used by the spawning herring for access to Lake Mattamuskeet is also the oldest of the four canals. This is consistent with a homing theory as a possible explanation.

Messieh (1977) used meristic counts as a basis for a study including the homing ability of the alewives. Eight meristic characters were examined and analyzed. More separation was found between groups of

fish during spawning than before spawning. Messieh felt that with the amount of overlap he found that the alewife was not as specific in homing to a smaller brook or stream as the shad or salmon, after entering the parent stream.

As a summary of the works presented, the evidence strongly suggests that the blueback and alewife do return to home streams for spawning. These fish have some mechanism for imprinting the home stream waters because a minimum of three to four years pass before the fish ever return to spawn.

Much of the home stream theory work has been done with alewives, but with the similarities of the alewives and bluebacks, I believe that the homing ability of the blueback would be equivalent to the alewife. The blueback herring spawning runs, in returning to natal waters, provide a genetic basis for the possible development of discrete local populations of herring that are discernible from other blueback populations. Any spawning isolation would be a result of natal waters serving as a boundary. Sharper boundaries would tend to heighten differences in local populations while crossover would tend to diminish differences.

As stated earlier, proteins selected for study in this paper came from the lens. The conservative nature of the lens proteins lends credence to any differences found in the protein patterns being genetic with little environmental influence. Environmentally influenced proteins could change rapidly and be affected by short periods of high stress such as capture methods or low amounts of dissolved oxygen in the water.

An application of Sokal's taxonomic distances was used to compare herring from the Chowan, Neuse, Meherrin, and Roanoke Rivers. Values utilized were percent occurrence of lens proteins for fish from each location. This data appears in Table 3 (bands five through eight), and the results of the analysis are in Table 6. Based on the distance values in Table 6, the protein patterns in fish from the Chowan and Meherrin Rivers were most similar. This could possibly be explained by the closeness of the two rivers as the Meherrin is a tributary of the Chowan. A chance for overlap and intermingling would exist between the populations of these two rivers as the fish spawning in the Meherrin must first traverse the greater part of the Chowan. The Neuse is the most geographically isolated of the river systems utilized. It is a tributary of the Pamlico Sound. The other three rivers fees into the Albemarle Sound. This isolation provides for less possibility of intermingling between fish in the Neuse and those of the Roanoke, Chowan, or Meherrin.

The calculated distance values correlate with geographic distance, increasing in value as the compared taxa grow farther apart. Geographic distance can be a genetically isolating mechanism resulting in less gene exchange between populations which allows for greater variance in gene frequencies between populations of a species. Isolation and genetic drift could account for the Neuse samples being the most divergent. Although Sokal's test indicated possible divergent trends in the populations, no protein bands were present or absent in a pattern that allowed positive population identification from the sample areas.

Table 6. Taxonomic distances calculated for different sample areas.

	Roanoke River	Chowan River	Meherrin River	Neuse River
Roanoke River	--	2.0040	2.543	3.8800
Chowan River		--	1.2099	3.1323
Meherrin River			--	3.2308

Another possible interpretation for the found degree of divergency in the patterns is that they resulted from likeness of environmental factors in the streams. Weinstein and Yerger (1976) suggested this as a possibility in their sea trout comparisons using serum proteins. This explanation is less plausible for the herring because of the conservative nature of the lens proteins.

The protein bands were also subjected to analysis in length-frequency classes. Length-frequency grouping is often used as an age approximation for fish. Eckroat (1971) worked with lenses of 9 natural populations of brook trout (Salvelinus fortinalis). The protein patterns were found to be independent of age using length-frequency groupings.

Eckroat and Wright (1969) worked with brook trout proteins and found the results not to be influenced by age of the fish. Hasler and Wright (1967) studied the serum proteins of white bass (Roccus chrysops), and the results were examined for age-dependency relationships. The serum proteins, less conservative than lens proteins, were found not to be influenced by age.

Using chi square analysis and length-frequency groupings for the blueback, there was no significant correlation between age and presence or absence relationships of the protein patterns.

The blueback lens patterns were tested for any significant influence of sex on the bands. No significant differences appeared in the protein bands that were attributed to sex.

Hasler and Wright (1967), in the study referred to earlier, tested

for sexual influences on serum protein bands in the white bass. Presence or distance of migration of the bands was not affected. One band present in males and females, stained darker in spawning females.

Huntsman (1970) compared results for sex differences in protein patterns of the northern hogsucker (*Hypentelium nigricans*) and the river carpsucker (*Carpiodes carpio*). No observable differences were found.

The results of the blueback study have indicated that lens proteins are too conservative for utilization as a population marker with this system of electrophoresis. The protein patterns do not allow natal stream identification of individual fish but would identify groups of fish. The group samples of bluebacks would have to spawn in one of the streams sampled and their protein pattern percentages bracketed by data of this study. The differences in the protein patterns may be slight because of some degree of intermingling of spawning sub-populations. This would reduce genetic variation by increasing the gene pool available to the spawning herring. Geographic analysis by Sokal's test has indicated that some degree of population divergence does exist.

Further study is needed before the extent of this divergence can be determined. Protein sources more influenced by factors such as environment may be desirable for study. Examples are muscle tissue or serum. The use of more than one protein source from each fish may be beneficial. The combination of results could be compared by statistical analysis for ascertaining the degree of divergence among the blueback populations.

SUMMARY

The geographically distributed protein patterns, tested by Sokal's test for taxonomic distance, showed some degree of divergence for the sample areas. D values for the closest river systems were lowest and d values for the farthest separated river systems were highest.

Length-frequency groupings, representing age brackets, had no significant correlation with the protein patterns. None of the bands were correlated with the sex of the fish. Year and location of capture had no significant correlation with the protein patterns.

The croaker, trout, and bluefish patterns were species specific allowing identification by the patterns. The lens protein system is too conservative to allow determination of the extent of some divergence in the sample areas.

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APPENDIX

Appendix Table 1. Percentage occurrence of bands by location, females, 1977

Location	Band 5	Band 6	Band 7	Band 8	N
1-Roanoke River	21	79	14	0	14
2-Chowan River	100	100	0	89	9
3-Neuse River	0	100	0	60	3
4-Meherrin River	100	100	100	0	6
5-Albemarle Sound	100	100	0	88	8
6-Blounts Creek	67	67	17	42	12

Appendix Table 2. Percentage occurrence of bands by location, females, 1978

Location	Band 5	Band 6	Band 7	Band 8	N
1-Roanoke River	83	91	96	96	23
2-Chowan River	0	0	100	86	7
3-Neuse River	100	100	100	100	15
4-Meherrin River	80	10	80	80	10

Appendix Table 3. Percentage occurrence of bands by location, females, 1979

Location	Band 5	Band 6	Band 7	Band 8	N
1-Roanoke River	100	100	100	100	5
2-Chowan River	0	100	100	82	1
3-Neuse River	0	100	100	100	9
4-Meherrin River	100	17	100	0	6

Appendix Table 4. Percentage occurrence of bands by location, males, 1979

Location	Band 5	Band 6	Band 7	Band 8	N
1-Roanoke River	100	100	100	100	3
2-Chowan River	0	100	100	60	5
3-Neuse River	0	100	100	100	7
4-Meherrin River	100	50	100	0	2

Appendix Table 5. Percentage occurrence of bands by location, males, 1977

Location	Band 5	Band 6	Band 7	Band 8	N
1-Roanoke River	0	90	20	0	10
2-Chowan River	100	100	0	0	2
3-Neuse River	100	0	100	100	3
4-Meherrin River	100	86	86	14	7
5-Albemarle Sound	100	100	0	75	4
6-Blounts Creek	0	100	50	0	4

Appendix Table 6. Percentage occurrence of bands by location, males, 1978

Location	Band 5	Band 6	Band 7	Band 8	N
1-Roanoke River	100	100	100	100	6
2-Chowan River	0	0	100	88	8
3-Neuse River	100	100	100	100	8
4-Meherrin River	100	0	100	60	5

Appendix Table 7. Percentage occurrence of bands by loation, both sexes, 1979

Location	Band 5	Band 6	Band 7	Band 8	N
1-Roanoke River	100	100	100	100	8
2-Chowan River	0	100	100	64	6
3-Neuse River	0	100	100	100	16
4-Meherrin River	100	25	100		8

Appendix Table 8. Percentage occurrence of bands by location, both sexes, 1977

Location	Band 5	Band 6	Band 7	Band 8	N
1-Roanoke River	12	84	17	0	24
2-Chowan River	100	100	0	73	11
3-Neuse River	50	50	50	80	6
4-Meherrin River	100	93	93	8	13

Appendix Table 9. Percentage occurrence of bands by location, both sexes, 1978

Location	Band 5	Band 6	Band 7	Band 8	N
1-Roanoke River	87	93	97	97	29
2-Chowan River	0	0	100	87	15
3-Neuse River	100	100	100	100	23
4-Meherrin River	87	7	87	73	15

Appendix Table 10. Percentage occurrence of bands by length of the males, 1977. N=30

	Length (cm)			
Band No.	22 N=4	23 N=17	24 N=7	25 N=2
1	100	100	100	100
2	100	100	100	100
3	100	100	100	100
4	100	100	100	100
5	100	53	29	100
6	100	82	71	50
7	75	24	71	50
8	25	18	29	50

Appendix Table 11. Percentage occurrence of bands by length of the males, 1978. N=27

Band No.	Length (cm)			
	23 N=5	24 N=14	25 N=7	26 N=1
1	100	100	100	100
2	100	100	100	100
3	100	100	100	100
4	100	100	100	100
5	80	57	86	[illegible]
6	0	50	86	[illegible]
7	100	100	100	100
8	60	93	100	100

Appendix Table 12. Percentage occurrence of bands by length of the males, 1979. N=17

	Length (cm)	
Band No.	24 N=8	25 N=9
1	100	100
2	100	100
3	100	100
4	100	100
5	25	33
6	100	89
7	100	100
8	63	89

Appendix Table 13. Percentage occurrence of bands by length of the females, 1977. N=54

	Length (cm)			
Band No.	23 N=19	24 N=22	25 N=7	26 N=6
1	100	100	100	100
2	100	100	100	100
3	100	100	100	100
4	100	96	100	100
5	79	64	43	33
6	84	86	86	100
7	21	23	14	0
8	42	32	57	67

Appendix Table 14. Percentage occurrence of bands by length of the females, 1978. N=55

	Length (cm)		
Band No.	24 N=6	25 N=18	26 N=31
1	100	100	100
2	100	100	100
3	100	100	100
4	100	100	100
5	83	67	81
6	17	56	84
7	67	100	97
8	67	94	97

Appendix Table 15. Percentage occurrence of bands by length of the females, 1979. N=31

Band No.	Length (cm)			
	23 N=2	24 N=9	25 N=9	26 N=11
1	100	100	100	100
2	100	100	100	100
3	100	100	100	100
4	100	100	100	100
5	50	44	1[illegible]	46
6	50	67	100	91
7	100	100	100	100
8	50	67	100	64