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SERUM PROTEIN ANALYSIS OF WALLEYES [1]

By Gerald U. Ulrikson [2] and Percy W. Laarman

Abstract

Walleyes, Stizostedion vitreum vitreum (Mitchill), were collected during 1969 spawning runs from three sites on Lake Erie; a tributary of Lake St. Clair (Thames River); Manistique Lake, Mackinac County; Muskegon River, Newaygo County; and Huron River, Washtenaw County.

Total protein and electrophoretic patterns of serum proteins were determined to identify sub-populations. Six major protein bands were detected for all the fish. Determinations were made for area units, percentage which each band was of the total area units, and milligrams protein per milliliter of serum. Analysis of the data showed the walleyes to be in four groups: I, Muskegon and Huron rivers; II, Sandusky Bay; III, Pointe Mouillee, Port Clinton and Thames River; IV, Manistique Lake. Only Sandusky Bay walleyes could be separated from the other three collections taken from Lake Erie and the Thames River. This suggests that the Sandusky Bay walleyes may be a genetically separate sub-population.

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[2] Dr. Ulrikson, formerly with the Michigan Department of Natural Resources, is in the Health Physics Division, Oak Ridge National Laboratory, Oak Ridge, Tennessee.

Introduction

The Michigan Department of Natural Resources has been concerned with regulation of walleyes, Stizostedion vitreum vitreum (Mitchill), in Michigan waters of Lake St. Clair and Lake Erie. To properly manage these fish, the need exists to determine whether they move throughout the lakes or if there are sub-populations inhabiting only Michigan waters. If sub-populations do exist and maintain their identity, then separate regulations may be necessary to properly manage the different stocks. The method of marking fish to determine movement in a large body of water requires large collections of fish, marking, and recoveries. This method is both time consuming and expensive.

The objective of this study was to utilize electrophoretic patterns of serum proteins of walleyes, collected from different geographical areas in Lake St. Clair and Lake Erie, to determine if sub-populations could be identified. If genetically different strains do exist, segregation would probably occur during spawning runs. The literature is well documented with reports on electrophoretic patterns from various tissues used to study the taxonomic relationship of different species of fish (Thurston, 1967; Tsuyuki et al., 1965; Tsuyuki, 1967; and Uthe et al., 1966). Booke (1964) reviewed the manner in which serum proteins may vary due to the physiological condition of the fish.

Methods

Walleyes were collected from seven locations during the spring of 1969. Three sites were in western Lake Erie at Sandusky Bay and Port Clinton, Ohio, and Pointe Mouillee in Michigan. One collection was made from the Thames River that flows into Lake St. Clair in Ontario. For further comparisons, Michigan walleyes were also taken from Manistique Lake (Mackinac County), Muskegon River (Newaygo County), and the Huron River (Washtenaw County). Collection dates and methods used to capture the fish were as follows: Thames River, 2 April, seine; Port Clinton, 21 April, seine; Pointe Mouillee, 23 April, gill net; Sandusky Bay, 5 May, seine; Muskegon River, 17 April, shocker; Huron River, 10 April, shocker; and Manistique Lake, 23 May, trap net. The fish were 15 to 21 inches long and of both sexes. However, most of the fish were in the 17- to 19-inch size range. Fish were transported to the Saline Research Station and placed in holding tanks for 2 weeks prior to collecting serum samples. The holding period was used in an effort to minimize the effects of handling stress on serum proteins.

Serum samples were taken by severing the caudal peduncle and collecting blood in centrifuge tubes. Samples were run the same day they were taken.

The gel electrophoretic method described by Davis (1964) was used for separation of the serum proteins. Three μliters of serum were used for each sample. Preliminary work showed this to be an optimum sample size. Each electrophoretic run was made with a constant amperage of 2.5 ma per tube. The electrophoresis was performed under

refrigeration at 4 C to prevent protein denaturation. Gels were prepared fresh before each electrophoretic run. At the end of the run, gels were stained for 0.5 hour with Amido-Black and then cleared in 7% acetic acid.

After clearing, the gels were run through a Photovolt Densitometer equipped with a gel carriage adapter. This was connected to a digital voltmeter program scanner (data acquisition system) which in turn was connected to an IBM keypunch. Optical density units for each band in the gels were punched on cards automatically during scanning of the gels. The cards served as input data for a computer program written to: (1) plot the optical density curves which reflect the amount of protein in the protein bands, (2) fix the base line thus separating true protein from background stain, (3) determine area units under the curve for each band and total area units for that scan, (4) determine what percentage of the total is made up by each band.

The total protein content of serum for each fish was also measured by the method described by Lowry et al. (1951). Optical density values were read on a Beckman DU spectrophotometer at 750 mμ. Lyophilized human serum was used as a reference to establish a standard curve which gave the relationship between quantity of protein and optical density values. By combining the percentages of each protein fraction obtained from electrophoresis and the spectrophotometric analysis of total protein, it was possible to measure the concentration (mg protein per ml serum) of each protein fraction present in the serum.

Results and discussion

The three units of measurement (area units, percentages and mg of protein) were highly correlated. The multiple correlation coefficient between milligrams protein per milliliter of serum and area units plus percentages was $R = 0.997$. Due to the high correlation among the three units of measurement, only per cent values were used to determine differences between collection sites. Per cent values were used for two reasons: (1) total protein reflected both in area units and in milligrams of protein is more subject to fluctuation than per cent values, whereas percentages would change only with a shift from one band to another, (2) the coefficient of variation of the means (variance divided by the mean) was slightly lower in per cent values, thus giving a better statistical parameter for analysis.

Six major serum protein bands were evident on the electrophoretic gels from all the walleyes. The "slowest-migrating" band (different proteins migrate at different rates) was designated as number I, and the most rapid migrating band as number VI. The average per cent values from each collection site for each band are shown in Table 1. The six measurements from each serum sample constitute a vector of multivariate data, and the observation vector from fish collected at one site may or may not have come from the same population as the observation vector from fish collected at another site. By using a technique of multivariate analysis called canonical analysis (Seal, 1964), the vector of six measurements may be transformed to a vector of two measurements which are independent of

each other, and the elements of this vector may be considered as the coordinate of a point on a two-dimensional plot. In addition, a circle of uncertainty (confidence limits) may be drawn around the transformed point of the mean vector. Recognizing the fact that the measurements of the per cent values in each of the bands are subject to statistical variation, these circles of uncertainty may be used to differentiate between walleyes from the various collection sites.

In Figure 1, the first two canonical variates from the mean vectors for each of the seven collection sites are shown along with their associated 95% confidence limits. From these data and an investigation of the general overlapping of the confidence limits, the seven walleye collections tend to fall into the following four groups: I, Muskegon River and Huron River; II, Sandusky Bay; III, Pointe Mouillee, Port Clinton and Thames River; and IV, Manistique Lake.

The widely separated locations of the Muskegon River and Huron River prevent natural mixing of the two walleye populations. Although the Huron River and lakes in this river system were stocked with walleyes in 1937, 1938 and 1939, the records indicate that the planted fish originated from Lake Huron. Therefore no explanation can be given for the similarity found in the Muskegon River and Huron River walleye populations.

Only Sandusky Bay walleyes could be separated from the other three collections taken from Lake Erie and the Thames River. This suggests that the Sandusky Bay walleyes may be a separate sub-population. Additional sampling would be necessary to confirm this hypothesis. From a geographical viewpoint the Thames River walleyes might be expected to maintain a distinct sub-population since the river flows into Lake St. Clair

and the other three collection sites were from western Lake Erie. However, the mixing of walleyes from the four collection sites is certainly possible by passage through the Detroit River as reported by Wolfert (1963).

The objective of separating four genetically different sub-populations of walleyes existing in Lake St. Clair and Lake Erie by electrophoretic patterns of serum proteins was not accomplished. Either genetic differences do not exist between each of the four spawning populations, or other tissue proteins or enzymes may be better parameters to investigate in an effort to separate genetic variations within a species.

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Gerald U. Ulrikson and Percy W. Laarman

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Table 1.--Analysis of optical density curves for serum proteins in walleyes from seven localities. Numbers are average per cent values of each band in the total. Number of fish given in parentheses.

Band	Sandusky Bay (18)	Pointe Mouillee (18)	Port Clinton (20)	Thames River (11)	Muskegon River (14)	Huron River (13)	Manistique Lake (10)
I	10.8	8.3	8.5	7.8	8.5	9.5	15.4
II	15.0	14.9	15.7	15.5	16.7	15.1	18.2
III	12.9	11.0	12.1	11.5	13.0	14.2	9.2
IV	7.1	7.8	6.6	7.9	9.8	12.8	8.2
V	52.5	56.3	55.1	55.3	48.9	45.8	47.2
VI	1.9	1.7	2.0	1.9	3.1	2.4	2.1

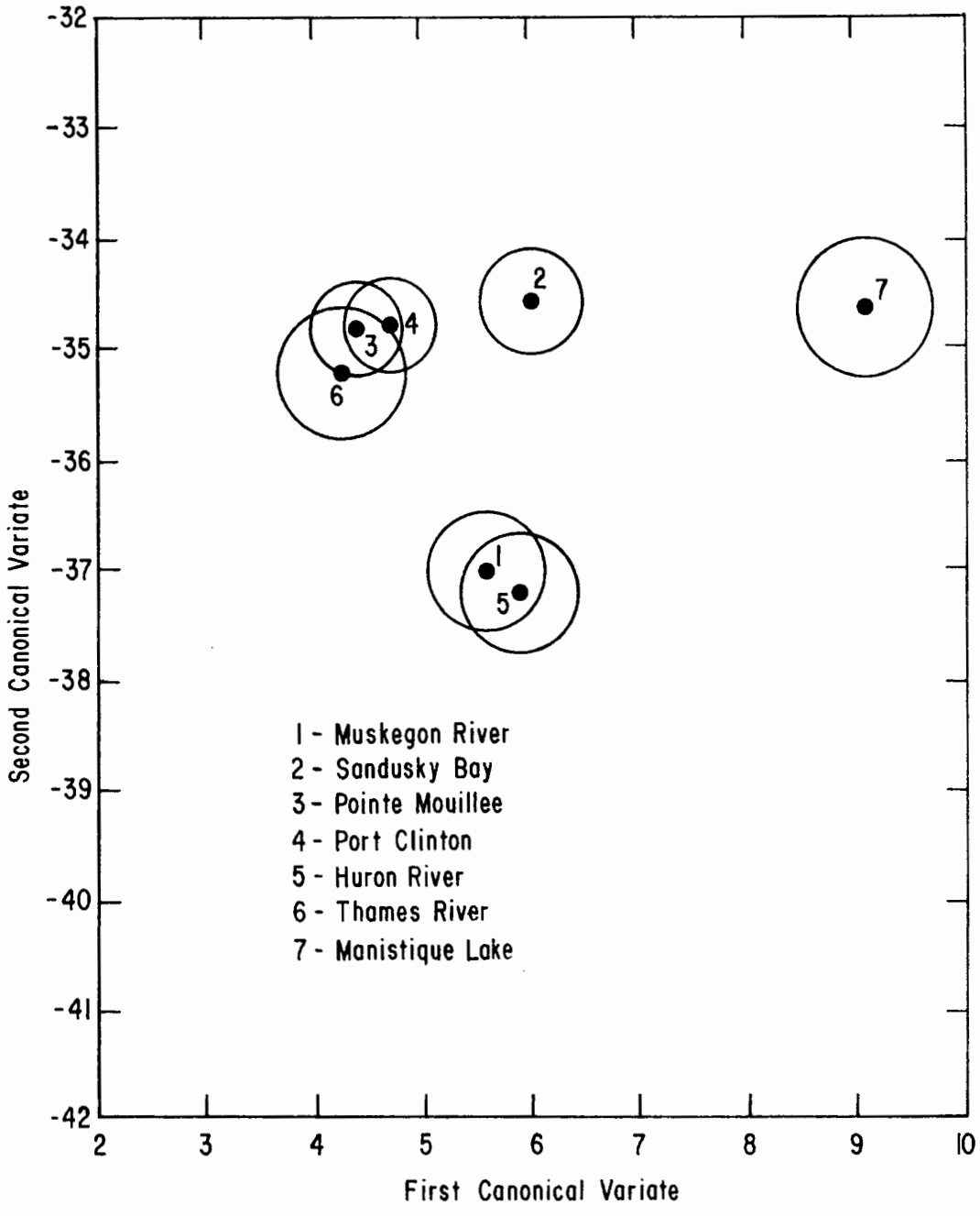

Figure 1.--The first two canonical variates from the mean vectors for seven collection sites. Circles denote 95% confidence limits.