An Analysis of Nuclear Eye Lens Proteins of King Mackerel, Scomberomorus cavalla, using High Performance Liquid Chromatography

DAVID L. NIELAND[1], GILBERT W. BANE[1]
DEBRA McMILLIN[2] AND GARY SHAFFFER[3]

[1]Coastal Fisheries Institute
Center for Wetland Resources
Louisiana State University
Baton Rouge, Louisiana 70803-7503

[2]Institute for Environmental Studies
Louisiana State University
Baton Rouge, Louisiana 70803-5705

[3]Coastal Ecology Institute
Center for Wetland Resources
Louisiana State University
Baton Rouge, Louisiana 70803-7505

ABSTRACT

The king mackerel, Scomberomorus cavalla, is a commercially and recreationally important species throughout its range. Recent analyses indicate a decrease in catches in the Gulf of Mexico. As a result, it has become apparent that a management plan must be implemented to allocate harvests of this resource. Among the more important pieces of information necessary to the formulation of a logical management plan are numbers and locations of stocks being fished. Based on tagging and allozyme data, hypotheses of two or three king mackerel stocks have been advanced.

Recent studies at the Coastal Fisheries Institute of Louisiana State University have applied the analysis of nuclear eye lens proteins by high performance liquid chromatography (HPLC) to the mackerel stock assessment problem. King mackerel from nine areas in the Atlantic and Gulf have been exampled. Analyses of ten protein peaks identified among the populations have resulted in the delineation of three groups within which no statistically significant differences were detected: 1) North Carolina, South Carolina and Florida Keys, 2) northwest Florida, Texas, Veracruz, Mexico and south Cuba, and 3) Louisiana and Yucatan, Mexico. Although the results of the HPLC research do not agree precisely with previous hypotheses, the discrimination of the "Atlantic" king mackerel group is most interesting. The confusing picture presented by the Gulf populations would be clarified by examining synchronously gathered specimens from each of the areas.

INTRODUCTION

The king mackerel, Scomberomorus cavalla, is distributed in the coastal pelagic waters of the western Atlantic Ocean from

Massachusetts, USA to southern Brazil. Throughout its range the species is harvested for both commercial and recreational purposes. However, it is in the waters of the western North Atlantic and the Gulf of Mexico where the king mackerel is most eagerly sought. Commercial landings in the United States alone number in the millions of pounds annually. Mexico has recently replaced the United States as the largest commercial producer of king mackerel with annual landings in the ten to 13 million pound range (Bane and Bane, 1984). The recreational catch, although difficult to quantify, contributes several more million pounds to the total annual harvest.

The tremendous pressure being exerted on this species has produced reports of decreased catches in both the commercial and recreational fisheries. Although there is some dispute over the extent to which the resource has been overfished in the Gulf of Mexico, it has become apparent that a management plan must be implemented. Future allocation of the catch by means of a fishery management plan has become not only a high priority management task, but also a volatile political issue. Cooperation among state, regional and national fishery management jurisdictions is imperative for the successful implementation of any plan.

In order to formulate a logical and equitable plan, many types of biological and fisheries data must be considered. Among the more germane questions encountered in the drafting of a management plan concerns the number and location of stocks being fished. Does the species behave as one panmictic population or has it become partitioned into two or more divergent demes? Investigations of this issue to date have produced conflicting hypotheses.

Based on an analysis of mark-recapture data, Williams and Godcharles (1984) inferred the existence of two stocks of king mackerel off the coasts of the United States, one in the Atlantic Ocean and one in the Gulf of Mexico. A scenario postulating the existence of two stocks of king mackerel in the Gulf of Mexico has also been advanced. Baughman (1941) first advanced this hypothesis based on anecdotal evidence of mackerel migrating northward at both the east and west ends of the Gulf. Allozyme polymorphisms detected at two genetic loci by May (1983) supply further evidence supporting the Gulf two stock hypothesis. However, May's data could not distinguish between king mackerels from the eastern Gulf and the Atlantic Ocean populations. The preliminary electrophoretic data presented by Grimes et al. (1985a; b) similarly argue for the existence of a western Gulf stock and a single combined eastern Gulf/Atlantic Ocean stock.

The Coastal Fisheries Institute (CFI) of Louisiana State University (LSU) is involved in king mackerel stock assessment research. Previous investigations have included analyses of king mackerel migratory groups in the Gulf of Mexico. One aspect of this research generated an innovative application of high performance liquid chromatography (HPLC) utilizing a methodology refined in our laboratory (R.J. Portier, unpublished). It was thought that analyses of nuclear eye lens proteins by HPLC would

prove valuable in the identification of king mackerel stocks. Research to date has shown this procedure to be a fast, reliable and reproducible method of examining eye lens proteins for intraspecific polymorphisms.

Nuclear eye lens protein polymorphisms have been used to identify subpopulations (stocks) within species of fishes (Smith, 1965; 1966a; Barrett and Williams, 1967; Smith and Goldstein, 1967; Eckroat and Wright, 1969; Peterson and Smith, 1969; Smith 1969a; Peterson and Shehadeh, 1971; Smith, 1971a; b; Smith and Clemens, 1973; Blake, 1976; Weinstein and Yerger, 1976a; Smith, 1978; Brenner and Irby, 1984). Electrophoretic techniques, principally those employing cellulose acetate and polyacrylamide as the supporting media, have received principal application in discerning genetically based differences in eye lens proteins. In these analyses polymorphisms are discriminated as variations in staining intensities or in frequencies of mobility patterns or by the occurrence of marker proteins (hence marker genes) within the population (Smith and Clemens, 1973). The pioneering investigations of Smith (1962; 1966b; 1968; 1969b; 1970; 1971c; 1983; Smith and Gilman, 1982) have considerably improved the resolution and precision of eye lens protein analyses. Consequently, the utility of eye lens protein analysis has been extended to considerations of phylogeny (Tsuyuki et al., 1968; Calhoun and Koenig, 1970; de Jong et al., 1981), taxonomy (Weinstein and Yerger, 1976b), and ontogeny (Cobb and Koenig, 1967; Zigman and Yulo, 1979; Benz, 1980; Smith and Gilman, 1982).

The eye lens nucleus is well adapted to protein studies. Among those qualities which make it a desirable tissue for study are: 1) high concentration and purity, ca. 35% protein and 65% water (Bloemendal, 1977), 2) resistance to denaturation ameliorates tissue handling procedures (Smith, 1983), 3) ease of isolation minimizes contamination from other tissues (Smith, 1983), 4) tissue turnover is minimized as the nucleus is nonmetabolizing and inert (Smith, 1983), and 5) physiological and environmental variables do not obscure the genetic bases of the proteins (Eckroat and Wright, 1969). However, caution is demanded when comparing eye lens proteins of fishes of various developmental stages. Progressive ontogenetic changes in soluble nuclear eye lens proteins (Cobb and Koenig, 1967; Zigman and Yulo, 1979; Benz, 1980; Smith and Gilman, 1982) should not be interpreted as indicating polymorphisms within or among populations. This problem can be avoided by an experimental design and sampling regime which ensures comparisons among individuals at the same developmental stage.

HPLC employs two essential elements: a mobile phase and a stationary phase. The mobile phase is a solvent or a mixture of solvents which carries an aliquot of protein extract through the system. The stationary phase is a separatory column which preferentially retains fractions of the extract for varying lengths of time. These retention times are functions of protein properties such as molecular weight, net electrical charge, chemical affinities, solubilities, etc. As the protein fractions elute from the column they are channeled to an absorbance

detector which monitors eluate (protein) concentration. The detector generates a series of electrical signals which is translated into a chromatogram by a data handling module. The chromatogram is a record of detector responses (proportional to protein concentration for a given peak) plotted versus time. The retention times of the response peaks, their relative heights, and their areas are the three types of data which may be produced during a chromatographic run. Each may be used in statistical comparisons of eye lens proteins within and among populations.

MATERIALS AND METHODS

Sample Collection and Preparation

Whole eyes or dissected eye lenses of adult king mackerel were obtained from nine areas in the western North Atlantic Ocean and the Gulf of Mexico. Collection localities and dates of collection are as follows: North Carolina, 13 January 1985; South Carolina, 7 June 1985; Florida Keys, 31 January, 1985; northwest Florida, June 1985; Louisiana, November 1984; Texas, July 1985; Veracruz, Mexico, January 1985; Yucatan, Mexico, January 1985; and south Cuba, May 1985. Using the HPLC system described below, which generates retention time, peak height, and peak area data, six individuals were analyzed from each population except for the northwest Florida population from which 24 fishes were analyzed. Many more individuals from each of the nine populations have been run on a less sophisticated system which yields only retention time and peak height data. These data are currently being translated into a format which is compatible with our statistical procedures.

All eyes or lenses were maintained frozen from time of collection to time of analysis. Prior to extraction the nucleus was isolated by removal of the lens kernel and vitreous materials. Individual nuclei were pulverized by 2 ml of an extraction medium with a hand-held, glass tissue homogenizer. The extraction medium, described by Smith and Gilman (1982), solubilizes both the water soluble (crystallin) and water insoluble (albuminoid) fractions of the nucleus. The homogenates incubated overnight at room temperature (ca. 24°C). Particulate matter was sedimented by centrifugation for 2 minutes at 12,800 x g in an Eppendorf microcentrifuge. Fifteen ul of supernatant fraction were injected per chromatographic run.

HPLC Equipment and Procedures

A gradient HPLC system (Waters Associates, Milford, MA, USA 01757) was used for analysis of eye lens proteins. The components of the system were: two model 510 solvent delivery systems (pumps), RCM-100 radial compression module, model 710B intelligent sample processor (WISP), model M-490 programmable multiwavelength detector, and model 840 data and chromatography control station. A Nova-Pak C_{18} Radial Pak separatory cartridge (Waters Assoc.) was employed in all analyses.

Reverse phase HPLC was executed using as the mobile phase the following solvents: Solvent A) 0.02% trifluoroacetic acid (TFA) in a 50:50 mixture of acetonitrile (CH_3CN) and water, and Solvent B) 0.02% TFA in water. The mobile phase was run according to the gradient program recorded below in Table 1.

Table 1. Mobile phase gradient program utilized in HPLC analysis of king mackerel eye lens proteins

Time (min)	Flow (ml/min)	A (percent)	B (percent)	Curve
Initial	1.5	0	100	*
1.0	1.5	0	100	6
4.5	1.5	40	60	4
12.0	1.5	40	60	6
12.5	1.5	0	100	6
18.0	Run Stop			

Elution profiles were graphically depicted as absorbance at detector wavelengths of 220 nm and 254 nm at a detector range of 0.1 AUFS (absorbance units full scale). Only those data gathered at the 220 nm wavelength will be considered in subsequent analyses due to poor detector response of proteins at 254 nm. All chromatographic runs were performed at ambient room temperature (ca. 24°C).

Statistical Analyses

After visual examination of chromatograms produced in each sample group, ten peaks were chosen for statistical analysis. The peaks selected were those which consistently appeared within one or more of the sample groups. These were identified by their mean retention times and ordinated as follows: Peak #1 = 2.24 min, #2 = 2.82 min, #3 = 3.32 min, #4 = 3.71 min, #5 = 4.19 min, #6 = 5.58 min, #7 = 5.93 min, #8 = 6.63 min, #9 = 7.11 min and #10 - 7.91 min. Not all peaks were represented in every population, but those seen within a sample group were invariably expressed in all individuals. The five to nine eye lens protein constituents per population is not inconsistent with previous electrophoretic studies (Cobb and Koenig, 1967; Benz, 1980). For each individual a record of peak retention times, peak heights and peak areas was tabulated.

In previous investigations (Bane and Nieland, 1985) on red drum, Sciaenops ocellatus, a correction factor to peak height was applied to each individual to compensate for differences in fish size. It was determined subsequently that fish size had no significant effect on protein peak height. In the current study, no correction factor could be supplied, as a size indicator

(standard or fork length) did not accompany all of the lenses used in the analysis, although all fishes were adult age. It is assumed that protein peak height is again independent of fish size in this study.

Statistical analyses were performed using simple linear regression, analysis of variance (ANOVA), and a series of linear contrasts designed to test specific hypotheses concerning subsets of the populations. The regression was done on peak height and peak area to determine whether the variables could be considered as separate measures of differences among the populations. All analyses were performed using SAS (SAS Institute, Inc. 1985).

RESULTS

The use of HPLC as a stock assessment tool is a novel application of a relatively new technology. As such, several questions deserve consideration. The first of these is whether chromatographic runs from a single individual may vary over time. Figure 1 presents the results of two runs of the same eye lens extract done over an interval of about two weeks. The near exact correspondence between the two tracings suggests that frozen storage does little to alter chromatographic results, at least over short time periods. Protein changes accompanying long term storage is currently being investigated.

The second question which must be addressed concerns within population variation in eye lens proteins. Figure 2(a-i) presents chromatograms of random trios of individuals from each population. The degree of conformance within each group is most striking, especially along the time (protein quality) axis. Variations in the Y direction (protein quantity) are the probable result of dilution differences created during eye lens processing. However, these quantity differences are parallel in nature and presumably introduce little within population variation.

An objective assessment of king mackerel stocks is most dependent upon identification of trends in similarities and/or differences among populations. Figure 3 depicts the eye lens chromatograms for a single randomly chosen individual from each of the nine sample groups. Several factors, such as pressure and temperature, can affect the efficiency of the chromatographic system and thus produce what appear to be differences in retention times. However, experience has shown that the initial peak (#1) in each population represents the same protein. Likewise, the ultimate peak (#10) in each case is the same protein in spite of discrepancies in retention times. Intermediate peaks align themselves accordingly. Visual examination of the arrays of peaks and valleys reveals patterns which, while sharing certain characteristics, show unique patterns. The most outstanding of these resemblances is that of the Veracruz and Cuba sample groups. Arguments could be made for similarities among several of the other groups.

Statistical analyses of peak number, peak height and peak area within and among populations disclose several facts of interest.

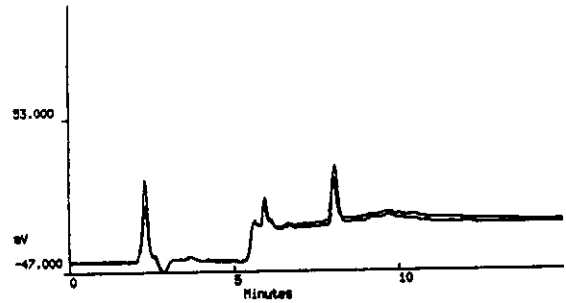

Figure 1. Comparison of chromatograms of a single king mackerel run on different days illustrating a minimum of day to day variation in runs.

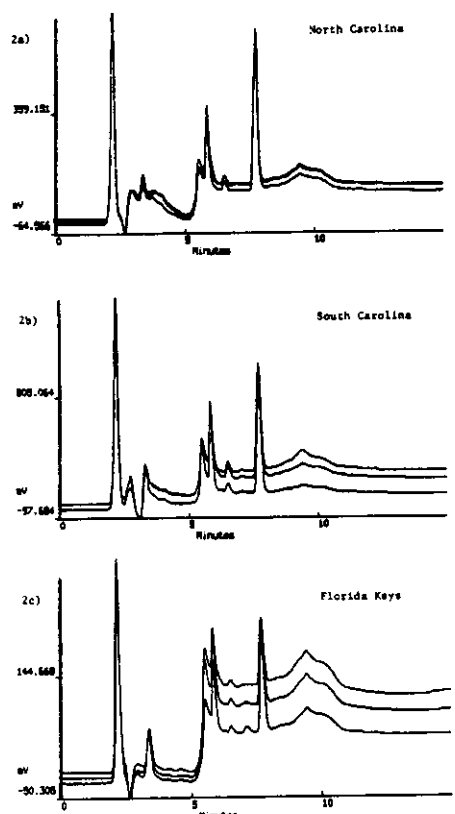

Figure 2 (a-c). Eye lens protein chromatograms for three adult king mackerels. Localities given above.

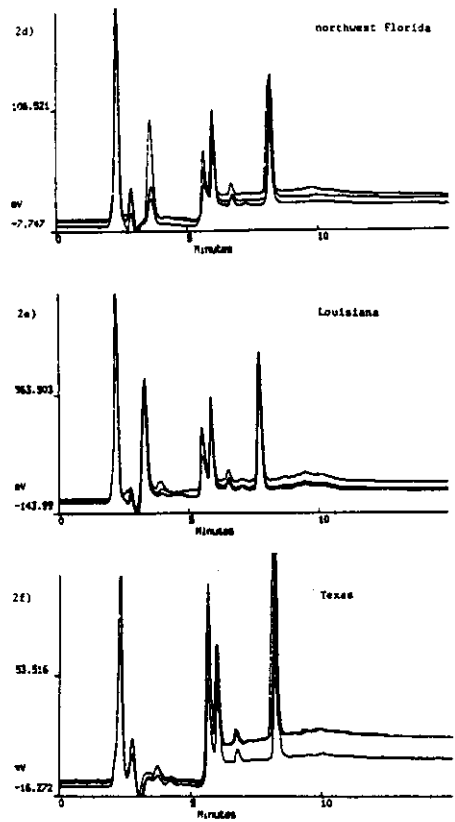

Figure 2 (d-f). Eye lens protein chromatograms for three adult king mackerels. Localities given above.

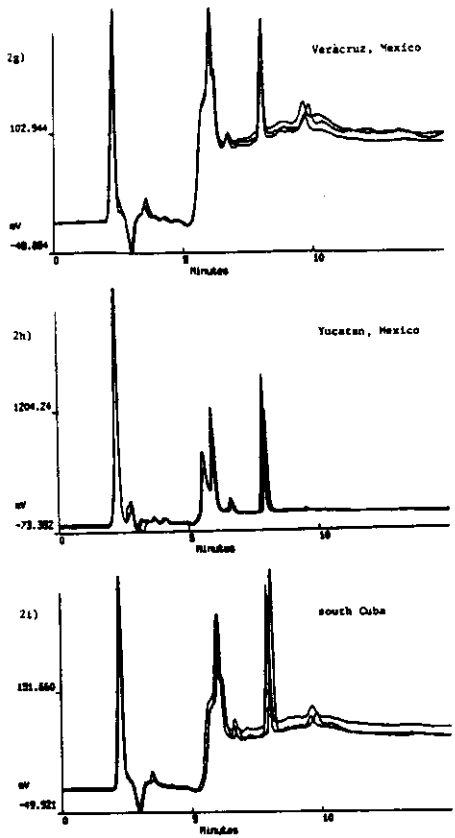

Figure 2 (g-i). Eye lens protein chromatograms for three adult king mackerels. Localities given above.

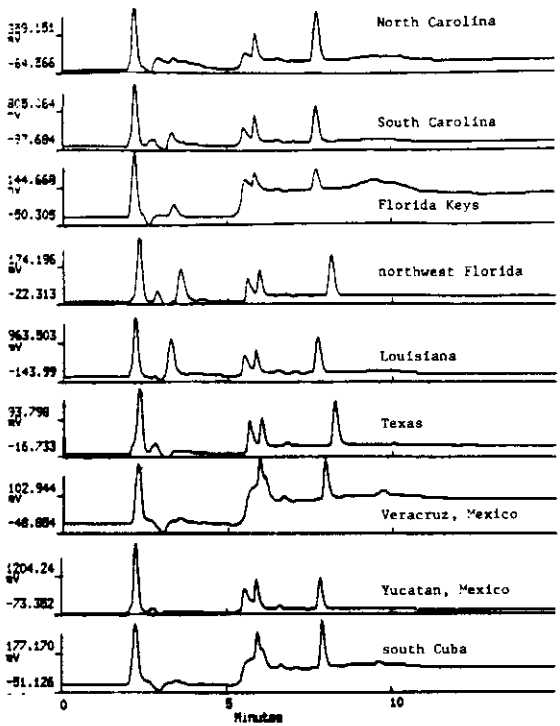

Figure 3. Comparison of representative eye lens protein chromatograms of king mackerel from nine populations. Localities given above.

The linear regression of peak height and peak area ($r = 0.97$, $p < 0.001$) indicates the two variables to be too closely related to one another to be treated as separate factors. Consequently, statistical analyses continued using only one of the variables, peak height. The ANOVA produced highly significant differences for both main effects: population ($F = 40.47$, $p < 0.0001$) and peak (protein) number ($F = 51.56$, $p < 0.0001$). The results of the linear contrasts are shown in Table 2.

Table 2. Sequence of linear contrasts comparing combinations of king mackerel populations

Linear Contrasts	F	p <
1) NC vs SC	1.66	0.1980
2) NC, SC vs FLK	0.00	0.9977
3) NC, SC, FLK vs NWFL	17.57	0.0001
4) NWFL vs TX	0.30	0.5815
5) NWFL, TX vs LA	160.12	0.0001
6) VC vs CU	0.71	0.4012
7) VC, CU vs NWFL, TX	1.96	0.1615
8) YU vs LA	1.30	0.2552
9) VC, CU, NWFL, TX vs YU, LA	287.30	0.0001
10) NC, SC, FLK vs YU, LA	158.48	0.0001

The initial contrast comparing North Carolina (NC) king mackerel with South Carolina (SC) kings was not significant. These two populations were pooled and contrasted against the Florida Keys (FLK) group, which also failed to differ significantly. The three "Atlantic" populations differed from the northwest Florida (NWFL) population (contrast 3), which failed to differ from the Texas (TX) population (contrast 4). The combined Texas and northwest Florida group was significantly different from the Louisiana (LA) king mackerels (contrast 5), which failed to differ from the Yucatan (YU) fishes (contrast 8). The Veracruz (VC) and Cuba (CU) populations did not differ significantly and when combined, did not differ from (contrast 6) the Texas and northwest Florida sample group (contrast 7). Comparison of the pooled Veracruz, Cuba, Texas and northwest Florida array with the Yucatan/Louisiana king mackerel produced a highly significant difference. Further comparison of the "Atlantic" group to the Louisiana/Yucatan populations (contrast 10) also yielded a highly significant difference.

DISCUSSION

Based on 72 specimens of king mackerel from nine populations, HPLC analysis of eye lens proteins has revealed the following three groups: 1) North Carolina, South Carolina and Florida Keys; 2) northwest Florida, Texas, Veracruz and Cuba; and 3)

Louisiana and Yucatan. While this scheme does not agree strictly with any previously published assessment of king mackerel stocks, general trends in eye lens protein makeup may be recognized. An "Atlantic" element, composed of fishes from North Carolina, South Carolina and the Florida Keys, is united by the absence of protein peaks number 4, 5 and 9 (Figure 4). The populations from northwest Florida, Texas, Veracruz and Cuba share the absence of protein peaks number 3, 5 and 9 (Figure 4). King mackerels from Louisiana and Yucatan are related more for the presence of nine protein peaks, seven of which are shared between the populations. Interestingly, in HPLC analyses of red drum, Louisiana fishes were also found to be unique from adjacent populations to the east and west (Bane and Nieland, 1985).

That the data do not supply a more consistent picture of king mackerel stock identity should not be surprising. While the current study shows the utility of the HPLC technique and the adaptation of HPLC data to statistical analyses, the results are of the most preliminary nature. Among the more glaring shortcomings is the time frame over which the fishes were collected. Considering the highly migratory habit of the king mackerel, comparisons of seemingly adjacent populations using specimens collected over a several month interval may yield little useful information. Implementation of a bimonthly simultaneous sampling in the nine regions would be the minimum required to produce any meaningful assessment of king mackerel stock structure. This intensive sampling regime is not limited to only HPLC analysis. Any other biochemical investigative procedure would require a similar intensive sampling scheme.

The purpose of this work, however, was not to solve all the questions of king mackerel stock structure in Atlantic and Gulf waters. We proposed only to demonstrate the utility of HPLC analysis of eye lens proteins as a stock assessment tool. To that end we have described a fast and reproducible technique for examining nuclear eye lens proteins for polymorphisms. Graphic and statistical evidence has shown that little within sample group variation of these proteins can be detected. Significant among population variation in lens proteins can be discerned using HPLF and this variation has great potential for delineating breeding stocks. We fully believe in the potential of HPLC to make a meaningful contribution to that body of knowledge concerning king mackerel stock structure.

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