Northeast Gulf Science

Volume 5
Number 1 *Number 1*

Article 11

10-1981

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DOI: 10.18785/negs.0501.11
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Recommended Citation

Skow, L. C. and M. E. Chittenden Jr. 1981. Differences in Hemoglobin Phenotypes Among Spanish Mackerel, *Scomberomorus maculatus*. Northeast Gulf Science 5 (1).
Retrieved from https://aquila.usm.edu/goms/vol5/iss1/11

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DIFFERENCES IN HEMOGLOBIN PHENOTYPES AMONG SPANISH MACKEREL, *Scomberomorus maculatus*[1]

The Spanish mackerel, *Scomberomorus maculatus,* inhabits continental waters of the western Atlantic Ocean from the Gulf of Maine to the Yucatan Peninsula (Colette and Russo 1979) and is seasonally common as far north as Chesapeake Bay (Bigelow and Schroeder, 1953). Spanish mackerel support important recreational and/or commercial fisheries in U.S. and Mexican waters (Lyles 1969; Deuel 1973; Doi and Mendizabal 1979; Trent and Anthony 1979).

Intelligent management of Spanish mackerel requires determination of how many populations make up the stock and of the population distributions and migrations. This task has been greatly simplified by recent studies (Collette et al. 1978) which show that *S. maculatus* ranges south only to Yucatan Peninsula and that *S. brasiliensis,* a new species formerly confused with *S. maculatus,* is distributed south of Yucatan. The present paper presents electrophoretic evidence for the existence of at least two populations of Spanish mackerel.

MATERIALS AND METHODS

Spanish mackerel were collected by hook and line off Port Aransas, Texas (37 fish) from 30 June to 29 July 1975 and off Beaufort, N.C. (35 fish) from 10 to 22 August 1975. Blood samples were taken after caudal amputation and immediately placed and stored on ice. Analyses were performed within 72 hours of collection.

To prepare samples for electrophoresis, serum was removed from the clotted blood by pipette and the clot was covered with two volumes of cold 0.85% NaCl solution. Clots were mechanically disrupted with a glass stirring rod and the freed erythrocytes collected in the supernatant. Erythrocytes were washed three times with cold 0.85% NaCl and lyzed in 4 volumes of distilled water. Cellular debris was separated from lyzates by centrifugation and the hemolyzates collected by pipette.

Hemolyzates were immediately analyzed by horizontal starch gel electrophoresis (Kristjanssen 1960) using tris-EDTA-boric acid buffer (Boyer *et. al* 1963) at 375V for 4 hours. Following electrophoresis, the gels were cut into mirror-image halves with a gel slicer. One gel half was stained for protein using Amido Black. The complementary gel half was scored for hemoglobin and then stained for heme peroxidase activity by immersing the gel in a 0.5% solution of dimethoxy benzidine which contained 0.5 ml of hydrogen peroxide. In all cases, the heme peroxidase activity coincided with the visible hemoglobin bands.

During the course of this study, we also searched for electrophoretic variants in serum proteins, serum esterases, and erythrocyte lactic dehy drogenase (LDH). The serum proteins and esterases were analyzed in a 1:1 aqueous dilution of the sera removed from clotted blood. LDH activity was typed in hemolyzate samples as prepared for hemoglobin analysis. Electrophoretic conditions were as described. Serum proteins were visualized by staining with Amido Black and serum esterase activity was demonstrated by the procedure of Roderick *et al.* (1971). Erythrocyte LDH activity was visualized by the method of Shows and Ruddle (1968).

RESULTS AND DISCUSSION

Hemoglobin Patterns

Spanish mackerel hemoglobins were

[1]Technical Article TA16060 from the Texas Agricultural Experiment Station.

resolved into simple electrophoretic patterns of one or two bands, designated f and s (Figure 1). The major band (f) made up about 80% of the total hemoglobin in the doublet pattern and was electrophoretically indistinguishable from the hemoglobin in the single band pattern. Therefore, we assume these hemoglobins to be identical. The presence of a second hemoglobin band (s) of lower concentration in the doublet pattern could be explained by several hypotheses. The most reasonable explanation for the observed phenotypes is genetic variation at one of the genes encoding a globin subunit, but the genetic basis of the polymorphism is not easily obtained from the data due to the small number of animals examined. We are confident that this band is not an artifact of preparation because the freqquency of the double band phenotype was independent of the time when fish were captured, and we could not generate the minor band by storing samples for up to three days after preparation. We also do not feel that the phenotype is a developmental expression of genes not normally expressed in adults, because the frequency of hemoglobin phenotype was not correlated with size or sex.

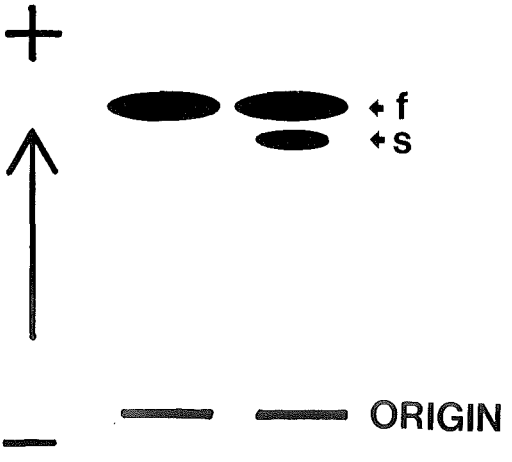

Figure 1. Diagram of starch gel electrophoretic patterns of hemoglobin from Spanish mackerel. The symbol *f* (fast) and *s* (slow) designate the two forms of hemoglobin found in this study.

Spanish Mackerel Populations

To determine whether the Spanish mackerel in this study represent separate populations, the data in Table I were subjected to a chi-square test for homogeneity. The value obtained (χ^2=16.76, 1 d.f.) is significant at α=0.001, leading us to conclude that Spanish mackerel populations off Beaufort, N.C. and Port Aransas, TX are genetically separated.

Table 1. Contingency test of Beaufort, N.C. and Port Aransas, TX observed hemoglobin phenotypes.

Location	Phenotypes f f	f s	Total
Beaufort			
observed	35	2	37 (.51)
expected	26.5	10.2	
Port Aransas			
observed	17	18	35 (.49)
expected	25.5	9.8	
Total	52	20	72

Preliminary findings of morphometric and meristic studies (Collette and Russo, personal communication) also indicate significant differences between fish from the Gulf of Mexico and the southeast Atlantic coast of the U.S. These findings support the suggestion (Beaumariage 1970; Wollam 1970) that Spanish mackerel occurring in the northern Gulf of Mexico and along the southeast Atlantic coast of the U.S. during summer form separate populations. It remains unclear how many populations occur in the northern Gulf of Mexico, however, because we collected only in the northwestern area. Spanish mackerel exhibit seasonal migrations along the Mexican coast (Doi and Mendizabal 1979) whose timing seems similar to well documented movements along the east and west coasts of Florida (many references including Earll 1883; Klima 1959). Springer and Pirson (1958) suggested that the Spanish mackerel that overwinter off Florida migrated in summer along the northern Gulf of Mexico to Texas, but that needs to be verified. A fundamental question that still requires clarification

is whether or not the fish that migrate along the west coast of Florida and along the east coast of Mexico are reproductively isolated and form separate populations.

During this study, several other proteins were examined for electrophoretic variation but were found to be unsuited for comparing the two populations because variants were absent or present at low frequencies or because the patterns were too complex for genetic analysis given the sample size. Serum protein patterns were highly polymorphic, and at least some variation (probably transferrins) was easily typed. Four or five electrophoretic bands were observed in single or double combinations, but the small numbers of fish collected precluded proper statistical analysis. Lactate dehydrogenase (LDH) activity in erythrocytes and α napthyl serum esterase activity were also examined. One animal (from Port Aransas) possessed a unique LDH phenotype. Serum esterase patterns were variable, but most differences appeared to be quantitative and the patterns were not analyzed further. These proteins may prove useful as genetic markers for Spanish mackerel populations if larger numbers of fish are available for analysis (LDH and transferrins) or when more information is available regarding the nature of the esterase electrophoretic variation.

ACKNOWLEDGMENTS

We are indebted to the following persons for assistance in collection of specimens and logistics: J. Stuckey and M. Van den Ayvle of Texas A&M University; C. Arnold and the staff of NMFS Port Aransas, TX. Laboratory; C. Manooch and personnel of NMFS Beaufort, N.C. Laboratory; E. Nakamura, NMFS Panama City Laboratory. Drafts of the manuscript were critically reviewed by Bruce Collette, Allyn Johnson, Eugene Nakamura, and Joseph Russo. Financial support was provided, in part, by the Texas Agricultural Experiment Station and by the Texas A&M University Sea Grant College Program, supported by the NOAA Office of Sea Grant, Department of Commerce.

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DOI: 10.18785/negs.0501.11