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RESULTS OF THE JACK MACKEREL SUBPOPULATION DISCRIMINATION FEASIBILITY STUDY

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ABSTRACT

A report is made on the feasibility of discriminating subpopulations of jack mackerel, *Trachurus symmetricus*, off of the southern California and Baja California coast. Histochemical, morphometric, and meristic characters are compared from four samples of approximately 200 fish each taken from three areas. The data are analyzed for homogeneity by chi-square tests. Heterogeneity was found only in anal fin ray counts. Recommendations for a comprehensive study are made.

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INTRODUCTION

Jack mackerel, *Trachurus symmetricus*, are found from the Gulf of Alaska to the Gulf of Tehuantepec, off the coast of southern Mexico, and as far as 1300 miles seaward (Blunt 1969). Within this range the maximum density is found from Point Conception to central Baja California (Frey 1971). Jack mackerel are differently distributed by age throughout their range. The center of abundance of eggs and larvae is from Point Conception to Cape San Quentin and nearly 500 nautical miles off the coast (Ahlstrom 1969).

For their first 3 to 6 years jack mackerel remain inshore, where they congregate in schools and are vulnerable to round haul gear. As they grow older and larger, they school less and inhabit offshore waters outside the range of the existing commercial fishery. The majority of female jack mackerel are mature at the end of their first year and essentially all are mature at age two (Wine and Knaggs 1975). Throughout the history of the fishery, the age mode of the catch has diminished until now the catch consists primarily of 1, 2, and 3 year old fish with 4 to 7 years becoming increasingly scarce.

This downward trend has concerned those who manage the fishery and has inspired the following questions: (i) what is the relationship between the exploited schools of fish in the southern California waters and the larger, non schooling, offshore fish, and (ii) what is the relationship between the exploited schools and those which are observed in the rest of the range? If the exploited population is recruited from surrounding populations and is the progeny of an offshore spawning stock, there is less to be concerned about than if there are separate spawning

stocks which must provide their own recruits.

To answer these questions, a study was funded by the Marine Research Committee in 1974 to assess the feasibility of discriminating possible subpopulations or separate spawning stocks. We used the classical technique of comparing morphometric and meristic characteristics and a relatively new technique of enzyme genetics analysis to test for population homogeneity.

METHODS AND MATERIALS

The jack mackerel used in our study were obtained from several sources. Two samples were taken from islands off Baja California and two samples were taken from southern California waters (Table 1). It was hoped that the great distances between sampling areas would be sufficient to demonstrate possible differences between suspected subpopulations.

TABLE 1. Source of Jack Mackerel Samples Collected for Use in Subpopulation Identification Study.

Sample group	Location	Date	Sample size	Fork length range (mm)	Fork length mean (mm)	Standard deviation	Approximate distance in nautical miles from: Catalina	Guadalupe	Cedros
I	Guadalupe Island	3/74	213	215-344	268	19	260		170
II	Cedros Island	3/74	197	134-233	157	16	350	170	
III	San Clemente Island	8/74	240	114-320	201	42	30	230	325
IV	Santa Catalina Island	10/74	326	204-245	231	25		260	350

Fish samples were collected by hook and line and blanket net under a night-light. When fish were taken in great numbers, they were held in the live fish holds of the research vessel ALASKA. When fishing was slow or finished for the night, blood sampling was initiated by placing a small number of fish in a dry bucket so that they would

become moribund. Blood was taken by heart puncture with an 18 gauge hypodermic needle and a Peel-A-Way heparinized blood sampler containing 2 cc of glycerol citrate solution (2% V/V) to prevent coagulation. Approximately 2 cc of blood was drawn so that each sample would be diluted to the same degree. Corresponding identification numbers were then placed into the fish's pharynx and on the blood sample; both were then placed into the freezer. Fish were frozen in groups of 25 to be used in subsequent morphometric and meristic studies.

Enzyme Genetics

The blood from 20 fish was tested for the presence of enzyme activity by the process of horizontal starch gel electrophoresis at the laboratory of Michael Soulé of the University of California at San Diego. Of 11 tests conducted, only phosphoglucose isomerase (PGI) showed sufficient polymorphism to be useful in a study of genetic variability as an indicator of subpopulation status. All of the blood samples were then tested for PGI phenotype by horizontal starch gel electrophoresis in our laboratory.

The freshly thawed blood was applied to pieces of Whatman no. 3 filter paper approximately 4 X 6 mm (0.16 X 0.25 inches) which were inserted into slits in a 13- X 18- X 0.6-cm (ca. 5- X 7- X 0.25-inch) starch gel made with Electro Starch and Poulik buffer, pH 8.6. Poulik tray buffer was used adjusted to pH 8.0 for the cathodal chamber and pH 7.0 for the anodal chamber. Solutions were readjusted to this level before each run for four or five runs and then replaced. Fifteen samples were run in a single gel. The optimum electric current was 60 milliamperes at 100 to 200 volts for 3 to 4 hours. During the run the gels were covered with a polyethylene sheet to prevent dehydration and the trays were placed in a refrigerator to prevent overheating. At

the completion of the run the gel was sliced to half thickness with piano wire and one of the half gels was placed, cut side up, in a plastic tray. The following zymogram development solution was then applied to the cut surface: 30 ml Tris/HCl 0.2 M, pH 8.0; 10 ml, MgCl 0.1 M; 4 ml Fructose-6-phosphate 0.018 M; 10 mg NADP in 1 ml of water; 30 units of G6-PDH in 1 ml water; 20 mg MTT in 1 ml of water; and 10 mg PMS in 1 ml of water. The zymograms developed in 1 hour of darkness incubated at 38°C (100.4°F). At that time the zymogram, which was manifested as blue lines, was interpreted. The PGI phenotype and allele frequencies for each area were tabulated to be used in chi-square tests of homogeneity.

Morphometrics and Meristics

In the laboratory whole fish samples were thawed and identified by the numbered card in their pharynx. A series of standard measurements was taken from the left size of the fish with dial calipers and recorded to 0.01 mm. The spines and rays of the dorsal and anal fins were counted and the otoliths were collected and subsequently read. The fin ray counts for each area were then tabulated for chi-square tests of homogeneity. Only groups I, II, and III were analysed in this phase of the study.

Statistical Methods

Before using observed PGI phenotype frequencies to test for population homogeneity, we used a chi-square test to compare them with the expected Hardy-Weinburg equilibrium frequencies.

We then used the chi-square test of homogeneity to evaluate the significance of differences in gene frequency, dorsal ray counts, and anal ray counts. The data were arranged in Rox X Column (R X C) tables and the expected number for each cell computed from the border totals.

The value for chi-square was then computed from the formula $\chi^2 = \Sigma(o-e)^2/e$ with o = the observed frequency and e = the expected, and degrees of freedom, df, = (R-1) (C-1). The probabilities associated with the various values of chi-square were determined from published tables. Cells with less than five expected occurrences were pooled with adjacent cells within each column.

RESULTS

As mentioned earlier, of the 11 substances tested in the screening process, only PGI showed useful polymorphism. Of the other enzymes sought esterase and phosphoglucomutase (PGM) showed low levels of polymorphism; lactase dehydrogenase (LDH) and malate dehydrogenase (MDH) were monomorphic; aldolase, glutamate-oxaloacetate transaminase (GOT), fumarase, glucose 6-phosphate dehydrogenase (G 6-PDH), and alpha glycerophosphate dehydrogenase (alpha GPDH) showed no results; and general protein was not considered to be diagnostic. Gary Sharp (InterAmerican Tropical Tuna Commission pers. commun.) indicates that positive results for some of these enzymes could probably be found using tissues other than blood.

The zymogram of PGI demonstrated five alleles which were designated as A, B, C, D, and E depending on relative speed of migration toward the positive pole, with A being the fastest (Figure 1). Heterozygotes displayed three bands, one for each allele and one at the approximate half-way point, which suggests a dimer structure. PGI activity was found at only one locus.

The observed phenotype frequencies do not differ significantly from the expected Hardy-Weinburg equilibrium frequencies (Table 2), computed from the observed PGI allele distribution (Table 3) and chi-square tests at this level of sampling support a null hypothesis of homogeneity (Table 4).

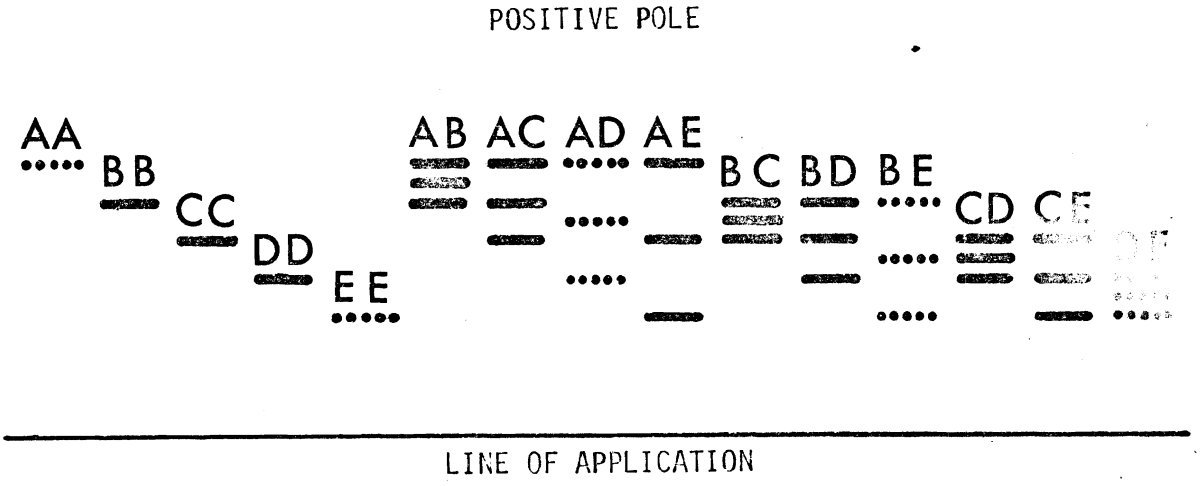

FIGURE 1. Diagram of phosphogluco isomerase zymograms from jack mackerel blood, separated by starch gel electrophoresis. Dotted lines indicate phenotypes which are possible but were not observed. Letters indicate each phenotype.

TABLE 2. Distribution of Phosphogluco Isomerase Isozyme Phenotypes Among Jack Mackerel from Southern California and Baja California Waters. Chi-Square Test of Null Hypothesis that the Phenotype Frequencies Follow the Hardy-Weinberg Equilibrim.

PGI phenotype	Sample group I Obs.	I Exp.	II Obs.	II Exp.	III Obs.	III Exp.	IV Obs.	IV Exp.
AA			0	.01	0	.04	0	.01
BB	11	6.09	4	4.88	8	6.34	12	10.14
CC	130	127.05	119	121.95	143	143.37	201	197.12
DD	0	.74	0	.41	0	.55	1	.44
EE							0	.01
AB			0	.47	1	.97	1	.53
AC			3	2.37	5	4.63	1	2.33
AD			0	.15	0	.29	0	.11
AE			0	.01	0	.02	1	.02
BC	47	55.60	52	48.79	58	60.29	85	89.42
BD	3	4.23	2	2.83	3	3.74	5	4.24
BE			0	.17	0	.33	0	.53
CD	22	19.29	16	14.17	20	17.77	17	18.66
CE			1	.79	2	1.56	2	2.33
DE					0	.10	0	.11
TOTAL	213		197		240		326	
Low frequencies pooled	14	10.93	10	12.05	11	12.25	11	10.66
X^2	2.67		.868		.930		1.490	
DF	3		3		4		4	

TABLE 3. Distribution of Phosphogluco Isomerase Isozyme Alleles from Jack Mackerel Taken in Southern California and Baja California Waters.

PGI allele	Sample group I	II	III	IV
A	0	.0076	.0125	.0046
B	.1690	.1574	.1625	.1764
C	.7723	.7868	.7729	.7776
D	.0586	.0457	.0479	.0368
E	0	.0025	.0042	.0046

TABLE 4. Chi-Square Test of Null Hypothesis that PGI Gene Frequencies are Homogeneous.

Gene	Sample group								Total
	I		II		III		IV		
	Obs.	Exp.	Obs.	Exp.	Obs.	Exp.	Obs.	Exp.	
A&B [1/]	72	73.98	65	68.43	84	83.36	118	113.23	339
C	329	331.07	310	306.20	371	373.03	507	506.70	1,517
D&E [2/]	25	20.95	19	19.37	25	23.61	27	32.07	96
Total	426		394		480		652		1,952

X^2 = 2.176, at 6 degrees of freedom. The null hypothesis is not rejected at the 0.05 level.

[1/] There was a total of 12 "A" alleles observed.

[2/] There was a total of 6 "E" alleles observed.

Morphometrics and Meristics

Morphometric and meristic data were compared to results obtained by Roedel and Fitch (Calif. Dept. Fish and Game, unpublished data) in 1954 who at that time examined 1,253 specimens taken from central and southern California. In their analysis, they observed no significant differences from one locality to another and hypothesized the samples to be from one homogeneous population.

After examination of the morphometric data and in consideration of Royce's (1957) arguments against direct comparison of proportions we decided that our morphological data were not suitable for subpopulation discrimination. A more meaningful treatment of our morphological data would be the use of multivariate analysis. However, to use this technique the sampled fish should all be in the same stage of growth (by length or age) and our samples did not meet this criterion (Table 1).

With respect to meristic counts, the number of first dorsal spines was uniformly eight with one exception of seven. The second dorsal always had one spine and the number of rays varied from 29 to 37. The chi-square test of homogeneity of the number of dorsal rays among areas produced the nonsignificant value of 15.13 at 12 degrees of freedom (Table 5). The anal fins all had three spines followed by 25 to 32 rays. The chi-square value was 30.05 at 8 degrees of freedom which far exceeds the allowed value and indicates that the null hypothesis, that the sample groups are homogeneous, must be rejected at the 0.05 level (Table 6). Further analysis comparing each group against each other suggests that they are all different, one from another. This is the only character which we examined that showed heterogeneity. The number of second dorsal rays and anal rays was positively correlated which is to be expected.

TABLE 5. Chi-Square Test of the Null Hypothesis that Second Dorsal Fin Ray Count Frequencies from Sample Groups I, II and III are Homogeneous.

Fin ray count	Sample Group I Obs.	I Exp.	II Obs.	II Exp.	III Obs.	III Exp.	Total
≥ 36 [1]	14	12.78	11	11.75	10	10.47	35
35	30	27.38	28	25.18	17	22.44	75
34	42	56.95	53	52.36	61	46.69	156
33	78	71.91	66	66.12	53	58.97	197
32	33	29.93	27	27.52	22	24.55	82
≤ 31 [2]	14	12.05	9	11.07	10	9.88	33
Total	211		194		173		578

X^2 = 12.803, at 10 degrees of freedom. The null hypothesis is not rejected at the 0.05 level.

[1] There were 4 counts greater than 36 observed.

[2] There were 5 counts less than 31 observed.

TABLE 6. Chi-Square Test of the Null Hypothesis that Anal Fin Ray Count Frequencies From Sample Groups I, II and III are Homogeneous.

Fin ray count	Sample group I Obs.	I Exp.	II Obs.	II Exp.	III Obs.	III Exp.	Total
≥ 30 [1/]	36	28.47	24	26.18	18	23.35	78
29	83	72.28	62	66.46	53	59.26	198
28	70	73.01	62	67.13	68	59.86	200
27	19	28.84	40	26.51	20	23.65	79
≤ 26 [2/]	3	8.40	6	7.72	14	6.88	23
Total	211		194		173		578

X^2 = 30.05, at 8 degrees of freedom. The null hypothesis is rejected at the 0.05 level.

X^2 (Groups I vs. II) = 13.72 (4 d.f.) Reject at 0.05 level.

X^2 (Groups I vs. III) = 16.19 (4 d.f.) Reject at 0.05 level.

X^2 (Groups II vs. III) = 10.53 (4 d.f.) Reject at 0.05 level.

[1/] There were 11 counts greater than 30 observed.

[2/] There were 6 counts less than 26 observed.

DISCUSSION

The results obtained from this feasibility study are encouraging. We have demonstrated that the jack mackerel off southern California and Baja California have a polymorphic protein in their blood which probably can be used in conjunction with proteins from other tissues to identify possible subpopulations or breeding stocks.

We feel that to compare optimally the relationship of the larger nonschooling fish, which occur offshore, with the smaller nearshore schooling fish, a serial sample of all year classes would have to be taken. A sample of hundreds of fish is necessary to trace possible trends in genetic equilibrium. Perhaps less conclusive results could be obtained if fewer fish were used. In order to produce the most conclusive results regarding geographic separation of stocks it would be necessary to sample mature fish during the height of spawning in case the stocks are separated only at this time. However, this does not necessarily reveal stocks which spawn at separate times at the same locality. The samples also should be taken during a relatively short period of time to avoid sampling the same stocks should they follow some migration route or mix after spawning.

The determination of sample size when using PGI gene frequencies can be made if the following requirements are observed and assumptions made: (i) a difference of at least 5% in the frequency of the dominant genes between areas is significant (Gary Sharp, InterAmerican Tropical Tuna Commission, pers. commun.) and, (ii) the power, the probability of rejecting the null hypothesis when in fact it is false, should be at least 0.80. The power of the test can be determined from tables published by Owen (1962) based on the value of λ, a noncentrality parameter for the noncentral chi-square distribution. The value of λ is determined from the following formula:

$$\lambda = \frac{n_1 n_2 (P_1 - P_2)^2}{P (1 - P) N} = \frac{n_1 n_2 \Delta^2}{P (1 - P) N}$$

where: λ = noncentrality parameter for the noncentral chi-square (χ^2) distribution

n_1 = size of the reference sample (alleles)

n_2 = size of the comparison sample (alleles)

P_1 = estimate of frequency of dominant allele of the population in area 1 based on reference sample

P_2 = estimate of frequency of dominant allele of the population in area 2 based on the comparison sample

P = average of P_1 and P_2 or $\frac{P_1 + P_2}{2}$

$N = n_1 + n_2$

From this formula it was determined that because the frequency of the dominant PGI gene is approximately 0.80, a homogeneous reference sample of 1000 fish (2000 alleles) and homogeneous comparison samples of 400 fish (800 alleles) are required to detect 5% changes in the dominant gene with a power of 0.80.

With respect to the morphometric study, a few very carefully made measurements of 50 to 100 fish of the same age group can be used to discern differences between subpopulations when compared through multivariate analysis.

Our comparison of meristic elements revealed suspected heterogeneity only with respect to anal fin ray counts. We believe that this feature deserves a more comprehensive examination in the future.

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REFERENCES

Ahlstrom, E.H. 1969. Distributional Atlas of fish larvae in the California current region: jack mackerel, *Trachurus symmetricus*, and Pacific hake, *Merluccius productus*, 1951 through 1966. CalCOFI Atlas, (2):v-xi, 1-187.

Blunt, C.E. Jr. 1969. The jack mackerel (*Trachurus symmetricus*) resource of the eastern North Pacific. CalCOFI Repts., 13:45-52.

Dando, P.R. 1974. Distribution of multiple glucosephosphate isomerases in teleostean fishes. Comp. Biochem. Physioc., 47(B):663-679.

Frey, H.W. (ed.) 1971. California's living marine resources and their utilization, Calif. Dept. Fish Game, Sacramento, 148 p.

Owen, D.B. 1962. Handbook of statistical tables. Addison-Wesley Pub. Co., Reading, Mass, 580 p.

Royce, W.F. 1957. Statistical comparison of morphological data. U.S. Fish and Wildl. Ser. Spec. Sci. Rept., Fish. (208):7-28.

Shaw, C.F., and R. Prasad. 1970. Starch gel electrophoresis of enzymes - a compilation of recipes. Biochem. Genetics, 4:297-320.

Wine, V.L., and E.H. Knaggs. 1975. Maturation and growth of jack mackerel, *Trachurus symmetricus*. Calif. Dept. Fish Game, Mar. Res. Tech. Rept., (32):1-26.