Restricted

INTERNATIONAL COMMISSION FOR THE NORTHWEST ATLANTIC FISHERIES

Serial No. 2711
(B.g.7)

ICNAF Res.Doc. 72/20
(also ICNAF SAC No. 72/19
- Revised)

ANNUAL MEETING - JUNE 1972

Biochemical Studies on the Stock Structure of Herring in the Gulf of Maine, Georges Bank and Adjacent Areas

by
Robert D. Lewis and George J. Ridgway
National Marine Fisheries Service Biological Laboratory
West Boothbay Harbor, Maine 04575

Introduction

The need for assessment and management of the herring stocks in the Gulf of Maine, Georges Bank and adjacent areas (ICNAF subareas 4X, 5Y, 5Z and 6, has become increasingly apparent in recent years. Increasing effort brought the landings from the area to a peak in 1968; subsequently, while high effort levels were maintained, the herring stocks in all sections of this area began to decline in abundance. Accurate assessments and optimal management require knowledge of the number and distribution of unit-stocks or sub-populations in the area. The stock structure has been investigated by a variety of means including: distribution of spawning and subsequent larvae distribution and drift (Boyar et al., 1971) (Iles, 1971); and meristics (Anthony and Boyar, 1968) (Iles, 1970).

Biochemical and immunogenetic methods also have shown promise for distinguishing the unit-stock structure of fishes. The use of biochemical methods for separating stocks is based on the knowledge that the structure of proteins is under genetic control. Nearly all living species are highly variable for a variety of genetically controlled forms of proteins including numerous enzymes. Differences in the frequencies of occurrence of these variant forms in groups of fish would provide evidence for reproductive isolations and the existence of separate stocks. We presented a paper summarizing serological and biochemical studies on the herring in the Gulf of Maine and adjacent waters at The Special Meeting on Biochemical and Serological Identification of Fish Stocks convened by ICES in 1969 (Ridgway et al., 1971). The results reported in that paper obtained with blood types, esterase types, and lactic dehydrogenase types supported the conclusion that the stock of adult herring on Georges Bank was independent of the stocks of adult herring spawning in the southwestern Gulf of Maine and off southern Nova Scotia. The results presented in this report have been obtained with an additional polymorphic enzyme system phosphohexose-isomerase.

The enzyme phosphohexose isomerase, also known as phosphoglucose isomerase, glucose phosphate isomerase, or D-glucose-6-phosphate ketol isomerase (EC 5.3.1.9) is found widely distributed in both plants and animals. It catalyzes the reversible conversion of fructose-6-phosphate to glucose-6-phosphate. Electrophoretic variants have been reported in mice (Carter and Parr, 1967), humans (Detterer et al., 1968), and rabbits (Welch et al., 1970).

This paper is concerned with the electrophoretic variants of phosphohexose isomerase (PHI) observed in adult herring and their distribution in hypothetical racial stocks in the Gulf of Maine, Georges Bank and the Mid-Atlantic Bight areas of the Northwest Atlantic.

Materials and Methods

All samples were taken from research cruises and commercial vessels between April 1970 and May 1971. The whole fish were held at -20°C. for no more than 2 months after which they were thawed and age, sex, spawning condition and morphometric data recorded. The heart and a .5 cc. cube of skeletal muscle were removed from each fish and placed in 1 cc. plastic vials. Sample and vial were quick frozen (immersed) in liquid nitrogen and stored at -40°C. Samples treated in this fashion showed no appreciable qualitative change after being stored for one year.

Equal parts of sample and distilled water were placed in a small mortar and ground with the aid of glass homogenizing beads. The homogenates were centrifuged at 20,000 g. for 30 minutes.

Horizontal starch-gel electrophoresis was performed using a discontinuous buffer system similar to that described by Ferguson and Wallace (1961). The gel buffer, pH 8.1, contained 3.63 gr. Tris and 1.11 gr. citric acid per liter, and the electrode buffer, pH 8.1, contained 1.4 gr. lithium hydroxide (LiOH) and 18.56 gr. boric acid per liter. A 12% starch-buffer solution was heated to 100°C., cooled to 85°C., degassed, and poured into a 13x22x.63 cm. mold which consisted of a .63 cm. acrylic plastic frame sandwiched between 2 sheets of plate glass.

After cooling for 1/2 hour at room temperature and 1 hour at 4°C. the glass top and frame were removed and the gel severed 6 cm. from the cathodic end to allow insertion of sample wicks. Thirty μ-liters of sample supernatant were placed on 5 x 7 mm. pieces of Beckman* #319329 paper wick. After insertion of sample wicks the gel pieces were pushed together and held in position by placing 5 mm. glass rods at each end of the gel. Each rod was anchored by a rubber band running under the plate glass and looped over each end of the rod.

Horizontal electrophoresis was performed using electrode trays and cloth wicks; the wicks were placed on the surface of the gel with the edge of the cathode wick 11 centimeters from the sample slit and the edge of the anode wick 3 centimeters from the sample slit. The gel and wicks were then covered with plastic film. Direct current was supplied by a Heathkit regulated voltage power supply. Electrophoresis was performed at 4°C. for 30 minutes at 165 volts across the entire system. The pieces of filter paper were then removed and electrophoresis was continued at either 350 or 110 volts until the borate-tris interface had migrated to the edge of the anode wick (4-4 1/2 or 16 hours respectively. The electrode trays were never reversed and the buffer was replaced after about 8 runs.

Using glass rods to control the depth of cut, gels were sliced with a stainless steel razor band and stained for PHI activity with a modified formula described by Detterer et al. (1968). Twenty-five ml. of a 2% agar solution cooled to 55°C. was mixed with 25 ml. .6 M tris HCl solution (pH 8.0) containing the following ingredients: 120 mg. Fructose-6-phosphate (disodium), 10 mg. NADP (Nicotinamide-adenine dineucleotide phosphate), 10 mg. MTT tetrazolium, 2 mgs. Phenozene methosulfate, and 12 units Glucose-6-phosphate dehydrogenase. The dry ingredients were mixed with the buffer no more than 5 minutes and the Glucose-6-phosphate dehydrognase no more than 1 minute before combining with the agar. This mixture was poured over the gel before the agar solution congealed and incubated at room temperature until PHI bands appeared (5-10 min.).

*Mention of trade names in this publication does not constitute an endorsement by the National Marine Fisheries Service

Results

PHI Types of Atlantic Herring

Figure 1 shows 15 of the 16 PHI variants which we have observed to date. PHI-zymograms of herring tissue extracts show at least a single dark staining "major" band, and two or more weaker components anodic to the major band. Individuals showing these patterns are considered to be homozygous. Other PHI zymogram patterns of herring contain three dark and varying numbers of lighter staining bands; these are considered to be heterozygous. This situation, which is similar to that found in mice (Carter and Parr, 1967), rabbits (Welch et al., 1970) and humans (Detterer et al., 1968) is consistent with a dimeric structure that is controlled by an autosomal locus. Those variants which we have observed can be explained as 16 of the 21 possible combinations of six codominant alleles (A through F) inherited at such a locus.

The allelic "zones" are easily identified by their migration distances on the zymogram. In our system the major isozyme band of individuals homozygous for alleles A, B, C or D migrate about 34, 24, 14 or 2 mm. respectively from the origin toward the anode and those homozygous for alleles E and F migrate 12 and 22 mm. respectively toward the cathode. Heterozygous phenotypes are identified by the location of the upper and lower major staining bands which always falls in one of these zones. The middle band, present in all heterozygotes, often occurs between these major zones as in the case when adjacent alleles are involved (AB, BC, etc.). In other heterozygotes (BD, BF, CD, etc.) the middle band occurs at the same position as an allelic zone.

Each isozyme pattern exhibits at least 2 minor bands situated anodally to the major band. These are easily observed above the single major band of the homozygous patterns and above all three major bands of the heterozygous BE, BF, and CF patterns. In the other heterozygous phenotypes only the 2 minor bands associated with the upper dark staining component are evident and the lower ones are either partially or completely masked by major components of similar mobility. These minor subbands are a frequent finding in isozyme studies and have yet to be fully explained. Detterer et al., (1968) suggest that in the case of human PHI they may be due to conformational isomers.

PHI activity was detected in the liver, gills, brain, eye, gonads, kidney, skeletal muscle, heart and intestine of adult herring. Quantitative differences between tissues from the same fish were not detected. However, the activity varied considerably with the greatest activity found in the skeletal muscle and the least in the eye.

Intra-area Comparison Between Observed and Expected Frequencies of PHI Phenotypes

Tables 1-5 show the distribution of the PHI patterns of 18 samples of adult herring from NE Georges Bank, SW Nova Scotia, SW Gulf of Maine, Massachusetts Bay and the Mid-Atlantic Bight, respectively. The expected distributions of the types according to the Hardy-Weinberg law of genotype distributions in large random mating populations are also given. Seventeen of the 18 samples revealed no significant differences between the observed and the expected genotype distributions, thus providing evidence for the validity of the proposed method of inheritance. Sample number 4 from the SW Gulf of Maine was the only sample which deviated from that expected from a random mating population. This deviation could result from a sampling of two or more sub-populations, a type 1 statistical error, or more likely, differential mortality among older fish which were predominant in this sample. Analysis revealed that 42% were 7 or more years of age and that the genotypes observed in these older fish differed significantly from those expected from a random mating population. The genotypes of fish less than 7 years of age did not differ significantly.

The eighteen samples were initially placed into four areas on the basis of available life history information (spawning areas and winter fishery). The four areas were NE Georges Bank, SW Nova Scotia, SW Gulf of Maine (Provincetown, Mass. to Boothbay Harbor, Maine) and the Mid-Atlantic Bight (Cape Cod to Cape Hatteras). However, when the observed phenotypes of the 7 samples from SW Gulf of Maine were pooled and compared with those expected from the Hardy-Weinberg equilibrium, they were significantly different ($P<.05$). The decision was then made to separate the Provincetown and Stellwagen (Walther Herwig 6-25-70) samples from the SW Gulf of Maine area because both had been taken at a time and place where mixing of Georges Bank and SW Gulf of Maine stocks seemed reasonably likely. Each of the resulting five areas or groups then were tested for intra-area heterogeneity and no significant ($P \leq .05$) differences were obtained (tables 1-5).

Tests for Inter-Area Differences

The Massachusetts Bay area was not compared with other areas because the sample totals (140 fish) were inadequate. Evidence for differences among the four remaining areas was sought by testing for inter-area differences in both the phenotypes and the alleles.

Table 8 shows the results of comparing the fit of observed phenotypes with those expected from the Hardy-Weinberg law when two different areas are paired. If two discrete populations are pooled their genotypic distribution may deviate significantly from the expected phenotypes calculated according to Hardy-Weinberg law. Two of the six possible comparisons show considerable though not significant deviation. These are SW Nova Scotia + SW Gulf of Maine and SW Gulf of Maine + Mid-Atlantic Bight comparisons.

Contingency Table Comparisons of Area Allele Numbers

Table 6 lists the number of frequency of alleles observed for each sample and area. A 2x5 contingency table comparison of two regions and five alleles (B, C, D, E, and F) for the six possible comparisons did not reveal any significant differences. However, it is apparent in Table 5 and Figure 2 that several of the alleles show very little differences between the populations yet in the 2x5 contingency table each contributes a single degree of freedom which raises the significance value of X^2 and masks the differences contributed by the differing alleles. When the genes are combined into two sets (fast migrating ABC and slow DEF) and a 2x2 contingency table constructed (Table 7) two of the six comparisons are significantly different. These pairs are NE Georges-SW Nova Scotia and SW Nova Scotia-Mid-Atlantic Bight. The NE Georges-SW Nova Scotia comparison shows highly significant (P.01) differences.

Analysis of Variance of Allele Frequencies from Spawning Areas

Angular transformation of the allele frequencies were used to compute the five alleles, two areas, two season factorial analyses of variance shown in Table 9. Samples from the three spawning areas were grouped according to spring and fall captures with two replicates for each season. To provide equal numbers of replications from each area which simplifies the computations, and to use samples which were taken at a time closer to the known spawning season, sample number 3 from the SW Gulf of Maine was excluded from these analyses.

As was expected, most of the variation in each of the three comparisons is due to the difference between alleles ($P<.001$), however interactions in two of the three comparisons are also significant. The interaction of the alleles and areas of the NE Georges-SW Gulf of Maine and NE Georges-SW Nova Scotia analyses are significant and highly significant respectively, indicating considerable genetic isolation between the stocks compared. Neither season nor any of its two interactions were significant sources of variation. However, the allele x season component of the NE Georges-SW Nova Scotia analysis is nearly significant ($.05<P<.06$). This interaction suggests that non-random genetic changes occurred among the herring populations occupying these areas between the spring and fall of 1970-1971.

Conclusions

The phosphohexose isomerase phenotypes of adult herring observed to date are 16 of a possible 21 combinations of six codominant alleles inherited at an autosomal locus. The results of the analysis of adult herring PHI isozymes supports the life history information available from earlier studies that the three major spawning groups (SW Nova Scotia, NE Georges, and SW Gulf of Maine) are discrete populations. Furthermore the overwintering fish in the Mid-Atlantic Bight area conform to the Georges Bank stock. However, more samples are needed to detect possible mixing of stocks in the Mid-Atlantic Bight area during winter.

References

Anthony, V. A., and H. C. Boyar. 1968. Comparison of meristic characters of adult herring from the Gulf of Maine and adjacent waters. Int. Comm. Northw. Atlant. Fish., Spec. Pub. 5:61-98.

Boyar, H. C., R. R. Marak, F. E. Perkins, and R. A. Clifford. 1971. Seasonal distribution of larval herring (Clupea harengus harengus L.) in the Georges Bank-Gulf of Maine area from 1962-1970. ICNAF Res. Doc. 71/100: 1-20.

Carter, N. D., and C. W. Parr. 1967. Isoenzymes of phosphoglucose isomerase in mice. Nature 216: 511.

Cochran, W. G. 1954. Some methods of strengthening the common X^2 tests. Biometrics 10: 417-451.

Detterer, J. C., P. O. Ways, E. R. Giblett, M. A. Baughan, D. A. Hopkinson, S. Povey, and H. Harris. 1968. Inherited variations in human phosphohexose isomerase. Ann. Hum. Genet., Lond. 31: 329-338.

Ferguson, K. A., and A. L. C. Wallace. 1961. Starch gel electrophoresis of anterior pituitary hormones. Nature 190: 629.

Iles, T. D. 1970. Vertebral numbers of the Bay of Fundy herring and the origin of New Brunswick sardines. Int. Comm. Northw. Atlant. Fish., Redbook 1970, Part III, p. 148-150.

Iles, T. D. 1971. The retention inside the Bay of Fundy of herring larvae spawned off the southwest coast of Nova Scotia. Int. Comm. Northw. Atlant. Fish., Redbook 1971, Part III, p. 93-103.

Lewontin, R. C., and J. Felsenstein. 1965. Robustness of homogeneity tests in 2xN tables. Biometrics 21: 19-33.

Ridgway, George J., Robert D. Lewis and Stuart W. Sherburne. 1971. Serological and biochemical studies of herring populations in the Gulf of Maine. Rapp. Proces. Verb. 161; 21-25.

Welch, S. G., L. I. Fitch, and C. W. Parr. 1970. A variant of phosphoglucose isomerase. Biochem. J. 117(3): 525-531.

Table 1. Distribution of PHI phenotypes of adult herring from N.E. Georges Bank.

Location	Sample no.		BB	BC	BD	BE	BF	CC	CD	CE	CF	DD	DE	DF	EE	EF	FF	(n) Total	X^2	df[a]
W. Herwig 41:45, 66:52 23 June 1970	1	obs.	1	10	---	2	---	22	5	7	1	---	1	---	1	---	---	50	.188	3
		exp.	1.0	9.4	.8	1.7	.1	22.5	4.0	8.0	.7	.2	.7	.1	.7	.1	---	---	.90<P<.95	
W. Herwig 41:37, 66:50 24 June 1970	2	obs.	1	18	1	2	---	41	12	14	2	---	4	---	2	---	---	97	2.549	6
		exp.	1.4	15.2	2.0	2.9	.2	42.2	11.2	15.8	1.3	.7	2.1	.2	1.5	.3	---	---	.80<P<.90	
W. Herwig 41:48, 66:02 13 Aug. 1970	3	obs.	1	16	3	1	---	46	12	18	2	---	---	---	1	---	---	100	5.364	6
		exp.	1.2	15.4	1.6	2.3	.2	49.0	10.5	14.7	1.4	.6	1.6	.2	1.1	.2	---	---	P = .50	
Alferas 41:23, 66:05 20 Aug. 1970	4	obs.	3	19	3	4	1	97	26	35	5	4	5	---	2	2	1	207	7.718	8
		exp.	1.4	22.3	3.3	4.0	.8	94.0	28.2	33.8	6.7	2.1	5.1	1.0	3.0	1.2	.1	---	.30<P<.50	
Total X^2																			16.019	23
		obs.	6	63	7	9	1	206.0	55	74	10	4	10	---	6	2	1	454	.80<P<.90	
Pooled X^2		exp.	4.7	62.2	8.1	10.9	1.5	207.6	54.1	72.4	10.1	3.5	9.4	1.3	6.3	1.8	.1		3.142	9
																			.95<P<.98	
Heterogeneity X^2																			12.877	14
																			.50<P<.70	

a. In X^2 tests with 5 or more degrees of freedom those phenotypes which had expected frequencies less than unity were grouped with related phenotypes to secure an expected greater than 1. (Lewontin and Felsenstein 1965). In tests with less than 5 degrees of freedom we followed Cochran's (1954) suggestions that expectations be at least 2 and that there be no more than 20% less than 5.

C 4

Table 2. Distribution of PHI phenotypes of adult herring from S.W. Nova Scotia.

Location	Sample no.		BB	BC	BD	BE	BF	CC	CD	CE	CF	DD	DE	DF	EE	EF	FF	Total	X^2	df
Ingomar	1	obs.	---	15	1	2	1	38	11	14	6	1	5	---	1	---	---	95	4.183	6
18 June 1970		exp.	1.0	12.2	1.9	2.3	.7	39.1	12.2	14.8	4.5	1.0	2.3	.7	1.4	.8	.1	---	.20<P<.30	
Ingomar	2	obs.	1	6	2	1	1	46	14	19	3	1	3	1	2	---	---	100	0.804	6
10 Aug. 1970		exp.	.3	7.4	1.2	1.5	.2	45.6	14.9	18.9	2.7	1.2	3.1	.4	2.0	.6	---	---	.99<P<1.00	
Port La Tour	3	obs.	---	16	5	2	---	49	24	26	2	---	5	---	4	1	---	134	6.281	8
14 Sept. 1970		exp.	1.0	14.3	2.9	3.6	.2	51.3	21.1	26.0	1.8	2.2	5.3	.3	3.4	.5	---	---	.50<P<.70	
St. Mary's Bay	4	obs.	1	15	3	2	---	39	7	19	3	2	5	1	2	---	---	99	7.038	6
7 May 197[illegible]		exp.	1.2	13.6	2.7	3.3	.5	37.6	12.3	18.5	2.5	1.0	3.0	.4	2.3	.6	---	---	.30<P<.50	
Total X^2																			18.306	26
																			.80<P<.90	
Pooled X^2		obs.	2	52	11	7	2	172	56	78	14	4	18	2	9	1	---	428	6.670	9
		exp.	3.4	48.3	8.4	10.8	1.7	172.9	60.3	77.5	12.1	5.3	13.6	2.1	8.7	2.7	.1	---	.70<P<.80	
Heterogeneity X^2																			11.636	17
																			.80<P<.90	

Table 3. Distribution of PHI phenotypes of adult herring from S.W. Gulf of Maine.

Location	Sample no.		AC	BB	BC	BD	BE	BF	CC	CD	CE	CF	DD	DE	DF	EE	EF	FF	n	X^2	df
Jeffreys Ledge	1	obs.	---	---	15	1	---	---	38	17	22	3	---	---	1	2	1	---	100	8.868	6
30 April 1970		exp.	---	.6	10.6	1.5	2.2	.4	44.2	12.6	18.0	3.3	.9	2.6	.5	1.8	.7	.1	---	.10<P<.20	
Seguin Is., Me.	2	obs.	1	1	11	2	---	1	44	11	17	4	2	2	---	2	2	---	100	3.499	6
4 May 1970		exp.	.6	.7	10.6	1.5	2.0	.6	43.6	12.5	16.5	4.6	1.0	2.4	.7	1.7	.9	.1	---	.70<P<.80	
Jeffreys Ledge	3	obs.	---	1	12	8	2	---	42	6	17	3	3	3	2	1	---	---	100	17.204*	7
10 Aug. 1970		exp.	---	1.4	14.6	3.0	2.9	.6	37.2	15.3	14.6	3.1	1.6	3.0	.6	1.4	.6	.1	---	.02<P<.01	
Pumpkin Ledge	4	obs.	---	1	4	1	1	---	31	8	10	1	---	3	---	---	---	---	60	.220	3
29 Sept. 1970		exp.	---	.3	5.7	.8	.9	.1	30.1	8.5	9.9	.7	.6	1.4	.1	.8	.1	---	---	.95<P<.98	
Jeffreys Ledge	5	obs.	---	---	18	2	---	---	40	14	17	2	---	2	1	4	---	---	100	6.130	5
6 Oct. 1970		exp.		1.0	13.1	1.9	2.7	.3	42.9	12.2	17.7	2.0	.9	2.6	.3	1.8	.4	---	---	.20<P<.30	
Total X^2																				35.921	27
																				.10<P<.20	
Pooled X^2		obs.	1	3	60	14	3	1	195	56	83	13	5	10	4	9	3	---	460	13.192	9
		exp.	0.7	3.9	55.1	8.6	10.7	1.9	197.6	61.6	76.7	13.8	4.9	12.0	2.1	7.5	2.7	.2	460.0	.10<P<.20	
Heterogeneity X^2																				22.729	16
																				.10<P<.20	

Table 4. Distribution of PHI phenotypes of adult herring from Provincetown - Stellwagen Bank area. (Massachusetts Bay)

Location	Sample no.		AC	BB	BC	BD	BE	BF	CC	CD	CE	CF	DD	DE	DF	EE	EF	FF	n	X^2	df
Provincetown 30 April 1970	1	obs.	---	---	14	2	---	---	52	13	16	---	---	1	---	2	---	---	100	.8063	4
		exp.	---	.6	11.8	1.3	1.7	---	54.0	11.8	15.4	---	.6	1.7	---	1.1	---	---	---	.80<P<.90	
W. Herwig 42°23',70°24' 25 June 1970	2	obs.	---	---	4	1	---	---	19	6	8	1	---	1	---	---	---	---	40	1.0046	3
		exp.	---	.2	3.6	5	.6	.1	20.3	5.7	6.4	.7	.4	.9	.1	.5	.1	---	---	P = .80	
Pooled X^2		obs.	---	---	18	3	---	---	71	19	24	1	---	2	---	2	---	---	140	1.4123	3
		exp.	---	.8	15.3	1.8	2.2	.1	74.3	17.5	21.8	.8	1.0	2.6	.1	1.6	.1	---	---	P = .70	

Table 5. Distribution of PHI phenotypes of adult herring from Mid-Atlantic Bight.

Location	Sample no.		BB	BC	BD	BE	BF	CC	CD	CE	CF	DD	DE	DF	EE	EF	FF	Sample size	X^2	df
Norfolk Canyon 9 Apr., 1970	1	obs.	---	12	1	1	---	32	8	12	1	---	1	---	3	1	---	72	4.895	
		exp.	.7	9.4	1.0	2.0	.2	32.7	6.7	14.1	1.4	.4	1.5	.1	1.5	.3	---	---	.30<P<.50	5
Del. 70-3 IV 1 May, 1970 39°59, 71°55	2	obs.	---	9	1	---	1	47	11	21	1	---	1	1	4	1	---	98	3.024	
		exp.	.3	7.6	.8	1.7	.2	47.2	9.7	21.5	2.8	.5	2.2	.3	2.6	.6	---	---	.50<P<.70	4
Pt. Judith, R.I 19 Jan. 1971	3	obs.	1	31	3	8	1	98	29	25	4	3	3	1	5	---	---	212	6.440	
		exp.	2.4	30.2	4.5	4.9	.6	95.8	28.2	30.9	4.0	2.1	4.6	.6	2.5	.7	---	---	.30<P<.50	6
Total X^2																			14.359	
																			.30<P<.50	15
Pooled X^2		obs.	1	52	5	9	2	177	48	58	6	3	5	2	12	2	---	382	12.434	
		exp.	3.2	47.4	6.0	9.0	1.1	175.6	44.8	66.5	8.1	2.9	8.5	1.0	6.3	1.5	---	---	.10<P<.20	9
Heterogeneity X^2																			1.925	
																			.90<P<.95	6

Table 6. Adult Herring PHI Alleles

	Sample Number	Number Observed A	B	C	D	E	F	Total (2n)	Frequency A	B	C	D	E	F
N.E. Georges (Table 1)	1	---	14	67	6	12	1	100	-----	.1400	.6700	.0600	.1200	.0100
	2	---	23	128	17	24	2	194	-----	.1186	.6598	.0876	.1237	.0103
	3	---	22	140	15	21	2	200	-----	.1100	.7000	.0750	.1050	.0100
	4	---	33	279	42	50	10	414	-----	.0800	.6740	.1010	.1210	.0240
Sub-Total		---	92	614	80	107	15	908	-----	.1013	.6762	.0881	.1179	.0165
S.W. Nova Scotia (Table 2)	1	---	19	122	19	23	7	190	-----	.1000	.6420	.1000	.1210	.0370
	2	---	12	134	22	27	5	200	-----	.0600	.6700	.1100	.1350	.0250
	3	---	23	166	34	42	3	268	-----	.0860	.6190	.1270	.1570	.0110
	4	---	22	122	20	30	4	198	-----	.1111	.6161	.1010	.1515	.0202
Sub-Total		---	76	544	95	122	19	856	-----	.0888	.6355	.1110	.1425	.0222
S.W. Gulf of Maine (Table 3)	1	---	16	133	19	27	5	200	-----	.0800	.6650	.0950	.1350	.0250
	2	1	16	132	19	25	7	200	.0050	.0800	.6600	.0950	.1250	.0350
	3	---	24	122	25	24	5	200	-----	.1200	.6100	.1250	.1200	.0250
	4	---	8	85	12	14	1	120	-----	.0667	.7083	.1000	.1167	.0083
	5	---	20	131	19	27	3	200	-----	.1000	.6550	.0950	.1350	.0150
Sub-Total		1	84	603	94	117	21	1920	.0011	.0913	.6554	.1022	.1272	.0228
Massachusetts Bay (Table 4)	1	---	16	147	16	21	---	200	-----	.0800	.7350	.0800	.1050	-----
	2	---	5	57	8	9	1	80	-----	.0630	.7120	.1000	.1120	.0130
Sub-Total		---	21	204	24	30	1	280	-----	.0750	.7286	.0857	.1071	.0036
Mid-Atlantic Bight (Table 5)	1	---	14	97	10	21	2	144	-----	.0972	.6736	.0695	.1458	.0139
	2	---	11	136	14	31	4	196	-----	.0561	.6939	.0714	.1582	.0204
	3	---	45	285	42	46	6	424	-----	.1061	.6722	.0991	.1085	.0141
Sub-Total		---	70	518	66	98	12	764	-----	.0916	.6780	.0864	.1283	.0157
Grand Total		1	343	2483	359	474	68	3728	.0003	.0920	.6660	.0963	.1272	.0182

Table 7. 2X2 Contingency Table Comparisons of Adult Herring PHI Alleles

	ABC	DEF	Total	χ^2 (corrected for cont.)	P
N.E. Georges S.W. Nova Scotia	706 620	202 236	908 856	6.408*	.01-.02
N.E. Georges S.W. Gulf of Maine	706 698	202 232	908 920	2.066	.10-.20
N.E. Georges Mid-Atlantic Bight	706 588	202 176	908 764	.106	.70-.80
S.W. Nova Scotia S.W. Gulf of Maine	620 688	236 232	856 920	1.146	.20-.30
S.W. Nova Scotia Mid-Atlantic Bight	620 588	236 176	856 764	4.139*	.02-.05
S.W. Gulf of Maine Mid-Atlantic Bight	688 588	232 176	920 764	.966	.30-.50

Table 8. Adult herring PHI inter-area Hardy-Weinberg comparison.

		AC	B	BC	BD	BE	BF	C	CD	CE	CF	D	DE	DF	E	EF	F	Total	Total χ^2	d.f.
NE Georges-	obs.	--	8	115	18	16	3	378	111	152	24	8	28	2	15	3	1	882	4.118	9
SW Nova Scotia	exp.	--	8.0	110.2	16.7	21.8	3.2	380.1	114.9	150.3	22.4	8.7	22.7	3.4	14.9	4.4	.3		.90<P<.95	
NE Georges-	obs.	1	9	123	21	12	2	401	111	157	23	9	20	4	15	5	1	914	7.717	9
SW Gulf of Maine	exp.	.6	8.6	117.2	16.7	21.6	3.5	405.2	115.9	149.1	24.0	8.4	21.3	3.4	13.8	4.4	.3		.50<P<.70	
NE Georges-	obs.	--	7	115	12	18	3	383	103	132	16	7	15	2	18	4	1	836	5.2446	9
Mid-Atlantic Bight	exp.	--	7.8	109.7	14.1	19.9	2.6	383.2	98.8	138.8	18.3	6.4	17.9	2.4	12.6	3.3	.2		.80<P<.90	
SW Nova Scotia-	obs.	1	5	112	25	10	3	367	112	161	27	9	28	6	18	4	--	888	15.138	9
SW Gulf of Maine	exp.	.7	7.3	101.6	16.7	21.2	3.5	370.4	120.0	151.8	25.4	10.2	25.0	4.2	16.2	5.3	.4		.05<P<.10	
SW Nova Scotia-	obs.	--	3	104	16	16	4	349	104	136	20	7	23	4	21	3	--	810	8.018	9
Mid-Atlantic Bight	exp.	--	6.6	95.7	14.5	19.8	2.8	348.1	105.2	144.2	20.3	8.0	21.9	3.1	14.9	4.2	.3		.50<P<.70	
SW Gulf of Maine-	obs.	1	4	112	19	12	3	372	104	141	19	8	15	6	21	5	--	842	15.039	9
Mid-Atlantic Bight	exp.	.7	7.1	102.5	14.6	19.7	3.0	373.1	106.5	143.2	22.0	7.7	20.4	3.1	13.8	4.2	.3		.05<P<.10	

Table 9. The analysis of variance of PHI allele frequency data.

Source of Variation	Degrees of Freedom	Mean Squares	Probability
NE GEORGES BANK AND SW NOVA SCOTIA			
Replications	1	0.2209	
Alleles	4	2,510.8565	P< .001**
Area	1	2.1827	
Season	1	0.2515	
Alleles x Area	4	12.8462	.01-.005**
Alleles x Season	4	7.1361	.05-.06
Area x Season	1	0.6033	
Alleles x Area x Season	4	1.8509	
Error	19	2.4875	
NE GEORGES AND SW GULF OF MAINE			
Replications	1	0.2756	
Alleles	4	2,688.1721	P<.001**
Area	1	0.4040	
Season	1	0.5108	
Alleles x Area	4	6.6421	.025-.05*
Alleles x Season	4	3.0694	.10-.20
Area x Season	1	0.9859	
Alleles x Area x Season	4	4.8928	.05-.10
Error	19	1.9158	
SW NOVA SCOTIA AND SW GULF OF MAINE			
Replications	1	0.2756	
Alleles	4	2,460.1322	P<.001**
Area	1	0.7076	
Season	1	2.2279	
Alleles x Area	4	3.7593	.10-.20
Alleles x Season	4	2.1814	
Area x Season	1	0.0462	
Alleles x Area x Season	4	6.1413	.05-.10
Error	19	2.1897	

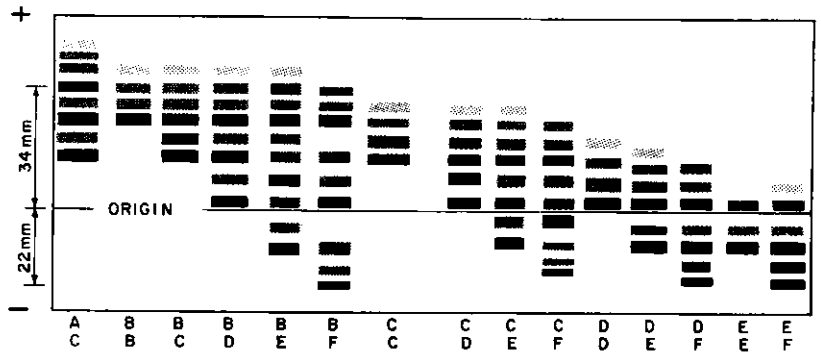

Figure 1. Phosphohexose isomerase enzyme phenotypes observed in adult herring from the N.W. Atlantic. (Since this figure was prepared the homozygote FF was observed in N.E. Georges Bank sample No. 5.)

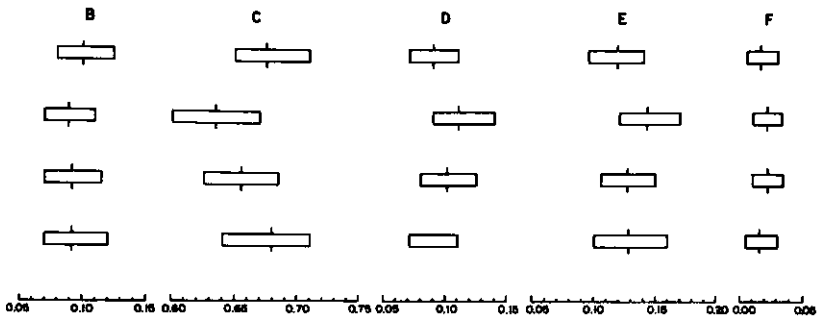

Figure 2. 95% Confidence Intervals for the Universal Frequency of PHI Alleles of Adult Herring

1. NE Georges Bank 2. SW Nova Scotia 3. SW Gulf of Maine 4. Mid-Atlantic Bight