STOCK IDENTIFICATION OF WEAKFISH, *CYNOSCION REGALIS*, IN THE MIDDLE ATLANTIC REGION

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ABSTRACT

The hypothesis that a single stock of weakfish, *Cynoscion regalis*, existed in the Middle Atlantic was tested. Using starch gel electrophoresis we identified two polymorphic loci (6-phosphogluconate dehydrogenase and malate dehydrogenase) out of a total of 25 protein loci surveyed. Statistical analysis of allelic frequencies revealed that the populations were statistically indistinguishable.

Weakfish, *Cynoscion regalis*, commonly reach sizes between 70 and 80 cm in length and 3.0 and 4.5 kg in weight. They occur from Cape Cod, MA to Florida but are most common in the Middle Atlantic region (Wilk 1979). Weakfish participate in a spring spawning migration into bays and estuaries. In the fall, the migrations reverse and fish move either offshore or to more southern waters to overwinter (Welsh and Breder 1923; Bigelow and Schroeder 1953; Wilk 1979). Spawning occurs from May to mid-July in northern estuaries (e.g., Delaware Bay and Gardiners Bay, NY; Shepherd and Grimes 1984) and from March to September in more southern waters (e.g., North Carolina; Merriner 1976).

Weakfish are an important commercial and recreational species and historically landings have fluctuated widely. From 1940 to 1949, commercial landings averaged 8,800 metric tons (t), with a high of 18,800 t in 1945. Between 1950 and 1969, annual catches declined to an average of 2,600 t, but a resurgence occurred when the catches rose to a 7,700 t average between 1970 and 1979 (Wilk 1981).

Recreational landings of weakfish have been similarly variable, and in some years have been estimated to be as large as the commercial landings (Murawski 1977). In 1965, catches only amounted to 1,000 t but increased to 7,100 t in 1970 (Wilk 1981). In 1974, recreational landings were approximately 9,100 t, or about 60% of the estimated total catch (Murawski 1977). Landings dropped to 5,000 t in 1979, and Middle Atlantic states accounted for 95% of the catch (Wilk 1981).

Several studies (Nesbit 1954; Perlmutter et al. 1956; Seguin 1960) have concluded that there were multiple stocks of weakfish in the Middle Atlantic region based upon mark recapture, scale circuli spacing, and morphological data, respectively. More recent studies have shown geographic differences in growth and reproduction of weakfish between Cape Cod, MA and Cape Hatteras, NC (i.e., northern fish lived longer, grew larger, and had a lower relative fecundity than southern fish; Shepherd and Grimes 1983, 1984). These life history differences could be due to environmental effects or could be indicative of discrete stocks (Shepherd and Grimes 1983). We hypothesized that a single panmictic population of weakfish exists in the Middle Atlantic region. In order to test this hypothesis, starch gel electrophoresis was used to identify protein variation for two polymorphic structural loci (malate dehydrogenase-2 and 6-phosphogluconate dehydrogenase) found among weakfish in this region.

MATERIALS AND METHODS

We sampled adult and juvenile (young-of-year) weakfish along the east coast of the United States from Buzzards Bay, MA to Cape Hatteras, NC (Fig. 1). Adult fish were caught in the fall of 1982 and summer of 1983 by the National Marine Fisheries Service bottom trawl survey cruises (Grosslein 1969). We also purchased adults in some locations

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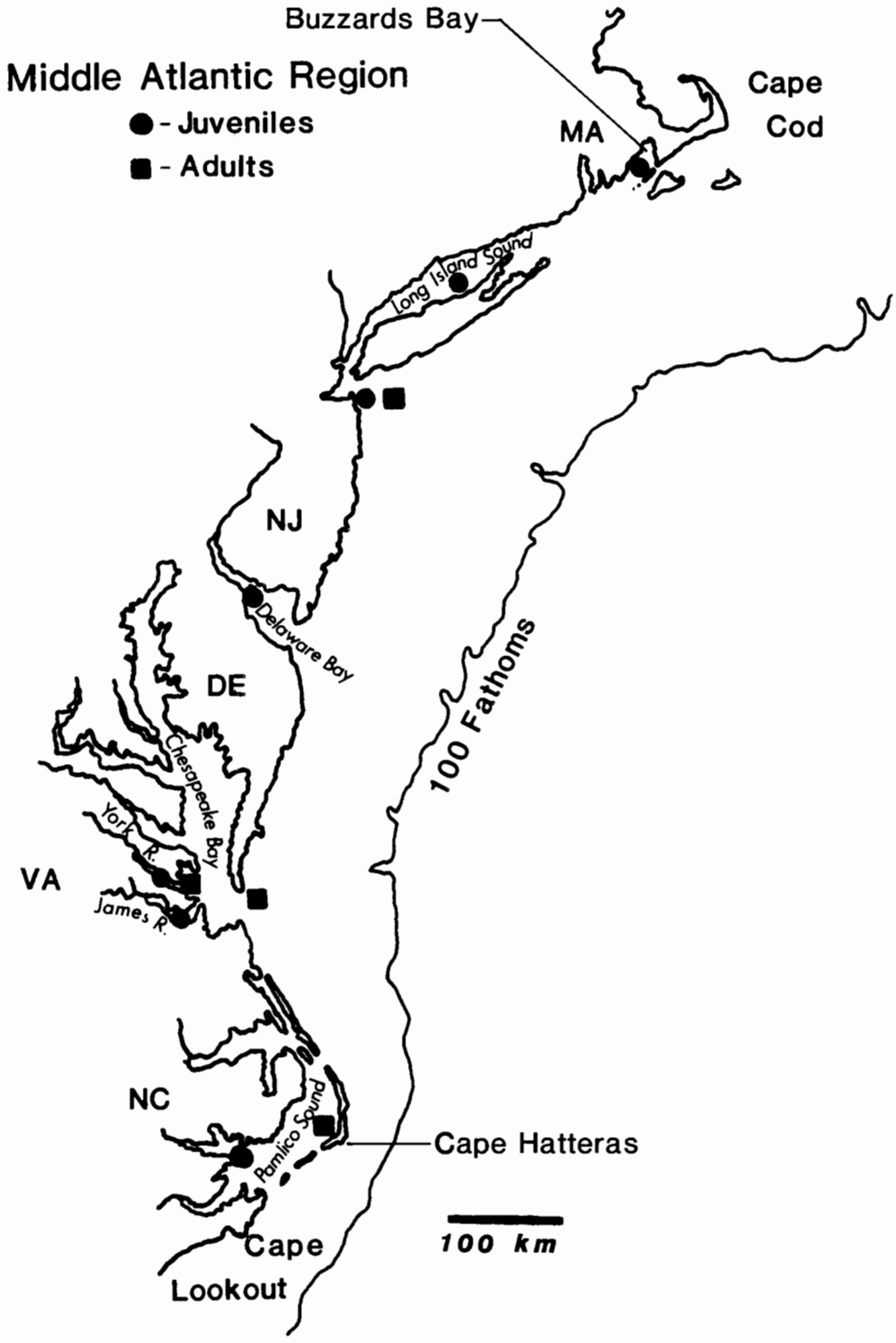

FIGURE 1.—Sampling localities of adult and juvenile weakfish sampled in the Middle Atlantic Region.

(i.e., Belford, NJ, the York River, VA and Cape Hatteras, NC) during the spring of 1982 and 1983.

We collected juvenile fish in their natal estuaries to minimize possible effects due to mixing after the fish migrated. Fish were trawled in Delaware Bay, NJ, Chesapeake Bay, VA, and Pamlico Sound, NC from August to October 1981 and 1982. We measured fish to the nearest mm total length (TL) and determined sex (when possible). Extracts of eye, liver, and skeletal muscle tissue were separately placed in centrifuge tubes and stored on dry ice. When this was not possible, we froze whole fish on ice and later removed the tissues in the laboratory. All tissue samples were stored at −8°C until analysis (for electrophoretic details see Crawford 1984).

Electromorph banding patterns were interpreted based upon the protein's subunit structure and previous studies with homologous enzymes. (Utter et al. 1974; Harris and Hopkinson 1976). We numbered loci from the anode to the cathode in ascending order. Allozymes were measured in millimeters relative to the most common homomeric electromorph which was designated 100 and numbered accordingly. The bands exhibited were consistent with reported information on their molecular structure (Manwell and Baker 1970). Allelic frequencies of polymorphic systems were calculated and examined for conformance to Hardy-Weinberg expectations (HWE) using Levene's (1949) method for small sample sizes ($N < 100$). For polymorphic loci that had null alleles the statistical procedures of (Speiss 1977) were followed to test for HWE. Sampling localities were compared by using a chi-square contingency test for haploid frequencies (Speiss 1977). We tested juvenile and adult allelic frequencies of polymorphic loci (excluding rare and null alleles) to determine whether significant differences in gene frequencies existed among 1) geographic location, 2) size/age (i.e., adults vs. juveniles) groups, and 3) sexes. Sampling locations were tested within regions, and if there were no significant differences among sampling locations, they were pooled. Pooled samples were then compared with all other regions to determine if there were regional differences. We calculated (averaged over the polymorphic loci) the genetic variation among the samples (F_{st}) (Hartl 1980). We obtained the percent polymorphic loci (common allele (p) < 0.950) and genetic distances (Nei 1972) using a BASIC computer program by Green (1979).

RESULTS

Weakfish from four populations (Long Island Sound, NY, Delaware Bay, NJ, York River, VA, and Cape Hatteras, NC) were initially screened by starch gel electrophoresis using 15 protein staining systems (Table 1) to identify polymorphic loci and calculate genetic distances. In samples obtained from other collecting localities only the polymorphic loci were evaluated.

The activity of the 15 enzyme (protein) staining systems was interpreted to reflect 25 structural loci of which only two (8%) were polymorphic, 6-phos-

TABLE 1.—Proteins and gel buffer-tissue combinations that provided the best resolution.

Protein systems	E.C.[1]	Tissue	Buffer	# of loci
Alcohol dehydrogenase	1.1.1.1	Liver	I	1
Aspartate aminotransferase	2.6.1.1	Muscle	IV	2
Esterase	3.1.1.1	Muscle	IV	2
α-Glycerophosphate dehydrogenase	1.1.1.8	Muscle	II	1
Glycerate dehydrogenase	1.1.1.29	Muscle	II	2
Isocitrate dehydrogenase	1.1.1.42	Muscle	I	1
Lactate dehydrogenase	1.1.1.27	Eye	II	3
Malate dehydrogenase	1.1.1.37	Muscle	I	3
Malic enzyme	1.1.1.40	Muscle	I	1
Muscle protein (nonspecific)		Muscle	II	3
Phosphoglucomutase	2.7.5.1	Eye	III	1
6-phosphogluconate dehydrogenase	1.1.1.44	Muscle	I	1
Phosphoglucose isomerase	5.3.1.9	Eye	III	2
Sorbitol dehydrogenase	1.1.1.14	Muscle	II	1
Xanthine dehydrogenase	1.2.3.2	Liver	IV	1

[1]Enzyme Commission number.
I - Aminopropyl, pH 6.0 (Clayton and Tretiak 1972).
II - Tris Citrate, pH 6.8 (Shaw and Prasad 1970).
III - Tris Versane Borate, pH 8.0 (Shaw and Prasad 1970).
IV - Ridgway, pH 8.5 (Ridgway et al. 1970).

phogluconate dehydrogenase (Pgd) and malate dehydrogenase (Mdh-2). In addition, three other loci—aspartate aminotransferase (Aat), phosphoglucose isomerase (Pgi), and xanthine dehydrogenase (Xdh)—exhibited rare alleles (i.e., common allele (p) > 0.950). Six-phosphogluconate dehydrogenase produced a single zone of allozyme activity on the starch gel that we interpreted as the product of a single gene locus. The heterozygotes displayed three bands which is typical of this molecule's dimeric structure (Manwell and Baker 1970). In weakfish, Pgd exhibited three alleles designated as 100, 98, and 96, a rare allele. Both juveniles and adults had similar frequencies of the most common allele (Table 2).

At the Mdh-2 locus, a dimeric protein product formed heteropolymers with the products of other Mdh loci. These heteropolymers occurred between the products of Mdh-1 and Mdh-2, and Mdh-1 and Mdh-3. The Mdh-2 locus was associated with liver, and the Mdh-3 locus is thought to be expressed in mitochondria (Thorne et al. 1963). This enzyme system also displayed a fourth isozyme band that migrated cathodally. This Mdh isozyme band is not reported in other similar studies and we do not know what protein loci it represented (Fig. 2). The Mdh-2 locus was polymorphic and exhibited four alleles: 103, 100, 97 (a rare allele) and a fourth null allele (*Mdh-2(N)*). Two fish homozygous for *Mdh-2(N)* were found. One was in a sample of juvenile fish from Spencer's Bay, NC and the other in an adult from Chesapeake Bay. The frequencies for the most common allele are found in Table 2. The uneven sample sizes (Table 2) occurred because of protein denaturation. The denatured samples indicated by streaks in the gels were excluded from analysis.

Allelic frequencies of three samples differed significantly from HWE for Mdh-2 (Table 2). This deviation reflected a deficiency in the number of heterozygotes which may have been due to the presence of the null allele (Selander 1970; Speiss 1977). We estimated null allele frequencies from the square root of the phenotype for Spencer's Bay (0.151) and Chesapeake Bay (0.146). Using the mean of the two values to estimate the null allele frequency for

TABLE 2.—Allelic frequencies of juvenile and adult fish for Pgd and Mdh including sample size, frequency of the most common allele and the standard error. All samples were collected in 1982 unless otherwise noted.

	Pgd(100)		*Mdh-2(100)*	
Location	*N*	Frequency (SE)	*N*	Frequency (SE)
Juveniles[1]				
Northern Region				
Buzzard's Bay and Long Island, NY	125	0.580(0.083)	98	0.576(0.041)
Sandy Hook, NJ	53	0.632(0.047)	47	0.617(0.050)
Delaware Bay, NJ (1981)	49	0.541(0.050)	46	0.511(0.052)**
Delaware Bay, NJ	91	0.527(0.037)	101	0.584(0.035)
Chesapeake Bay Region				
James R., VA	38	0.605(0.056)	48	0.510(0.051)***
York R., VA (1981)		—	47	0.543(0.073)
York R., VA	89	0.584(0.037)	67	0.612(0.060)
North Carolina Region				
Far Creek, NC (1981)	28	0.536(0.066)	38	0.645(0.055)*
Far Creek, NC	94	0.500(0.036)	98	0.602(0.035)
Spencer's Bay, NC	43	0.430(0.053)	45	0.547(0.052)
Wysocking Bay and Far Creek, NC	64	0.428(0.044)	63	0.571(0.044)
Adults[2]				
Northern Region				
Belford, NJ (1983)	11	0.636(0.103)	29	0.500(0.066)
Chesapeake Bay Region				
Virginia	121	0.620(0.044)	138	0.554(0.038)
Southern Region				
North Carolina	57	0.596(0.065)	59	0.636(0.063)

*HWE χ^2 0.05 = 3.841, df = 1.
**HWE χ^2 0.01 = 6.635, df = 1.
***HWE χ^2 0.001 = 10.828, df = 1.
[1]Geographic comparisons among regions of allelic frequencies for juveniles; χ^2 = 1.942, P > 0.05, df = 2 (Pgd); χ^2 = 2.268, P > 0.05, df = 2 (Mdh).
[2]Geographic comparisons among regions of allelic frequencies for adults; χ^2 = 0.566, P > 0.05, df = 2 (Pgd); χ^2 = 3.020, P > 0.05, df = 2 (Mdh).
+Represents a pooled sample, where samples <10 were combined.

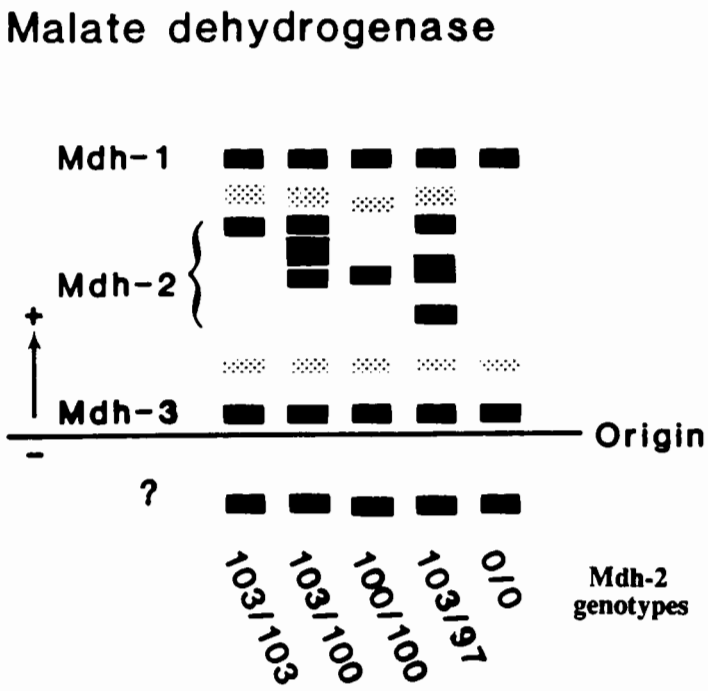

FIGURE 2.—Diagram of observed isozyme pattern for Mdh. The dark bands are protein products of presumptive loci and the lighter bands are heteropolymers formed from among the products of different loci.

localities that significantly deviated from HWE caused the chi-square values to become nonsignificant. At a frequency of 0.149 (if assumed throughout the sampling range) the expected number of individuals homozygous for the null allele among all the samples is 20, yet only two were found.

Our analyses indicated that there were no significant differences of allelic frequencies among sampling locations or among geographic regions (Table 2). The value of F_{st} was 0.046 and Nei's (1972) genetic distances were <0.003. No significant differences in allelic frequencies between juveniles (mean total length = 113 mm) and adults (mean total length = 310 mm) existed (Pgd: $\chi^2 = 2.622$, $P > 0.05$, df = 1; Mdh-2: $\chi^2 = 0.001$, $P > 0.05$, df = 1).

Comparisons between male and female fish indicated significantly different allozyme frequencies for Mdh-2 but not for Pgd. The frequency of the common allele (100) at the Mdh-2 locus was 0.518 ± 0.033 (SE) for 114 males and 0.637 ± 0.035 (SE) for 95 females, and these frequencies were significantly different ($\chi^2 = 6.024$, $P < 0.05$, df = 1). No differences were found at the Pgd locus ($\chi^2 = 1.785$, $P > 0.10$, df = 1).

DISCUSSION

Our study of allelic frequencies from populations of *C. regalis* along the east coast between Cape Cod, MA and Cape Hatteras, NC identified no statistically distinguishable differences. Nei's (1972) genetic distances are quite small and the F_{st} value (0.046) is low; both indicate little genetic variation among the populations. Nonsignificant allelic frequency comparisons among geographic locations and size/age classes were consistent with population homogeneity. A comparison of allelic frequencies at the Mdh-2 locus showed a significant difference between sexes. We are unable to explain this difference and cannot discount sex linkage or sexual selection as possible causes. Alternatively, with the numerous chi-square tests used in the analyses a Type II statistical error may have occurred.

Sample populations at several locations showed a heterozygote deficiency at Mdh-2 causing deviations from Hardy-Weinberg equilibrium. Several factors may cause heterozygote deficiencies (e.g., inbreeding, Wahlund effect, selection against heterozygotes, scoring biases, and null alleles; Speiss

1977), but the presence of null heterozygotes seems the most likely explanation. The low number of individuals observed to be homozygous for the null allele suggests that it may be lethal for these individuals (Speiss 1977).

Previous investigators have suggested that two or three distinct stocks of weakfish occur in the Middle Atlantic region (Nesbit 1954; Perlmutter et al. 1956; Seguin 1960). Nesbit (1954) examined distances between circuli on scales and conducted a marking study using celluloid belly tags. He tagged 5,789 fish and 7.5% were returned when the fish were eviscerated. Thirty-six percent of the returned tags were from retail dealers and consumers providing little information regarding actual recapture location. Nesbit concluded that the fishery consisted of two stocks. Perlmutter et al. (1956) examined intercirculi distances, fin rays, age, and growth data as well as Nesbit's (1954) data and concluded that there were northern and southern spawning weakfish populations.

Seguin (1960) performed a univariate analysis of morphometric and meristic data on juvenile weakfish and separated Middle Atlantic weakfish into three segments: 1) New York, 2) Delaware (and possibly Virginia), and 3) North Carolina. She reported "a north-south trend in regression coefficients" which may have been associated with environmental gradients (e.g., temperature) and clinal variation in the characters. Meristic characters, however, may be influenced by temperature (Barlow 1961) and intercirculi distances are related to growth rates that can vary geographically (Lux 1972; Shepherd and Grimes 1983; Harris and Grossman 1985). Because growth is affected by many environmental factors (e.g., temperature and food availability), it may not be indicative of genetic discontinuity (Joseph 1972).

Our results suggest that weakfish populations in the Middle Atlantic are not sufficiently distinct, genetically, to be considered as separate stocks (i.e., reproductively isolated). Weakfish perform extensive spring and fall migrations that could permit ample gene flow between populations. There are no obvious isolating mechanisms and only a small number of migrants would be needed to cause allelic frequencies to converge and make the population homogenous (Hartl 1980).

In conclusion, the results of this investigation do not support the findings of earlier studies that distinct stocks of weakfish are present in the Middle Atlantic. Even though there do not appear to be genetically discrete weakfish populations, there are variations in the population parameters (Shepherd and Grimes 1983, 1984). The ability of a population to sustain a harvest is largely dependent upon its growth, mortality, and fecundity. These life history parameters are used in fishery assessments (e.g., dynamic pool and stock-recruitment models). Use of northern weakfish growth parameters would predict overly optimistic yields for southern fisheries, and an incorrect stock-recruitment relationship. Therefore, as a practical matter it is probably best to manage weakfish as discrete northern and southern units. These units may not be reproductively independent, and the effects of fishing (particularly recruitment overfishing) are likely to be imposed upon the entire population.

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