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Population Structure of the White Perch, Morone americana, in Lower Chesapeake Bay as Inferred from Mitochondrial DNA Restriction Analysis

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POPULATION STRUCTURE OF THE WHITE PERCH, *Morone americana*, IN LOWER CHESAPEAKE BAY AS INFERRED FROM MITOCHONDRIAL DNA RESTRICTION ANALYSIS

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by

Brian W. Bowen

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APPROVAL SHEET

This thesis is submitted in partial fulfillment of the requirements for the degree of

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DEDICATION

This thesis is dedicated to my parents. To my father, John R. Bowen, for providing guidance, encouragement, and support. To my mother, Veronica A. Bowen, for teaching me patience and faith.

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ABSTRACT

White perch (Morone americana) populations in lower Chesapeake Bay are defined with mitochondrial DNA restriction analysis. A total of 123 individuals from the James, York, Rappahannock, and Potomac tributaries are analyzed with three informative restriction enzymes. The frequency of clone types in the James, Rappahannock and Potomac drainages differs significantly from a pooled mean frequency, due to the presence of unique clone types confined to each of these tributaries. This data suggests that migration between drainages is sufficient to prevent microevolutionary divergence, but too low to impact stock integrity. The four drainage basins of lower Chesapeake Bay must therefore be managed on an independent basis.

POPULATION STRUCTURE OF THE WHITE PERCH, *Morone americana*, IN LOWER CHESAPEAKE BAY AS INFERRED FROM MITOCHONDRIAL DNA RESTRICTION ANALYSIS

INTRODUCTION

The white perch, Morone americana is a semi-anadromous (Mansueti, 1961) percoid species, native to temperate Atlantic estuaries from Nova Scotia to South Carolina (Thoits, 1958). It is abundant in brackish and tidal fresh water, where it feeds on benthic invertebrates and small fish. During late spring, white perch ascend tidal rivers to spawn in fresh water. After migrating upstream as much as 80 kilometers, white perch deposit demersal semi-adhesive eggs in shallow water and along gravel bars (Mansueti, 1961). Subsequently, juvenile perch use tidal fresh water as a nursery and feeding ground (St. Pierre and Davis, 1972).

Like the congeneric striped bass (Morone saxatilis), white perch can complete their reproductive cycle in fresh water. During the latter half of the twentieth century, white perch invaded the Great Lakes, where they now compete and hybridize with the congeneric white bass (Morone chrysops) (Larsens, 1954; Scott and Christie, 1963; Todd, 1986). White perch also have been stocked into lakes of New England (Nichols and Breder, 1927), New York (Dence, 1952), and Nebraska (Hergenrader, 1980).

In upper and lower Chesapeake Bay, white perch have traditionally supported a modest fishery of about one

million pounds annually. The long term decline in striped bass stocks, however, continues to enhance the economic importance of white perch and escalating pressure on white perch stocks is such that overfishing can now be recognized as an impending possibility. It is incumbent upon management personel to implement guidelines for the continued vitality of this resource while the fishery remains healthy. To formulate a management strategy for this species, fisheries scientists require fundamental life history data, including growth rate, fecundity, and population structure. Previous work has defined many of these relevant aspects of white perch biology (Bath and O'Connor, 1982; Wallace, 1971; Taub, 1969; Mansueti, 1964; Miller, 1963; Thoits, 1958). However, stock definition within Chesapeake Bay remains uncertain (Morgan, 1971). By defining populations of white perch in lower Chesapeake Bay this study may provide data upon which management decisions can be based.

White perch population structure is also of interest from an evolutionary perspective. This species occupies a narrow zone between two contrasting habitat types, marine and fresh water, which are reflected in contrasting population types. Populations of fresh water fish are physically restricted to a single drainage. In contrast, populations of marine fishes are confined by large scale climatic or geographic barriers. In general, the potential for dispersal is much greater in the marine habitat, and

population structure reflects this fact (Ehrlich, 1975; Avise, 1987). Populations of marine fishes are geographically broad, such that an entire species may be contained within a single population. On the other hand, populations of fresh water fish are defined by immutable geographic barriers, such that each historically non-overlapping drainage contains a distinct phylogenetic unit.

Diadromous fish are a notable exception to this pattern. Population structure in these fishes combines features from both habitat types. The catadromous American eel (Anguilla rostrata) spends most of it's life confined to a single freshwater drainage basin. Upon reaching maturity, it returns to the ocean to spawn and die in the Sargasso Sea. This remarkable reproductive strategy results in a panmictic population which encompasses the entire species (Avise et al. 1986). In this case, a marine reproductive strategy produces a marine population structure in a predominantly fresh water species. Conversely, spawning site fidelity in anadromous species may impose a fresh water population structure on a predominantly marine species. In the semi-anadromous white perch, dispersal of eggs and larvae is constrained by a fresh water reproductive strategy. Adults inhabit brackish water (five to fifteen parts per thousand) but can tolerate high salinity, and may use a coastal route for dispersal and colonization. Indeed, their presence in every major estuary on the east coast lends credence to this mode of

dispersal. The white perch therefore exists on the boundary between two distinct ecospheres. As such, it occupies a narrow zone between contrasting evolutionary landscapes.

White perch are reported to occasionally occur in high salinity coastal waters (Woolcott, 1962). Mansueti and Scheltema (1953) estimated a preferred salinity range of 5 to 18 parts per thousand. However, twenty years of VIMS survey data indicate that in Chesapeake Bay the white perch rarely occurs above 14 parts per thousand. In the James, York, Rappahannock and Potomac tributaries, this preferred salinity range does not normally extend into the bay (Stroup and Lynn, 1963; see Fig. 1). With this study, salinity is considered as a barrier to migration between tributaries of lower Chesapeake Bay. If such is the case, then these drainage basins should contain genetically distinguishable populations, isolated from one another by a barrier of high salinity water.

To define populations of white perch, I used the technique of mitochondrial DNA (mtDNA) restriction analysis. This technique is rapidly gaining acceptance as the most sensitive assay currently available for population discrimination. Mitochondrial DNA is a maternally inherited cytoplasmic DNA molecule of approximately 17,000 nucleotides or 17 kilobases (kb). The mitochondrial genome evolves at a rate five to ten times faster than nuclear DNA (Brown, 1979) possibly due to the absense of a "proof reading" repair function during DNA replication (see Brown,

1981). Purified mtDNA is analyzed by restriction enzymes, which cleave the double stranded molecule at specific four, five, or six nucleotide sequences. Resulting fragments can then be separated with standard horizontal gel electrophoresis. By analyzing restriction fragment patterns, one can detect base substitutions or other genetic changes at restriction sites. By assaying a large number of restriction sites, sequence divergence between phylogenetic units can be directly estimated. The presence or absense of a restriction site may also be recognized as a qualitative character, such that restriction fragment data can be analyzed within a cladistic framework. Phylogenetic units defined by restriction site patterns are resolvable maternal lineages, or matriarchal twigs of an evolutionary tree (Avise, 1986). In this study we define maternal lineages of white perch in lower Chesapeake Bay. On the basis of these lineages, a population structure can be inferred.

MATERIALS AND METHODS

White perch were collected on the VIMS monthly trawl surveys between September, 1984 and May, 1985. The sampling regime of this ongoing program allowed collection of specimens over a distance of at least 30 kilometers in each river basin. Small numbers of gravid females were collected at eight kilometer intervals on the James, York, Rappahannock and Potomac Rivers and immediately stored on wet ice. Ovaries were removed within 24 hours and stored at -20 ^{o}C. Mitochondrial DNA was subsequently isolated by the phenol extraction technique of Chapman and Powers (1984) with the following modifications: (1) RNAse was used to remove RNA contamination from some samples; (2) Sucrose layer in mitochondrial isolation steps was omitted. Once isolated, DNA samples were stored in sterile water at -20 ^{o}C. Restriction digests were accomplished following manufacturers specifications. Initially, a few samples from each river system were digested with six restriction enzymes (Bgl I, EcoR I, EcoR V, Hind III, Sma I, and Xba I) to determine which endonucleases might be informative. Endonucleases were considered informative if they produced two or more restriction patterns in the populations under analysis. In this study, three enzymes were deemed

informative: Sma I, EcoR I, and EcoR V. Subsequently, 22 to 45 specimens from each river were examined with these three enzymes. Digestion fragments were separated on 1% agarose gels, and molecular weights were scored with a one kilobase ladder supplied by Bethesda Research Laboratory. Gels were stained with ethidium bromide by the method recommended by Maniatis et al. (1985) and photographed under ultraviolet illumination.

For each of the three informative restriction endonucleases, distinct fragment patterns were assigned letter designation. The most frequent pattern is designated A, with the other patterns following in order of descending frequency.

Statistical analysis of fragment patterns was accomplished with a chi-square test of heterogeneity (Sokal and Rohlf, 1981). With this method one may test for significant differences in clone type frequency among subpopulations. To quantify the migration rate between drainages, I employed the log linear relationship described by Slatkin (1985). This method relies on the frequency of private genotypes (i.e. genotypes confined to a single locale) to estimate the level of gene flow between subpopulations.

RESULTS

A minimum of 22 individuals per drainage were analyzed with the three informative enzymes (Sma I, EcoR I, and EcoR V). A total of seven restriction fragment patterns were observed (Fig. 2a). For each endonuclease, the most frequent pattern (type A) was found in all drainages. Alternate restriction patterns were confined to a single drainage at frequences of 10 to 23 percent (Table 1). In all cases the alternate patterns were a single restriction site removed from the common clone type (Fig. 2b).

During the restriction pattern analysis, evidence supporting the existence of multiple mtDNA length polymorphisms within individual specimens (heteroplasmy) was observed (Fig. 3). Size heteroplasmy has been reported previously in lower vertebrates (see Bermingham et al, 1986, for review) but not to the extent evidenced in white perch. In every individual of a given clone type I observed restriction fragments shared by all individuals, and alternate fragment sizes that seemed to cluster around the most commom size fragment. To some degree, I was able to determine relative frequencies of these size variants by the intensity of corresponding electrophoretic bands. However, the evolutionary implications of these size variants, particularly the dynamics of vertical transmission, remain uncertain (Chapman et al, 1982; Avise

and Lansman, 1983). For this reason, size polymorphism data was excluded from this analysis.

The six enzyme survey revealed no fixed differences between restriction site patterns for the four drainages of lower Chesapeake Bay. However, with each of the three polymorphic restriction enzymes we found a clone type common to all four drainages, and one or more unique clones confined to a single drainage (Fig. 4). For example, Sma I pattern B was found at a frequence of 23% in the James River, but was not found in any other drainage (Table 1). Likewise, EcoR V pattern B was found in the Potomac River at a frequency of 11% but was not found in any other drainage. The absence of fixed differences in restriction site pattern suggests that these groups have not been isolated over a microevolutionary time scale. However, the presence of unique clones within three of the four tributaries suggests that contemporary gene flow is extremely low.

A chi-square test of heterogeneity (Sokal and Rohlf, 1981) indicates that clone frequencies in the James, Rappahannock, and Potomac drainages differ significantly from a pooled mean frequency. The presence of unique clone types in these three drainages is significant at alpha levels of 0.01, 0.01, and 0.07, respectively (Table 1). As no unique clones types were observed in the York River, this drainage was not statistically distinguishable. The

high alpha level (0.07) in the Potomac sample set may reflect small sample size from this drainage.

Slatkin (1985) described a log linear relationship between the frequency of private genotypes (i.e. genotypes confined to a single population) and the number of migrants per generation. With this relationship, private genotypes may be used to estimate gene flow under the following equality:

$$\ln(P(I)) = a \ln(Nm) + b$$

where, $a = -0.505$
$b = -2.440$

I = occupancy number; the number of populations in which a genotype is present.

P(I) = conditional average frequency; mean genotype frequency in those populations in which it occurs.

Nm = number of migrants in populations samples.

For this data set, $P(I) = 10.67$. The estimated number of migrants (Nm) is 0.15 in a total sample of 123 individuals. This corresponds to a migration rate per generation of 0.0013. By Slatkins estimate, 1.3 individuals per thousand are migrants, a level much too low to impact fisheries management strategy.

Slatkins equation is derived from isozyme data, and is based on rates of evolution in the nuclear genome. As previously noted, mtDNA accumulates genetic changes more rapidly than the nuclear genome. If new genotypes arise five to ten times more rapidly in the mitochondrial genome

then an estimate of migration based on these mitochondrial genotypes could be conservative by an order of magnitude. However, even a ten fold increase in Slatkin's estimate of migration (up to one migrant per hundred individuals) is not sufficient to perceptably alter stock dynamics within each drainage. Migration is still too low to impact fisheries management strategy.

Wright (1931,1940) demonstrated that if $4NM >> 1$, then no divergence should be expected between populations with a diploid genome, where N is the effective population size and M is the migration rate. In a haploid system (such as mtDNA) the relationship is adjusted to $NM >> 1$ (Chapman et al, 1982). With Slatkin's estimate of migration, and a conservative estimate of effective population size ($N = 10,000$) the results of Wright's inequality, $NM = 13$, suggests that no evolutionary divergence should be expected between white perch populations. With a higher estimate of effective population size, or a higher migration rate,the results are even more emphatic. Migration is too low to impact stock integrity, but is sufficiently high to prevent population structuring of the type observed in fresh water species.

DISCUSSION

To date, population surveys undertaken with mtDNA restriction analysis have relied on relatively small sample sizes. Specimens are routinely analyzed with 15 to 20 restriction enzymes such that an accurate estimate of sequence divergence between populations can be made with a small number of individuals. In this study, I have selected a small number of restriction enzymes that show restriction pattern variation and tested a large number of individuals (22 to 45 from each tributary) with these enzymes. I suggest that this is an appropriate approach for fisheries research. Testing for differences in clone frequency can be used to elucidate the kind of recent or short term isolation that defines fish stocks. In several respects, this approach is analogous to isozyme analysis. With conventional protein electrophoresis, fixed differences in allozyme mobility are seldom available to demonstrate long term isolation between fish stocks. Significant differences in isozyme frequency are usually the basis for stock definition. Likewise, significant differences in mtDNA clone frequency can be construed as evidence of short term or "ecological" isolation.

While management personnel address pragmatic questions of stock definition, the fishery biologist may be concerned with evolutionary as well as ecological time scales.

Fortunately, resolution at both levels is possible with a properly designed study. To address the question of divergence on a microevolutionary scale, six to ten individuals from each suspected population can be analyzed with a complete battery of up to twenty restriction enzymes. At this stage, enzymes that reveal two or more restriction fragment patterns can be identified as useful for frequency analysis. If fixed restriction pattern differences exist between populations, then such evidence of long term isolation precludes the need for further analysis. Lacking this conclusion, a larger sample (up to 30 individuals per population) can be analyzed with a few "polymorphic" enzymes to detect frequency shifts. The larger (statistically significant) sample size, analyzed with a small number of restriction enzymes, allows one to efficiently focus on evidence of population substructuring.

For conservation biologists concerned exclusively with stock definition, analysis of clone frequency shifts may be sufficient. However, screening with a complete battery of restriction enzymes is necessary to identify the ones useful for frequency analysis. With an initial survey of six restriction enzymes, this study falls short of an ideal design. I was able to identify three useful enzymes in our initial survey, but further screening may have provided additional data.

Tributaries of lower Chesapeake Bay do not contain separate populations in the microevolutionary sense, but

they do contain separate stocks in the fisheries sense. Previous work supports this hypothesis. Woolcott (1962) used meristic and morphometric characters to compare white perch in major drainages of Chesapeake Bay. Based on pectoral fin measurements, scale counts, body depth and head depth, he concluded that the major tributaries of Chesapeake Bay contain semi-isolated populations. Mansueti (1961) reached the same conclusion based on a tag-recapture study in the Patuxent tributary.

Mitochondrial DNA restriction analysis indicates that a low level of migration exists between the tributaries of lower Chesapeake Bay. Sequence divergence between drainages is essentially zero, much less than that observed in comparable surveys of fresh water fishes. Bermingham and Avise (1986) detected an mtDNA sequence divergence of 6.1 to 8.7% between Lepomis species in historically separate drainages. These genetic distances are based on fixed restriction site differences and are clearly divergent on a microevolutionary scale. Evidence for isolation between white perch populations is based on clonal frequencies, not fixed clonal differences. If one dismisses the possibility that white perch populations are younger that concordant fresh water percid populations, one may infer that migration has historically occurred between adjacent drainages. For this reason, white perch populations in the tributaries of lower Chesapeake Bay have not diverged to the same extent as fresh water teleost populations.

Three of the four tributaries examined contain at least one unique clone at significant frequency. White perch in these drainages have been isolated to the extent that mutations have arisen and increased to moderate frequencies. Each private genotype is a single restriction site removed from the common pattern, suggesting a relatively recent common ancestry. Significantly, these unique clone types have not spread to adjacent drainages, indicating that white perch populations are more structured than those of comperable marine teleosts.

With a six enzyme survey, I did not find mtDNA genotypes confined to the York River. However, the York River is flanked on both sides by demonstrably isolated drainages. The presence of unique clones in three of the four tributaries indicates that gene flow between drainages is too low to impact fisheries management strategy. I therefore conclude that the major drainages of lower Chesapeake Bay contain distinct stocks which must be managed on an individual basis. If one tributary becomes depleted from overfishing, it is not likely to be replenished by migration from other tributaries. The exhausted stock must recover without significant natural input from other areas. St. Pierre (1975) offers circumstantial evidence to support this view. He documented a drastic drop in white perch abundance in the James River during 1971. While he could not elucidate the causes, he noted that white perch abundance in adjacent

drainages remained high. The depressed white perch stock took at least four years (or two generations) to recover, implying that significant input from other drainages was not a factor in repopulation.

St. Pierre (1972) suggested that fluctuations in white perch abundance may be the product of high annual mortality (69%), irregular year class strength, and a short generation time (two to three years). These factors may occasionally combine to depress white perch stocks by at least one order of magnitude. Such dramatic changes in population size are likely to have a profound effect on genetic diversity and genotype frequence. Rarer genotypes may be extinguished. Alternately, a few distinct lineages may form the basis for subsequent repopulation. While essentially a stochastic process, this bottlenecking is likely to induce rapid changes in genotype frequencies. Under the conditions described by St. Pierre (1972), new genotypes could rapidly increase to significant frequency.

As a benthic grazer, the white perch is probably not confined by rigid zones of food availability. The data presented here supports the idea, initially suggested by Mansueti (1961), that dispersal is limited by a barrier of high salinity water. While their center of abundance adjusts to seasonal fluctuations in salinity profile, white perch are seldom found above 14 parts per thousand. It is well documented that white perch can tolerate full salinity

(Thoits, 1958), but they prefer a much lower salinity level.

If salinity is the barrier to migration in the lower bay, then such a barrier should not exist between drainages in the upper bay. Under normal conditions, salinity levels in upper Chesapeake Bay remain well below 12 parts per thousand. The obvious inference is that the potential for disperal is greater, and perch in the upper bay should constitute a single population. Existing mtDNA restriction data support this hypothesis (Mulligan, 1987).

The salt water - fresh water interface is not a physical constant. Rather, it is a dynamic phenomenom, modified by precipitation, tidal cycle, and large scale climatic factors. During late winter and early spring, the potential range of the white perch is expanded by seasonal precipitation. This annual shift in salinity profile could produce occasional opportunities for dispersal. However, at this time of year mature perch are ascending the rivers to spawn in fresh water, diminishing the likelyhood of interestuarine migration. Nonetheless, occasional aberrations in salinity profile could provide a mechanism for gene flow between white perch stocks. The barrier of high salinity water is effectively relaxed during atypical periods of extremely heavy rainfall. For example, Chesapeake Bay is flushed out with fresh water several times per century by tropical storms, and major shifts in fauna have been observed. After tropical storm Agnes in

1970, white perch were captured at the mouth of Chesapeake Bay (John A. Musick, personal communication). Over a period of weeks, the salinity gradient was restored, but the opportunity for migration between tributaries is apparent. These rare dispersal opportunities may be sufficient to prevent divergence on a microevolutionary scale. However, over the ecological time scale that concerns fisheries biologists, the evidence indicates that James, York, Rappahannock and Potomac drainages contain separate stocks of white perch.

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TABLE 1. Frequency data for three informative restriction enzymes

TABLE 1. FREQUENCY DATA FOR THREE INFORMATIVE ENZYMES

ENZYME	TRIBUTARY	CLONE TYPE	NUMBER	CLONE FREQUENCY WITHIN DRAINAGE	CHI-SQUARE LEVEL OF SIGNIFICANCE
Sma I	*James	A	20	67%	<.01
		B	7	23%	
		C	3	10%	
	York	A	22	100%	
	Rapp.	A	22	100%	
	Potomac	A	28	100%	
EcoR I	James	A	36	100%	
	York	A	33	100%	
	*Rapp.	A	26	90%	.01
		B	3	10%	
	Potomac	A	45	100%	
EcoR V	James	A	25	100%	
	York	A	22	100%	
	Rapp.	A	21	90%	
		C	1	5%	
		D	1	5%	
	*Potomac	A	25	89%	.07
		B	3	11%	

* Denotes statistical significance

FIGURE 1. Average summer and winter salinity profiles for Chesapeake Bay.

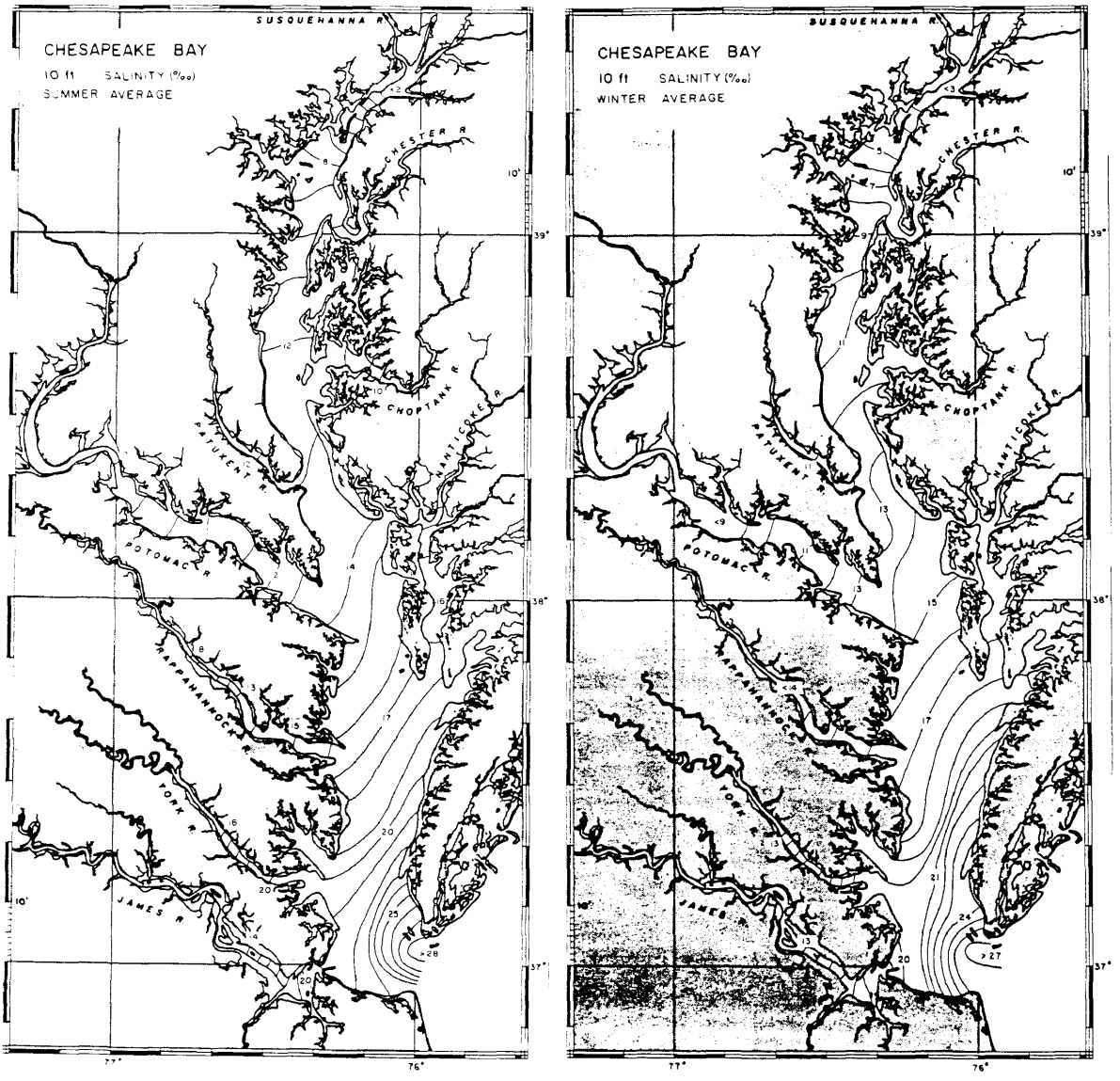


Figure 1. Salinity gradient in Chesapeake Bay for summer and winter, averaged over the period 1949 - 1961. From Stroup and Lynn, 1963.

FIGURE 2 A. Diagram of restriction fragment patterns from white perch in lower Chesapeake Bay
B. Parsimony network indicating the relationship between clone types for the three informative enzymes

a)

SMA-I			ECO R-I		ECO R-V			
A	B	C	A	B	A	B	C	D
						14.0		
				10.0				11.0
			9.0					
					7.8		7.8	
			7.0	7.0				
	6.5				6.3		6.3	6.3
5.8	5.8	5.8						
		5.0						
4.7		4.7						
3.9	3.9							
					3.1	3.1		
							2.7	
1.4	1.4	1.4						
			(1.0)					

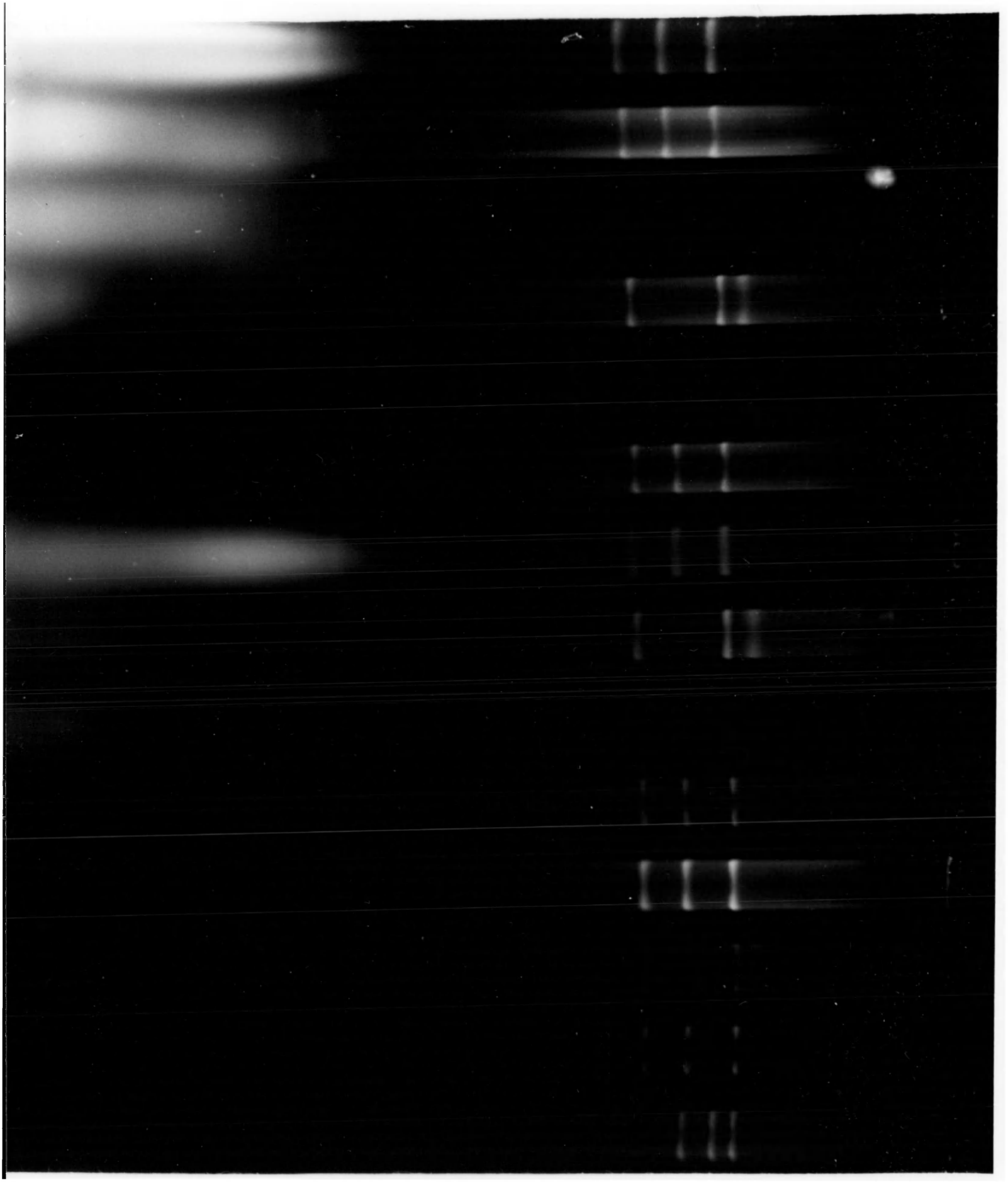
b)

SMA-I	ECO R-I	ECO R-V
A--->B ↑ | C	A--->B	B<---A--->D ↑ | C

a) Restriction fragment patterns for three informative enzymes. Clone types are alphabetized in order of descending frequency. For each restriction enzyme, type A represents the most frequent restriction pattern. Number to left of each band is approximate size of restriction fragment in kilobases.

b) Parsimony network indicating the relationship between clone types for the three informative enzymes. In all cases, rarer clone types are a single mutation event (restriction site gain or loss) removed from the common type A. Arrow indicates direction of restriction site loss.

FIGURE 3 A. Sma I restriction patterns for individuals from the James River
B. Sma I restriction patterns for individuals from the York River



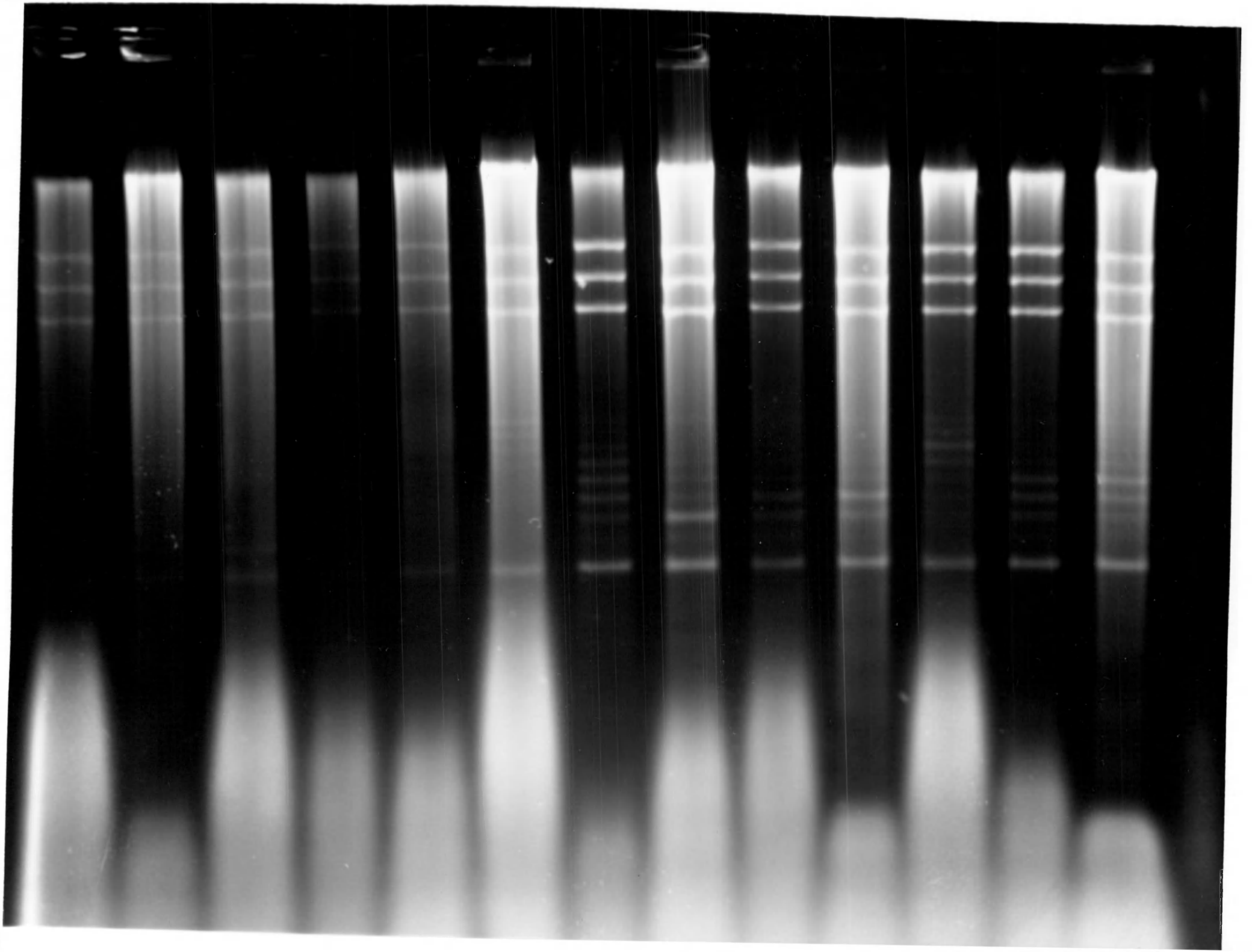


FIGURE 4 Distribution of clone types in lower Chesapeake Bay

Figure 4. Distribution of clone types within lower Chesapeake Bay