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Molecular evolution in the fish genus *Morone*

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City University of New York, 1987

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MOLECULAR EVOLUTION IN THE FISH GENUS *MORONE*

by

Isaac Wirgin

A dissertation submitted to the Graduate Faculty in
Biology in partial fulfillment of the requirements for the
degree of Doctor of Philosophy, the City University of
New York

1987

This manuscript has been read and accepted for the Graduate Faculty in Biology in satisfaction of the dissertation requirement for the degree of Doctor of Philosophy.

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Table of Contents

List of Tables

List of Figures

I. The Biology of Striped Bass and the History and Future Applications of its Stock Discrimination

The striped bass Morone saxatilis is an anadromous percoid of great recreational and commercial value. The native range of this inshore species included the Atlantic coast from the St. Lawrence River, Canada to the St. Johns River, Florida (Raney, 1952). In the Gulf of Mexico, the striped bass was known to occur from the Suwanee River (Wooley and Crateau, 1983) or Tchefuncta River (Pearson, 1938) in northwest Florida to Louisiana (Raney, 1952). However, Matlock et al. (1984) have presented evidence that they were endemic to Texas waters, apparently becoming extinct there in the 1930's. Striped bass from the Navesink River in New Jersey were introduced into the San Francisco Bay region of California in 1879 and 1882 and now occur from south of Los Angeles, California, to the Columbia River in British Columbia (Forrester et al. 1972). Since the mid-1960's, rapid advances in the hatchery culture of striped bass in the southeastern United States have permitted the annual production of large numbers of fingerlings for release in natural systems (Bayless, 1968 and Stevens, 1966). In response to recreational demands, the recent introduction of striped bass into freshwater impoundments has greatly expanded the species range across the U.S., with at least 100 lake and reservoir fisheries established by 1981 (Axon and Whitehurst, 1985).

Despite an historically intensive and long-running fishery, relatively little was known about the life history of the striped bass before the 1930's (Merriman, 1941). Although recognized to ascend rivers for spawning purposes, it was not clear whether coastal striped bass did so in a random fashion or if distinct stocks existed that utilized individual river systems. Concentrated research on Atlantic striped bass was initiated in response to questions raised by the pronounced increases in striped bass landings that occurred over a wide area of the Atlantic coast during 1936 and 1937 (Vladykov and Wallace, 1952). These studies included efforts towards determining the source of this increase in population size and at evaluating the stock composition of the coastal striped bass stock. Synoptic studies of a somewhat smaller magnitude were carried out on the San Francisco Bay striped bass population at about the same time (Scofield, 1931 and Clark, 1934).

Long-term records show a strong instability for the size of the Atlantic coastal stock. Data from a Massachusetts (Cuttyhunk) sportsmans club and a Long Island (Montauk), New York commercial fishing family suggest a general decrease in striped bass abundance from 1865 to 1937, after which the population suddenly expanded (Koo, 1970). Later, Van Winkle et al. (1979) stated that "Commercial fisheries data since the 1930's indicate substantial yearly fluctuations, superimposed on a longer-term increasing trend, on the size of Atlantic coast striped bass populations." Recently, the trend towards larger Atlantic catches has dramatically reversed, commercial catches in the Chesapeake region declining 93% between 1973 and 1983, with similar declines experienced in other Middle Atlantic and New England states (Boreman and Austin, 1985). Commercial landings declined from a record high of 6.7 thousand metric tons in 1973 to a record low of 0.8 thousand metric tons in 1983. In addition, based on a survey of recreational fishermen, estimates of the total landings by sport fishermen from Maine to North Carolina declined from 1.3 million fish in 1979 to 128,000 fish in 1984 (USDOI and USDOC, 1986). Although comparable catch statistics for 1985 and 1986 will be unavailable due to the establishment of a federal moratorium curtailing fishing effort on the coastal migratory stock, this ominous trend may have been reversed. Both personal observations and anecdotal reports received by agencies coordinating tag return data (J. Waldman, personal communication) suggest rejuvenated catches in the northern half of the coastal stock's range.

Interestingly the coastal striped bass stock has traditionally been supported by an occasional (every six to seven years) exceptional year class thought to be invariably of Chesapeake origin (Koo, 1970). Mechanisms responsible for Chesapeake year class strength at the larval or juvenile stage currently are under intensive investigation in many laboratories. Kohlenstein (1981) has demonstrated that a minimum of three years is required for recruitment of this Chesapeake cohort into the migratory coastal stock. Following an initial population explosion when this exceptional Chesapeake year class is recruited into the fishery, population numbers soon decline as fishing and natural mortality take their effect in the absence of a major resupply. The last great Chesapeake year class was 1970 (determined by the annual Maryland juvenile index), and as a result as the 1970's progressed this fishery rapidly dwindled.

While year class production in the Chesapeake has remained relatively poor in the early and mid-1980's, several consecutive years of outstanding breeding success in the Hudson River (Young, 1984), perhaps combined with increased long distance migration by Hudson fish in a northerly direction, may have resulted in a rejuvenation of a portion of the coastal fishery. The beneficial effects of this population explosion have not been felt in the southern half of the stock's range, either due to behavioral restrictions on migratory patterns, or perhaps the relatively young age of the Hudson component of this stock. Therefore, concern in the Chesapeake region for the health of the striped bass fishery remains heightened. As a result of the great historical fluctuations shown in both the overall size of the coastal migratory stock and in separate oscillations in the magnitude of the individual spawning populations, the striped bass has been the focus of a multitude of studies that attempted to delineate individual unit stocks contributing to the mixed coastal fishery.

The anadromous life history of the striped bass has dictated an optimal management strategy largely on a population basis. Factors impinging on one population may not be felt by inhabitants of adjacent breeding systems. However, due to significant genetic mixing, insuffficient temporal isolation among populations, or the possible effects of stabilizing selection in reducing the extent of genetic variability, the degree of detectable genetic polymorphism among stocks of striped bass has remained relatively low. Efforts to identify stock-specific characteristics for use as management tools have bridged five decades. During this span the number of techniques utilized in stock discrimination has expanded, reflecting the many biochemical and computerized advances that have occurred in the biological sciences. By chronicling the numerous studies comprising the 50 year history of striped bass stock discrimination and in focusing on this single species, the advantages, theoretical limitations, and technical difficulties of these approaches and their applicabilities to comparable problems with other species can be appreciated. Brief descriptions of individual studies of striped bass stock discrimination are provided, highlighting the overall results and certain of the strengths and weaknesses of the approaches taken. This discussion is intended to serve as an update of the excellent general review of stock discrimination methods by Ihssen et al. (1981).

Efforts at stock discrimination within the Atlantic coastal migratory fishery have accelerated in recent years, not only in response to greatly reduced catches, but also because of the occurrence of potentially harmful contaminants in several of the major contributory spawning systems. For both reasons, identification of ancestry of striped bass in the mixed coastal fishery would provide data which could allow better regulation of specific spatial or temporal units within the overall migratory stock.

While the overall migratory behavior of the coastal stock has long been understood (Merriman 1941), several finer points still require clarification. These include: 1) the ultimate winter destination(s) of the various adult components of the coastal stock; 2) do fish natal to one spawning system visit other breeding areas at non-spawning times; 3) is the migratory range of adults representative of the various spawning systems dependent on their relative population sizes?

Coastal striped bass are acknowledged to undertake an overall southerly migration in the fall, however, the final destination of individual components of the mixed stock during the winter season are not known. Commercial landings of large numbers of adult striped bass were common off the Outer Banks of North Carolina in the late 1960's and early 1970's (personnal observation); a period of exceptional Chesapeake recruitment into the coastal fishery. Based on tag returns from striped bass released off the North Carolina coast, Holland and Yelverton (1973) postulated that three distinct groups of fish overwinter off the coast of North Carolina; a somewhat sedentary Roanoke, North Carolina contingent, a group that enters the Chesapeake in the spring, and a unit that participates in the northerly coastal migration in the spring. McLaren et al. (1981) and John Waldman (personal communication) reported winter recaptures of a small number of Hudson tagged fish (during the spawning system) from coastal southern New Jersey and Delaware. Early studies hypothesized that the Hudson may serve as an overwintering site for some Chesapeake spawned fish (Raney, 1952 and Vladykov and Wallace, 1952). Do contingents of striped bass which overwinter in the individual spawning systems, the Chesapeake Bay proper or the Hudson, represent fish spawned exclusively in that system? Based on isozyme data, Grove et al. (1976) have hypothesized that "southern" spawned fish enter the Hudson shortly after the completion of spawning. Is inter-breeding system migration a common occurrence at certain times?

When individual spawning systems undergo several consecutive years of outstanding year class production, it has been conjectured that the coastal migration of its inhabitants extend beyond its normal range, potentially populating river systems beyond its customary migratory limits. Davis (1966) has raised this issue for several rivers in Maine. Recent returns from the Canadian Maritimes of Hudson River tagged fish representative of major Hudson year classes support this contention (John Waldman, personal communication). Unique spawning system specific genetic tags would permit answering all these questions.

Unfortunately for fishery managers, the relative contribution of the various spawning systems to the coastal stock is probably not a static value. Van Winkle and Kumar (1982) stated that the proportional contribution of the major spawning systems can vary greatly from year to year, dependent on their relative year class strengths. For example, initial estimates of Chesapeake Bay contribution to the coastal migratory stock approached 90% during the period shortly after the last major Chesapeake year class was produced in 1970 (Berggren and Lieberman, 1978), with the remainder assigned to either the Hudson or Roanoke, North Carolina rivers. This estimate was achieved through discriminant analysis based on a index of five morphometric and meristic characters. More recent estimates (Saila et al. 1983 and Fabrizio et al. 1985) also based on discrimant analysis of morphometric and biochemical characters indicated a substantial increase in the Hudson's contribution, from less than 10% in the early 1970's to approximately 50% in the mid-1980's. In view of the recent repeated successful recruitments (1980-1983) in the Hudson (Young, 1984), together with continued year class failures in the Chesapeake system (Boreman and Austin, 1985), Van Winkle and Kumar's (1982) claim for the instability of relative breeding system contributions appears substantiated.

Interest in striped bass stock discrimination has also intensified lately in the southeastern U.S. Following severe reductions of Gulf coast striped bass populations in the 1950's and 1960's, resulting from damming and pollution of the major drainage systems, considerable effort has been applied toward their repopulation with native Gulf coast striped bass. However, due to earlier stockings of Atlantic striped bass in Gulf coast rivers, the need has developed to identify pure Gulf striped bass in mixed Gulf fisheries to assess the progress of these efforts and to serve as broodstock.

For the west coast, no studies that document efforts to discriminate between the two stocks that occur in California and Oregon have been published. The degree of mixing between these populations remains unknown. There is morphological and behavioral evidence of polymorphism in the collective San Francisco Bay-Delta striped bass stock that appears to show some geographic cohesion (NMFS/SWFC, 1984) but these differences are seen between offshore and Bay-Delta samples, not between the two major spawning systems; the San Joaquin and Sacramento rivers. Given the hypothesized effects of the periodicity of water diversion on the removal of juveniles from the two California spawning systems, the discrimination of San Joaquin versus Sacramento adults would aid in evaluating the magnitude of this problem in relation to adult population size.

Although the aim of distinguishing unit stocks of a species is relatively unambiguous, this is not true for the concept of the unit stock itself. Unit stocks are here defined as randomly mating populations of individuals which are either temporally or spatially isolated from similar intraspecific aggregates. As a result of their reproductive isolation, each stock may develop genotypic differences. These genotypic differences may eventually be reflected in phenotypic differences. Variation in genotypic frequencies may result from random factors or differential selective pressures. Phenotypic variability may also arise from the immediate impact of differential environmental conditions. Manifestations of divergence can include morphological, physiological, behavioral, biochemical, and genetic characters, certain of which may be useful in demonstrating the existence of separate unit stocks and in identifying the ancestry of unknown individuals in a mixed fishery. We divided efforts to identify and discriminate between striped bass stocks into two categories: behavioral and morphological, and genetic and biochemical approaches, each of which are then subdivided by the distinct techniques applied.

Behavioral and Morphological Approaches

Tagging Studies and Catch Data

Tagging studies have helped frame questions of stock identification. Merriman (1941) confirmed through tagging experiments that distinct stocks of striped bass contribute to the Atlantic coastal fishery. His study indicated that there is a mass migration northward in springtime of striped bass from the Middle Atlantic states to about as far north as Cape Cod, with a return migration in autumn. This movement produced little or no mixing with striped bass stocks found near the two extremes of their Atlantic coast range. The concept of migratory central Atlantic populations surrounded by more sedentary riverine stocks to the north and south was verified by the numerous mark-recapture studies that followed.

Several tagging studies, including Merriman's (1941) were of primarily coastal fish and these provided evidence that striped bass from certain river systems mixed in the Middle Atlantic coastal region. At least two of these studies suggested that the relative contributions from spawning rivers to the coastal stock were not stable, but instead reflected the effects of dominant year classes. This data was based on tagging studies encompassing approximately thirty years.

Schaefer (1968) found that a significant number of striped bass tagged in the mid-Long Island Atlantic surf prior to 1961 were recaptured from the Hudson River. However, returns from fish tagged in later years were overwhelmingly of southern origins, a result which the author largely attributed to migration of the dominant 1958 Chesapeake year class. Similar results were obtained by Alperin (1966) for striped bass tagged in Great South Bay, Long Island, New York.

Other workers, in response to an increasing body of meristic and morphometric data that suggested the existence of discrete populations, tagged fish on their riverine spawning grounds under the assumption that individual stocks would be isolated from each other at that time. Stock integrity was supported as striped bass returned to their natal rivers with no straying. For example, as part of their tagging effort in the Potomac River during 1959-1961, Nichols and Miller (1967) found that of 37 fish tagged during spawning season and recaptured during subsequent spawning seasons, none were recovered from another breeding system, while 32 were taken from the Potomac. The remaining five fish recaptured during the spawning season were either taken in the ocean or in the Chesapeake Bay proper. Mansueti (1961) found that of striped bass tagged in Maryland tributaries to Chesapeake Bay during the spawning season, six out of eight recaptures during subsequent breeding seasons were taken in the general region of previous spawning. Massman and Pacheco (1961) reported that 94% of spring recaptures of striped bass tagged in the James, York, and Rappahannock Rivers in Virginia during previous spawning seasons were recaptured from the river system in which they were tagged. Almost all other spring recaptures occured beyond the confines of other spawning systems. In total, it would appear that Chesapeake striped bass are 95-100% accurate in their homing tendency. McLaren et al. (1981) point to a need to tag adult fish at spawning time in the Hudson to gain an understanding of the movements of the Hudson stock. However, they do not tabulate their recaptures on a seasonal or monthly basis, prohibiting an evaluation of the homing tendency of the Hudson population. Other studies supportive of homing include Vladykov and Wallace (1952) and Hassler et al. (1981). Evidence for the localized nature of striped bass populations to the north and south of the migratory mid-Atlantic stocks was provided by Beaulieau (1962) and Dudley et al. (1977), respectively.

Tagging studies were initially conducted to determine which spawning systems contribute to the mixed coastal fishery. Vladykov and Wallace (1938) and Merriman (1941) were the first to point to a Chesapeake-Delaware Bay and Hudson River origin for a majority of the coastal stock. Tagging efforts within the Chesapeake (Pearson, 1938; Vladykov and Wallace, 1953; Mansueti, 1961; Massman and Pacheco, 1961; Nichols and Miller, 1967; and Moore and Burton, 1975) all pointed to an exodus of a small number of adult striped bass from the Chesapeake, however, perhaps not in sufficient numbers to support the then massive coastal fishery. By reanalyzing the results of earlier Potomac River tagging studies on the basis of age and sex, Kohlenstein (1981) felt that enough age-III females left the Chesapeake to support a majority of the predominantly female (Schaefer, 1968a, 1968b) coastal striped bass fishery. The idea of a limited impact of the Hudson (Clark, 1968 and McLaren et al. 1981) and Roanoke Rivers, North Carolina (Trent and Hassler, 1968 and Hassler et al. 1981) to the coastal stock was obtained from additional tagging results. Despite several decades of tagging Roanoke striped bass within their natal stream, no returns have been obtained from the coastal fishery north of the entrance to Chesapeake Bay.

The limits of movement, and the coherence of west coast striped bass populations were described by Clark (1936), Morgan and Gerlach (1950), Calhoun (1952), and Chadwick (1967). A synopsis of conventional tagging studies conducted on striped bass is provided in a report by the Hudson River Foundation (1985).

A relatively new type of mark, coded magnetic wire microtags, has been employed successfully on Pacific salmon (Hankin, 1982), and is currently being used to mark releases of juvenile striped bass from the Con Edison Hatchery on the Hudson River. These minute, gun-injected tags carry binary coded information, and are magnetized to allow detection in live fish through the use of a portable sensor. In the Hudson program the microtags were originally inserted in the internostril region as in salmonids, but poor retention (Texas Instruments, 1975) necessitated a change to cheek muscle insertion (EA Science and Technology, 1985a). Problems associated with this approach are that fish must often be fin-clipped or marked externally to serve as a check on the metal tag detector, and that recaptures must be sacrificed to recover the microtags. Of course, this tag will not be detected by the average recreational fisherman.

Catch data may also provide evidence of separate stocks. Koo (1970) described fluctuations in Atlantic commercial landings that paralleled fluctations in Chesapeake landings following a two year lag, thereby suggesting a Chesapeake ancestry for the majority of the coastal fishery. Catches of striped bass in North Carolina displayed a pattern independent of that observed in the Chesapeake. Van Winkle et al. (1979) concluded that the unique periodicities displayed by commercial catches from North Carolina supported consideration of striped bass from that region as a separate stock from those of the Hudson River and Chesapeake Bay.

In summary, these five decades of tagging studies have framed the overall patterns for migration of the coastal stock. First, the various spawning systems which contribute to the coastal stock were identified. Second, the concept of homing in striped bass was advanced. Experimental evidence was limited to at most two tributaries of the Chesapeake. No detailed studies investigating the homing of Hudson River striped bass have been attempted. Third, the idea of periodic fluctuations in the relative contributions of the major spawning systems to the coastal fishery was proposed. However, no attempt to quantify the relative contributions of the various spawning stocks to the overall coastal fishery was undertaken. A major failure inherent to design of all these studies must be emphasized which severly compromises many of the conclusions which have been reached. To definitively track the movements of representatives of the major spawning systems during their life histories, these fish must be tagged as spawning adults during the breeding season or as premigratory juveniles (or yearlings) on their natal streams. In most cases this requirement was not met. Frequently, many, but not all of the fish tagged in a study did meet this requirement. However, the data presentation was not subdivided to allow the reader to determine which fish were tagged

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Meristics and Morphometrics

Major efforts to distinguish striped bass stocks on a morphological basis began in the 1930's. These studies attempted to distinguish "races" of striped bass, a level of differentiation that was considered lower than the subspecific rank (Raney and Woolcott, 1955). Synopses of striped bass stocks identified through meristic and morphometric studies, together with tabular data summaries are presented in Westin and Rogers (1978) and Setzler et al. (1980).

Vladykov (1934), in a study on the variability of taxonomic characters in fishes, noted differences in dorsal and anal soft ray counts that led him to conclude that North Carolina striped bass formed a distinct and separate population from those of the Chesapeake region. He also reported that at least three different populations existed within the Chesapeake Bay system: Upper Bay, Potomac River, and James River.

Truitt and Vladykov (1937) found little diagnostic value in their vertebral and gill raker counts of Chesapeake striped bass. They did present mean values for dorsal and anal fin spine and soft ray counts that differed slightly by region. Raney (1952) later pointed out that such data would have been more useful if displayed as frequency distributions which would allow the reader to evaluate how much overlap in counts existed among systems. Vladykov and Wallace (1938) described soft ray counts of the second dorsal and anal fins and concluded that there are two main populations in the Chesapeake: The Upper Chesapeake, and the James River, with some indication of a third distinct population in the Potomac River.

Raney and Desylva (1953) investigated the existence of distinct Hudson River and Chesapeake Bay stocks by employing a variety of meristic characters in the first two age classes of striped bass, under the assumption that premigratory fish would be restricted to their natal rivers. While they found little variation in fin spine counts, they did achieve a measure of discrimination with counts of the soft rays of the dorsal, anal, and pectoral fins. However, these counts yielded incomplete partitioning that varied yearly. For example, for the aggregate 1936, 1949, and 1950 collections, dorsal soft ray counts of 11 or less separated 71% of known Hudson River striped bass from the 62% of the Chesapeake stock that possessed 12 or more. When the somewhat anomalous 1936 sample was disregarded, correct classification of 76% of Hudson specimens resulted. Higher separatory levels were yielded from employment of a character index devised to maximize variation in several fin ray counts. The character index utilized was the total of the dorsal, anal, and pectoral soft rays. It provided the highest and most consistent level of discrimination across numerous pooled year classes, with 85% of Hudson River fish showing an index of 55 or less and 72% of Chesapeake striped bass at ≥56.

In a similar study based on the same character index, Raney, Woolcott and Mehring (1954), found that the Delaware River striped bass stock was intermediate to the Hudson and Chesapeake populations, but that its affinities were closer to the latter. Their other findings included that Albemarle Sound striped bass are similar to Chesapeake fish but are still endemic, and that striped bass from some South Carolina rivers were similar to the geographically adjacent Albemarle Sound stock in dorsal and anal fin ray counts, but somewhat lower in pectoral and lateral line scale counts, an indication of considerable endemism.

Raney and Woolcott (1955) compared lateral line scale and fin ray counts between striped bass of rivers of the southeastern United States, Their primary findings were that the Santee-Cooper Rivers in South Carolina and St. Johns River in Florida harbored distinct populations, although specimens from the geograpically intermediate Georgia rivers were not sampled.

Raney (1957) investigated the presence of populations within the tributaries of the Chesapeake, examining only nonmigratory age 0+ striped bass to assure a known river of origin. Using the character index employed in previous studies in addition to counts of individual fin elements, Raney concluded that there are three Chesapeake subpopulations; James River, York-Rappahanock rivers, and Upper Bay. Lewis (1957) examined the same problem using gill raker counts of the 1955 year class, and found a breakdown of Chesapeake striped bass similar to that of Raney (1957). Lewis (1957) also concluded that the Hudson River supported a distinct striped bass stock.

In a study of striped bass morphometrics Lund (1957) found four distinct Chesapeake stocks, in the James, York, Rappahannock, and Potomac rivers, in addition to a differentiated Hudson River population. Measurements used included body depth, caudal-peduncle depth, prepelvic distance, predorsal distance, and head length.

Murawski (1958) examined lateral line scale counts of striped bass from Florida to Canada and found distinct populations in several southeastern rivers, Albemarle Sound, and the Delaware, Hudson, and St. Lawrence rivers. Within the Chesapeake, his conclusions were consistent with the results of Lewis (1957) and Raney (1957).

Raney and deSylva (1953) initially hypothesized the possible existence of multiple Hudson subraces; upstream and downstream, based on the aforementioned meristic index. Raney (1957) further elaborated on Hudson heterogeneity.

A different approach, stepwise linear discriminant analysis of five meristic and morphometric characters was used by Berggren and Lieberman (1978). Their data collection program involved measuring 13 characters for each fish from which the five best discriminators were selected. Counts and measurements used were: 1) ratio of snout/internostril width, 2) a scale growth ring ratio, 3) the character index of Raney and DeSylva (1953), 4) a gill raker count, and 5) lateral line scale count. This approach produced a resolution of approximately 75% between Hudson River, Chesapeake Bay, and Roanoke River striped bass specimens. These workers used these differences to determine ancestry of striped bass in the mixed fishery in the various coastal zones. They concluded that the vast majority of all coastal fish were of Chesapeake descent, with the Hudson impact restricted to its immediate environs.

Van Winkle and Kumar (1982) reanalized the meristic and morphometric data generated from the earlier Texas Instrument study (Berggren and Lieberman, 1978) to obtain estimates of relative stock composition. Fourteen variables were derived from the same five characters used in the earlier study by not only using the measured characters but also their squares and cross products. Both a stepwise discrimant analysis and maximum likelihood analysis were performed on this data set. Roanoke fish were deleted from the analysis due to their presumed negligible impact on the coastal fishery north of the Chesapeake Bay (Hassler et al. 1981). A confusion matrix indicated that approximately 21%

of Hudson spawning stock was misclassified as Chesapeaake and 20% of the Cheaspeake spawning stock was misclassified as being of Hudson ancestry. Relative contribution of the Hudson stock was shown to vary between 10 and 50% dependent on year class strength.

A study by Saila et al. (1983) to estimate relative contributions of striped bass stocks to the Rhode Island coastal trap fishery combined three approaches: isoelectric focusing of eye lens proteins, morphometrics, and elemental composition of otoliths, the latter two of which were combined to increase stock resolution. Otoliths were analyzed for metal cations using atomic absorption spectrophotometry. Of five trace elements examined, only cadmium and copper were found to display sufficient among sample variability to be included in the discriminant analysis. This analysis also employed three morphometric variables: head length, internostril width, and jaw length. The percent contribution of metal uptake analysis to the overall levels of stock discrimination obtained (94% correct classification of the Hudson River verification subset and 83% for the Chesapeake), was not provided. Application of the trace element and morphometric discriminators to the Rhode Island mixed fishery indicated 67% Chesapeake and 33% Hudson contributions, while the isolelectric focusing component performed on the same fish suggested a 50% contribution from each stock. However, the value of both estimates are questionable since sampling in Rhode Island was performed over a 10 day period and therefore, may not represent yearly contributions. As suggested by Clark (1968) it is likely that striped bass travel in contingents, with a single coastal zone visited by multiple contingents of fish of heterogenous ancestry during the course of a single season. Indeed, a later isoelectric focusing study by Fabrizio et al. (1985) showed significant temporal fluctuation by season off Rhode Island in the annual composition of the striped bass catch.

Scale Morphology

The growth rings or annuli on striped bass scales were used by Merriman (1941) as a stock discriminator. Based on the assumption that different growth rates would occur in young striped bass over a wide latitudinal range as the result of environmental variation in nursery areas, and that striped bass don't migrate until approximately age II, Merriman analyzed growth information on scale samples from the dominant 1934 year class in migratory striped bass from Cape Cod Bay, Connecticut, and Long Island and compared patterns to those obtained from two nursery regions Chesapeake Bay and Currituck Sound, North Carolina. He found patterns in all migratory fish to resemble closely that encountered in the Chesapeake but different in comparison with specimens from the other nursery area, Currituck Sound. However, he noted that the overlap of growth patterns between populations as a major drawback to this approach. Tiller (1950) later found a close coincidence in body length and calculated length at the first annulus between striped bass of a single dominant Chesapeake year class and those from Long Island, New York. He considered this evidence supportive of the Chesapeake as a major contributor to the Middle Atlantic fishery.

An updated scale morphology approach was employed by Taub (1975) as a striped bass stock discriminator. Although predicated on the same principle defended by Merriman (1941), that striped bass of different stocks will show environmentally-based differences in their scales, Taub examined characteristics of the circuli rather than the annuli. A surface profile analyzer was used to detect scale surface microstructure. Five features were recorded, including intercirculus spacing and average circulus height. Linear discriminant analysis permitted classification accuracies of Hudson and Chesapeake striped bass of between 65-70% on training sets with slight size biases. Inasmuch as there were definite but varying correlations between circulus height and fish size for the two stocks examined, Taub estimated that correction for fish size heterogeneities might produce classification rates exceeding 70%.

Parasites

Sindermann (1983) reviewed the value of parasites as natural tags for marine fish. This approach has only recently been applied on striped bass. Hogans (1984) examined intestinal parasites of striped bass captured in a survey of the Kouchbouguac River, New Brunswick. Although located near the mouth of the St. Lawrence River, striped bass from the Kouchbouguac River harbored three helminth species found in Chesapeake striped bass but not in any fish species in Canadian waters. A follow-up tagging study on about 60 subadult striped bass taken in the river's winter fishery resulted in tag returns from the mouth of Delaware Bay and from the Wye River, Maryland in addition to a number of Canadian returns (USFWS/NMFS 1984). It remains to be seen if a problem common to the parasite technique, that is whether parasite species are specific to individual fish stocks to a sufficient degree, will allow development of this approach in striped bass.

In summarizing the results of the non-genetic approaches outlined above several points should be emphasized. Maximum discrimination of spawning stocks approached 80% based on indices of multiple characters or their derivatives. The development of discriminant analysis and the maximum likelihood approach provided a sophisticated statistical methodology which maximized resolution among spawning stocks, and permitted an estimate of relative contribution to the coastal fishery based on a battery of independent stock discriminators. The development of training sets has gained favor is an approach to validate the resolving power of the various discriminators. The major study in this regard, Berggren and Lieberman's does have several shortcomings which are frequently overlooked. First, collection of Hudson spawning stock specimens began in early March, two months prior to the beginning of the breeding season, thereby potentially including representatives of other spawning systems in this reference collection. Second, these workers concluded that during the September-October period of 1975, 24% of 82 fish taken off southern Massachusetts were of Roanoke ancestry. This data is extremely disturbing in view of Hassler et al.'s tagging results which have never reported a return from the Massachusetts area.

An additional area worthy of further investigation warrants mentioning. Blaxter has pointed to the temporal instability of meristic counts. Yet, no study has been conducted over a long period of to determine the stability of meristic counts in striped bass within a single or multiple spawning systems. Currently, Raney and deSylva's (1953) Chesapeake 1954-1955 data set provides the only opportunity to evaluate this question.

Genetic and Biochemical Techniques

Striped bass have been the focus of a multitude of genetic studies that attempted to discriminate among stocks of the species. The success of these efforts is predicated on several historical events that shape the genetic makeup of a population. First, changes in the DNA (chromosomal rearrangements, DNA additions or deletions, point mutations) must have occurred independently in different populations. Second, sufficient time must have elapsed since the isolation of separate breeding units to allow for these genetic changes to have become common enough to be of diagnostic value. Third, interbreeding as the result of insufficient temporal or spatial partioning of populations must be negligible. And fourth, individual stock size must have remained sufficiently large to avoid the "flushing" effects of a sudden genetic bottleneck which potentially could eliminate rare genotypes. It should be added that while bottlenecks may serve to eliminate rare genotypes and thereby reduce overall genetic variability in a population, they can also aid in stock discrimination through founder effects in which heretofore uncommon genotypes become predominant in a population. As a result of a drastic decline in population size, the affected stock may acquire genotypic frequencies which differ dramatically from adjoining isolated populations.

The success of any genetic approach in differentiating populations is primarily dependent on the existence of sufficient levels of detectable natural variation and its partitioning along geographic bounds. However, the absence of measurable discriminatory genetic variation among populations does not preclude the possible existence of separate stocks. In the case of recently isolated populations or moderate gene flow a genetic approach might prove uninformative while a conventional tagging study could demonstrate the existence of discrete stocks. It should be emphasized that not all biochemical techniques are purely genetic although they may receive genetic influences. Other governing factors include the environment, and ontogenetic changes in physiology.

Immunology

Radio-immuno assay is a procedure which may be used to determine relative genetic similarity between taxa. It has gained widespread use in determining distance between extinct species heretofore refractory to biochemical analysis (Lowenstein, 1985). The applicability of this technique to stock discrimination is based on the premise of inherited stock specific differences in the structure of particular antigens. Its primary appeal in this regard stems from the large number of samples which may be surveyed for a modest cost. Individual antigens must be purified and then used to prepare their specific antibodies. The degree of reactivity between these prepared antibodies and the same antigen from fish of different hypothesized populations may then be quantified to provide a measure of relatedness. Immunological tests to discriminate among striped bass populations have recently been attempted by Simon and Schill (1984). Serum albumin was purified and albumin antibodies were prepared from striped bass from the Monck's Corner hatchery. Reactivity between these antibodies and the antigen from Monck's Corner and Choptank River, Maryland striped bass was quantified. As expected, lesser reactivity was observed when antigens were combined with antibodies from specimens of the other stock. While the immunological approach may indicate which fish are more closely related, its application in stock discrimination may be hindered by intra-stock polymorphism. Additionally, the genetic basis for structural differences in the particular antigen and therefore the degree of antigen-antibody reactivity are complex. Post-translational modification of antigen molecules may result in seasonal or ontogenetic variation in antigen reactivity. As a result, quantification of genetic relatedness among different populations may be difficult. The immunological approach will determine relative relatedness between individuals, however, it will not indicate the molecular history responsible for the degree of divergence observed. Most importantly, there is no reason to expect this approach to be more sensitive in detecting genetic variability in protein molecules than observed via conventional electrophoresis. This expectation is based on the assumption that the variability in isozyme loci is representative of that encountered throughout the nuclear genome.

Cytogenetic Studies

Cytogenetics, the study of chromosome number, morphology, and behavior, has been applied to numerous fish species (Gold 1979). Kerby (1972) and Rachlin et al. (1978) used conventional karyotypic analysis to explore both chromosome number and morphology in striped bass from the York (Virginia) and Hudson rivers, respectively. While both workers reported an invariant chromosome number of 48, Rachlin and his co-workers found two characteristic karyotypes in Hudson River fish that varied in chromosomal size and type. Considering the enormous potential detrimental effects from variability in chromosome number or morphology, it is hard to envision extensive gross chromosomal polymorphisms in natural populations.

Studies of this type are complicated by the influence of developmental stage (Barsiene, 1978) and the occurrences of intraindividual polymorphism (Hartley and Horne, 1982). Recent improvements to karyotypic techniques have been made that permit the staining and visualization of specific chromosomal landmarks, thereby providing additional sensitivity beyond the detection of differences in chromosome size and shape. These approaches include the determination of the chromosomal distribution of nucleolus-organizer regions which code for ribosomal proteins (Phillips et al. 1986), the chromosomal distribution of replication bands (Delaney and Bloom, 1984), G-bands resulting from Giemsa staining of trypsin digested chromosomes (Blaxhall, 1983), and C-bands specific for constitutive heterochromatin (Zenzes and Voiculesca, 1975). However, considering their limited success in the detection of interspecific polymorphisms in other fish species, it is uncertain whether these newer techniques would provide significant further resolution of unit stocks of striped bass.

Protein Electrophoresis

Many genetic studies involving striped bass and other fishes have focused on an indirect examination of base sequence changes in the nuclear genome through analysis of its protein products. Electrophoretic analysis represents a biased analysis of overall genetic variability in a species in that only polymorphisms at structural loci are potentially detectable. Genetic differences at structural loci (those genes coding for protein products) between populations are reflected in amino acid substitutions in these proteins (usually isozymes). Only those variant forms of the isozyme that carry amino acid substitutions which specifically affect either the pH dependent net charge or molecular size may be detectable via electrophoresis. Thus, for the average protein only about 30% of all nucleotide substitutions will be detectable via this methodology (Nei, 1975; Wilson et al. 1977). An advantage of protein electrophoresis is that it is not always necessary to sacrifice specimens. Sufficient protein product has been obtained in another percoid, the largemouth bass, Micropterus salmoides, from such low risk tissue sample sites as the caudal fin and scale-epithelial tissue and from blood plasma (Carmichael et al. 1986). Reviews of protein electrophoresis as applied to fishes include de Ligny (1969), Utter (1974), Allendorf and Utter (1979), and Smith and Fujio (1982).

Morgan et al. (1973) reported success in characterizing discrete populations of striped bass in the upper Chesapeake Bay by discontinuous polyacrylamide gel electrophoresis of five serum proteins including a transferrin locus. They found striped bass of the Elk, Potomac-Patuxtent, and Choptank-Nanticoke rivers to form discrete breeding units, with the Elk River population the most distinct. It has since been found that serum proteins are subject to considerable ontogenetic and environmental influence, and that transferrin is quite labile upon extended storage. In addition, complex serum protein electropherograms are often uninterpretable due to inconsistent staining, resolution, and the multiplicity of bands. Sidell et al. (1980) repeated much of Morgan et al.'s (1973) work, however they were not able to identify unique populations within the Chesapeake. Transferrin proved totally monomorphic among striped bass from seven major spawning systems that occur along most of the length of the Chesapeake Bay. Twelve serum protein bands were consistently resolved and occurred at frequencies of less than 100%. Of the two isozymic loci investigated, glycerol-3-phosphate dehydrogenase (α-GPDH) proved polymorphic but no significant location-specific differences could be found, while isocitrate dehydrogenase (IDH) provided inconsistent resolution. Sidell et al. (1980) concluded that Chesapeake striped bass were genetically homogeneous, perhaps due to significant gene flow between fish of the different river systems. Hitron (1974) also examined allelic products at the transferrin locus and concluded that the Hudson River contained a single population of striped bass.

Otto (1975) screened 28 isozymic loci in striped bass from the Hudson, four tributaries of the Chesapeake Bay, the Maine coast, and 18 loci in striped bass from California. Although he reported striped bass to be among the most monomorphic vertebrate species investigated, low levels of variation were observed at five loci (α-GPDH, IDH, esterase 3, and phosphoglucomutases 1 and 3), with polymorphism (at the 5% level) detected at three of these. Although genetic distance between his hypothesized populations was small, the author concluded that significant differences existed between the Hudson and aggregate Chesapeake samples. His reported frequency differences among populations were insufficient to aid in stock identification

In an extensive survey using starch gel electrophoresis, Grove et al. (1976) in conjunction with other Texas Instruments' studies screened twenty-eight enzyme systems involving 52 genetic loci in striped bass from the Roanoke River, four tributaries of the Chesapeake Bay (Potomac, Rappahannock, Choptank, and Elk Rivers), and the Hudson River. Of the 52 loci investigated, only two proved polymorphic: αGPDH and IDH. Slight differences were noted in frequencies of αGPDH among populations, while Hudson and Roanoke striped bass displayed a unique allele at the IDH locus in very low frequencies (0.065 and 0.025 (below polymorphic level considered significant), respectively). Frequencies of polymorphic alleles showed a clinal distribution. The authors concluded that the genetic structure of striped bass populations is one of the most homogeneous ever studied.

Analysis of isozymic genotypes may have limited utility in discriminating among discrete spawning populations of striped bass within a reservoir system. Rogier (1985) found only 3 of 56 loci polymorphic in striped bass captured from two tributaries (the

Roanoke and Dan Rivers) of Kerr Reservoir (Virginia-North Carolina), which did not allow discrimination between fish from the two rivers. This is not surprising in view of the recent origin (1950's) of this self sustaining reservoir population and the potential mixing it has recently afforded the two striped bass populations.

Isoelectric Focusing

Isoelectric focusing is a variant of conventional electrophoresis whereby proteins are separated on the basis of their isoelectric points instead of their net charge or molecular size. Isoelectric point refers to the pH at which a protein has no net charge. The isoelectric point of any protein is a function of its primary amino acid structure, and therefore is genetically determined. This technique may be used to separate isozymes or any structural protein, with the potential scoring of the relative amount of protein in each band determined via scanning densitometry. However, while isoelectric mobility of individual protein components is solely genetically determined, the concentration of individual bands can have both a genetic and non-genetic component. Isoelectric focusing has the advantage of providing sharper resolution of protein bands than conventional electrophoretic techniques. However, studies comparing the sensitivities of isoelectric focusing and conventional protein electrophoresis have suggested no increased levels of detection of polymorphism through the former technique (Ramshaw et al. 1979; Coyne, 1982; and Bussman, 1984).

While isoelectric focusing has seen only limited application to fish stock discrimination, several such studies have recently been conducted on Atlantic coast striped bass using eye lens proteins. Saila et al. (1983) and Fabrizio et al. (1985), both failed to detect differences in mobility, and hence, exclusively genetic polymorphisms among the bands resolved and scored. However, Saila et al (1983) and Fabrizio at al. (1985) did report differences between Hudson and Chesapeake-Roanoke samples in the relative abundance of six eye lens proteins as quantified by scanning densitometry, which, when combined with four morphometric characters allowed an estimation of the contributions of these stocks to the mixed coastal fishery. Calculated contributions of Hudson spawned fish to the coastal Newport, Rhode Island trap fishery via this methodology varied between approximately 10% and 50% dependent upon the month of sampling. While this approach permitted these calculations for single years, densitometric analysis of isoelectrically focused eye lens proteins is complicated by non-genetic factors. The relative abundance of these proteins is influenced by the environment (Leveille et al. 1984). Moreover, Smith and Gilman (1982) reported that the expression of eye lens proteins in fish varies with age, a finding consistent with results from similar studies on other vertebrates (Bours, 1980; and Bindels et al. 1983). Although Fabrizio et al. (1985) attempted to accommodate the bias introduced by size differences among their samples, the utility of this set of protein markers towards the discrimination of a range of sizes in the coastal striped bass fishery remains questionable. De Jong (1986) in summarizing evolution in eye lens crystallins concluded that this class of proteins evolves quite slowly.

A variation of isoelectric focusing, two dimensional electrophoresis has seen application in the detection of intraspecific variation in other animal species but not yet in fishes (Leigh-Brown and Langley, 1979; Aquadro and Avise, 1981). While this technique would be effective in separating potentially informative markers, the complexity of data interpretation would strongly limit the number of fish which could be examined, and intersample comparisons would be very difficult because of the inclusion of samples on different gels. Both isoelectric focusing and two dimensional electrophoresis present the additional problem of difficulty in the genetic interpretation of obtained banding patterns.

DNA fingerprinting

A recent development in human genetics shows promise for fish stock identification. "DNA fingerprinting" generates Mendelian inherited genetic markers that "are as unique to each individual as are the whorls on the finger pads" (Lewin, 1986). Fragments of chromosomal DNA are generated by restriction endonucleases and are localized on gels by hybridization with complementary radioactive probes containing part of the human myoglobin gene. The DNA fragments which are detected in the unknown samples contain multiple copies of tandem repeats of these unique DNA sequences termed minisatellites. These minisatellites are scattered about the entire genome. Interindividual polymorphism in the length of these DNA fragments results from their having variable numbers of copies of these repeated sequences. By considering a composite profile of many of these hypervariable DNA fragments for each individual, all persons can be uniquely identified (Jeffreys et al. 1985). Given the protein monomorphism exhibited by striped bass, "DNA fingerprinting" provides the potential for examing a hypervariable portion of the nuclear genome. Should genetic techniques which examine extrachromosomal DNA (mtDNA) demonstrate polymorphic markers, they would still not provide information on the ancestral lineage of the nuclear genome. The vast majority of our genetic information is encoded within the nuclear genome. Thus, by considering information provided by both of these approaches, we will maximize our resolution in the area of stock identifiction and have a handle on the extent of diversity in the nuclear genome.

Ironically, the extreme variability revealed by the fingerprint technique may be too sensitive for adoption to stock identification if every fish proves different. However, by considering only a small number of the observed DNA fragments, rather than the entire profile, it may be possible that stock specific differences can be detected. Assuming that an appropriate DNA probe for fish can be developed, the technical demands of this procedure are far less rigorous than encountered in the analysis of mtDNA. Samples may be as small as a drop of blood, and their preservation may not be burdensome inasmuch as human genotypes have been determined from a four year old bloodstain (Lewin, 1986).

Application of this technology seems well suited to situations where hatchery production is being used to augment natural reproduction of striped bass. If, as is sometimes the case, a very small number of parents are used as broodstock, a manageable number of marker parental genotypes could be determined, which could allow monitoring of their progeny in natural populations. The hypervariability of this marker would permit specific genetic tags for the progeny of specific matings performed at the hatchery.

In summary, genetic approaches have added little to our understanding of the structuring of striped bass populations. Protein electrophoresis, which in certain in other species has provided a wealth of information regarding the genetic architecture of stocks, has proved of no value in the identification of striped bass populations. The applicability of the other approaches outlined above is probably restricted due to the technical limitations in surveying a large number of samples.

Conclusions

What have been the results of 50 years of striped bass stock discrimination? Two trends are evident, at least in regard to the Atlantic coast. One is a winnowing of our accepted notions of existent stocks from those of a single coastal population, to those of the major estuaries, to perhaps those of individual rivers within an estuarine system. This resductionist trend reflects two related influences. The first is that perception of stocks is largely a function of the resolution power of the techniques employed. Increasingly sophisticated techniques have allowed movement somewhat nearer the ultimate baseline of identifiers for populations from individual rivers. Variation in the species can be detected below the level of individual rivers, but does not assist the unit stock concept. A second influence is that new techniques that probe the genome focus attention, as a result of their genetic bases, on the lowest useful manifestation of the stock concept in an anadromous species, that of a river's breeding population.

The second trend is more subtle. Although levels of resolution have slowly increased with the introduction of new techniques and the occasional unification of new techniques with traditional ones, no approach appears to yield 100% separation between individuals from the stocks, that is, the ability to unambiguously identify the river of origin for each striped bass. Maximum resolution between Hudson and Chesapeake training sets reported to date approximates 75-80%. Significant progress has occurred though, in the move towards the cataloguing of genotypic information on populations. Early attempts at stock discrimination in striped bass, such as the numerous morphometric and meristic studies outlined in these studies were clearly based on phenotypic characters. Many more recent efforts that attempted to blend morphological and biochemical characters also relied heavily or completely on measurements of the phenotype. Examples include the study by Grove, Berggren, and Powers (1976) which, in addition to protein electrophoresis, employed morphometric measurements; and Fabrizio et al. (1985) whose ostensibly biochemical approach was heavily dependent on morphometrics for its resolving power (80%), and which utilized an apparently largely environmentally and developmentally influenced biochemical technique, the concentration of individual eye lens proteins separated by isoelectric focusing.

Other approaches taken have been more purely genotypic, such as karyotyping, protein electrophoresis, and isoelectric points of proteins. None of the phenotypic or genetic techniques appear to have consistently exceeded 90% resolution between stocks, and most have yielded results in the 70-80% range. Given that genetic approaches have not greatly improved the ability to discriminate between striped bass populations, where then is the progress, particularly since most genotypic techniques are so much more complex? The answer lies with the long term utility of the techniques. Phenotypes are the product of environmentally influenced genotypes. It has been demonstrated that several environmental factors including temperature, salinity, and oxygen may affect meristic counts in early life history stages (Blaxter, 1984). Since the environment fluctuates across many time scales, especially annually, the generation of each new cohort within a population, even with the same genetic makeup, may differ substantially in morphometric measurements and meristic counts. Such intrapopulational variation may thereby prohibit any characterization of the population, that is, phenotypic stock discrimination will demand knowledge of the appropriate characters for each year class involved. Sensitivity to this problem was shown by Raney and others in the studies they conducted in the 1950's on the Atlantic coastal populations in which comparisons were made between members of a single year class or with year class effects considered.

While phenotypically influenced stock discriminators do provide signficant information, knowledge of genotypes, however, offers freedom from the need for repeated yearly sampling merely to readjust the stock identifying character, albeit at the price of added complexity. By focusing efforts at finding genotypic characters, information of a relatively fixed type will accumulate, allowing allocation of resources to more specific questions.

An additional byproduct of the cataloguing of genetic profiles of striped bass populations has recently been advanced by Meefe (1986) in regard to endangered fishes. In these times when natural striped bass populations are being augmented by hatchery production (various tributaries of the Chesapeake Bay, the Hudson River, the Apalachicola River in Florida), genotypic characterizations of potential broodstock provides a mechanism to maintain or restore the requisite genetic variation in these populations.

Unfortunately, just as advanced techniques are permitting the development of a "library" of genotypic information on the individual stocks of striped bass, indiscriminate hatchery plantings of non-native stocks are confusing the genetic makeup of natural populations. For example, Wooley and Crateau (1983) have documented the introduction of Atlantic coast striped bass into the Apalachicola drainage system of the Gulf of Mexico. Striped bass from the Hudson River were stocked in the Maine's Kennebec River in 1985 (EA

Science and Technology 1985b). Striped bass of the designated "Brookneal" strain reared in Virgina hatcheries have been released in the Navesink River in coastal northern New Jersey. Beyond the important question of whether striped bass stocks from other populations will be as well adapted to their new environments as the local stocks they are meant to supplement, continued hatchery introductions of non-native fish would lead to dilution or eradication of the geographical component to intraspecific variability necessary to allow use of genetic approaches. The potential also exists for such genetic scrambling to decrease the effectiveness of the phenotypic approaches as they measure the product of the environment on the genetic baseline

Future Applications

Future applications of stock discrimination techniques to striped bass will likely focus on specific management problems. Primary will be regular estimates of the contributions of various mid-Atlantic river systems to the coastal striped bass stock. Substantial differences among the estimates of Berggren and Lieberman (1978), Saila et al. (1983), Fabrizio et al. (1985), and the analysis by Van Winkle and Kumar (1982) suggest the need for regularly updated estimates of the stock composition of Atlantic coastal striped bass on a year class basis. This is due to the discordant relative year class strength in the two major contributors to the coastal stock.

Reliance on a genotypic approach is to be preferred, since if stock identifiers are already known, all efforts can be applied toward obtaining a truly representative coastal sample, without needing to obtain "training sets" of specimens representative of individual river systems. Thus, large numbers of fish could be sampled over a wide range of age classes, from numerous coastal locations, over their entire period of availability. Other questions concerning Atlantic striped bass that could receive new stock discrimination efforts include the identification of those fish found in non-spawning rivers (the Connecticut River, various river systems of northern Massachusetts, New Hampshire and Maine), and of winter aggregations that occur sporadically in the coastal nearshore zone between New York and North Carolina (Holland and Yvelterton, 1973)

Dependable estimates of coastal stock contributions might allow refinements to current striped bass management strategies. It is not hard to envision a coupling of coastal stock contribution data to juvenile abundance indices that are routinely taken for striped bass spawning systems that occur in New York, Maryland, Virginia, and North Carolina. Thus models of relative stock contribution could be constructed based on differential young-of-the-year indices and subsequent abundance estimates in the coastal mixed-stock fishery. If the stock makeup of individual year classes of subadult and adult striped bass that occur along the coast could be partitioned with sufficient confidence to allow correlation with their original juvenile abundance indices, then it is conceivable that a calibration could be effected that would allow realistic estimates of future individual stock contributions based soley on juvenile sampling. After several years of calibration, assuming constant fishing and natural mortality, it would be possible to predict relative contributions on the basis of relative juvenile indices.

Future work in Atlantic coast striped bass stock discrimination may also need to take into account another historically significant stock, that of the Delaware River. The Delaware River appeared to be a major spawning area for striped bass which supported a somewhat differentiated stock (Merriman, 1941) based on the meristic studies of Raney et al. (1954), Lewis (1957) and Murawski (1958). However, the population crashed by 1960 presumably due to gross pollution in the lower reaches of the river (Chittenden 1971). There is recent evidence of renewed natural reproduction of striped bass from the Delaware based on the capture of juveniles and yearlings for several consecutive seasons (Himchak, 1981; Himchak, 1985). However, transport of striped bass eggs and larvae from the Chesapeake through the Chesapeake and Delaware Canal cannot be ruled out as a source for the juveniles and yearlings encountered in some of the upper reaches of the Delaware. A second possibility is that striped bass spawning in the Delaware are emigres from the Chesapeake. However, Kohlenstein (1982) has ruled out that possibility on the basis of the non-migratory behavior of young Chesapeake striped bass. If there is an expanding remnant population, it may be that recent stock identification attempts have been at least somewhat confused by these Delaware spawners.

Other applications of stock discrimination of striped bass will include the identification of hatchery-produced individuals inasmuch as cultured striped bass are being produced in ever increasing numbers for purposes of stock restoration or compensation for power plant induced mortality. Regardless of whether future hatchery production of striped bass is performed with native stock only, it is clear that assessments of hatchery contributions to the wild population will be necessary. Two approaches beyond microtagging appear to best lend themselves to this need to identify what is essentially a substock capable of displaying behavioral or morphological differences from the wild population. One is where phenotypic differences between hatchery- and wild-produced juveniles are utilized, even if hatchery fish are of the native stock. Phenotypic variation should occur because temperature, feeding and other possible differences will almost certainly differ significantly between the artificial and natural environments, differences that could show in a variety of meristic or morphometric parameters. Perhaps the most practical method because of its ease of application and lack of harm to the fish is the scale profile technique of Taub (1975).

A second way to identify hatchery individuals in the wild would be to use a purely genotypic approach, made particularly feasible because genotypic information could easily be obtained from all broodstock. The manipulation of allelic frequencies to tag fish stocks has already been successfully applied via protein electrophoresis to salmonids (Seeb et al., 1986). Low degrees of polymorphism detected by protein electrophoresis in striped bass would suggest a different approach to genetic tagging in that species, such as mtDNA analysis. Although most easily implemented where the number of broodfish employed is low, if a mtDNA approach was used the number of genotypes needed to serve as a baseline could be reduced with only female fish considered, since mtDNA is maternally inherited.

II. Mitochondrial DNA Analysis of the Atlantic Coastal Stock of Striped Bass: Fisheries Consequences and an Evaluation of Molecular Results

mtDNA evolution

The use of mitochondrial DNA to explore evolutionary relationships among taxa representative of the appropriate divergence times has gained widespread use as a tool to both quantify the extent of their genetic distance and to clarify phylogenetic relationships among these groups (Avise and Lansman, 1983; Brown, 1983; Brown, 1985). The potential use of this molecular approach in stock identification of commercially or recreationally significant species was highlighted at a recent stock discrimination workshop sponsored by the National Marine Fisheries Service (Panama City, Florida; Nov., 1985). The appeal of this approach stems primarily from the rapid rate of evolution displayed by the maternally inherited mtDNA molecule. This is in part due to an increased mutation rate resulting from a lack of a mitochondrial repair system and an absence of an editing function associated with its DNA polymerase. Additionally, functional constraints placed on the mtDNA molecule are lessened due to its multiplicity of copies (Brown, 1983). In comparison to the nuclear genome, it has been observed that in primates the mtDNA base sequence evolves approximately five to ten times more rapidly (Brown et al. 1979). As a result, comparisons of mtDNA sequence conservation even between closely related taxa have yielded sufficient differences to allow for estimates of the extent of their genetic divergence, and therefore have permitted a reconstruction of their recent evolutionary past. In contrast, comparative examinations of the products of the nuclear genome at the level of low (conspecific) taxonomic divergence have frequently failed to yield significant information. This observation is particularly applicable to populations of anadromous and marine fishes where electrophoretically detected, geographically defined allelic frequency differences are commonly absent, resulting from gene flow, life history patterns, or some intrinsic environmental factor(s) (Gyllensten, 1985). Therefore, mtDNA should provide an excellent model for exploring and quantifying the genetic similarity between populations which only recently have become reproductively isolated, or which experience common selective pressures on the nuclear genome. Although the rate of base substitution at individual nucleotide positions within the mitochondrial codons has been shown to vary substantially (Brown, 1983); individual genes within the mitochondrial genome of animals have not been shown responsive to selective pressures. Therefore, genotypic frequencies are believed to represent a product of exclusively random factors. Given sufficient time since descent from a common ancestor, two reproductively isolated lineages should show substantial sequence divergence. However, it should be noted that due to the import of many nuclear encoded gene products into the mitochondrion, mtDNA change may not be totally independent of genomic DNA control.

In a series of papers dealing primarily with mammalian evolution, Brown et. al. (1979 and 1982) have attempted to quantify this rapid rate of mtDNA sequence change. The mtDNA sequence in a group of interspecific primate comparisons was estimated to have diverged at a rate of approximately 2% per base per million years between currently isolated gene pools in primates. This estimate was based on a time scale obtained from protein sequence data for primates. If the mitochondrial genome averages approximately 16,500 base pairs in vertebrate species (Brown, 1983), two isolated populations would differ at approximately 330 nucleotide positions after one million years. However, by the passage of approximately 15 million years all nucleotide positions which are apparently not under selective constraint (about 30%) would have undergone substitution, and therefore a dramatic reduction in nucleotide substitution rate is observed. The gap between nuclear and mitochondrial genomic substitution rates narrows rapidly after 15 million years (Brown, 1983).

The applicability of this mtDNA substitution rate to other non-primate taxa is predicated on two factors. First, the mtDNA mutation rate must be constant and equal in all eukaryotic taxa. Intertaxa differences in mtDNA mutation rate could be accounted for by variation in the structure or function of unique mitochondrial DNA polymerases. To date this has not been demonstrated. Replication of mammalian mtDNA is done by gamma-polymerase, an enzyme that is prone to a higher rate of nucleotide misincorporation than the major nuclear DNA replication enzyme, alpha-polymerase. In addition, gamma-polymerase lacks the ability to edit its newly synthesized mtDNA product (Brown, 1983). Second, the rate of fixation of these mutations must not vary among different taxa. This second assumption is based on an absence of functional or selective constraints on a minority of the mtDNA base sequence. This is due to a multiplicity of copies of the mtDNA molecule per cell. A vertebrate cell contains many, often thousands of mtDNA molecules (Avise and Lansman, 1983). Thus, each cell has the potential luxury of having several copies of the mtDNA change without disrupting its normal cellular function. As a result, the initial fixation rate of base substitutions should be a product of random factors. However, Brown (1985) has speculated that the rate of base substitution in the mitochondrial genome may not be totally independent of events involving nuclear genomic products. This is due to the interaction of nuclear and mitochondrial encoded gene products.

Within taxa, even in the face of extensive migration disrupting the establishment of significant genotypic frequency differences between hypothesized populations, one would still anticipate substantial levels of conspecific mtDNA sequence polymorphism, although not necessarily partitioned along geographic bounds. Given the presumed universality of this mtDNA substitution rate and the absence of selective constraints on a portion of the mitochondrial genome among all higher eukaryotes, one would predict given sufficient evolutionary time, an abundance of mtDNA sequence polymorphism within all taxa. This also assumes that there is an absence of selective pressures on approximately 30% of the mitochondrial genome in all vertebrate species. This is a reasonable assumption considering the fact that nucleotide substitutions in the protein genes (and there are virtually no intergenic spaces in the mtDNA) occur most frequently in the third positions of codons, since most of these changes are silent.

The applicability of the mtDNA approach to problems in fisheries

Potential utilization of this wealth of mtDNA sequence polymorphism as a tool to discriminate among individuals representative of different breeding systems seems obvious. This abundance of natural variation would serve as an innate genetic marker to uniquely identify representatives of different reproductive units. The absence of sexual recombination in conjunction with the maternal inheritance of the mtDNA molecule (Gyllensten et al. 1985; Lansman et al. 1983) permits an effective tracing of different ancestral maternal lineages in natural populations. Thus, individuals could easily be assigned maternal ancestry. It would be particularly effective in situations where female migration is negligible.

The obvious applicability of this approach to problems in the field of fisheries management has been appreciated. One of the fundamental problems in the effective management of both marine and anadromous species is a determination of the number of unit stocks which contribute to a mixed fishery, i.e., one supported by multiple breeding populations, and the identification of individuals representative of the contributory units. Given this information, it would be possible to estimate the relative contribution of the different spawning systems to the overall mixed fishery. This knowledge of population architecture provides the managers of this resource potential flexibility in establishing regulations, permitting conservation of threatened stocks and exploitation of those deemed of satisfactory size and health. Even exploitation of a mixed coastal fishery could be optimized to maximally harvest a component deemed in satisfactory condition, while simultaneously conserving the threatened component of the resource.

Since the concept of unit stock is predicated on the existence of discrete breeding units within a species' distribution, these isolated systems, given sufficient evolutionary time, will have accumulated either unique forms or frequencies of polymorphic genetic markers. These differing frequencies of genetic markers will result from either distinctive selective pressure, or from the differential action of random factors in the separate spawning systems. Thus, a genetic identification of discrete unit stocks is predicated on the existence of significant genotypic frequency differences between hypothesized populations. While protein electrophoresis has served as an effective genetic tool to identify population units for certain anadromous species (particularly Pacific salmon; see review in Utter et al. 1974), a lack of natural protein variation have precluded the adoption of this approach for most marine and anadromous species.

Enzymes, called Type II restriction endonucleases generally recognize specific 4, 5, 6, or 8 base pair sequences in the mtDNA molecules and cleave the molecule at or near one of these recognition sites. If the circular mtDNA has a single recognition site for a given restriction endonuclease the circle is cut and linearized. A single DNA fragment results. If two or more recognition sites are present, the number of restriction fragments generated will equal the number of restriction sites cleaved. The number of fragments produced is quantified, and the size of individual fragments determined via gel electrophoresis. Small differences (one or two base pairs) in the size of individual mtDNA fragments can easily be recognized given the selection of the appropriate electrophoretic gel porosity. Enzyme recognition sites, and thus the number and size of fragments generated may be either added or deleted by single base substitutions. If two fish representative of different breeding systems have identical recognition sites, the number and size of mtDNA fragments generated by the action of a single restriction endonuclease or a battery of these enzymes will be identical. However, given sufficient time, mutation and fixation can result in the accumulation of different recognition sites between reproductively isolated populations. If so, the number of enzyme recognition sites will differ, which will result in an alteration in the number and size of mtDNA fragments.

Restriction endonuclease analysis of mtDNA allows for a rapid sampling of base sequence conservation of random (not shown definitively) stretches of the mtDNA base sequence among individual fishes. The more restriction enzymes used the closer one gets to the sequencing the molecule. Obviously, the ultimate strategy would be to sequence the entire molecule, however, logistical constraints prohibit the adoption of this approach to even a modest number of samples. Almost, this same effect can be achieved by moderate use of four-or-five-base-cutting enzymes which sample a large portion of the mitochondrial genome per digest.

Intraspecific mtDNA diversity in fishes

To date this methodology has been applied to several taxa of fishes with varying results. Avise and his group (Bermingham and Avise, 1986; Avise and Saunders, 1984) have demonstrated substantial levels of mtDNA sequence divergence in both intra-and-interspecific comparisons in the sunfish genus Lepomis. However, sunfish populations probably constitute the only aquatic group in which mtDNA sequence evolution has proceeded at a rate comparable to that observed in most terrestrial vertebrate taxa (see reviews by Avise and Lansman, 1983; Brown, 1983; Brown 1985). Avise et al. (1986) were unable to differentiate between panmictic populations of American eels (Anguilla rostrata), however they were readily able to distinguish between American and European eels (Anguilla anguilla). In very small, but geographically widely isolated samples of skipjack tuna (Katsowonus pelamis), Graves et. al. (1984) found surprisingly low levels of sequence divergence. Both intra-and-interspecific investigations on various salmonid species have been undertaken by two groups of workers. Wilson et al. (1985) were able to uniquely identify via their mtDNA profiles, isolated conspecific populations of rainbow trout and steelhead (Salmo gairdneri) which differ greatly in life history. An interspecific comparison between rainbow and cutthroat trout revealed a nucleotide divergence value considerably less than observed in comparable primate comparisons. Finally, Birt et al. (1986) were unsuccessul in differentiating between anadromous and landlocked populations of Atlantic salmon (Salmo salar). Surprisingly, utilizing eleven restriction enzymes, these workers only reported a single base substitution separating populations which have been isolated for a minimum of 10,000 years with little or no opportunity for gene flow. Table 1 summarizes these mtDNA results in fishes.

In summary, except for Avise's work with sunfish, studies have indicated substantially lower levels of mtDNA divergence among comparable taxa in fish than reported in terrestrial vertebrate species comparisons. Most of these authors have invoked either recent divergence times or the ameliorating effect of migration to explain the lack of mtDNA genotype partioning based on geographic isolation. However, when considered jointly, these observations cast doubt on the applicability of Brown et al.'s (1979) substitution rate for the comparison of fish taxa. Does mtDNA of the lower vertebrates, or perhaps aquatic species diverge at a slower rate than that observed among primate species?

Goals of this Study

1) Do striped bass populations harbor geographically partioned mtDNA variation. Can restriction endonuclease analysis of mtDNA be used as an effective management tool to discrimate among striped bass of mixed ancestry in the coastal fishery?

2) To determine whether the coastal migratory stock is supplied with striped bass representative of distinct Mendelian populations. Do the populations of the three major spawning systems consist of significantly different gene pools, and therefore constitute recognizably different stocks? Protein electrophoresis studies conducted in the past have failed to answer this question.

3) Will a composite mtDNA restriction genotype reveal fixed differences among the three major spawning systems, and therefore permit unequivocal identification of ancestry of individual striped bass? If so what percentage of fish from each of the individual breeding systems will afford this opportunity?

4) Does the Chesapeake contain a single homogeneous population or do several different recognizable populations of striped bass inhabit the bay? If so, are these separate populations restricted to individual tributaries or is the Chesapeake divided into separate zones (i.e., lower vs. upper Bay), each of which support unique mtDNA genotypes?

5) Are there population subdivisions in the Hudson system? Although the potential for spatial isolation seems minimal, temporal staggering of distinct female spawning aggregates is possible. It is known that large spawning female striped bass spend only a very short time within the confines of the river, and therefore the potential exists for visitation by several distinct pulses of ripe females, each with individual female ancestral lineages. Based on significant differences in meristic counts for the 1954 and 1955 year classes, Raney (1957) has hypothesized on the possible existence of two separate populations within the Hudson.

6) Do spawning populations exhibit temporal stability in mtDNA genotype frequencies from year to year? Within a season is there temporal variation in mtDNA genotypic frequencies?

7) What is the extent of mtDNA sequence divergence among striped bass representative of the major breeding systems? How does this level of mtDNA variation compare to that reported in other terrestrial and aquatic species?

8) Are individual spawning systems marked by mtDNA sequence monomorphism or is each system occupied by organisms displaying a range of mtDNA genotypes?

9) Does the use of four and five-base cutters provide added resolution in stock discrimination not observed by the exclusive use of six-base cutters? Considering the low levels of protein variation observed in striped bass, and the concordance of relative levels of protein and mtDNA sequence variation reported in other organisms (Avise and Saunders, 1984; Kessler and Avise 1985a); the added sensitivity provided by four-base cutters seems necessary. Is all variation restricted to large fragments which can easily be generated via the six-base-cutter route, or do the small DNA fragments provide added information needed in stock discrimination. If six cutter digests provide sufficient discriminatory power this might permit the use of hybridization probes using frozen tissue as advocated by Gonzalez-Villasenor et al. (1986) for stock identification.

10) Does the Connecticut River support its own genetically distinct population of

striped bass? The Connecticut River has no known spawning population of striped bass, yet there is an annual run of subadults in the middle reaches of the river (Kynard and Warner, 1987). These fish have been observed and counted far upstream from the mouth of the Connecticut in fish lifts at the Holyoke Dam, Massachusetts, (river km 119) and the Turner Falls Dam, Massachusetts, (river km 172) during annual Atlantic salmon and American shad surveys. In 1980-82, 80-90% of these fish were age II with about 60% male (Kynard and Warner, 1987). Merriman (1941) hypothesized that at one time all major northern rivers had striped bass spawning activity, although no specific reference was made to the Connecticut River. Considering the small size of these fish, the limited mobility of subadult striped bass, the upstream distance of the fish lifts from the mouth of the Connecticut River, and the distance (~150 km) from the mouth of the Connecticut River to the closest known spawning system (the Hudson), the question arises as to the ancestry of these fish. Are these fish migrants from one of the major breeding systems or do they represent the descendants of a remnant Connecticut River population?

Protocol

Collections

Table 2 lists the dates and sites of striped bass collections. Adult striped bass (except for the Montauk coastal collection) were sampled at breeding times in the major spawning rivers. This was done to insure their being native to the rivers in which they were collected. An exception was the sample collected at Troy, New York during the month of July. Young-of-the-year and yearling striped bass obtained at various sites in the Hudson were assumed of Hudson ancestry due to the inability of southern stocks to undergo oceanic migrations till a later age (Kohlenstein, 1981). All fish (with the exception of Montauk samples) were transported to our laboratory alive and maintained in holding tanks until processing.

We have selected Montauk Point, New York as the site for coastal sampling based on several considerations. First, Montauk has long supported a significant sport and commercial striped bass fishery. Many different types of commercial gear (haul seines, trawling, pound nets) are employed over a long season (April-late November) offering the potential sampling of varied sizes and temporal contingents of fish. Second, even during periods of critical declines in striped bass landings, Montauk still offers sample availability. Third, Montauk is beyond the limits of the immediate confines of the New York Bight, and thus does not attract a disportionate share of Hudson River fish (Clark, 1968). Characterizations of individual fish for the Montauk collection are displayed in Table 3.

Standard lengths and sex determinations were made on samples where possible. It should be noted, however, that for various reasons it was not possible to sex all fish. Scales were retained for future ageing analysis from all fish. It should be noted that the vast majority of our Chesapeake samples are male. This was due to a lack of females at the time of our collections (resulting from the extremely small number and large size of Chesapeake females then available). While sex differences in mtDNA genotype frequencies have not been observed, we may have been sampling only single cohorts from an array of multiple contingents which comprise a single tributaries spawning aggregate. Ideally, collections would have been conducted over an extended period of time, sampling multiple age classes.

mtDNA Purification and Characterization

All mtDNA was obtained from freshly sacrificed striped bass. The details of the procedure are outlined in Wirgin et al. (manuscript submitted) and the next section of this study. Approximately 10 grams of tissue were homogenized per sample. Differential centrifugation was used to isolate a mitochondrial pellet which was further purified by application to a preformed sucrose gradient. Following mitochondrial lysis, the mtDNA was purified via multiple rounds of CsCl density gradient ultracentrifugation. This was followed by butanol extraction, overnight dialysis, alcohol precipitation, and resuspension in TEN buffer (10mM Tris, 1mM Na_2EDTA, 10mM NaCl; pH 8.0). mtDNA was stored at -20^0 until used for digests. Sufficient mtDNA was obtained from each fish for approximately 50 individual restriction digests

Digest conditions followed manufacturer's (Bethesda Reseach Labs, Boehringer-Mannheim Biochemicals; International Biotechnologies, Inc; and New England Biolabs) recommendations with the exception that digest times were extended to 4-6 hours. Approximately 5-10 ng of mtDNA were used per digest. Generally 50-100 times the recommended number of enzyme units were used for each digest to prevent possible partial digestions. Restriction digests were immediately followed by end-labeling, alcohol precipitation, and resuspension in loading buffer (TBE) and stop load (50% glycerol, 0.025% xylene cyanol, and 0.025% bromphenol blue. In total, 12 restriction enzymes were utilized in characterizing the mtDNA genotypes of these fish. Not all enzymes were used in screening all striped bass. However, a minimum of 6 restriction digests were performed on each fish.

mtDNA fragments were end-labeled in an ice bath with a cocktail of all four $\alpha^{32}P$ end-labeled nucleotides and visualized by autoradiography. All radionucleotides were purchased from New England Nuclear. Labeling was done using the large (Klenow) fragment of E. coli DNA polymerase I purchased from International Biotechnologies, Inc. Fragments obtained from most digests were aliquoted and separated on both agarose and polyacrylamide gels to visualize and score a range of fragments of widely different molecular sizes. Gels were dried prior to autoradiography. For all four and five-base cutter digests a minimum of two gels of different concentrations were utilized to maximize the percentage of the genome which could be effectively scored. Molecular sizes of all striped bass mtDNA fragments were determined by comparison of their relative mobility to the appropriate phage or plasmid DNA standards (*agarose gels*- λ-Hind III; or λ-Hind III-Eco RI ; *acrylamide gels*-λ-Hind III-Ecc RI; PBR-Pst I-Dde I; or θ174-Hae III).

Percentage of shared fragments between individuals was calculated by the procedure of Upholt (1977). An estimate of inter-individual mtDNA divergence per base (p) was also determined via the methodology of Upholt (1977).

Mapping Procedures

Relative cleavage sites on the striped bass mtDNA molecule for different restriction enzymes were determined by performing a series of double digests. In most cases single digests were followed by a round of alcohol precipitation to remove undesired buffer constituents before commencing second digests. Sites were assigned following the rationale outlined in Maniatis et al. (1982). Relative ordering of cleavage sites was determined from fragment lengths and numbers by an extensive series of single and double digests. Gels were organized so that the products of the appropriate single digests were run in adjacent lanes to those containing the fragments resulting from double digests. This permitted a determination of which fragments generated by single digests had restriction sites for the second enzyme. In total, 12 restriction endonucleases were used for mapping. mtDNA used for mapping was obtained from two striped bass collected off Rye, New York in the western Long Island Sound.

Dissociation and analysis of D-loop mtDNA

D-loop DNA is a non-covalently bound segment of DNA (500-1000 bases) which is complimentary to the heavy strand of vertebrate mtDNA (see Figure 1). The D-loop containing region is the only major non-coding sequence in the animal mitochondrial genome. It has been termed the control region of the mtDNA molecule by Attardi (1985) due to its role in the initiation of heavy strand replication and promotion of both heavy and light strand transcription. Sequencing of D-loop DNA in rats (*Rattus norvegicus* and *R. rattus*) has revealed the conservation of several domains that are capable of folding into cloverleaf structures believed of importance in the processes of mtDNA replication and transcription (Brown et al. 1986). Yet, despite its functional importance, the D-loop region is believed the most rapidly evolving segment of the mtDNA molecule.

D-loop DNA was isolated from three striped bass, each representative of one of the three different common mtDNA size classes. This was done to determine whether length variation in mtDNA could be localized to the D-loop region. Single stranded D-loop DNA ($\approx$20 ng) was thermally dissociated from double stranded mtDNA by heating at 80^0 C for 5 minutes (Brown et al., 1978). The separated single stranded D-loop DNA was then end-labeled with α^{32}P-dideoxy ATP using terminal transferase (2 units- New England Nuclear) following the methodology outlined by Yousaf et al. (1984). The labeled D-loop DNA was alcohol precipitated and resuspended in 90% formamide (Bethesda Research Labs) in 1 x TBE and 0.02% bromphenol blue and 0.02% xylene cyanol (Maniatis et al. 1982). The DNA was heated to 90^0 C for 5 minutes, quick chilled on ice, and immediately loaded on a 5% denaturing polyacrylamide gel containing 5 M urea. Gels were vacuum dried and autoradiography was performed. D-loop DNA sizes were estimated by comparison of relative mobilities to end-labeled, single stranded ø174 digested by Hae III and Hind III-Eco RI double digested λ DNA standards.

Results

General

Table 4-A-C depicts the mean size and number of striped bass mtDNA fragments obtained from the various restriction enzymes used in this study. Our strategy was to use several four and five base cutters because by producing multiple fragments, they would enable us to screen a sizeable proportion of the mitochondrial genome with a limited number of restriction enzymes. We have calculated a mean striped bass mtDNA size for the coastal stock based on all digests performed on the reference spawning stocks of 17,860 base pairs. In total, 155 fragments were visualized, representing 730 base pairs. This constitutes 4.1% of the entire striped bass mtDNA nucleotide sequence. Four-base-cutters produced a mean of 26 fragments, five-base-cutters 21 fragments, and six-base- cutters 5 fragments.

Base Substitutions

Despite the use of several four and five base cutters, base substitutions, as revealed by the addition or deletion of mtDNA fragments, were extremely rare among the population comparisons conducted. None of the six base-cutters revealed any base sequence divergence. The five base-cutters Ava II and Hin FI also failed to reveal any nucleotide sequence divergence despite generating a large number of mtDNA fragments (42). The four base-cutter Rsa I, revealed the greatest amount of base sequence heterogeneity among the individuals sampled. Four rare genotypes were observed, each in a single fish. All other striped bass displayed the common Rsa I genotype. Three of the unique Rsa I genotypes are exclusively found in the Rappahannock River while the fourth genotype was observed in a single fish from the Roanoke River. Taq I also generated a rare restriction genotype observed in only one fish from the Roanoke River. These five rare genotypes uniquely identify these individuals. It is interesting to note that all five rare genotypes are found in populations at the southern extreme of the range of the coastal migratory stock. Striped bass apparently harbor the lowest level of intraspecific mtDNA sequence diversity for any animal species surveyed in depth to date ($p=0.0004$).

Length Polymorphisms

Macro size variation

Five different lengths of the mtDNA molecule were observed in the populations surveyed. Each of these lengths differed by approximately 100 base pairs from the next largest size.

Therefore, the largest genotype was approximately 400 base pairs larger than the smallest.

All restriction enzymes revealed this length polymorphism with the exception of the six-base-cutter Xba I. Xba I generates a set of large comigrating bands which could easily mask 100-400 base pair variation in fragment length. Table 4-A-C lists the fragment number which displayed this molecular length variation for each enzyme. The difference in molecular size (kb) among the polymorphic fragments generated for each single enzyme digest was consistent for almost all enzymes used. In all cases this molecular length variation was seen in a single mtDNA fragment for each restriction enzyme used. With the exception of the four-cutter Mbo I, all alternate sizes of the polymorphic fragment were clearly seen between adjacent non-polymorphic fragments (see Figures 2 and 3 for representative digests depicting major length variants). For Mbo I, length variants appeared as doublets or comigrating bands. For the different length genotypes generated by Mbo I, therefore, different fragments appeared as doublets. This observation was confirmed via densitometry using an LKB Laser Densitometer Model 2222-010.

The fact that all length polymorphisms are recognizeable as single fragments suggests that the large additions or deletions of mtDNA responsible for this macro-size variation are all confined to a single region of the mtDNA molecule. All of these macro-size variants were visualized on agarose gels. The different classes of size variants were most easily scored, and molecular size determinations were most accurate when using four-or-five-base cutters. When using some six-cutters such as Pvu II, the polymorphic fragment was quite large (about10,000 bp), sometimes making it difficult to size the variants accurately.

Three of the length genotypes were common among the populations surveyed, while two were rare. The frequencies of the five genotypes in the various populations are presented in Table 5. Table 3 depicts the frequencies of major length genotypes in our coastal collection off Montauk, New York. It should be noted that all populations displayed intrapopulation mtDNA size heterogeneity. No single length genotype was fixed in any population. However, the frequencies of these length variants did differ signficantly among the individual rivers and overall systems surveyed. Table 6 provides a matrix of Chi Square values for all possible site comparisons. It should be noted that in many cases cell size for this analysis was very small.

Systems comparisons of macro-length variants

On the overall systems level, it may be seen that when comparing the Chesapeake total to the Hudson total, highly significant differences are observed. In addition the Chesapeake total also differs significantly from that observed in the Roanoke. However, no significant differences are observed when comparing Hudson to Roanoke totals suggesting a closer relatedness between these two systems.

Subsystem comparisons

If we divide the Chesapeake system into two major components; Upper Bay (C & D Canal and Upper Bay-Aberdeen) or (C & D Canal, Upper Bay-Aberdeen, and Choptank River) and Lower Bay (James River and Rappahannock River), we find that both of these Chesapeake subsystems considered independently differ significantly from Hudson totals. However, it should be noted that the magnitude of this difference is far greater when comparing Upper Bay to Hudson River totals. This observation suggests a closer relatedness between Hudson River and lower Chesapeake Bay stocks. Surprisingly, when comparing Upper and Lower Chesapeake Bay frequences no significant differences were observed. Similarily, no signicant differences were found when comparing Upper and Lower Chesapeake Bay frequencies independently to that observed in the Roanoke total.

Intrasystems comparisons

No significant differences were observed when comparing the various tributaries of the Chesapeake Bay. Similarly, the Hudson stock proved homogeneous regarding this length characterization. No significant differences were noted between consecutive year collections in the Hudson or Roanoke. Sample sizes were too small to make this comparison in the Chesapeake system. A more meaningful temporal comparison would have been to compare consecutive year classes from the same system.

Connecticut River derivation

mtDNA length frequencies in the Connecticut River were shown to differ highly significantly ($p < .001$) from those reported for the Chesapeake total precluding a Chesapeake origin for Connecticut River striped bass. With the exception of the James and Rappahannock Rivers in the lower Bay, all other single sites listed for the Chesapeake showed highly significant differences in mtDNA length frequencies when compared to the Connecticut

River. However, both the Hudson and Roanoke apparently share common genotypic frequencies with those observed in the Connecticut. It should also be noted that both the Hudson and Connecticut rivers share in common a rare length genotype which would suggest exchange between these two systems. Due to an absence of a unique Connecticut River length genotype; sharing of a rare genotype, and similar major length genotypic frequencies, migration of Hudson spawned fish to the Connecticut is postulated.

Micro size variation

One four-base cutter, Taq I, revealed micro-size variation in two small fragments visualized on 6% acrylamide gels. Frequencies of both of these minor length variants were easily recognizable and determined. Although both of these length genotypes were quite rare, they do prove useful in stock discrimination. Ten striped bass, of upper or mid-Chesapeake origin, displayed a unique Fragment # 17 for this enzyme (see Figure 4). Table 7 depicts the distribution of these Taq I fragments among Chesapeake sites. It should be noted that these microvariants were not found in either lower Chesapeake tributary; the James or Rappahannock rivers. Most importantly, these rare micro genotypes were also absent from the Hudson and Roanoke systems.

This rare fragment was 16 bp smaller than the common fragment we have observed to be 452 bp. Those striped bass exhibiting these unique micro size variants represented all three different major length genotypes. This suggests that this micro-length addition or deletion of DNA may map to a molecular site independent of the major length variation. Fortuitously, one of these Chesapeake striped bass possessed the largest major length genotype (1), common to Hudson, but only observed in two Chesapeake samples. Possession of this rare, but exclusively Chesapeake Taq I micro size variant, allowed for discrimation of this single Chesapeake fish from all Hudson samples.

In addition, one fish of Chesapeake ancestry also exhibited length variants in two other smaller Taq I fragments of 204 bp and 196 bp. These small length variants differed by 5 bp and 1 bp respectively from the common genotypes for these fragments. Despite this small size difference, these variant Taq I fragments were easily identifiable on the appropriate acrylamide gel concentrations. All of these micro-variants were unique to the Chesapeake system and therefore allowed for unequivocal identification of ancestry of their carriers.

They proved extremely valuable in stock identification.

Heteroplasmy

Heteroplasmy refers to the expression of more than one mtDNA genotype in a single individual. The additional forms of the mtDNA molecule must be in sufficient copy number to allow for their detection. It is generally agreed that the minority form of the molecule must be present at a level approximating 1-5 % of the majority form to achieve the threshold of detectability via current technqiues of DNA visualization (Avise and Lansman, 1983). Heteroplasmy may result from any one or a combination of three possible events; if a mutation occurred during development in a somatic cell line, if the prefertilized egg from which the individual fish developed was not fixed for a single form of the mtDNA, or if there is a paternal contribution to the developing egg which differs from the maternal form.

We have observed a substantial level of heteroplasmy for the major length variants in our study (≈20%). These individuals, in addition to exhibiting the normal complement of fragments for any given enzyme digest, also presented at least one extra DNA band. Some fish exhibited three heteroplasmic bands. This extra or heteroplasmic band(s) was always adjacent to the DNA fragment which revealed length variation among the populations surveyed. In all cases the molecular size of this extra heteroplasmic band corresponded to that of other length variants observed among other coastal striped bass surveyed. No heteroplasmy was observed for fragments resulting from base substitutions, highlighting the low frequency of generation of this form of mtDNA variation in striped bass. Densitometric analysis of heteroplasmic bands revealed absorption values ranging from 5% to almost 50% of adjacent non-heteroplasmic DNA fragments. In several cases it became necessary to use the densitometer to determine a length genotype for samples due to the similar DNA concentrations of the heteroplasmic and designated length genotype fragment. This observation of heteroplasmy was not restricted to a single spawning system. All three major spawning systems, the Connecticut River, and the coastal Montauk collection contained striped bass displaying this phenomenon. In fact, the incidence of heteroplasmy was greatest for the Montauk sample. See Table 8 for the frequency of heteroplasmy in the various populations. In all cases, samples which we called heteroplasmic displayed this phenomenon as expected for all restriction enzymes used, with the exception of Xba I and Mbo I. In addition, two samples showed more than one heteroplasmic genotype. No heteroplasmy was observed for base substitutions or minor length variants.

We have not used heteroplasmy as a tool in stock discrimination; however, there are frequency differences between populations. In the absence, of molecular data detailing the transmission genetics of the heteroplasmic state within the cellular population of individual fishes, we have no conception of the duration of time that this condition is maintained within individuals or between generations. Studies have recently been reported in crickets on the transmission genetics of heteroplasmy (Rand and Harrison, 1986), however, it would be inappropriate to extrapolate these results to striped bass. Given the frequency of heteroplasmy in adult striped bass; and the number of progeny produced from a single hatchery female, it does seem possible to conduct similar studies of heteroplasmy transmission in striped bass.

Cleavage mapping

In total, thirty-six restriction sites were mapped for the thirteen enzymes used in this segment of the study. It should be emphasized that not all sites have been mapped for all twelve of these enzymes. For enzymes such as Ava II and Taq I which generate a multitude of fragments, only those sites flanking the larger fragments have been mapped. Map positions are shown in Figure 5. The overall distribution of restriction sites was symmetrical, with all regions of the mtDNA molecule represented.

Sites of length variation

We did not attempt to map the site(s) of minor length variation. However, major length variation (100-400 bp) all mapped to a single region of the mtDNA molecule. By comparing those fragments which reveal major length polymorphism (Ava II-3, Eco R I-4, Hind III-4, Pvu II-1, Sac I-1) to our restriction map, it was discovered that major size variability was contained in a single region between map sites 1000 and 2900. We did not proceed to double digest mtDNA using the above enzymes with sites in this region, with the purpose of further localizing the site of length variation.

D-loop analysis

D-loop DNA has been reported in many vertebrate species (Brown, 1985). Its presence is revealed by a prominent bulging triple-stranded structure resulting from the synthesis of a short stretch (≈500-600 bp) of non-covalently bound single-stranded DNA complementary to the light strand (see Figure 1). D-loop DNA displaces the heavy strand in this region (Kasamatsu et al. 1971). The D-loop DNA is this short segment of single-stranded DNA believed to function as a primer in heavy strand DNA synthesis. The entire D-loop area has been termed the control region due to its presumed role in mtDNA replication and transcription (Attardi, 1985). The D-loop region is the site of many known major DNA additions or deletions resulting in mtDNA length diversity (Brown, 1983)

We were interested in characterizing D-loop DNA from striped bass representative of the different mtDNA size classes to determine whether they possessed differences in the size of their individual D-loop DNAs which could be correlated with their mtDNA length genotype. If positive correlations were found, this would provide strong evidence localizing the macro length variation to the D-loop region.

By running denaturing gels we observed three distinct classes of single stranded DNA molecules in all three samples. The two least mobile DNA bands of approximately 1370 and 940 nucleotides seemed to represent single DNA species. Although in both cases DNA bands were broad, each band appeared comprised of a single size class of single stranded DNA. However, the third, most mobile class consisted of a minimum of two distinctly different DNA sequences, differing in size by approximately 40 bases (670 to 633 nucleotides). We assumed that this third class of DNA bands was the D-loop DNA based on its size. All three striped bass samples showed identical banding patterns in this zone. Therefore, it did not appear that mtDNA major length variation could be mapped to the D-loop region.

Conclusions

Stock Identification

Mendelian populations, and therefore unit stocks are commonly identified by significant differences in genotypic frequences among sites sampled. Given sufficient time since their isolation, individual stocks will accumulate significant differences in their gene pools as a result of random factors or differential selective pressures. If hypothesized distinct stocks do not exhibit substantial differences in genotypic frequences, one plausible explanation is insufficient time since reproductive isolation.

Despite a wealth of protein studies addressing the problem of striped bass stock discrimination, to date none of these workers have demonstrated the existence of discrete unit stocks contributing to the coastal migratory stock (Otto, 1976; Grove and Powers, 1976). Morgan et al. (1973) claimed to be able to differentiate distinct populations among the various tributaries of the upper Chesapeake Bay by polyacrylamide gel electrophoresis of several protein products. However, subsequent work by Sidell et al. (1980) was unable to duplicate Morgan's results. A lack of detectable protein variation has precluded the demonstration of unique unit stocks. Protein monomorphism in striped bass is not soley restricted to the Atlantic coastal migratory stock. It is also observed among southeast (R. Wattendorf, pers. comm.), freshwater (Rogier et al. 1985), and Pacific populations of this species (Otto, 1976; Wirgin and Grossfield, unpublished data).

Based on isoelectric focusing of eye lens proteins, Fabrizio et al. (1985) and Saila et al. (1983) claim to be able to differentiate between representatives of these spawning systems. However, these workers were unable to find structural differences in these proteins among individuals surveyed. They did, however, observe significant differences in the concentration or expression of these protein products. The genetic basis for these protein concentration differences is not clearly understood. While these observed concentration differences may reflect frequency differences at underlying regulatory loci, an equally plausible explanation may be differential environmental or developmental cues impacting monomorphic regulatory loci. We have also examined eye lens proteins via isoelectric focusing in striped bass populations. No structural protein differences were detected. Observed concentration differences were correlated with age and eye lens weight of the individual fish (Wirgin and Grossfield, unpublished data).

Signficant differences in mtDNA major length genotypes have permitted us to differentiate between Hudson and Chesapeake stocks of striped bass. In addition, significant mtDNA genotypic differences are reported for Chesapeake versus Roanoke populations. This is the first demonstration of significant genetic differences between the heretofore hypothesized Mendelian populations.

However, in comparing Roanoke to Hudson samples we did not observe significant differences. Even given a larger sample size it is unlikely that this technique will reveal significant differences between the Hudson and Roanoke systems. This similarity between Hudson and Roanoke samples is not totally unexpected. Earlier meristic studies by Raney and DeSylva (1954) suggested a genetic affinity between Hudson and Roanoke stocks.

mtDNA as an Innate Genetic Tag to Uniquely Identify Individuals.

Base Substitutions

One pivotal question concerns the utility of this approach in identifying individuals of unknown ancestry in the coastal fishery. The rarity of detected base substitutions limits the applicability of mtDNA sequence diversity as an effective tool in stock discrimation. Rsa I and Taq I permit the detection of 3 unique variants in the Rappahannock River (21%) and 2 unique variants in the Roanoke River (9%) respectively. Obviously, the striped bass with these base substitutions may be uniquely assigned as to river of ancestry. The Hudson River did not support any striped bass with unique base substitutions.

Macro Size Variants

Until very recently, almost all mtDNA vertebrate population surveys estimating genetic distance recorded substantial levels of base substitutions (Brown, 1985). In addition, intraspecific vertebrate mitochondrial genomic size was thought to be quite stable with the exception of small additions and deletions (1-10 bp) reported in human populations (Cann and Wilson, 1983). Large scale interspecific variation in mtDNA length among closely related taxa, however, had been reported (Brown, 1983; Brown, 1985).

Surprisingly, several recent studies have reported considerable variability in the overall length of the mtDNA molecule within both invertebrate and vertebrate species. A single isofemale strain of a species of fruitfly; Drosophila mauritiana, did exhibit major polymorphism in mitochondrial genomic size. Two variants differing in size by approximately 500 bp were reported (Solignac et al. 1984). Harrison et al. (1985) have observed three discrete mtDNA sizes in conspecific populations of northeastern U.S. crickets. Bermingham et al. (1986) and Densmore et al. (1985) have reported multiple size classes of mtDNA in bowfin, tree frogs, parthenogenetic lizards (31 classes) respectively. While the crickets, bowfin, and tree frogs exhibited moderate levels of recognition site gain or loss in addition to length variation, the lizards revealed unexpectedly low levels of base substitution accompanying these length polymorphisms ($p= 0.006$). It should also be noted that two distinct models of length polymorphism were detected in the lizard work; continuous and discrete variation. Two classes of discrete variants were observed differing in molecular size by 35 bp. Twenty nine continuous size variants in a sample size of 92 individuals were reported with a maximum molecular size range of 370 base pairs. Interestingly, the existence of these two different classes of length variants (discrete vs. continuous) suggests the likely operation of more than one molecular mechanism responsible for the generation of length variation.

Our work with southeastern U.S. populations of striped bass has also disclosed extensive length polymorphism (four discrete classes) in the fish mitochondrial genome accompanying unexpectedly low levels of base substitution. These discrete size classes of length variants differed from each other by approximately 100 bp. In total, considering both the coastal and southeastern populations, we have detected six discrete classes of length variants in striped bass mtDNA each differing in size by approximately 100 bp. Both in our striped bass work and Bermingham et al.'s (1986) studies on bowfin, all demes investigated displayed intrasystem heterogeneity for length variants.

Major mtDNA discrete length variation does offer some potential for stock discrimination. Three easily identifiable length variants were commonly encountered in the reference populations surveyed in addition to the two rare variants reported. The largest of these three genotypes (1) was observed in 32% of Hudson River striped bass. This A genotype was noted in two (3%) Chesapeake Bay striped bass: one from the Choptank River and one from the C & D Canal. Due to micro-variation in a Taq I fragment, however, the single sample from the Choptank River was unequivocally distinguishable from all Hudson River striped bass. Therefore, all Hudson River striped bass possessing this genotype (32%) were distinguishable from all Chesapeake Bay striped bass except for the single specimen from the James River.

In addition, two (3%) of Hudson River striped bass displayed a rare large length genotype (1-A) also noted in a single Chesapeake sample from the Rappahannock River. However, the single Rappahannock striped bass bearing this genotype was uniquely identifiable due to its possession of the rare aforementioned Rsa I base substitution not observed in the two Hudson River fish. By scoring all major length variants a total of 35% of

Hudson River striped bass were uniquely identifiable from all Chesapeake samples except for the single specimen from the C & D Canal.

. It should be noted that differences did exist in the frequencies of one of the other two major classes of length variants (2 & 3) between the Hudson and Chesapeake stocks. The second largest common genotype, (2); was observed in 57% of Chesapeake striped bass while 33% of Hudson striped bass displayed this genotype. The third common genotype, (3); was seen in about equal frequency between Chesapeake and Hudson samples; 33% and 32% respectively. If we limited our focus to the relative frequencies of these two genotypes (2 and 3) in the Upper Chesapeake Bay (Aberdeen and C & D Canal) to Hudson River, we found more dramatic differences than when considering the Chesapeake Bay in total. We have reported frequencies of 71% and 25% for the (2) and (3) genotypes respectively in the upper Chesapeake Bay. Although these frequencies differ considerably from those observed in the Hudson (33% (2), 32% (3)), these differences are not significant (Chi square value=2.211, df=1).

In comparing Roanoke to Chesapeake samples, major length variation allows us to uniquely identify 17% of Roanoke striped bass. If we include rare Rsa I and Taq I base substitution variants exclusively detected in Roanoke collections, 26% of Roanoke striped bass may be uniquely identified in this mixed sample.

When comparing Roanoke to Hudson River striped bass, major length variation is of little ultility in stock discrimination.

Micro Size Variants

Small additions or deletions in the mitochondrial genome of humans (Aquadro and Greenberg, 1983 and Cann and Wilson, 1983) and domestic cattle (Hauswirth et al. 1984) have been documented. However, as reviewed by these workers, small addition and deletion events in humans were about one-fifth to one-half as frequent as base substitutions. In addition to major length variants, Densmore et al. (1985) also observed occasional minor length variants (1-9 bp) among lizards. This mode of mtDNA evolution has not been reported in any fish mtDNA studies to date.

Despite running relatively high concentration polyacrylamide gels (6-8%) in our southeastern striped bass study, micro size variants were not detected.

In this current study, small addition/deletion events were detected at two Chesapeake spawning sites at moderate frequencies by the enzyme Taq I (see Table 7). These micro size variants were viewed in three different Taq I fragments. One sample exhibited two Taq I fragments displaying microvariation. These variants differed by 1 to 16 base pairs from the common size reported for these fragments. Despite the use of several other four-and five-base-cutters, no other restriction enzyme yielded fragments displaying these small differences. This is not unexpected, however, unless the sequence containing these small added/deleted DNA tracts is found in a small restriction fragment (10-300 bp), there is little chance of its being detected. It is conceivable that other small addition or deletion events may have occurred at other isolated sites throughout the striped bass mitochondrial genome. The use of a battery of other four-cutter-enzymes could result in the detection of further microvariants, thereby improving the resolving ability of this approach.

Micro-size variants were only observed in the Chesapeake Bay. In total, 19% of Chesapeake striped bass exhibited unique Taq I micro variants. These Taq I micro-genotypes were reported in 36% of Upper Bay (Aberdeen) and 60% of Choptank River samples. No micro variants were detected in the two lower Chesapeake Bay rivers sampled; the James and Rappahannock rivers. Surprisingly, despite close geographic proximity, we also failed to observe this genotype in any of our samples from the C & D Canal. In total, 48% of Choptank River and Upper Bay (Aberdeen) striped bass sampled displayed this genotype. Scoring Taq I micro-variants can aid in distinguishing Upper Bay and Choptank River vs. lower Chesapeake Bay (James and Rappahannock) striped bass. By using loglinear analysis of multiway frequency tables as outlined in the BMDP statistical software package (M. Brown, 1985), it was possible to take into account both macro-and-micro variation in differentiating populations. Based on a combination of both of these criteria we were able to distinguish upper from lower Chesapeake Bay fish. Both the Hudson and Roanoke Rivers failed to yield any micro variants. Thus, this micro genotype could be used to uniquely identify individual Chesapeake Taq I micro variants from all Hudson and Roanoke Rivers striped bass.

Micro variants were observed in representatives of all three common classes of major length variants. A similar distribution of micro variants among major length variants in lizards was reported by Densmore et al. (1985). This suggests that the major and minor DNA addition/deletion events occurred at different sites in the mtDNA molecule. It also suggests that the frequency of events generating mtDNA length variation in striped bass must be quite high.

Cleavage mapping, sites of length variation, and D-loop analysis

Our observation of symmetrical restriction site distribution in striped bass mtDNA agrees with that reported in the only mtDNA fish map published to date, for the common killiefish, Fundulus heteroclitus, by Gonzalez-Villasenor et al. (1986). Only Densmore et al. (1985) have reported asymmetry in the distribution of mtDNA restriction sites, that occurring in several species of lizards in the genus Cnemidophorus. This finding of symmetry is significant when adopting various statistical approaches in analyzing restriction data for the purpose of quantifying mtDNA sequence divergence between taxa. All approaches assume randomness in restriction site distribution, and therefore estimates of mtDNA divergence based on nucleotide differences at restriction sites should reflect the degree of sequence similarity found throughout the entire mitochondrial genome. A clumping of restriction sites would not provide random sampling of the complete genome and therefore bias divergence estimates. This map should provide us with a tool to probe the striped bass mitochondrial genome for the localization of the various known mitochondrial genes.

We have mapped the major length variation in striped bass mtDNA to a single region of the molecule between map site units 1000 and 2900. Most inter-and intra-specific macro-size variation in mtDNA reported to date has been localized to the D-loop region (for reviews see Brown, 1983; Brown, 1985; and Attardi, 1985). However, our analysis of isolated D-loop DNA from individuals of the different mtDNA size classes failed to show variability in the number, sizes, or concentrations of their D-loop DNA bands. Although multiple species of D-loop DNA were observed, no differences between individuals were detected. Thus, our major length variation does not appear to be within the D-loop region. Densmore et al. (1985) and Brown et al. (1978) also failed to reveal differences in the length or number of D-loop DNA bands among conspecific individuals (in parthenogenetic lizards and humans respectively) using an identical approach. These workers, however, did report differences in the relative concentration of each D-loop size class among conspecific individuals. Clayton (1982) has hypothesized that D-loop DNA may have multiple sites for the initiation and termination of its synthesis within an individual organism. Thus, D-loop DNA's of different sizes may be produced by the use of different combinations of these sites. This may explain our detection of multiple size classes of D-loop DNA.

Mode of mtDNA evolution in striped bass

One of the most striking features in these population comparisons is the acute lack of base substitutions encountered, in contrast to the unusually high frequency of length variants. In addition, heteroplasmy was routinely detected. This combination of mtDNA characteristics to date has only been seen in parthenogenetic lizards. What hypotheses may be advanced to explain these unique findings?

1) Marine and estuarine organisms generally display lower levels of genetic discontinuity and polymorphism than that observed in terrestrial and freshwater systems.

Gyllensten (1985) has reviewed the extent of protein polymorphism in freshwater versus anadromous and marine species of fishes. He has pointed out that the severe genetic discontinuities in allelic frequencies traditionally encountered among populations of freshwater species, are generally absent in anadromous and marine systems. He has indicated that the vast majority of protein polymorphism exists within systems, rather than between them. This analysis suggests that both anadromous and marine species of fish harbor levels of protein polymorphism comparable to that observed in freshwater systems. Yet, the effects of mixing resulting from some level of migration between spawning systems precludes a buildup of significant allelic frequency differences.

In accord with this analysis, though, using mtDNA as a genetic marker, Bermingham and Avise (1986) and Avise et al. (1984) detected substantial genetic differentiation of several southeastern U.S. freshwater sunfish and bowfin populations. It is interesting to note that earlier protein studies on sunfish populations revealed comparably high levels of polymorphism. (Avise and Smith, 1974). A mtDNA analysis of freshwater rainbow trout populations displayed larger sequence divergence values than observed among populations of steelhead which are anadromous members of this same species (Salmo gairdneri) (Wilson et al. 1985). Therefore, it would appear that freshwater species of fishes display more genetic discontinuity than observed within the anadromous or marine fauna. This is to be expected in terms of potential isolating mechanisms, however, this does not explain an overall lack of genetic polymorphism within a species.

In contrast, this current study, our other report on southeastern U.S. striped bass populations, and the work of Graves et al. (1984) on skipjack tuna highlight the absence of mtDNA sequence diversity among the individuals examined. Despite sampling respresentatives of two oceans, Graves et al. (1984) were unable to distinguish between Atlantic and Pacific populations of skipjack tuna. Gene flow around Cape of Good Hope may be weakly hypothesized to explain a lack of genetic differentiation between the two oceans. Avise et al. (1986) also reported a lack of mtDNA differentiation among American eel populations which contrasted sharply with the protein data for the same species supplied by Williams and Koehn (1984). While Avise et al. (1986) argued that mtDNA monomorphism in eels resulted from panmictism (all populations sharing a single spawning site), it is difficult to reconcile mtDNA monomorphism with abundant levels of protein polymorphism. Perhaps in this case the two genomes (mitochondrial and nuclear) experienced separate selective pressures or stochastic histories. In summary, marine and anadromous fishes apparently harbor among the lowest levels of base sequence diversty in the mitochondrial literature to date. This report on striped bass populations is even more startling in view of the screening of an unusually large number of mtDNA fragments for variants by the use of four and five-base-cutters.

For this discussion it is most interesting to note that not only was it impossible to differentiate among populations by scoring base substitutions, however, most surprisingly, little or no mtDNA polymorphism was detected even within populations. Obviously, it is impossible to genetically distinguish among populations if polymorphisms are non-existent. An absence of genetic differentiation between populations may be explained on the basis of gene flow or migration among populations.

What factors may be responsible for reduced levels of genetic polymorphism within a species? These factors either must affect the mutation rate, or the rate at which these mutations are fixed in the populations. Specific factors to consider might include; a reduced mutation rate, recent origin of the species or a severe population bottleneck, small population size, or purifying selection for a single best fit genotype. Are any of these factors particularly operative in the marine and anadromous environments to lower levels of observed genetic variation? At this point, it would be difficult to dismiss any of these factors as potentially responsible for reduced levels of mtDNA polymorphism.

In contrast, Saunders et al. (1986) detected a major break in mtDNA clonal diversity distinguishing Florida Atlantic and Gulf populations of horseshoe crabs (Limulus poylphemus) from those inhabiting the more northern tier of its North American distribution. Yet despite the acknowledged "great age" of this species, very little mtDNA sequence diversity was noted within the two reported "clones". These workers advanced "deterministic evolutionary forces" such as temperature constraints, differential major ocean currents, and the limited motility of horseshoe crabs as a possible explanation for the observed major mtDNA frequency break. However, these authors posed an intriguing, but equally plausible hypothesis to explain the observed mtDNA frequency discontinuity in the absence of deterministic forces, and therefore solely reliant on the operation of stochastic factors. Using this stochastic model with limited gene flow, computer simulations were able to generate mtDNA frequency breaks similar to those observed in the horseshoe crab populations.

In summary, in describing distributions of mtDNA genotypes in populations of marine and anadromous fauna, depressed levels of mtDNA sequence variation would seem the rule. While mixing certainly would explain a lack of population differentiation, it does not explain the lack of mtDNA sequence polymorphism. As demonstrated by Saunders et al. (1986), given sufficient mtDNA polymorphism in the presence of limited gene flow, even marine species displaying continuously distributed populations may develop major mtDNA genetic breaks.

2) Phylogenetic considerations: the lower vertebrates in general, and fish in particular, display lower levels of mtDNA polymorphism and greater levels of length variation and heteroplasmy than observed in the higher vertebrates.

Sequence divergene

Virtually all comparisons of mtDNA in higher vertebrates have yielded generous estimates of sequence diversity (see reviews in Avise and Lansman, 1983; Brown, 1983; Brown, 1985). It was generally accepted that mtDNA sequence divergence in primates exceeds that observed in the nuclear genome by approximately an order of magnitude. Avise and Lansman (1983) have commented that mean values of nucleotide sequence divergence between conspecific terrestrial individuals generally range from 0.3 to 4%; and on occasion approach 10%. Similarly, Kessler and Avise (1985) reported mean mtDNA nucleotide differentiation even among highly conservative avian congeners ranging between 3 and 6%. Despite a great conservation of mitochondrial genomic content and organization, the proliferation of base sequence diversity must prove either neutral or beneficial among the higher vertebrates.

However, with the exception of Avise et al.'s (1984), Avise and Saunders (1984), and Bermingham and Avise's(1986) studies on sunfish populations, conservative estimates of mtDNA sequence variation have been the rule among populations of fishes (see Table 1 and Table 15). Based on the results included in these tables, we have calculated mean mtDNA nucleotide sequence divergence values for conspecific and congeneric comparisons as 3.3 and 20.4%. However, if we exclude sunfish from these calculations, mean intra-and-interspecific values drop to 0.5% and 5.2% respectively. This conservative trend certainly is evident among all striped bass populations surveyed where our value of conspecific mtDNA diversity was $p=0.0004$

The question arises as to the applicability to the lower vertebrates of Brown et al.'s (1979) molecular clock calibration for mtDNA sequence divergence of $p=0.02$ derived exclusively from primate data. Intertaxa differences in nuclear genomic base substitution rates have recently been reported between rodents and man. Findings by Wu and Li (1985) have disputed the universality of the rate-constancy hypothesis of molecular evolution initially proposed by Zuckerkandl and Pauling (1965). These workers found evidence for higher rates of nucleotide substitution in rodents than in man in nuclear coding regions for eleven genes, resulting from elevated mutation rates. Wu and Li (1985) speculated that an increased mutation rate in rodents could be attributed to a shorter cellular generation time, and thus more rounds per unit of time of potentially mutagenic replication. Britten (1986), in reviewing the mass of literature describing the extent of interspecific nuclear genomic divergence using either DNA sequence data involving neutral changes or from DNA-DNA hybridization studies, concluded that the rates of DNA sequence evolution differ significantly between taxonomic groups. He observed that the rate of DNA sequence change decreased markedly during primate evolution, and that this slowdown was not attributable to dynamic population histories of periodic bottlenecks and explosions; or gene flow between small and large populations. Instead, Britten (1986) hypothesized that a reduction in the mutation rate resulting from an alteration in DNA replication, repair, or transposition mechanisms was responsible for this reduced rate. Unfortunately, while it is known that the mitochondrial genome undergoes multiple rounds of unedited and unrepaired replication per cell cycle (Rabinowitz and Swift, 1970), it is not known whether the number of these rounds of replication are constant across diverse taxa.

The non-universality of a single molecular clock in comparisons among all taxonomic groups has very recently been extended into mtDNA sequence studies by Vawter and Brown (1986). These workers reported similar levels of single copy nuclear DNA and mtDNA sequence divergence in interspecific comparisons between species of sea urchins. This contrasted with the much enhanced levels of mtDNA divergence when compared with the nuclear genome encountered in vertebrate species. From these results it is clear that the rate of change of these two distinct subcellular genomes is under separate control, and that the mechanisms responsible for these rates may differ on a taxon by taxon basis Thus, it may be necessary to define boundaries for accepted molecular clocks for each of the two genomes.

The rate of mtDNA base sequence change in the lower vertebrates appears lower by an order of magnitude when compared to that observed in higher vertebrates. Birt et al. (1986) reported no mtDNA variation between allopatric anadromous and landlocked populations of Atlantic salmon (Salmo salar) despite a minimum divergence time of 10,000 years. Only a single base substitution was reported among the individual salmon examined. Both this current study and our past work with southeastern striped bass populations, in combination with Densmore et al.'s (1985) thorough analysis of mtDNA transmission in populations of parthenogenetic lizards suggests an alternative mode of mtDNA evolution in the lower vertebrates. Certainly, a much slower rate of individual base substitution is observed (p=0.004-0.006). This observation suggests that selection may be operating on the vast majority of the base sequence to preserve an already "best fit" genotype. While mutational changes in the mtDNA molecule are thought to be selectively neutral (Brown ,1983), the maternal inheritance of the molecule provides a potentially excellent vehicle to transmitt a tightly linked, selectively advantageous genotype (Saunders et al. 1986).

Length variation

Both our striped bass studies, and the work on lizard populations report a division of mtDNA genotypes into both discrete (striped bass & lizard) and continuous (lizard) major length classes. Bermingham et al. (1986) also reported length polymorphisms in treefrog and bowfin populations although the types or number of classes of variants is not clear from their report. While this major length variation has been mapped to a single site in the striped bass mitochondrial genome; two adjacent sites in the control region or D loop of the lizard genome have been identified as targets for these major additions and deletions. We have not determined whether this single site in the striped bass molecule lies immediatedly adjacent to the D-loop region. While Densmore et al. (1985) have pointed to the correlation of a background nuclear triploid condition in the parthenogenetic lizards as possibly signficant in the generation of major mtDNA length variants, this obviously is not the case in the striped bass, bowfin, or treefrogs which all have diploid genomes. Does the possession of major length polymorphisms confer any selective advantage to these species? Considering their presumed location in the non-coding control region; site of initiation of DNA replication and transcription, it is difficult to conceive of any advantage which they may bestow.

In addition, it should be noted that both the lizards and striped bass experienced minor addition/deletions events at other genomic sites. While such events have been reported in comparisons of human populations (Aquadro and Greenberg, 1983 and Cann and Wilson, 1983), their frequencies only approached one-half to one-fifth that observed for base substitutions among these conspecific populations. Between striped bass populations the number of different addition/deletions events exceeded the frequency of base substitutions. Similar findings were reported for lizard populations. While this finding is unique to these studies, it should be noted that perhaps the execution of exhaustive methodologies in both these studies contributed greatly to the uncovering of these minor variants.

Heteroplasmy

Finally, both the lizards and striped bass exhibited substantial levels of heteroplasmy (10-20%). Rand and Harrison (1986) have recently reported similar levels (15%) of heteroplasmy for length variants among cricket populations supporting earlier reports for this condition among invertebrate populations of Drosophila mauritiana (Solignac et al. 1983; Solignac et al. 1984). This condition had been anticipated in animal populations as a forerunner to interindividual variation (Avise and Lansman, 1983), but until recently had gone undetected among conspecific vertebrate comparisons. An absence of heteroplasmy in the presence of substantial levels of sequence variability would suggest that the fixation of novel mitochondrial lineages is a very rapid process. Considering the large number of higher vertebrate conspecific comparisons contained in the literature not reporting this condition (with the major exception of domestic cattle; see Hauswirth and Laipis, 1982; and Hauswirth et al. 1984), the recent frequent observation of this condition in invertebrate and lower vertebrate populations suggests a significant difference in the mode of mtDNA evolution within these taxa. Not surprisingly, heteroplasmy for both lizards and striped bass is evidenced for length variants. Considering the frequency of molecular length polymorphisms encountered in these populations, the observation of this form of heteroplasmy conforms with earlier expectations of cellular mtDNA heterogeneity preceding population polymorphism. It also suggests a lower mutation rate for base substitutions than size variants.

Models have been devised examing the transmission genetics of mtDNA, both within cell lineages and between animal generations (Chapman et al. 1982). Additionally, Rand and Harrison (1986) have recently reported experimental results on the transmission of heteroplasmy between two generations of crickets. Heteroplasmy can initially arise from two different sources; either from mutations in a female lineage or from a distinct paternal contribution to the zygote (assuming the existence of paternal leakage). It has been suggested that a signficant selective advantage for one heteroplasmic genotype (Hauswirth and Laipis, 1982) or differential amplification of mtDNA molecules during oocyte development (Piko and Matsumoto,1976) could explain rapid genotypic shifts. However, if selection does not operate to maintain this heteroplasmic state, then a series of stochastic factors control the ultimate frequencies of the multiple mtDNA forms in subsequent cell cycles, and therefore the transmission of the heteroplasmic state to the next generation. These random factors include; the mutation rate for mtDNA (base substitutions versus length variants), the effective mtDNA population size in the cell (based on the number of mitochondria per cell and the number of DNA molecules per mitochondrion), and finally the number of cell generations per animal generation. Clearly the larger the cellular population of mtDNA molecules, or the smaller the number of cell generations per animal generation, the greater the probability of transmission of heteroplasmy to the next generation. Rand and Harrison (1986) have reported that fixation or loss of heteroplasmy in individual crickets would require 120-510 animal generations. This prediction based on empirical results obtained from two generations assumes a random drift model. These results agree quite closely with Attardi's (1985) prediction of 20 to 200 animal generations to obtain a pure mtDNA population from a mixed state. Although mechanisms have been proposed to explain the generation of major length variants in lizards (Densmore et al. 1985), experimental data is lacking. Most importantly, no estimate exists of a mutation rate for length variants. Considering the limited number of classes of length genotypes observed in striped bass, it is possible that individual mutant length genotypes may be introduced in a single fish or lineage multiple times.

Once again, it is not clear what advantage or disadvantage possessing multiple forms of the mtDNA molecule would confer. Obviously, heteroplasmy at some level must precede mtDNA diversity in a population. The maintenance of such a high frequency of heteroplasmic individuals within populations of lower vertebrates suggests that this condition may not be purely transitory, but is in fact characteristic of these taxa. If this were true, then heteroplasmy would mark all descendants of certain ancestral maternal lines for generations. Given the absence of selective pressure against this condition, the percentage of heteroplasmic individuals within fairly large populations would remain relatively constant over time. If this were the case, then heteroplasmy could then be used as a genetic marker to uniquely identify striped bass carrying this innate tag. However, in our current analysis we have refrained from using heteroplasmic frequency differences as a tool in population discrimination. While Rand and Harrison (1986) have estimated the retention of heteroplasmy for crickets as hundreds of generations, similar estimates for fishes are currently lacking. A survey of heteroplasmic conditions over several generations in a species of fish would be required before an estimate of fixation or loss rates could be achieved. Considering the number of striped bass hatcheries currently in operation and the incidence of heteroplasmy in this species, it would be logistically possible to follow the dynamics of heteroplasmic transmission between animal generations with large numbers of progeny available. By quantifying the extent of variation of heteroplasmy among progeny, an estimate of the number of generations to fixation (or elimination) of the heteroplasmic state could be obtained. This approach was used by Rand and Harrison (1986) with cricket populations.

In summary, characterizing conspecific comparisons of some mtDNA sequences in lower vertebrates we note the following novel features generally not encountered in the higher vertebrate taxa: extremely low levels of base substitution, moderate amounts of small addition/deletion events, division of mtDNA genotypes in a species into either numerous continuous or discrete major size classes, and finally elevated levels of heteroplasmy. If we examine the results of either congeneric or confamilial comparisons of mtDNA evolution in these same lower vertebrate taxa are similar trends apparent? In congeneric and confamilial mtDNA comparisons in the salmonid fishes Berg and Ferris (1984) and Wilson et al. (1985) reported sequence divergence values ranging between 2% (rainbow-cutthroat trout) to 10% (brown-brook trout). These diversity figures are rather conservative when contrasted to those obtained for closely related primate species (9% to 19%; Brown, 1982). Our work with the other members of the genus Morone suggests moderate levels of interspecific sequence diversity.

It is apparent that substantial levels of mtDNA sequence divergence have been generated between congeneric and confamilial species of fishes, however, at a much slower rate than detected among primate comparisons. The possibility must be considered that the rate of mtDNA change is not constant throughout the evolution of lower vertebrate taxa. The evidence suggests that mtDNA sequence change proceeds much more rapidly at the time of adaptive radiation. This period of rapid change is then followed by an extended interval of stabilizing selection.

3) Reasons inherent to the life history of striped bass.

Several factors inherent to the biology of striped bass serve to foster decreased levels of genetic diversity. Despite the publication of a vast number of studies conducted to explore the extent of structural protein diversity among striped bass populations (Otto, 1976; Grove et al. 1976; Berggren and Lieberman 1978; Sidell et al. 1980; Rogier et al. 1985; Saila et al. 1983; Fabrizio et al. 1985; Wirgin and Grossfield, unpublished data), little or none has been found. Our work using mtDNA further extends this finding. It has been shown that relative levels of mtDNA base sequence diversity generally follow that found in protein variability studies (Avise and Saunders, 1984).

In evaluating reasons for this paucity of genetic variation in striped bass perhaps an examination of pertinent aspects of the life history of this species will provide some insight into this genetic monomorphism:

Drastic fluctuations in population size.

The older literature (Merriman, 1941; Raney, 1952; and Koo, 1970) and observations on the current situation (Boreman and Austin, 1985) both highlight the historical drastic changes in abundance experienced by the striped bass populations comprising the coastal migratory stock. Historically, this fishery has been primarily supported by recruitment from unusually large year classes produced at intervals ranging from seven to an undetermined number of years (at least for the Chesapeake component). As these large year classes age, obviously the number of individuals comprising their reproductive populations decline dramatically. In addition, males dominate the spawning grounds. During periods of relatively large abundance (1974-1975), Wilson et al. (1976) reported a male to female ratio of approximatley 4:1 during spawning on the Potomac River. Currently, with population sizes in the Chesapeake drastically reduced, the ratio and actual numbers of spawning females on the various tributaries of the Chesapeake is greatly diminished (personal observation). Therefore, it is possible that a very small number of unusually old females are currently responsible for year class production in each tributary. Given that mtDNA is strictly maternally inherited, and therefore only the number of spawning females need be considered in determining effective population size, it is conceivable that presently and in the distant past, small population size has dramatically reduced the pool of mtDNA diversity. If this "flushing" effect occurs at frequent intervals over a species evolutionary past, it serves to compound the elimination of genetic diversity.

Striped bass are a relatively new species.

Given the uniformly low levels of protein polymorphism presented above, in conjunction with the paucity of mtDNA sequence divergence, striped bass may represent a recently evolved species, in which insufficient time has elapsed since the speciation event for the buildup of substantial genetic variation. Based on our calculated maximum diversity value in striped bass mtDNA of $p=0.0004$, maximum divergence time of striped bass populations from a common ancestor would be postulated as 20,000 years. Critical to this argument is the overall paucity of genetic diversity within this species, rather than the lack of geographically partitioned variation. An absence of significant genetic differences could be explained by the effects of the most recent glacial event (12,000 years ago). Of course, the validity of this hypothesis is dependent on the applicability of the base substitution rate observed in primate comparisons to the lower vertebrates. Kessler and Avise (1985) have proposed the same argument, that parallel decelerated rates of mtDNA base subtitution and small protein-electrophoresis distance values among selected avian genera is compatible with the theory that avian taxa have relatively recently shared common ancestry. Two independent genomes; contrasting greatly in inter-and-intragenic structure, employing autonomous replication and transcription systems, differing in repair ability and functional constraints, still display extremely low concordant levels of genetic polymorphism within particular taxa. This is a strong argument in favor of recent speciation in striped bass.

However, Brown (1985) has advanced an interesting, yet unproven counterargument that should be considered. The rate of substitutional events in the mitochondrial genome may not be independent of control by the nuclear genome. The rate of mtDNA evolution may be dependent on the rate of nuclear DNA change. Therefore reduced nuclear genomic variation may impact the mitochondrial genome. In view of the import of numerous nuclear encoded gene products into the mitochondrion, there is considerable interaction between these and the mitochondrial genome. Considering the hypothesized priming of mtDNA replication by RNA, and the nuclear coding for RNA polymerase, genomic polymorphism resulting in diversity in this enzyme could alter the rate of mtDNA change. Without molecular data on the presumed interaction between the two genomes it is impossible to evaluate the validity of this attractive alternative.

Sex ratio of the coastal migratory stock.

When Chesapeake contributions to the coastal stock were high (about 90%; Berggren and Lieberman, 1978) 85 to 90% of the migrating segment was female (Schaefer 1968a, 1968b, and Holland and Yelverton 1973, Oviatt, 1977). In addition, few young males leave Chesapeake Bay (Kohlenstein, 1981). This predominance of migratory females in the coastal fishery, in conjunction with the exclusively maternal transmission of the mtDNA molecule presents the opportunity for considerable mtDNA gene flow among populations if the homing ability of striped bass is less than perfect. To date the accuracy of striped bass homing for most of the major spawning rivers has not been quantified.

Synopsis of reasons to explain the paucity of genetic diversity in striped bass

In summary, it would appear that no single reason may be advanced to explain the unique mode of mtDNA evolution in striped bass. In our opinion three distinct phenomenon are working in concert to produce this observation. First, mtDNA sequence change in the lower verebrates is probably slower than that observed in the primates. The only exception to this hypothesis is Avise's work with sunfish. All other lower vertebrates exhibited reduced levels of mtDNA diversity, although not of the same magnitude as observed in striped bass. Second, elevated levels of mtDNA length variation and heteroplasmy (with the exception of herds of dairy cows) seem to be restricted to the invertebrates and lower vertebrates. However, even at this level, intertaxa differencess in susceptibility to these events are likely. It may be argued that the discovery of heteroplasmy and minor length variation may be more a function of the abilities of the different laboratories to discern these molecular changes than a reflection of differences in biology. Yet workers in Avise's lab reported heteroplasmy and length variation in bowfin, but none was observed in hundred of sunfishes. As added proof we have observed both heteroplasmy and length variation in all of the species in the genus Morone. Third, the recent evolutionary past of the striped bass probably contributed to its paucity of genetic variation. Both mtDNA and protein data reinforce this hypothesis. Once again, if we look at all other Morone species, moderate levels of mtDNA sequence divergence are observed. This suggests that the deceleration of evolutionary change detected in striped bass is a result of historical events which have occurred after radiation of the genus.

III. Mitochondrial DNA Analysis of Southeastern Striped Bass Populations

Endemic Southeastern Striped Bass Populations

Atlantic coast systems

Endemic populations of striped bass in the southeast (excluding North Carolina) have historically received little attention, perhaps due to the localized nature of their small to moderate sized sport and commercial fisheries. Atlantic populations of striped bass extend as far south as the St. Johns River in northern Florida. Many of the major rivers in South Carolina, Georgia, and extreme northern Florida still support small, yet robust riverine populations of striped bass (see reviews by McLane, 1958; McIlwain, 1980). The absence of oceanic captures supports the hypothesis of a non-migratory behavior of these populations (Dudley et al. 1977).

The Florida Fresh Water Fish and Game Commission has allocated considerable resources to study and maintain the endemic striped bass population in the St. Johns River. Despite the impact of significant fluctuation in population size, in part due to the inacessablity of fish to traditional spawning areas in tributary streams, a small natural population has been maintained in the St. Johns. Part of the Commisssion's effort has centered on restocking the St. Johns River as described in Table 9. Initial stockings were progeny of broodstock obtained from the Nassau River in northern Florida and the St. Marys River on the Florida-Georgia border. Subsequent broodstock has been collected either in the St. Johns itself or from Lake Talquin in northwest Florida. Until recently, Lake Talquin was exclusively populated from fish derived from Monck's Corner, South Carolina broodstock . Natural reproduction has not been observed to date in Lake Talquin (Charles Mesing, pers. comm.). Starting in 1983, Gulf fish were also introduced into Lake Talquin. However, after that date, selection of broodstock from Lake Talquin was planned to include only older year classes, thereby hopefully reducing the possibility of the introduction of Gulf fish into the St. Johns system. To date, no Gulf coast striped bass are known to have been introduced into an Atlantic coast river system.

Gulf coast systems

Along the Gulf Coast, the historical distribution of striped bass extended from the Ochlockonee River in Florida (McLane, 1958) to at least the Tchefuncta River in Louisiana (Pearson, 1938). It has been hypothesized that separation of Atlantic and Gulf Coast populations occurred following the closing of the Suwanee Straits in the late Pleistocene about 15,000 years ago (McLane, 1958). Movement of striped bass around the southern tip of the Florida peninsula is unlikely due to temperature constraints.

At one time, the distribution of Gulf coast striped bass may have been continuous, occupying most major coastal rivers extending from northwest Florida to the Mississippi River. However, many of these populations have been extinct since the late 1950's and 1960's because the construction of various dams prevented access to spawning sites, the adverse affects of pesticides from agricultural sources, or other possible factors. Following these environmental perturbations, remnant populations may have been restricted to the Apalachicola River in Florida (Crateau et al. 1980), the Pascagoula River in Mississippi (McIlwain, 1967), and perhaps the Alabama River in Alabama (McIlwain, 1980).

Stocking programs were initiated in the late 1960's to restore viable sport fisheries in many of these impacted Gulf coast river systems. Virtually all states in the region have participated, resulting in a restoration of runs and even a westward expansion of the species' range into Texas (Matlock et al. 1984). Initially, attention was focused soley on enhancing population size in the various river systems with little regard to the preservation of the genetic integrity of any remnant Gulf Coast striped bass populations. In all cases, initial stockings were comprised of striped bass of Atlantic Coast ancestry, with the Monck's Corner, South Carolina or Edenton, North Carolina hatcheries serving as suppliers of these fish. Monck's Corner broodstock is acquired annually from natural spawning populations from either the Santee or Cooper rivers in South Carolina. Edenton broodstock is obtained from native populations in the Roanoke River, North Carolina.

Particular attention, by both federal and state agencies, has been focused on the Apalachicola River in northwest Florida, perhaps due to the historically large size of its run and popularity of its sport fishery. The Apalachicola system has served as a model for the restoration of striped bass runs along the Gulf coast. Wooley and Crateau (1983) have estimated population size in the upper Apalachicola River for adult striped bass above 381 mm (TL) as approximately 1500-2000 fish for the spring of 1981. Unfortunately, populations surveys were not conducted earlier. Also, this estimate did not consider striped bass in the upper reaches of the Flint and Chattohoochee Rivers, the major tributaries of the Apalachicola River. It has since been demonstrated that these two tributaries serve as the major spawning sites for the Apalachicola system (Charles Mesing, pers. comm.). In 1980 and 1981, 43% of striped bass sampled in the Apalachicola River were believed to be of Gulf ancestry, based on lateral line scale counts (Wooley and Crateau, 1983).

In terms of flow, the Apalachicola is Florida's largest river. The Apalachicola River originates from Lake Seminole created by construction of the Jim Woodruff Lock and Dam. Both the Flint and Chattahoochee Rivers flow into Lake Seminole (Wooley and Crateau, 1983) (see Figure 5). Sport fisheries for striped bass currently exist at the base of Woodruff Lock and Dam in the early spring and in the thermal refuges in Lake Seminole during the summer and fall months. Recent reports based on electroshocking results indicate that striped bass range up the Flint River at least as far as Albany, Georgia, and the Chattahoochee River to the Columbia Lock and Dam (C. Mesing, pers. comm.) Since its large drainage system extends into Florida, Alabama, and Georgia, officials from all three states and the U.S. Fish and Wildlife Service participate in decisions regarding conservation and restoration of its natural resources.

Several years after the completion of the Woodruff Lock and Dam in 1957, striped bass fishing declined dramatically. In response, stocking of the Apalachicola system started in 1966 and has continued to the present (see Table 10). Initial stockings consisted of fish of Atlantic origin. However, by 1980, policy had shifted in favor of attempted preservation of a remnant Gulf coast strain since it was believed better adapted behaviorally and physiologically to Gulf system conditions (Wooley and Crateau, 1983). It is believed that striped bass of Gulf lineage are better adapted to utilization of thermal refuges. As a result, from 1980 to the present, all stockings in the Apalachicola were striped bass of presumed Gulf origin produced in the Welaka National Fish Hatchery, Florida from Gulf identified broodstock collected in the Apalachicola system. Striped bass of Gulf ancestry were identified by high lateral line scale counts (>63 or 65). These fish are reared and held in the Warm Springs, Georgia Hatchery prior to stocking.

<u>The Problem</u>

A major objective of the United States Fish and Wildlife Service in the southeast is the restoration of a self-sustaining run of striped bass of Gulf ancestry in the Apalachicola system. However, except for lateral line scale counts, no marker exists to identify striped bass of Gulf lineage in the Apalachicola. The heritability of lateral line scale counts in striped bass has never been determined. In addition, with the exception of lateral line scale counts, no evidence exists confirming the continued presence of a Gulf strain in the Apalachicola system. Our major aim in this study is to determine if we see a unique mtDNA genotype in the Apalachicola which we have not seen in any Atlantic system. This finding would confirm the continued existence of representatives of a Gulf strain of striped bass.

In light of the introduction of Atlantic striped bass into the Apalachicola system, a mechanism is necessary to differentiate between native Gulf and introduced Atlantic fish for use as broodstock. Studies by Raney and Woolcott (1955) and Barkuloo (1970) before the introduction of Atlantic striped bass in the Apalachicola system had demonstrated significantly higher lateral line scale counts exhibited by Gulf coast striped bass (Mean= 66.7, Range= 63-72) than found in southeastern Atlantic striped bass (Mean= 54 in Florida, 58 in South Carolina, Range= 51-60), with no overlap in counts observed. Therefore, all individual fish could be uniquely identified to lineage based on this single criterion. However, differences in lateral line scale counts between the two races seems to have been erroded either as a result of the introduction of Atlantic fish or perhaps a change in environmental conditions. Crateau et al. (1980) reported a decrease in counts in Apalachicola striped bass to a mean of 62.4. An examination of lateral line scale counts in several hatchery year classes from matings between two Gulf coast parents with high counts (>65) resulted in a substantial reduction in the mean count for the progeny. (E. Crateau, pers. comm.). Significant temporal instability of lateral line scale counts in Cheaspeake and Hudson striped bass among individual year classes was reported earlier by Murawski (1958). Recent efforts to distinguish between Gulf and Atlantic striped bass by conventional proten electrophoresis and isoelectric focusing of various tissues have been unsuccessful (R. Wattendorf, pers. comm.; this study).

As a result of the decrease in lateral line scale counts of Gulf fish there has been increasing difficulty in recognizing suitable broodstock for Gulf hatchery production at the Welaka hatchery. Demands for fingerling striped bass of definite Gulf ancestry have increased annually outstripping the hatchery supply. In view of this difficulty, and in the absence of definitive evidence demonstrating increased viability of Gulf selected fish in the Apalachicola system, controversy has mounted regarding the advisability of continued use of Gulf coast brood stock in repopulating the Apalachicola or other Gulf systems. Do fish with high lateral line scale counts also harbor uniquely Gulf genotypes?

The first question to be addressed in resolving this issue is whether a unique Gulf coast strain of striped bass currently exists based on mtDNA genotype. If a Gulf coast strain of striped bass does exist, what is the extent of genetic divergence between it and Atlantic stocks? This can quantified by an estimate of the extent of base sequence divergence in mtDNA.

Our second major concern deals with the question of whether some innate genetic tag exists that will allow for unequivocal identification of fish of Gulf ancestry. If the answer is affirmative, it would permit identification of broodstock of Gulf ancestry for hatchery production. This innate tag would allow for the determination of the current relative abundance of Gulf versus Atlantic fish in the Apalachicola River system. This question could be expanded to include the relative contribution of the Atlantic versus Gulf lineage to annual year class production in the Apalachicola system. Natural reproduction in the Apalachicola system has recently been confirmed (1985), with spawning perhaps occurring at several geographically disparite sites (C. Mesing, pers. comm.). Is this production resulting from broodstock of Gulf or Atlantic ancestry? In addition, considering the large distance between observed spawing areas in the Apalachicola system, a real possiblity exists for genetic subdivisions of striped bass of exclusively Gulf lineage. Differences in growth rate and survivability of the different strains in the wild could also be ascertained.

Materials and Methods

Sample Collections

Sample collections are separated into two divisions; a reference collection and naturally reproduced representatives of the 1985 year class from the Apalachicola system. Fish comprising the reference collection have been assigned presumptive ancestry based on lateral line scale count or known lineage. In the absence of any other system to differentiate ancestry of these fish, we had to rely on scale counts. The reference collection consists of striped bass obtained from several locales; the Monck's Corner Hatchery in South Carolina, the St. Johns River on the Atlantic coast of Florida, multiple sites from the Apalachicola system on the Gulf coast of Florida, and two different year classes from the Warm Springs Hatchery in Georgia. Warm Springs Hatchery fish represent progeny obtained from matings of Gulf coast broodstock collected from the Apalachicola system. Based on lateral line scale counts of sampled fish or their parents, both year classes of fish obtained from Warm Springs and adult fish collected from the Apalachicola River were of presumed Gulf ancestry. Characterizations of individual fish are listed in Table 11.

Characterizations of the naturally reproduced 1985 Apalachicola year-class fish are presented in Table 12. These young-of-the-year striped bass were collected from two different sites within the Apalachicola system. Their lateral line scale counts were not uniformly high, ranging between 56 and 65; with a mean of 61. The potential exists for natural reproduction from both Atlantic and Gulf parents within the Apalachicola system, and therefore these fish were not included within the reference collection. Standard lengths and lateral line scale counts of all fish were either determined in our laboratory or generously supplied by U. S. Fish and Wildlife Service or Florida Game and Fresh Water Fish Commission personnel.

In addition to those samples enumerated herein, it should be noted that we have determined mtDNA genotypes for striped bass from the various spawning systems contributing to the coastal migratory stock including; the Roanoke River, North Carolina, various tributaries of the Chesapeake Bay, multiple sites from the Hudson River, the Connecticut River, and a single Atlantic coastal site. Detailed genotypic frequencies for these sites are reported elsewhere. Therefore, our Atlantic collection should not be construed as limited to the St. Johns River and the Monck's Corner Hatchery.

Fish were shipped to our laboratory alive and all mtDNA preparations were conducted on freshly killed specimens. Sources of mtDNA included heart, liver, muscle, and where possible, gonads. All of these tissues have proven to be an excellent source of purified mtDNA. Muscle preparations were treated separately from those obtained from other tissues. Until the lysis of purified mitochondria was initiated, all preparatory steps were conducted at 4° C or on ice baths.

Mitochondrial Isolation

The protocol we have adopted for mitochondrial isolation has been modified from Wodtke (1974) for the isolation of mitochondria in eels. Tissues were washed several times in 150 mM KCl, 50mM Tris-Cl pH 7.4, 2 mM Na_2EDTA to minimize red blood cell contamination. The tissue was minced to fine pieces in this buffer and then transferred into MSTE buffer (225 mM mannitol, 75 mM sucrose, 50 mM Tris-Cl pH 7.4, 2 mM Na_2EDTA). A Waring blender was used to disrupt the tissue for a pulse of about 20 seconds and aliquots were then thoroughly homogenized with a Dounce homogenizer. Homogenates were brought to approximately 10% (wt. of tissue/ vol.) with more MSTE buffer and centrifuged 3-4 times for ten minute intervals at 2500 RPM in a Sorvall GSA rotor at 4° C. The supernatants were retained after each spin and precipitates containing nuclei and other cellular debris were discarded. Once a relatively clear and debris free supernatant was obtained, it was spun at 9500 RPM in a GSA rotor at 4° C for 50 minutes. The resulting red-brown mitochondrial pellet was resuspended in MSTE buffer and respun at 9500 RPM. The mitochondrial pellet was once again resuspended in 15 ml of MSTE buffer and layered on top of a preformed sucrose step gradient (1.5 M and 1.0 M sucrose) in TE buffer (10mM Tris-Cl pH 7.4, 1mM Na_2EDTA). This gradient was then centrifuged at 25,000 RPM for one hour at 4° C in a Sorvall AH 627 rotor (37 ml buckets) in a Sorvall OTD 65 ultracentrifuge. Purified intact mitochondria appeared at the interface of the two sucrose solutions. The top layer was removed by vacuum and discarded. The mitochondrial interface was removed by using "shorty" (Bellco) serological pipets. This mitochondrial fraction was then diluted approximately 4:1 with MSTE buffer and centrifuged for 15 minutes at 13,000 RPM in a Sorvall SS-34 rotor. The resulting mitochondrial pellet was retained for lysis.

Mitochondrial DNA Isolation

The final mitochondrial pellet was resuspended in 3 ml of STE buffer (100 mM NaCl, 50 mM Tris-Cl pH 8.0, 10 mM Na_2EDTA). Twenty % SDS (BDH) was added to a final concentration of 2% to lyse the mitochondria. The lysate was allowed to incubate at 37° C for 20 minutes during which clearing was observed. The degree of clarity indicated lysis. One-sixth volume of saturated CsCl in TE buffer was added to the lysate. The lysate was then stored at 4° C for at least one hour to allow for formation of a Cs-dodecyl-S04 complex containing proteins and membranes. This lysate was centrifuged at 12,000 RPM in a Sorvall SS-34 rotor for 15 minutes. On occasion, it was necessary to respin the lysate several times to obtain a debris free supernatant.

Ethidium bromide was then added to the resulting clear supernatant to a final concentration of 800 ug/ml and solid CsCl to a buoyant density of 1.57-1.58 g/cm^3. All buoyant densities were determined by refractometry. The solution was then centrifuged at 36,000 RPM for 48-72 hours at 20°C in a Beckman SW 40 or Sorvall TST 60.4 swinging bucket rotor. Upon completion of centrifugation, the mtDNA either appeared as a very faint band 2-3 millimeters below the brightly fluorescent nuclear DNA band, or in many cases was not visible at all. The mtDNA was recovered via side puncture of the centrifuge tube several millimeters below the nuclear DNA band. The buoyant density of this mtDNA containing fraction was adjusted to 1.35 g/cm^3 through the addition of more STE. This fraction was then layered on top of a preformed CsCl step gradient consisting of a bottom layer of 0.7 ml (1.7 g/cm3) and a top layer of 2.5 ml (1.4 g/cm3) in a 4.4 ml ultracentrifuge tube. Both added layers contained ethidium bromide at a concentration of 200 ug/ml. This gradient was centrifuged at 45,000 RPM in a Sorvall TST 60.4 or Beckman SW 65 rotor for 3.5 hours at 21°C. The bottom 1.4 ml volume was recovered via bottom puncture. 0.8 ml of CsCl (1.55 g/cm3) containing ethidium bromide at a concentration of 200 ug/ml was layered on top of the sample and centrifuged for 20 hours at 36,000 RPM in a TST 60.4 rotor. Usually two bands were visible. The bottom band consisted of closed, superhelical mtDNA. The top band contained relaxed mtDNA and sometimes nuclear DNA contamination. Both bands were retained for testing. During this final centrifugation, tubes manufactured by Sarstedt were used because of the superior visibility which they allowed. Beckman tubes were found to develop stress lines which hindered visualization of DNA bands. This difficulty with Beckman tubes was also noted by Densmore et al. (1985)

Ethidium bromide was removed through four to five steps of extraction with butanol containing saturated CsCl in TE buffer. CsCl was extracted through overnight dialysis using the multiwell BRL Dialysis Apparatus Model 1200 MD. Dialysis buffer contained 50 mM NaCl, 10 mM Tris-Cl pH 7.5, and 1 mM Na_2EDTA. The DNA was then alcohol-precipitated in the ultracentrifuge in the 17 ml small buckets for the Sorvall AH627 rotor at 4^0 C at 25,000 RPM. This step was done twice, once with 100% EtOH with 0.3 M NaAc and a second time with 70% EtOH. The DNA precipitate was then air dried (several hours), and resuspended in 200 ul of TEN buffer (10 mM Tris-Cl pH 7.4, 1 mM Na_2 EDTA, 1mM NaCl).

DNA was frozen at -20^0 C for storage for later use. Sterile procedures were strictly adhered to in all DNA handling. Under these conditions the mtDNA is stable for a minimum of several years.

<u>Restriction Endonuclease Digest Procedures</u>

All digests were performed according to manufacturer's specifications (BRL, IBI, or NEB). Approximately 1-8 units of enzyme were used per digest. An excessive overdose of certain enzymes was seen to generate partial digests. Digestion times generally were 4-6 hours. This varied somewhat from enzyme to enzyme. DNA concentrations used per digest varied between 2 and 10 ng. Digest conditions for all restriction enzymes were optimized specifically for use with striped bass mtDNA.

<u>Separation and Visualization of Fragments</u>

Fragments were separated by vertical gel electrophoresis. We found electrophoresis in the vertical mode to allow for finer resolution of mtDNA fragments. This required the use of one or two sandblasted plates when agarose was the separating medium. Large fragments resulting from six-base endonuclease digestions were separated on 1.5 mm thick agarose gels (0.8-1.8%) made in TBE buffer (8.9 mM Tris, 8.9 mM Boric acid, 0.25 mM Na_2 EDTA pH 8.3). Fragments generated by four-and-five-base endonuclease digests varied greatly in size, and therefore both agarose and polyacrylamide gels were used in their separations. Four to 12% polyacrylamide gels (1.0 mm thick) allowed for the separation of the anticipated smaller fragments due to their more restrictive porosity. In the case of many four base cutter digestions, three different gels (1.5%-12%) were run per enzyme digest in an attempt to resolve as many DNA fragments as possible.

The large fragment of DNA polymerase I (Klenow fragment obtained from IBI) was used to end-label the 3' termini of the DNA fragments with a cocktail of all four alpha-32P deoxynucleoside triphosphate precursors at 10° C in an ice bath (Maniatis et al. 1982). This was followed by alcohol precipitation, drying under vacuum, and resuspension in three volumes of TBE buffer and one volume of stop load (50% glycerol, 0.025 g bromophenol blue, 0.025 g xylene cyanol/100 ml). All gels were vacuum dried on homemade apparatus or the Hoeffer large slab gel dryer. Agarose gels were dried at 80^{0} C for 40 minutes, while polyacrylamide gels were dried at 65^{0} C for one hour. Fragments were visualized by autoradiography with and without intensifying screens. The absence of intensifying screens permitted much finer resolution of mtDNA bands although times of exposure may extend beyond seven days.

Mobility of all mtDNA fragments was determined either manually or through use of a sonic digitizer (Science Accessories Corporation, Southport, Connecticut). Sizes of all mtDNA fragments were determined by comparison to those of the appropriate known DNA standards (λ- Hind III, λ-Hind III + Eco RI, or ØX174-Hae III) which were run in adjacent lanes on all gels. Where needed, relative quantification of DNA concentrations in individual bands was determined by use of an LKB Ultrascan XL Laser Densitometer.

Results

Overview

mtDNA isolated for this study was of sufficient purity to allow for easy comparisons of fragments down to approximately 40 base pairs. Smaller fragments were observed but not scored due to poor resolution resulting from chosen gel porosities. For all enzymes, at least 96% of the entire striped bass mitochondrial genome was scored. A total of 168 mtDNA fragments were generated and scored by the ten restriction endonucleases used in this study. A relatively large percentage of the mitochondrial genome was surveyed by the use of a moderate number of 4, 5, and 6 base restriction enzymes. Six-base cutters produced an average of six fragments, five-base cutters 19 fragments, and four-base cutters 26 fragments.

A total of 740 base pairs were screened for polymorphisms, representing 4.3% of the mean Florida striped bass mtDNA genomic size of 17,463 base pairs (bp). The total number of mtDNA fragments produced for each enzyme, and size of each fragment found among Florida populations is depicted in Table 13. We do not intend to indicate a degree of accuracy beyond the capabilities of our system. All fragment sizes reported are means based on sizes determined from individual enzyme digests for all fish sampled. Representative standard deviation values for a 2815 bp fragment were ± 22 bp with a coefficient of variation of 0.008. Similarly, typical standard deviation values for a smaller 1054 bp fragment were ±14 bp with a coefficient of variation of 0.013. Considering the large number of size determinations reported, we feel that these estimates are fairly accurate. For those mtDNA fragments which proved polymorphic, Table 13 provides the mean size found for that polymorphic fragment only for fish collected from the Monck's Corner Hatchery.

Length Polymorphisms.

Reference Collection

Length polymorphisms refer to differences in the overall length of the circular mtDNA molecule among the individuals surveyed. These molecular size differences are revealed only after digesting the mtDNA molecule with a restriction enzyme, sizing the individual fragments obtained, and summing their total. Length polymorphisms should be revealed by all restriction enzymes used. Assuming that the mtDNA size difference among samples is attributable to an insertion or deletion of DNA at a single site in the molecule, only one, or perhaps two, mtDNA fragments generated by each enzyme digest will reveal size heterogeneity. If insertion or deletion events occur independently at several scattered sites in the mtDNA molecule, then several different fragments may display size heterogeneity for each restriction enzyme used. Also, if the region of insertion or deletion lies entirely between two adjacent recognition sites, molecular size heterogeneity will only be observed in a single fragment.

mtDNA molecular size for each individual fish was obtained based on the mean of all total fragment sizes determined for each restriction enzyme digest performed on that sample. Among the populations surveyed, four different sized molecules were encountered. The mean molecular size obtained for each genotype is as follows: A= 17,883 bp, B= 17,683 bp, C= 17,554 bp, D= 17,485 bp.

All restriction enzymes used, except for Xba I, produced mtDNA fragments displaying size heterogeneity (see Figure 7). Table 14 shows for each restriction enzyme, which fragment (Fragment 1= largest fragment) displays the size heterogeneity, and the alternate sized fragments observed among the fish surveyed. In this study, all mtDNA length variation was contained in a single fragment for all restriction enzymes used. With the exception of the fragment displaying size heterogeneity, all other mtDNA fragments exhibited identical electrophoretic mobility.

Aha III, Ava I, Ava II, Hind III, HinF I, and Taq I all produced single fragments which clearly showed differences in their size (see Figure 8). For each of these enzymes, one fragment was definitively present in four different sizes. All of these fragments displaying size heterogeneity were in a region of the agarose gels where size differences were obvious and could be accurately measured. All four alternate sizes of a polymorphic fragment were bracketed between immediately adjacent larger and smaller fragments respectively. Surprisingly, polyacrylamide gels (6-12%) did not reveal any length polymorphisms of small fragments for any restriction enzyme despite the large number of fragments scored on this medium. This contrasts to our results obtained with the Atlantic coastal migratory stock where Taq I micro-length variants aided considerably in stock discrimination.

Mbo I

The largest mtDNA genotype (A) displayed an extra band of 1450 bp between fragments 2 and 3. None of the other restriction length phenotypes for this enzyme displayed an additional band between these two fragments. However, all of the other restriction phenotypes (B, C, D) did display a single fragment which was much darker than any adjacent fragments. However, for each phenotype (B, C, or D) a different fragment was darker. This would suggest that for these bands multiple copies of DNA of identical size for that fragment were present. Densitometric analysis confirmed that these particular fragments showed approximately double the absorption of nearby fragments. Since, in this case, densitometric absorption reflects the amount of DNA present, it was assumed that the darker bands contained approximately double the amount of DNA of adjacent bands. This observation can be explained by two DNA fragments migrating to the same point in the gel. Therefore, Mbo I restriction phenotypes for molecules B, C, and D had comigrating bands for fragment 4 (1262 bp), fragment 5 (1163 bp) and fragment 6 (1092 bp) respectively. The difference in size among these four Mbo I fragments is consistent with the difference in mean molecular size among the four genotypes calculated on the basis of digests for all other enzymes.

Rsa I

For three of the genotypes; B, C, and D; length polymorphisms in Fragment 2 were clearly evident between Fragments 1 and 3. The size fragments obtained were 2630 bp (B), 2469 bp (C) and 2369 bp (D). Fragment 2 for genotype A, however, did not appear as a separate entity on the gel between fragment 1 and fragment 3. Instead, for genotype A, fragment 2 comigrated to the same position as fragment 1. Densitometry confirmed its presence at that position.

Msp I

Fragment 5 displayed a length polymorphism; however, only two of the presumed largest alternate sizes were detected. Genotypes A and B displayed fragments of 1219 bp and 971 bp respectively. None of the other fragments appeared as doublets or comigrants.

Distribution of Length Genotypes among Populations

Reference Collection

The distribution of length genotypes among reference populations is depicted in Table 11. Genotype A, the largest, was found exclusively in two striped bass presumed native to the Apalachicola River. It was not seen in any Atlantic collection from this study or our work with the coastal migratory stock. Genotype B was observed in three out of nine wild fish taken from the St. Johns River. Genotype C was seen in the other six fish from the St. Johns, all yearlings from the 1984 year class at the Warm Springs Hatchery, and one large female from the Chattohoochee used for 1986 hatchery year class production. Genotype D, the smallest, was observed in all 1983 year class striped bass from the Warm Springs Hatchery, the second large hatchery destined Chattohoochee female, and one female collected from below the Woodruff Dam.

1985 Apalachicola Year Class Collections

Distributions of the mtDNA length genotypes within the naturally reproduced 1985 Apalachicola year class are shown in Table 12. One individual displayed the unique A genotype, while six fish displayed the C and D length genotypes respectively.

Base Substitutions

Base substitutions refer to single or multiple base changes in a recognition site or potential recognition site for a particular restriction enzyme. Base changes in the sequence of an existing recognition site will render that site refractory to cleavage by that enzyme. Base changes in the sequence of a potential recognition site have the ability to make that site susceptible to cleavage if the appropriate sequence has been created. In either case, the number and size of mtDNA fragments generated is altered.

Base substitutions were very rare among the populations surveyed. Only two enzymes, Xba I and Rsa I, uncovered base sequence divergence among the individuals examined. Both of these, however, generated polymorphisms which proved very useful in stock discrimination.

Reference Collection

All fish examined, except the 1984 Warm Springs year class and three fish collected below Woodruff Dam displayed identical restriction patterns (Xba I-Pattern-1) for the restriction enzyme Xba I: six fragments of 5524, 3478, 3405, 3165, 1714, and 121 bp (Figure 7). The 3478 and 3405 bp fragments initially appeared as a doublet but were subsequently resolved. Also, all striped bass we have examined in the coastal migratory stock are monomorphic for Xba I-Pattern-1. In contrast, all 1984 Warm Springs fish and three Apalachicola fish displayed a 5 fragment pattern (Xba I-Pattern-2): 5524, 4954, 3478, 3405 and 121 bp (Figure 7). Therefore, an Xba I restriction site was lost in the 4954 bp fragment in this later group of striped bass. The sum of the 3165 and 1714 bp fragments (4879 bp) approximates that of the missing 4954 bp fragment. In summary, the minority

Xba I (Pattern-2) genotype was only seen in some striped bass of putative Gulf lineage.

Rsa I also uncovered base sequence divergence among the individuals examined. All striped bass, except two collected in the wild from the Apalachicola River (A length genotype), exhibited a 29 fragment pattern for this enzyme. These two Apalachicola striped bass had a 1498 bp fragment, which was clearly absent from all other fish. These Apalachicola fish, in addition, were missing the 822 and 677 bp fragments, present in all other fish. Thus, a recognition site was lost in the 1498 bp fragment.

1985 Apalachicola Year Class Collection

The two different Xba I genotypes were detected in this group of fish. Most striped bass with the C length genotype had the minority Xba I Pattern-2. In addition, most fish with the D length genotype displayed Xba I Pattern-1. Distributions of these genotypes is seen in Table 12.

Heteroplasmy.

Heteroplasmy refers to within-individual differences in mtDNA. Two or more forms of the mtDNA molecule may coexist within an individual, a condition designated as heteroplasmy. Both forms will be visualized, if the minority form is in sufficient copy number. Avise and Lansman (1983) indicate that a minority mtDNA form must constitute at least 1-5% of the total mtDNA present to be detected. However, one form may be more intensely labelled than the other, depending on their relative concentrations. The DNA concentration of the minority heteroplasmic and the majority band should equal the DNA concentration of adjacent non-heteroplasmic bands. This is determined densitometrically. Obviously, only those fragments exhibiting either base substitutions or length variation will reveal these alternate bands.

We only considered a fish heteroplasmic for a length polymorphism if it exhibited an extra band for all restriction enzymes used. If supernumery bands were only evident in a fish for digests with a single restriction enzyme, we assumed that these extra bands resulted from incomplete digestion.

Heteroplasmy was clearly seen for all enzymes examined in one fish from the St. Johns and a single young-of-the-year striped bass from the Apalachicola. This represents 4.7% of all fish sampled. For example, an Ava II digest of the aforementioned St. Johns fish, in addition to generating the expected full complement of fragments for this enzyme, all of approximately equal labelling intensity, also yielded an alternate sized fragment 3, of reduced labelling intensity. Densitometry demonstrated the absorption and thus concentration of this band to be 43% of neighboring bands on the gel. For HinF I, a similar observation was recorded for fragment 1. Absorption of the minority fragment was 38% of adjoining bands. In all cases tested, when considered jointly the sum of absorption of the heteroplasmic band and its matching majority band approximated that observed in adjacent non-heteroplasmic fragments.

Discussion.

Overview.

The lengths of the mtDNA genome we have reported for striped bass of the southeast; 17,833, 17,683, 17,554 and 17,485 base pairs (bp), fall within the range of 15,700-19,500 base pairs generally observed in other animal species (Brown, 1983). This compares with a mean molecular size of 17,820 bp for all striped bass collected from the various breeding systems (Hudson, Chesapeake and Roanoke) contributing to the Atlantic coastal migratory stock. In contrast, Ferris et al. (1984) reported a mitochondrial genomic size of 16,900 base pairs in skipjack tuna (Katsowonus pelamis) and Berg and Ferris (1984) observed a slightly smaller size of 16,670 base pairs in several species of salmonids. These values agree closely with the size reported by Araya et al. (1984) for carp (16,400 base pairs). Different restriction enzymes were used in conducting these various studies, generating widely differing numbers of mtDNA fragments, some perhaps not resolvable or scoreable on the gel concentrations employed. However, assuming these earlier estimates to be accurate, the striped bass appears to have a considerably larger mitochondrial genome (800-1200 base pairs) than observed in other fish species to date. By using many four and five base cutters, we have generated DNA fragments which are much easier and accurately sized than the largest fragments generated in six base cutter digests. It is the largest DNA (>5000 base pairs) fragments for which the error factor in sizing is greatest. If anything, our estimates of mean molecular size may be low due to the increased possibility of non-scored comigrating bands arising as a result of four-base-cutter digests.

It has been demonstrated by DNA sequencing and physical mapping in several other organisms that the vast majority of the animal mitochondrial genome is functional, with few non-functional intervening sequences between coding regions. Conservation of this feature of genomic organization into fish has been demonstrated by Araya et al. (1984). The opportunity for base sequence divergence is greater when functional constraints on a particular sequence are minimal. Striped bass, with a considerably larger genome than many other fish, possibly contain a DNA insert with lessened functional constraints. This would offer the potential for considerable intraspecific sequence divergence, and could result in levels of sequence divergence substantially higher than observed in many other taxonomic comparisons. This expected high level of variation, however, was not detected in striped bass.

Length Polymorphisms

Early reports indicated that major mtDNA size variation among conspecific or congeneric comparisons is quite rare. mtDNA genomic length, particularly within species, was thought to be very stable. However, microvariation (several base pairs) in length of the molecule was observed in conspecific comparisons among humans (Cann et al. 1984; Aquadro and Greenberg, 1983). Macrovariation in genomic length has recently been reported. Harrison et al. (1985) described three distinct size classes of mtDNA that differ from each other by 300 bp within and across two closely related species of crickets. Major length polymorphisms were also observed in species of parthenogenetic and bisexual lizards by Densmore et al. (1985). However, unlike the cricket study, a minimum of 37 different length variants were observed among 92 lizards examined. This suggests that the frequency at which length variations occur can be very high. In considering the presence of continuous and discrete length variants in different taxa, it suggests that mulitple mechanisms may be responsbile for the generation of this variation. In addition, these lizards exhibited surprisingly few base substitutions. The cricket study in addition to length polymorphisms did reveal a moderate level of base substitutions. A mechanism for generating this length variation in lizards was postulated by Densmore et al. (1985) involving multiple sites for the initiation and termination of D loop synthesis.

We have observed four different sized mitochondrial genomes which differ in length from the largest molecule by 200, 329, and 398 base pairs. Unlike the lizard study, we found these length polymorphisms to fall into four discrete classes. This mtDNA size polymorphism together with two base substitutions allows for the unequivocal discrimination of individual striped bass representative of all of the populations investigated. The largest genotype, A (17,833 bp), was only found in wild fish from the Apalachicola River. We have not observed this genotype in any of our South Carolina or St. Johns collections of striped bass or our other work on the Atlantic coastal migratory stock. At the least, this length genotype serves to uniquely identify fish of Apalachicola ancestry among the Florida and South Carolina fish we have examined. All individuals with this length genotype (A) also displayed rare Xba I genotypes.

It may be suggested that this genotype is unique to Gulf strain striped bass and therefore confirms the existence today of a distinct, genetically separate Gulf Coast population. However, it would be informative to examine fish collected from several of the small, yet healthy riverine populations in South Carolina and Georgia, and to increase our sample size from the St. Johns. Any of these systems could have served as "seed" populations for a Gulf coast strain, and could therefore still harbor rare Atlantic genotypes that may not have been seen in the St. Johns collection. This is particularly true in light of the drastic population fluctuations experienced by the St. Johns and the possible resulting elimination of rare genotypes. It should also be noted that the A genotype was not observed in either year class of Warm Springs fish despite their Apalachicola heritage.

The B (17,683 bp) genotype was only seen in 33% of St. Johns River fish. It was not observed in any wild fish or their descendents from the Apalachicola system. However, it is a common genotype exhibited by the Atlantic coastal migratory stock. The B genotype is distinct from that displayed by the 1984 year class at Monck's Corner. If the B genotype is absent in the Santee and Cooper Rivers, South Carolina populations which serve as broodstock for Monck's Corner hatchery production, this genotype may serve as a marker to differentiate Monck's Corner transplants from native fish in the St. Johns system.

The C (17,554 bp) genotype was seen in 66% of St. Johns striped bass, in all fish representative of the 1984 year class at Warm Springs, in all Monck's Corner fish, and one Chattohoochee female. For this study, it is of no value in discriminating Atlantic from presumed Gulf fish. The C genotype is also commonly found in the Atlantic coastal migratory stock. The presence of this genotype in the St. Johns may have resulted from stocking of Monck's Corner fish or may reflect naturally occurring mtDNA polymorphism in the system. Despite the riverine nature of this population, the historical potential for gene flow from northern populations is real.

The smallest genotype, D (17,485 bp), was observed in all 1983 year class striped bass from Warm Springs, one large female from the Chattahoochee River, and one fish collected from below Woodruff Dam. The absence of this genotype from all southeast Atlantic collections in this study allows for unequivocal identification of these fish within any of the populations examined in this study. Yet, this genotype is also common within the Atlantic coastal stock. While the presence of this genotype may serve to identify fish within southeastern populations, it is questionable whether its bearers are descendants of an original Gulf strain. Considering the small number of discrete size classes of mtDNA observed within these populations, implying a common mechanism for their formation, it is possible that the D genotype arose independently in Gulf coast populations. A more definitive answer to this question must await molecular work on the mechanism whereby these length variants are formed and the rate at which they are generated.

We have examined the relationship between mtDNA length genotypes and lateral line scale counts using the Student's t-test for unequal sample sizes based on all fish collected. Significant differences in mean lateral line scale counts were noted between length genotype A and all other length genotypes (length A-length B, $t=2.92$, $p=0.05$; length A-length C, $t=2.42$, $p=0.05$; length A-length D, $t=3.38$, $p=0.01$). However, it should be cautioned that mean lateral line scale counts for genotypes A and B were based on small sample sizes. While the selection of a striped bass with a high lateral line scale counts increases the possibility of identifying a fish with a uniquely Gulf mtDNA genotype, its does not insure that fact. One large female collected from the Chattahoochee with a scale count of 66 still displayed the C genotype.

Base Substitutions.

Early studies showed that base substitutions occurred two to ten times more frequently than additions or deletions in natural populations of humans (Cann and Wilson, 1983). However, base substitutions were surprisingly rare within the striped bass populations we surveyed. The proportion of shared fragments was calculated between all populations compared for all restriction enzymes used. Upholt's (1977) calculation was then used to determine p; the number of base substitutions per nucleotide separating representatives of the different populations. Sequence divergence between southeast Atlantic populations was non-existent. Maximum sequence divergence in comparing southeast Atlantic versus Gulf striped bass was $p=0.0004$ for four base cutters, $p=0.0000$ for five base cutters, and $p=0.0011$ for six base cutters. We calculated a weighted p value considering all restriction enzymes used of $p=0.0004$. This value is the lowest reported for spatially separate populations of any animal species, below even the 0.006 reported for populations of lizards (Densmore et al. 1985). Remember, that our p value in striped bass was obtained through the use of four-base-cutters which should have optimized our potential recognition of base sequence divergence.

Two possible but contrasting hypotheses may be offered to explain this paucity of sequence divergence. First, given the brief time period, a minimum of ~15,000 years since isolation of the Gulf strain, the low levels of sequence divergence detected are consistent with the time elapsed. Brown et al. (1979) reported an absolute rate of mtDNA evolutionary change among mammalian species of 0.02 substitutions per base pair per million years. Based on this rate, over 15,000 years, a p value of 0.0003 would be predicted in comparing Atlantic versus Gulf striped bass. This value closely approximates our calculated p value of 0.0004.

Avise and Bermingham (1986) have proposed that the Apalachicola drainage may have served as the boundary between "eastern" and "western" fauna based on discontinuities in mtDNA genotypes in bowfin and three species of sunfishes. Phillips et al. (1981 and 1982) also reported major intraspecific isozymic frequency breaks in largemouth bass (Micropterus salmoides) at the Apalachicola system. Given the potential origin of striped bass as a purely freshwater form, it is possible that the discontinuties in striped bass mtDNA sequence genotypes may have resulted from reproductive isolation during a period of freshwater existence and therefore predated the closing of the Suwanee Straits.

In addition, the levels of nucleotide divergence among all our Atlantic collections in the coastal migratory stock are far below what might be expected. The extent of conspecific mtDNA sequence polymorphism within individual Gulf and Atlantic river breeding systems is exceedingly rare when compared to the levels of variation observed within even small populations of spatially restricted terrestrial organisms (Kessler and Avise, 1985). Assuming the applicability of this substitution rate for striped bass, one may conclude a very recent origin for these populations. It could be argued that the mutation rate of striped mtDNA is unusually low or that selection is operative to maintain best fit genotypes. Even if mixing were invoked to explain the low levels of divergence among populations, one would still expect much higher levels of mtDNA diversity within populations. All the protein electrophoresis and isoelectric focusing work on striped bass reports exceedingly low levels of genetic variation, which supports a recent origin hypothesis.

However, a second explanation for this lack of sequence divergence must be considered. In light of the extensive length polymorphisms and moderate levels of heteroplasmy observed, an alternate mode of mtDNA evolution may be proposed for the lower vertebrates. Rather than accumulate extensive conspecific base substitutions, length variation could serve as the primary mode of evolutionary change. The results reported for lizards, most salmonids, and skipjack tuna support this contention for the lower vertebrates. In this case, the stated rate of base substitutions calculated based on primate data would be inappropriate for comparisons among fish populations.

The only two base substitutions observed, however, were very valuable in stock discrimination. Xba I- Within the reference collection only the 1984 Warm Springs year class and the two Apalachicola fish with the largest length genotype (A) exhibited a five fragment pattern for this enzyme. Based on mtDNA length, the 1984 Warm Springs fish were indistinguishable from the predominant St. Johns genotype or that observed in all Moncks Corner striped bass. However, by considering their Xba I restriction genotype these 1984 Warm Springs striped bass could be distinguished from all Atlantic collections we have examined. In view of the source of broodstock for the production of these striped bass, an examination of more wild Apalachicola striped bass should uncover more individuals bearing this genotype. Application of the Student t-test failed to reveal significant differences in mean lateral line scale counts between striped bass exhibiting Xba-I genotypes 1 & 2 (t=1.48)

Rsa I- Two wild striped bass (Length genotype A) from the Apalachicola River were already identifiable based on the unique length of their molecule. In addition, their Rsa I restriction phenotype consisting of 28 fragments, including one of 1498 bp, was unique among the populations surveyed. This Rsa I pattern was not found in any of our Atlantic collections.

Year class production

In 1985, stocking of the Apalachicola system was halted for a one year period to determine whether natural reproduction is currently occurring in the river. In the absence of stocking, collection of young-of-the-year fish confirmed the existence of natural reproduction. During November, 1985 young-of-the-year striped bass were collected at four widely separated geographic sites within the Apalachicola system. These included 1) the Chatohoochee River near the Columbia Lock and Dam, approximately 45 miles above Lake Seminole; 2) in Lake Seminole proper; 3) in the upper Apalachicola River below the Woodruff Dam; 4) five miles from the mouth of the Apalachicola River at Apalachicola, Florida (Charles Mesing, pers. comm.). However, several questions arises as to the ancestry of these fish. Are they descendants of Gulf or Atlantic strain lineage? Do Atlantic transplants reproduce in Gulf systems? If they do, how successful are they in relation to their Gulf strain counterparts? If these young-of-the-year striped bass are all of Gulf ancestry, do they represent the progeny of a single breeding system, or considering their wide geographic distribution, does the Apalachicola system harbor multiple breeding populations?

We have demonstrated that of thirteen young of the year striped bass examined from the Apalachicola system, seven could unequivocally be assigned Gulf lineage on the basis of a unique Xba I genotype. In addition, length genotype (D) permitted the determination that six of the remaining seven fish were also of Gulf ancestry based on our reference collection. However, it should be emphasized that while we did not encounter the D length genotype in our southeastern Atlantic collection, it is very common within the Atlantic coastal migratory stock. Considering the earlier stocking of the Apalachicola system with fish from the Edenton Hatchery (broodstock obtained from the Roanoke River), it is very possible that the D length genotype was introduced via transplantation, and therefore does not represent uniquely Gulf ancestry.

A significant precaution in the interpretation of this data should be noted. The mitochondrial DNA genotype of an organism may not be always indicative of its nuclear genotype. By focusing on the mitochondrial genotype we are ignoring all paternal contributions to genotypic frequencies. However, we are not suggesting that we have detected "genetically pure" Gulf striped bass, only that their mtDNA differs from that observed in any Atlantic collected fish. As such we believe that it may be used as a genetic marker to discriminate among striped bass whose mtDNA lineage differs. In the absence of detectable variability in the products of the nuclear genome, mtDNA currently affords the only opportunity to distinguish among striped bass of diverse lineages. The fact that we have observed unique mtDNA genotypes in the Apalachicola system does not necessarily mean that these fish also harbor a distinctly Gulf nuclear genotype. Nor do these results imply that these striped bass are better adapted to existence in a Gulf environment. Data demonstrating a selective advantage for particular mtDNA genotypes is lacking.

The possibilty exists that nuclear and cytoplasmic genomes have different evolutionary dynamics. Ferris et al. (1983) and Powell (1983) reported that in hybrid zones between species of mice (<u>Mus domesticus</u> and <u>M. musculus</u>) and fruit flies (<u>Drosophila persimilis</u> and <u>D. pseudoobscura</u>) respectively, individuals with the nuclear genome or morphological characteristics of one species, displayed the mitochondrial DNA pattern of the other species. In contrast, Gyllensten et al. (1985) observed concordance between between allozyme frequencies and mtDNA genotypes in a hybrid swarm of cutthroat trout. With the exception of a single unexplained fish, Avise et al. (1984) also found concordance between mtDNA and allozyme genotypes in hybrid swarms between subspecies of sunfishes (*Lepomis macrochirus macrochirus* and *Lepomis macrochirus purpurescens*). While it would be informative to explore the relationship between allozyme and mtDNA genotypes in striped bass, the lack of protein polymorphism in this species makes this venture impossible. While isozyme loci may not be characteristic of the entire nuclear genome, the compelling evidence on a large number of isozymic and structural protein loci (Grove et al. 1976; Otto, 1975; Rogier et al. 1985) suggests that the striped bass nuclear genome is exceedingly monomorphic. In the absence of other viable genetic tags, mtDNA currently is the only moderately polymorphic genetic approach in striped bass stock discrimination.

Another practical implication of this work should be considered. While we have demonstrated the probable continued existence of a Gulf strain of striped bass through the discovery of a unique mtDNA tag, we still have no information concerning the nuclear constituents of these "Gulf" fish. mtDNA is maternally inherited independently of the nuclear genome. Since the introduction of Atlantic striped bass in the Apalachicola system started in 1965 (see Table 10), approximately four generations have elapsed. During this period of time; any remnant Gulf fish have had an opportunity to mate with transplated Atlantic fish. It is impossible to estimate what the probability of these remnant fish interbreeding with Atlantic striped bass has been. However, it is likely that the original Gulf nuclear genome has been diluted to some degree in those striped bass carrying the Gulf mtDNA tag. Considering the fact that the vast majority of genetic information is encoded in the nuclear genome, a quantification of the percentage of a "Gulf" nuclear genome in these fish would prove beneficial in terms of future management decisions. While isozyme analysis has not proved informative in this regard, new technologies have recently been developed, and used in evolutionary biology, which will allow us to directly measure the extent of base sequence diversity in the nuclear DNA. These include "DNA fingerprinting" and restriction fragment length polymorphism analysis. Adoption of these approaches in characterizing southeastern striped bass should be a high priority item for future research.

Heteroplasmy

Considering the extensive congeneric and conspecific mtDNA polymorphisms reported in most comparisons it is surprising that until fairly recently heteroplasmy has for the most part gone undetected. Substantial levels of heteroplasmy would be expected where mtDNA diversity is high. Recent work on natural populations of lizards (Densmore et al. 1985) and crickets (Harrison et al. 1985) have reported moderate levels of heteroplasmy in these organisms. In both these cases heteroplasmy was evident for fragments displaying length polymorphisms. We have only observed heteroplasmy for two fish in this study. We have also observed this phenomenon at low frequency in representatives of the Atlantic coastal migratory stock. Heteroplasmy has only involved length variants. In all cases, the additional fragment was one exhibited by other individuals in that particular population. We have also observed heteroplasmy for base substitutions in our work with the other *Morone* species, and in one fish heteroplasmy was observed for both a base substitution and a length polymorphism.

Management Implications

1) Although mtDNA evolution in striped bass does not follow the same mode or rapid tempo as evidenced in terrestrial and freshwater conspecific populations, enough genetic variability is present to identify ancestry of selected groups of fish. However, for those individual restriction enzymes which reveal mtDNA polymorphisms, variant genotypes are not fixed in separate geographic locales. This necessitates examing a large number of fish or alternatively compiling population specfic composite genotypes based on multiple enzyme digests.

2) Three native striped bass taken from the Apalachicola system exhibited a larger size mtDNA than seen in any of our Atlantic Coast collections. They also displayed unique Rsa I and Xba I genotypes. It is likely that these individuals were descendants of the relict Gulf coast or a currently very rare Atlantic population.

3) All representatives of the 1984 Warm Springs Hatchery year class displayed Xba I genotypes not observed in any of our Atlantic coastal collections. It is likely that these fish are also descendants of an ancestral Gulf population. Seven of thirteen 1985 Apalachiola year class fish exhibited this Xba I genotype.

4) Several different mtDNA genotypes are shown by striped bass with high lateral line scale counts currently inhabiting the Apalachicola River system. Whether these several maternal lines constitute a single interbreeding population is not clear. These could represent one or more different stocks. We found three different mtDNA size classes in our small collections from the Apalachicola and its hatchery reared descendants. If the sampling program was expanded, possibly more polymorphisms would be revealed within Gulf populations. Traditionally, striped bass in the Apalachicola have been viewed as a single stock. Given the length of the system, the wide geographic disjunction of possible spawning sites, and spatial partitioning due to an apparent behavioral preference for thermal refuges, the potential for population subdivision of even native Gulf populations is great. Our data supports this hypothesis. Further work is clearly needed.

5) The St. Johns exhibited a mix of genotypes. This is to be expected because of the mixture of striped bass which it has received. Three St. Johns fish did exhibit a unique genotype not found in any other Florida striped bass, suggesting the presence of continued natural reproduction in the St. Johns, stocking from a different source, or a new mutation.

6) All hatchery spawned striped bass from a single year class exhibited identical mtDNA genotypes. Considering that a single or pair of mothers was used in hatchery production for each year class, this was to be expected. In the absence of additional mutation, selection of the appropriate maternal parent as broodstock would allow for innate genetic tagging of all progeny of any mating and would permit their unique identification if released in the wild. Obviously this assumes that a maternal parent can be found whose mtDNA genotype differs from that commonly encountered in the wild and does not confer upon its descendents a selective disadvantage. The effects of mtDNA genotypic variation on fitness have yet to be demonstrated.

A perfect application of this strategy has recently arisen in Lake Talquin in northwest Florida. Florida authorities are interested in determining the relative survivability, growth, and condition of Gulf versus Atlantic striped bass in this system. One Gulf and one Atlantic maternal parent could be selected as broodstock on the basis of both lateral line scale count and differing mtDNA genotypes. In the absence of natural reproduction, all fish from that year class could be unequivocally identified as to lineage for the duration of their existence, and therefore their relative performances in Lake Talquin could be determined.

7) The Santee and Cooper rivers in South Carolina annually supply broodstock for the Monck's Corner Hatchery, the production from which is utilized throughout the United States. Both of these populations are presumed to harbor a relatively small number of spawning adults. An examination of a sufficient number of adult females from these two spawning systems, would provide an inventory of all possible mtDNA genotypes carried by Monck's Corner produced striped bass. As a result, Monck's Corner reared striped bass could be easily monitored when released into other populations.

8) We have demonstrated a unique Gulf mtDNA tag in some striped bass in the Apalachicola system. The potential exists for the continuted existence of a small number of striped bass of original Gulf lineage in some of the river systems in Alabama, Mississippi, and Louisiana despite the stocking of Atlantic fish for the last two decades. Before stocking hatchery reared fish from the Apalachicola system in these western Gulf systems, it would prove informative to survey these systems for any other unique Gulf mtDNA tags. This opportunity will be lost forever after the introduction of Apalachicola fish.

IV. The Use of mtDNA to Explore Evolutionary Relationships in the Genus Morone

Introduction

The fish genus, Morone contains six species in its worldwide distribution. The eastern Atlantic-Mediterranean is represented by two species, while four species are found in North America. In total, these fish inhabit a wide spectrum of aquatic habitats, including totally fresh water, brackish, and marine environments. Some of these species are of enormous economic importance, while others are of marginal commercial value in exclusively local fisheries. In a similar fashion, several of these species are held in great esteem by the sport angling community, while others have little or no appeal. As a result of this dichotomy in commercial and recreational significance, several members of the genus have been intensely studied, leaving little mention of other members in the literature.

North American Species

Among the North American representatives of the genus Morone, perhaps the most familiar and popular is the striped bass, Morone saxatilis. The endemic Atlantic distribution of this anadromous species extends from the St. Lawrence River in Canada to the St. Johns River in Florida (Raney, 1952). Historically, its natural range has also included the major Gulf of Mexico drainage systems extending from the Suwanee River in northwest Florida in an easterly direction to at least Louisiana (Wooley and Crateau, 1983). In the past century, the continental distribution of striped bass has been increased through transplantation, to include the Pacific coast of North America from British Columbia to extreme southern California (Forrester et al. 1972). In addition, successful hatchery propagation has allowed for the introduction of these fish into numerous freshwater impoundments throughout much of the U.S. south and southwest (Axon and Whitehurst, 1985). The striped bass commands a great deal of interest among both sport and commercial fishing interests. As a result of this economic importance (Koo, 1970), declining Atlantic coast population sizes (Boreman and Austin, 1985), and the release of environmental pollutants in known major breeding and nursery grounds, striped bass are among the most intensely studied species in the fish world (see reviews by Westin and Rogers, 1978; and Setzler et al. 1980).

Sympatric with the striped bass along much of its Atlantic range, the historical distribution of the white perch, Morone americana, extended along the Atlantic coast of North

America from the Maritime Provinces of Canada to approximately South Carolina (Scott and Christie, 1963). Native landlocked populations of this otherwise anadromous fish are also found in many of the lakes of the extreme United States northeast and in the Canadian provinces of New Brunswick and Nova Scotia (Scott and Christie, 1963). As a result of the construction of various locks and canals in mid-and-western New York State in the first quarter of the 19th century, white perch were also introduced into Lake Ontario (Scott and Christie, 1963) and subsequently spread into Lake Erie (Larsen, 1954; Busch et al. 1977; and Todd, 1986) in the late 1940's and early 1950's. White perch were also accidentally introduced into a flood control reservoir in eastern Nebraska in the early 1960's from which representatives escaped to form populations in the Platte, Missouri, and Little Blue River drainages (Hergenrader and Bliss, 1971; and Hergenrader, 1979). Historically, this species has been of moderate economic importance, particularly in the Chesapeake region resulting from its great abundance (Woolcott, 1957). Due to steadily decreasing numbers of Chesapeake spawned striped bass in recent years, and its rapidly expanding distribution, the white perch has taken on added commercial and sporting significance.

The yellow bass, Morone mississippiensis and the white bass, Morone chrysops, both exclusively freshwater species, are frequently sympatric in their distributions. The endemic range of yellow bass is restricted to the narrow confines of the Mississippi River drainage extending from southern Minnesota to Louisiana. Minor populations are also found in the backwaters of eastern Texas and perhaps Oklahoma (Lee et al. 1980 and Olney et al. 1983). Perhaps due to their relatively small size (<275mm) yellow bass attract very minor sport and almost no commercial interest.

While the white bass is sympatric with the yellow bass over a significant portion of its range, its distribution is considerably more extensive including the Mississippi drainage and the Great Lakes east to the St. Lawrence River (Woolcott, 1957). As a result of its sport fishing appeal, the species has been transplanted into many major reservoirs in virtually every southern state (see Lee et al. 1980 for the broad confines of its current distribution). Commercial fisheries for white bass are quite extensive in the Great Lakes. In addition, white bass males are used in the hatchery production of the popular striped bass x white bass hybrids which have been extensively stocked in the U.S. southeast to support a sizeable recreational fishery. This hybrid has been so successfully stocked in the southeast that there is concern over their ecological competition and potential genetic introgression with the white bass and striped bass parental stocks in the area (Avise and Van Den Avyle, 1984).

European-African Species

The two eastern Atlantic representatives of the genus Morone are sympatric over a great deal of their natural ranges, which include the Atlantic coast of western Europe, the Mediterranean coast of Europe east to the Black Sea, and the Atlantic coast of northwest Africa (Woolcott, 1957). The extremes of their ranges differ, however, in that populations of the European sea bass, Morone labrax, extend further north along the shores of western Europe to include the British Isles and the coast of Norway. The northern distribution of spotted sea bass, Morone punctatus, terminates at the Bay of Biscay in France, yet its range extends further south along the west African coast than that of M. labrax. It is not clear from the current literature what is the southern terminus of its distribution. Both species are primarily marine, although they inhabit brackish systems seasonally. Little is known about the biology of the spotted sea bass. In contrast, due to its considerable commercial and sport-fishing importance, the European sea bass has been widely studied by various investigators (Kennedy and Fitzmaurice, 1972; Holden and Williams, 1974; Ottaway and Simkiss, 1979). The European sea bass has been rated "Britain's most valuable sporting sea-fish" (Kelley, 1979). In addition to its importance as a sport fish in British coastal waters, recent years have witnessed an increase in the commercial importance of M. labrax (Claridge and Potter, 1983). There is a localized commercial fishery for spotted sea bass in some nations of western Africa (D. Dorfman, personal communications). In addition, both species support considerable aquaculture operations along the Mediterranean and southwestern Atlantic coast of Europe. (see Table 15 for a synopsis of Morone characterizations)

The Problem

Interspecific relationships among the six current Morone representatives have long been questioned. Although members have been placed simultaneously in as many as six genera (Morone, Roccus, Dicentrarchus, Labrax, Lepibema, and Chrysoperca), most recent analyses have advocated monogeneric classification (Berg, 1949; Bailey et al. 1954; Woolcott, 1957; and Waldman, 1986). Consequently, all six species are generally recognized as congeners united under the synonym Morone, proposed by Mitchill in 1814 and given priority to by

Berg (1949), Bailey (1951), and Whitehead and Wheeler (1966). However, perhaps through the influence of Whitehead and Wheeler (1966), who advocated the retention of a tri-generic division for these fish, M. labrax and M. punctatus are still considered to belong to the genus Dicentrarchus in much of the European community. Based on the observation of derived shared dental characters, Waldman (1986) has recently argued that the genus Morone is indeed monophyletic.

While the majority of recent osteological evidence suggests that these species comprise a single genus, Woolcott (1957) originally proposed that subgenera may be defined within the genus Morone using combinations of skeletal characters. Waldman (1986) employing dentition patterns, extended Woolcott's earlier findings by including M. punctatus in his analysis. Waldman defined three sister taxa within the genus confirming Woolcott's earlier determination. By combining morphological data, life history patterns, and geological events, these workers attempted to reconstruct the phylogenetic history of the Morone species.

Insight into the extent of genetic relatedness among Morone species may be inferred from the results of extensive efforts at intrageneric hybridization conducted at multiple southeastern U.S. fisheries facilities. Using striped bass females, viable striped bass x white bass, striped bass x white perch, striped bass x yellow bass, and F_2 striped bass x white bass hybrids have been artifically produced on a routine basis (Westin et al., 1978). Avise and Van Den Avyle (1984) and Todd (1986) have commented on the potential occurrence of naturally reproduced striped bass x white bass hybrids and backcrosses in the Savannah River, Georgia and Cherokee Reservoir, Tennessee; and white bass x white perch hybrids in Lake Erie respectively. If we accept the premise that there is a direct relationship between the tendency to hybridize and the extent of genetic relatedness, we could surmise that interspecfic genetic divergence at least between several pairs of Morone species is relatively low. Yet, in contrast, sunfishes which readily hybridize in nature (Childers, 1967), exhibit high levels of congeneric protein (Avise and Smith, 1974) and mtDNA diversity (Avise and Saunder, 1984).

We have examined the extent of interspecific mtDNA sequence divergence within the genus Morone also to answer several related questions:

First, does the extent of mtDNA divergence among Morone species fall within the range of intrageneric values reported in the literature for interspecific comparisons made among terrrestrial and freshwater taxa? Several evolutionary studies of mtDNA divergence in fishes have recently been reported in the literature. Do the estimates of Morone mtDNA divergence fall within the range observed in other species of fishes? By acquiring representatives of all six species in the genus we have the unique opportunity to explore genetic distance relationships between taxa which last shared a common ancestor at the time of continental divide about 150 million years ago. We have reported depauperate levels of mtDNA sequence divergence both between and within spatially isolated southeastern U.S. and Atlantic coastal populations of striped bass (Wirgin et al.,manuscript submitted). Do these low levels of differentiation reflect an exclusive feature of the striped bass life history or biology, or do all the Morone species reflect this rate deceleration. Does the rate of mtDNA evolution slow down among the lower vertebrates in general and in the fishes specifically? An alternative hypothesis might be retardation of mtDNA sequence change at the subspecific level, followed by a rapid acceleration once speciation has been achieved.

A second question which the data may address is whether the molecular evidence supports the intrageneric divisions based on morphology proposed by Woolcott (1957) and Waldman (1986). By scoring the presence or absence of mtDNA fragments in all six Morone species, a large body of independently derived characters may be examined in an attempt to reconstruct the Morone phylogenetic history. Six taxa may be arranged to form 945 cladograms (Felsenstein, 1978). Therefore the statistical odds on random congruence between the results of morphological and biochemical analysis are extremely small and would serve as evidence that they are sensitive to either identical or at least correlated phenomena that allow for the identification of evolutionary pattern. Incogruence would suggest that the techniques are not sensitive to the same phenomena, or that one or both techniques have been incorrectly applied. While the necessary outgroups have not been included in this analysis, are there characters shared by all Morone species which would support the monophyly proposed for this genus by Waldman (1986) on the basis of shared derived dentition patterns?

Regardless of whether morphological and biochemical congruence is indicated, restriction endonuclease analysis of mtDNA would permit development of an estimate of molecular distance between species. While estimates of absolute difference between species may be informative to the construction of classification, the utility of this analysis would be greatly increased by introducing the time element and calculating rates of divergence. Brown et al. (1979) proposed a rate constant for mtDNA evolution based on primate data which has been adopted across all animal taxa by most workers. Recently the universal applicability of this mtDNA rate determination has been questioned (Vawter and Brown, 1986). Additionally, Britten (1986) has shown that the rate of neutral mutations in the nuclear genome differs by a factor of five among between taxonomic groups. Correlation of molecular divergence calculations to the probable geographic events that have fostered differentiation within the genus Morone would allow for division of distance estimates by a temporal factor to yield estimates of the rate of molecular evolution. This would permit an evaluation of the applicability of the accepted rate of mtDNA change to the genus Morone. While this would be informative at the species level, further refinements at smaller time scales could be accomplished by incorporating estimates of differentiation we have obtained at the population level within striped bass. If the molecular clock is constant both between and within taxa, a rate estimate based on any known time frame should be acceptable.

Restriction endonuclease analysis of mtDNA provides an excellent tool to quantify the extent of genetic divergence between taxa whose divergence time has not exceeded 15-20 million years (Brown et al. 1979). The above time limitation should be qualified by noting that it was derived exclusively from primate protein sequence data, and therefore may be inappropriate for lower vertebrate comparisons. Quantification of mtDNA sequence divergence avoids the problem of scoring characters which are not exclusively genetically derived.

A word of caution in the interpretation of this data should be acknowledged. We have reported conspecific variation in the length of the mtDNA molecule in striped bass (Wirgin et al. manuscript submitted). Both large (100-400 base pair) and small (1-5 base pair) differences in mtDNA length were observed. We do not know if this observation is prevalent in the other Morone species. In striped bass the large variants were mapped to a 1900 base pair sequence in the mtDNA molecule, while the site(s) of minor length variation(s) is(are) unknown. It should be realized that interspecific heterogeneity in the size of the mtDNA molecule introduces a bias toward overestimation of mtDNA sequences diversity. If the additions or deletions are limited to a single molecular site, then only a single or small number of fragments even if flanked by identical restriction sites must be scored as non-shared due to the alteration of electrophoretic mobility resulting from either a DNA addition or deletion within that fragment. If primarily six-base-cutters are used in the estimation of sequence divergence, a substantial error may be introduced due to the small number of fragments scored. By using four and five-base-cutters, a large enough number of fragments is scored, so that the error introduced by size heterogeneity reflected in a single or small number of fragments is minimized.

Past Studies Using Mitochondrial DNA

The use of mtDNA as a tool in quantifying both interspecific and intraspecific evolutionary relationships has revealed extensive sequence divergence in almost all mammalian taxa compared (see reviews by Avise and Lansman, 1983; Brown, 1983: and Brown, 1985). This has included a wide range of organisms from mice to humans. The use of this technology however, has seen limited use among the lower vertebrates until very recently. The question arises, therefore, as to whether the lower vertebrates exhibit a rate of sequence divergence similar to that observed among mammalian taxa. Avise and Saunders (1984) and Bermingham and Avise (1986) have calculated the extent of both intra and interspecific mtDNA sequence divergence among nine species of sunfish, Lepomis. They observed substantial sequence differences (mean p value for all species= 0.206) among the populations and species sampled. Several investigators have examined mitochondrial sequence divergence in salmonid species. Berg and Ferris (1984) reported rather conservative levels of mtDNA sequence divergence among four species of trout and salmon (subfamily: Salmoninae) A more recent study on other species in the salmonid family however, revealed more extensive mtDNA polymorphism (Wilson et al., 1985). Work in our laboratory by Wirgin et al. (manuscript submitted) showed extremely low levels of mtDNA sequence divergence among both coastal and southeastern populations of striped bass. In reviewing the literature on lower vertebrates therefore, we find instances of both marginal and extensive mtDNA sequence evolution (see Table 16).

Material and Methods

Sample Collections

Characterizations of individual fish are listed in Table 17. Through the efforts of Dr Gerald Posner of the City College of New York we were able to obtain live European sea bass (M. labrax) from the Casablanca, Morocco City Aquarium. Unfortunately, we do not know the ultimate site of origin of these fish. Surprisingly, these fish revealed considerable intraspecific variation, and therefore a detailed history of their ancestry would have proved beneficial. Spotted sea bass were collected from two different sites in Senegal. Small fish (4-8 cm) were seined at a single site near the mouth of the Senegal River at the city of St. Louis. Two larger fish were purchased already dead from commercial fishermen in Dakar Harbor. Utilizeable mtDNA was purified from these two fish.

Most samples of North American representatives of the species were shipped to our laboratory in New York City alive, and all mtDNA preparations were conducted on freshly killed specimens. With the exception of the two specimens of M. punctata from Dakar, all DNA preparations were conducted on fish which had been sacrified in the previous eight hours and maintained on ice. Sources of mtDNA included heart, liver, muscle, and where possible, gonads.

Laboratoy procedures

Mitochondria were purified by differential centrifugation, followed by a single sucrose step gradient on the ultracentrifuge to further purify the mitochondrial fraction. Mitochondria were then lysed in SDS. Multiple steps of cesium chloride density gradient ultracentrifugation were executed to obtain ultrapure mtDNA. Purified mtDNA was butanol extracted, dialyzed, and alcohol precipitated to remove unwanted constituents.

All digests were performed according to manufacturer's specifications (BRL, IBI, or NEB). Approximately 1-8 units of restriction enzyme were used per digest. Digest times generally were 5-6 hours. This varied somewhat from enzyme to enzyme. DNA concentrations used per digest varied between 5 and 10 ng.

For all restriction digests performed, both an agarose (1%-1.8%) and at least a single polyacrylamide gel (4%-6%) were used to separate and visualize DNA fragments. For Hin FI and Taq I digests which generated a multitude of bands, in addition to the agarose gel, two polyacrylamide gels of different concentrations were run (4% and 8%).

All DNA fragments were visualized through end-labeling with Klenow fragment of DNA polymerase (IBI) using a cocktail of all four alpha P^{32} nucleotides (NEN), followed by autoradiography. mtDNA fragments were sized either manually or through use of the IBI Gel Reader and Cyborg software package by comparison to the appropriate digested phage or plasmid DNA standards (Lambda-Hind III; Lambda-Hind III-Eco RI; PBR-Pst I-Dde I; and øX 174-Hae III).

Data analysis

mtDNA fragments displaying coincident electrophoretic mobility were considered homologous. Therefore, they were assumed to be of equal size and to possess identical flanking restriction enzyme recognition sequences. Each DNA fragment was considered a discrete character. For all subsequent analyses, raw data was the proportion of shared DNA fragments in any two species comparison. This analysis of shared fragments was then used in two distinct forms of data analysis. Our first task was to obtain an estimate of genetic divergence for each comparison. The proportion of shared fragments for each intertaxa comparison for all restriction enzymes used was formally summarized in the F statistic originally proposed by Upholt (1977) where $F=2N_{XY}/(N_X+N_Y)$. N_X and N_Y equal the number of fragments in species N and Y respectively. N_{XY} is the number of fragments shared by these two species and can be computed in total for all enzymes used. This F value was then employed to calculate an estimate of intertaxa nucleotide sequence divergence (p value) by employing the methodology of Upholt (1977) where $p=1-\frac{(-F+(F2+8F)^{1/2})}{2}^{1/n}$.

n is equal to the number of bases in that restriction enzyme's recognition site. p values were calculated individually for each class of restriction enzyme (4, 5, or 6-base cutters). If as suggested by George (1982), there is a significant difference in sequence divergence estimates for four versus five and six base enzymes, utilizing a weighted p estimate for all three enzyme classes should minimize this effect. It has been suggested by John Avise's group (Lansman and Avise, 1983) that the potential for comigration of non-homologous fragments resulting from the use of four-base cutters may inflate estimates of genetic relatedness, however, W. M. Brown's group strongly advocates the use of the four-base-cutter approach.

In addition, this data was used to generate a matrix of absence or presence information for each restriction fragment which was compiled for each species. This information was used to search for the most parsimonious trees using the Wagner criteria. In constructing an evolutionary tree it is necessary to choose among a large number of alternative trees. The most parsimonius tree is that which implies the minimum amount of evolutionary change between operational taxonomic units. Thus, the most parsimonius tree is the tree with the smallest number of streps. A framework for an understanding of the theoretical basis and applicabilty of this approach may be obtained from Kluge and Farris (1969). Wagner networks were generated from this discrete character data set (absence-presence of restriction sites) by using the METRO search algorithm contained in the PHYLIP phylogenetic analysis package distributed by Dr. Joe Felsentein of the University of Washington. The original algorithm was developed by Metropolis et al. (1953) and recently described by Kirkpatrick et al. (1983). This program randomly searches for the most parsimonious network by testing all possible networks for the grouping which requires the least number of steps in its construction. The final output of the program consists of a single network which satisfies this requirement. Other possible phylogenies which may tie the displayed network (in terms of number of steps required) are not presented. It should be noted that one assumption of this approach as outlined in the METRO program description is violated by restriction fragment data; "that different characters evolve independently". Clearly, the presence of individual fragments is correlated with the absence or presence of other fragments. A base substitution at one restriction site has the potential to impact the electrophoretic pattern of two other distinct DNA fragments, and therefore two discrete data points. Data was input into METRO eight times with varying orders of species entry. The phylogenetic tree presented is unrooted due to the absence of any outgroups in this analysis. Selecting an outgroup was not attempted due to the acknowledged lack of unanimity regarding the position of the genus Morone among the lower Percoid fishes (Waldman, 1986); plus the potential unavailability of candidate species for mtDNA restriction analysis.

This absence-presence data was input into the BOOTM program (also in PHYLIP) to place confidence limits on the phylogenies generated. The bootstrap methodology employed in BOOTM is fully explained by Felsenstein (1985). This analysis samples this discrete character data repetitively recording groups of species that form monophyletic subsets. This process is repeated multiple times and the number of times a particular subset appears monophyletic is recorded. The greater the percentage of times a monophyletic grouping is supported, the greater the confidence in that phylogeny. Felsenstein suggests that significant evidence in support of monophyly is the occurence of a particular subset in at least 95% of bootstrap estimates. These confidence levels may then be inserted onto the tree branches generated via the METRO algorithm. This data was input into BOOTM eight times with at least 100 replicates specified per run. It should be noted that restriction fragment data generated by Hin FI was treated independently by both METRO and BOOTM due to the large data set generated by the other restriction enzymes. In addition, Taq I data was only analyzed for the four North American species.

Results

In total 555 mtDNA fragments were scored for the six Morone species. The number of fragments obtained for each restriction enzyme digest for each species is indicated in Table 18. We have not presented the size of the individual fragments due to the large number generated and limited confidence in values obtained from relatively small sample sizes for each species. Also, we have not attempted to determine whether extensive interpecific mtDNA length variation exists within the genus. When studying levels of intraspecific mtDNA sequence divergence in which most mtDNA fragments display identical electrophoretic mobility, it is easy to recognize those individual fragments whose mobility is altered by small additions or deletions of DNA, particularly when using four-or-five-base cutter enzymes. In comparing two individuals for mtDNA size differences, it is simple to recognize genomic size differences when all bands between the individuals are identical except for one. In the case of this interspecific study, base sequence divergence between individuals was so great that very few bands lined up. As a result, it was very difficult to determine if there were small differences in gemomic size between individuals representative of the different species. While the sizes of individual fragments may be summed to arrive at a total genomic length for that organism, the sum of the error in the sizing process for individual fragments may exceed the extent of intertaxa genomc length diversity. Of course, large sample sizes can increase the confidence levels in the sizing of individual fragments, and thus the calculated total genomic size. The likely occurence of interspecific length variabilty may be inferred, however, from the intraspecific length variability and heteroplasmy which we have reported for striped bass (Wirgin et al. manuscript submitted) and similar observations which we report currently for white perch. If we assume a mean generic Morone mtDNA length of 17,500 base pairs, we have sampled approximately 0.03% of the genome.

While it was not the intent of this study to quantify the extent of congeneric or conspecific length variation, several cases of length polymorphisms and heteroplasmy became readily apparent through casual inspection of individual banding patterns. In addition one conspecific base substitution proves particularly informative. These were:

1) Hind III revealed a 195 base pair difference between two distinct gentoypes in M. labrax. The third largest fragment had two alternate sizes of 2905 bp and 2710 bp respectively. Therefore, we know length variation is not restricted to North American Morone species. The molecular mechanism responsible for generation of these length variants predated the split of the European-African Morone species from the North American species.

2) Hind III exposed length variation in yellow bass. Once again the third largest fragment exhibited two distinct sizes of 2770 bp and 2320 bp, a difference of approximately 450 bp.

3) Hind III also clearly displays interspecific length heterogeneity between yellow bass and white perch. On agarose gels, yellow bass have a six band pattern, while the white perch has only five bands (see Figure 10). On acrylamide gels each species has a single band of different size (white perch=110 bp; yellow bass=310 bp). On the agarose gel, bands 1,2,4, and 5 in the perch pattern display identical mobility as yellow bass band 1,2,5, and 6. Thus, these four fragments are approximately equal in size between the two species. Band 3 (2390 bp) from the perch does not line up with either of the two remaining yellow bass bands (band3=2710 bp, band 4=1910 bp). If we add the molecular sizes of yellow bass bands 3 and 4, they exceed that of white perch band 3. Thus, the yellow bass genome is more than 2000 bp larger than that encountered in white perch.

As a result of small sample size for each species, often collected from a single site, we did not focus on quantifying the extent of intraspecific sequence divergence. Base substitutions were rare within the species surveyed. Pvu II did, however, reveal an obvious substitution in white perch. All four perch collected in the James River, Virginia displayed a four fragment pattern for this enzyme. Of five perch sampled from New York waters (Hudson

River or Long Island), one displayed the same four fragment pattern as encountered in Virginia, the other four fish displayed a three band pattern for this enzyme. Apparently fragments 2 (2950 bp) and 4 (1280 bp) in Virginia fish arose as a result of the addition of another Pvu II site in fragment 2 (4193 bp) in New York fish. This base substitution would be effective in distinguishing New York from James River white perch. It is extremely interesting in light of the absence of a similar magnitude of sequence diversity in its sympatric congener, the striped bass, in New York versus Chesapeake waters.

The extent of congeneric mtDNA sequence diversity

We have calculated an overall estimate of mtDNA sequence divergence among all possible *Morone* species comparisons of approximately 10% ($p=0.101$). We have computed mean sequence divergence estimates for North American versus Afro-European *Morones*, a mean of all possible comparisons of North American *Morones*, and an intra-Afro-European *Morone* comparison of; $p=0.123$ (S.D.=0.013), $p=0.075$ (S.D.=0.016), and $p=0.0876$ respectively. Clearly, the intercontinental comparison yields almost double the diversity than observed among North American species.

Within the North American *Morone* species most interspecfic comparisons yield comparable divergence estimates with the exception of the *M. americana*-*M. mississippiensis* comparison ($p=0.0432$) whose value is almost half the mean divergence observed for all North American species ($p=0.075$). Within the genus these two species appear most closely related based on this distance estimate. Tables 19-A-C illustrate the proportion of shared fragments and calculated p values for all possible *Morone* comparisons based on 5-base-cutter, 6-base-cutter, and weighted 4-5-and-6 base-cutter digests respectively.

Lansman et al. (1981) and Avise and Lansman (1983) have advocated a qualitative approach in using restriction patterns derived from individual mtDNA digests to attempt to resolve the phylogenetic history of closely related, recently diverged taxa. Often by examing the gain or loss of individual restriction sites it is possible to reconstruct the molecular events that have led to the divergent genotypes. Thus, one clade on a network may be distinguished from other assemblages by the presence or absence of a single or small group of restriction sites. However, in the case of these *Morone* comparisons, it is not possible to adopt this approach due to the great diversity of mtDNA restriction patterns and the large number of site differences involved in the interconversion of fragment patterns.

Figure 9 illustrates the Wagner network derived from the discrete presence-absence restriction site matrix and generated via the METRO program. Numbers along the branches indicate the proportion of times that this phylogeny was supported in the bootstrap analysis derived from the BOOTM program. It should be remembered that this network is unrooted.

Discussion

Estimates of base sequence divergence in mtDNA are believed to solely reflect the relative time since any two taxa last shared a common female ancestor. Surveys of mtDNA sequence differentiation among mammalian species invariably yield generous levels of diversity. Congeneric divergence values between terrestrial taxa typically range between 8 and 25% (Lansman et al. 1981). For instance Brown (1982) has estimated the extent of sequence divergence among a series of closely related primate species with values ranging between 9 and 19%. Fresh water congeners apparently also harbor substantial levels of mtDNA sequence diversity. Avise and Saunders (1984) have determined a median p value equal to 21% for nine species of sunfishes in the genus Lepomis. In contrast, Kessler and Avise (1985) have reported relatively depauperate levels of mtDNA differentiation among avian species with p values ranging between 3 and 6%. All these studies conclude that differences in the extent of mtDNA differentiation among larger taxonomic groups represent signficant differences in the time since their descent from a common female ancestor. These workers all adhere to a common rate of mtDNA base substitution of ≈2%/Myr originally proposed by Brown et al. (1979) based on estimates of primate mtDNA divergence using protein and fossil data to estimate actual times of divergence required to calculate rates.

We have previously reported exceedingly low levels of mtDNA sequence diversity among striped bass populations (Wirgin et al. manuscript submitted). The mean congeneric mtDNA divergence value which we observed among the Morone species is approximately 10% which falls within the lower range of mtDNA diversity values reported for terrestrial and fresh water organisms. In addition, this value is in the range of divergence estimates (3-10%) for subfamilial comparisons of Salmominae supplied by Berg and Ferris (1984). This would suggest that the exceedingly low divergence values obtained in the conspecific striped bass mtDNA analysis primarily reflected pecularities to striped bass biology or its life history.

If we now apply Brown's mtDNA base substitution rate constant to the individual p values derived from the Morone intrageneric comparisons, average times of divergence range between 4 and 6 million years with the exception of the yellow bass-white perch comparison which yields a divergence time of 2 million years ago. This close genetic relatedness betwen M. americana and M. mississippiensis was originally noted by Woolcott (1957) who was unable to separate these two species using osteological characters. If we only consider the intercontinental diversity estimates (p=12%) that were obtained when comparing M. labrax and M. punctatus to the four North American species an inconsistency between the molecular based estimate of divergence time (6 million years) and that predicted by geological events (30-60 million years) becomes apparent. It has been argued that the applicability of mtDNA diversity estimates are only applicable if common ancestors were last shared within a period of 15 million years. In those cases where this limiting divergence time has been exceeded, a sequence diversity estimate of approximately 30% should have been realized. In the case of the Afro-European Morone species versus the North American Morones species, therefore, a divergence estimate in the range of 30% is predicted. This suggests a recalibration of the molecular clock is in order, or a reconsideration of the events leading to the formation of these species.

Both Woolcott (1957) and Waldman (1986) propose similar scenarios for the evolutionary history of the genus Morone. These two investigators hypothesized that a common ancestor which roamed both sides of the Atlantic each gave rise to a single North American and Afro-European Morone. They them speculated that two branches arose in the North American ancestral stock, each of which later bifurcated, resulting in an anadromous and freshwater form. One branch contained yellow bass and white perch and the other branch white bass and striped bass. In the eastern Atlantic a similar bifurcating event ocurred, giving rise M. labrax and M. punctata. Our network confirms the M. mississippiensis-M. americana and M. labrax-M. punctata groupings. However, a similar relationship for M. chrysops and M. saxatilis is not reveaed, nor do all four North American species come off a common ancestor.

A practical application of this analysis should be mentioned. In recent years, the Apalachicola system in northwest Florida has been stocked with the female striped bass x male white bass hybrid. However, representatives of the reciprocal cross; male striped bass x female white bass, have entered this same system by escaping from an adjoining watershed. It has been noted that the two hybrids exhibit greatly different mobilities in the Apalachicola system through conventional tagging studies (C. Mesing, pers. comm.). mtDNA would provide a readily identifiable tag to mark representative hybrids of these two crosses to track their movements in this system.

V. Isoelectric Focusing of Striped Bass Eye Lens Protein

Introduction

In view of the lack of protein polymorphism detected in natural populations of striped bass by conventional electrophoretic procedures (Otto, 1975; Grove et al. 1976; Sidell et al. 1980; Rogier et al. 1985; this study), we sought to determine if cryptic protein variability might be revealed by the use of biochemical techniques that focus on protein characters other than net molecular charge or size. We chose isoelectric focusing as an alternative approach to explore for protein variability in striped bass due to its known ability to finely resolve distinct molecular forms from complex heterogeneous mixtures. In other organisms, it has been shown that electromorphs can harbor multiple allelic states, that can be recognized by altering electrophoretic conditions or by denaturing techniques (Singh, 1983; Coyne and Felton, 1977). However, it should be recognized that most attempts to detect cryptic variation have failed to uncover substantially elevated levels of protein diversity (see review by Selander and Whittam, 1983).

Additionally, isozymes, the primary focus of most electrophoretic studies, may not be representative of the entire gamut of nuclear encoded gene products, and thus may harbor uncharacteristically low levels of protein diversity (Rogier et al. 1985). Thus, we have chosen eye lens proteins as a potential system in which to explore for polymorphic protein products. Given the number of genetic loci coding for eye lens proteins (Piatigorsky, 1984), and the number of dimeric, oligomeric, and multimeric protein aggregates which can be formed, a wealth of protein products may be screened for potentially valuable genetic markers.

Our primary aim in this study was to investigate the extent of intraspecific variation of eye lens proteins in striped bass by isoelectric focusing to evaluate if this approach could be used as an effective tool in striped bass stock discrimination. Our efforts in this regard fell into two categories. These included an estimate of the amount of genetically determined variability in the primary structure of the resolved protein bands. These polymorphisms are revealed by differences in the mobility (i.e., the isoelectric points) of the individual protein bands. Secondly, we were interested in quantifying densitometrically the absolute amount of protein in each band. This information could also be used as a marker to discriminate among individuals representative of different spawning aggregates. However, it should be noted, that protein concentration of individual bands probably reflects a combination of genetic, environmental, and ontogenetic factors. Populations sampled included representatives of the three major spawning systems contributing to the Atlantic coastal migratory stock; the Hudson River, the Chesapeake Bay, and the Roanoke River, North Carolina. In addition, the extent of divergence between Atlantic and Gulf coast populations was determined. This was done in an effort to utilize this approach to provide information necessary for the management of this resource.

Preliminary data generated in our laboratory and work by others (Saila et al. 1983; Fabrizio et al. 1985) had suggested that conspecific differences in protein concentration for individual eye lens bands was common. However, studies with other organisms have reported that concentration differences in eye lens protein bands might be correlated with the age of the individual (Bloemendal, 1977; Bours, 1980; Bindels et al. 1983; Krichevsdaya et al. 1984). In fact, mechanisms to account for these events have been postulated including differential gene expression and post-translational modification. As a result, we sought to determine if the protein concentration profiles which we obtained from striped bass could be correlated with the age of the fish. Could age dependant changes in isoelectric focusing patterns be observed? To answer this question, we sampled a minimum of 12 striped bass representative of three different age classes from the Hudson system, to evaluate if their focusing patterns could be correlated with age of the fish.

The use of eye lens proteins as a system for the identification of conspecific genetic variation has several advantages which were outlined by Saila et al. (1984) and Fabrizio et al. (1985). Crystallins, which comprise approximately 80-90% of all the water-soluble proteins in the lens (Piatigorsky, 1984), provide a rich pool of multiple protein aggregates to score for inter-individual differences. However, the banding patterns obtained may be complex resulting in difficulty in their interpretation. This is due to the multimeric aggregates of crystallin monomers observed as discreet bands. Work reported by Piatigorsky (1984) has somewhat clarified the relationship between lens crystallin protein products and the gene families which code for their subunits. As a result of these findings, the identification of individual protein bands, and an understanding of their genetic basis, should pose less of a problem in data analysis than previously. The isoelectric points and molecular weights of bovine crystallins and their monomers are reported in the literature (Righetti and Caravaggio, 1976), thus aiding in protein identification. Another advantage to this approach is that eye lens proteins are not directly in contact with the circulatory system; they are nourished by the aqueous humor (Blomendal, 1977). Thus, the impact of environmental factors that can alter the properties of the crystallins are reduced (Eckroat and Wright, 1969). The vertebrate lens can be considered a reservoir of protein information because there is no replacement of old cells (De Jong and Hendriks, 1986). Thus, lens nucleus becomes a particularly valuable source of information. Crystallins are highly stable and resist denaturation even after days without refrigeration (Smith, 1965,1971).

Intraspecific variation of eye lens proteins has been reported for different species of fishes including brook trout (Eckroat and Wright, 1969); several species of seatrout (Weinstein and Yerger, 1976); bluefin (Smith and Clemens (1973); and yellowfin tuna (Smith and Gilman, 1982); blue whiting (Bussman, 1984); ocean whitefish (Smith and Goldstein, 1967); and channel rockfish (Smith, 1971). Initial studies on striped bass were reported by Saila et al. (1983) and Fabrizio et al. (1985).

Yet, despite findings of intraspecific polymorphisms, it has been surmised that crystallin genes are highly conserved, evolving at a rate of only 1%/17 million years (Wilson et al. 1977) to 3-10% per million years (de Jong and Hendricks, 1986). Current thinking maintains that structural or functional constraints are responsible for such slow rates of change.

The vertebrate lens is a tissue primarily composed of a single cell type, the lens fibers (Garber et al. 1984). Lens fiber are cells devoted to the specialized production of a single class of structural protein products, the crystallins. The crystallins can be defined as well-ordered structural proteins required for proper refraction, transparency, and plasticity of the lens (De Jong, 1981). Protein concentration in fish lenses is approximately 50% (De Jong, 1981). The vertebrate lens is compsed of four types of proteins; the crystallins, the albumenoids, actin, and vimentin (De Jong and Hendriks, 1986; Smith and Gilman, 1982). There are four major immunologically distinct crystallin families; alpha, beta, gamma, and delta crystallin (Quax-Jeuken et al. 1985). Crystallin diversity has probably been generated by alternative RNA splicing and gene duplication (Piatigorsky, 1984). Each crystallin family consists of several polypeptides with related amino acid sequences.

Alpha crystallins comprise 35-45% of total water-soluble proteins in young mammals and 20% in sub-mammalian species. (Bindels et al. 1983). Alpha crystallins are multimeric proteins that are made up of four subunits; alpha_{A2}, alpha_{B2}, alpha_{A1}, and alpha_{B1}. Alpha_{A2} and alpha_{B2} are the primary products of homologous genes (Skow and Donner, 1985), while their deamidation produces the other alpha crystallin monomers; alpha_{A1} and alpha_{B1} (Delcour and Bouchet, 1978). The A and B subunits share molecular weights of about 20,000 daltons with approximately 60% sequence homology (De Jong, 1981). They occur in molecular aggregations in a ratio of approximately 3 to 1, respectively (Tardieu et al. 1986). The alpha crystallins have been shown to consist of 40 to 50 subunits (Tardieu et al. 1986). The alpha crystallins account for 75% of total protein synthesis in the lens (De Jong and Hendriks, 1986). Alpha crystallins have isoelectric points that range between 4.8 and 5.0.

Beta crystallins comprise 33-50% of mammalian lens proteins. They are the best conserved of the lens proteins during evolution (Bindels et al. 1983). Beta crystallins are multimeric proteins with 2 to 6 subunits with isoelectric points ranging between 5.1 and 7.0 (De Jong, 1981).

The gamma crystallins are monomeric with an isoelectric point range between 7.1 and 8.1 (Bindels et al. 1983). They appear in all vertebrates with the exception of birds and reptiles. The delta crystallins are tetramers composed of two closely related gene products found only in birds and reptiles. Their isoelectric points range from 4.9-5.3 (De Jong and Hendriks, 1986; Piatgorsky, 1984).

Materials and methods

Sample collection

Populations sampled for stock identification

Striped bass were collected from various tributaries of the Chesapeake Bay. These sites included the James and Rapahannock Rivers on the western shore of the bay, the Choptank River on the eastern shore, and the Chesapeake and Delaware Canal at the northern terminus of the Chesapeake. The following sites along the Hudson River were sampled; Westway, NYC; 125th St., NYC; Cornwall; Claverack; and Troy. These five widely separated sites covered almost the entire known distribution of striped bass in the Hudson. Fish were also sampled from the Roanoke River in North Carolina. Southeastern U.S. collection sites included two locations in Florida, the Apalachicola River and the St. Johns River. In addition fish were sampled from the Monck's Corner hatchery in South Carolina. For a complete listing of sampling sites, dates of collection, and sample size see Table 20.

Striped bass sampled for age-related changes

For the aspect of this study dealing with age related changes in the expression of the various eye lens proteins, all fish were taken from the Hudson River. Young-of-the-year fish were collected with a 200 ft. beach seine from the area of Nyack, New York in September, 1986. Older fish were collected via hook and line or drifting gill nets. The fork length of all fish was used to determine their age based on the relationship derived by the Texas Instrument working group specifically for Hudson River striped bass and detailed in Westin and Rogers (1978) page 62. Approximately 12 fish of the following three age brackets were examined in this analysis 1) young-of-the year; 2) 2-3 year olds; and 3) 4-7 year olds.

Sample preparation

Intact eyes were extracted from freshly sacrificed fish, and lenses were blotted on paper towels to remove any extraneous contaminating materials. Lenses were then stored at -40° C until further processing. Lenses were weighed and frozen by the addition of an excess of liquid nitrogen, and then pulverized in an equal volume (wt:vol) of 0.01M Tris-HCl, pH 7.5 buffer and allowed to stand at 4° C for 12 hours. The homogenate was then centrifuged in an Eppendorf minicentrifuge at maximum speed for 15 minutes at room temperature, and the supernatant was retained. The sample was then dialyzed against five liters of 0.05M Tris-HCl, pH 7.6 buffer to remove salts using the multi-well BRL Dialysis Apparatus (Model 1200 MD). Samples were then diluted 5:1 using homogenization buffer and refrozen at -40° C for future use. Removal of salts from the samples greatly improved resolution of protein bands.

Gel Preparation

Monomer concentrations were T=5%; C=3%. Gels were made up as follows. 0.5 mg of riboflavin and 70 mg of ammonium persulfate were mixed with double distilled water to a final volume of 100 ml. This riboflavin- ammonium persulfate solution was made fresh daily. Four ml of this solution were added to 4 ml of a 0.6% bisacrylamide (Bio-Rad Laboratories.) monomer stock solution and 4 ml of a 69.4% acrylamide (Bio-Rad Laboratories) monomer stock solution. The acrylamide stock solution was made fresh every two weeks and filtered through a Whatman #4 circular filter under vacuum before storage. Four ml of a solution containing; 2.0 g of sucrose, 1.2 ml of LKB ampholyte of pH range 3.5-9.5 or 4.0-6.5, and water to volume (4 ml) was added. 16 ul of TEMED (N,N,N,N-Tetramethylethylenediamine-Bio-Rad Laboratories) was added to intiate polymerization; and the complete solution was immediately dearated. Gels were then poured. Photopolymerization was complete in about15 minutes. Gels were then stored at 4° C prior to use in a humidity chamber. All gels were used within a two day period from the time of polymerization.

Ultra-thin gels (0.25-0.5 mm) were cast horizontally by capillary action between two thin glass plates following the instructions contained in LKB manual 1818-P. Gels were cast on FMC GelBond PAG plastic support film which allowed for ease of future manipulations, including densitometry of gels. The GelBond film was made to adhere to the lower glass plate in the cassetted by the addition of several drops of water which were spread evenly by the use of a roller. To aid in the separation of gel casting cassettes, the top glass plate was siliconized using dimethyldichlorosilane (5% in chloroform; Sigma Chemicals).

Isoelectric focusing

All gels were prerun at 400V, 15mA, and 9W for 5 minutes. Isoelectric focusing was performed on a horizontal LKB Ultraphor unit using an LKB Macrodrive 5000 V Power Supply. The dimensions of gels were 260 mm x 130 mm x 0.2-0.5 mm. A running temperature of approximately 4° C was maintained during electrofocusing by cooling plate connection to an external Lauda bath. Two separate pH gradients were utilized to screen all samples. Initial screenings utilized a wide pH 3.5-9.5 gradient; however, actual intraspecific comparisons were made using a pH 4.0-6.5 gradient. This narrower gradient allowed for optimal resolution of most protein bands of greatest interest.

Six ul (about 40 ug of total protein) aliquots of samples were applied to the gels via the use of uniformly sized filter paper applicators (LKB). Gels were run under constant limits of 2000 V, 20 mA, and 10 W for approximately 45 minutes or until prestained standard marker proteins reached a set point. All gels contained prestained isoelectric point markers, pI 4.7-10.6 (EM Products or BDH Chemicals Ltd.) as standards to allow for determination of isoelectric points of all sample protein bands.

An aqueous 11% tricholoracetic acid-3.5% sulphosalicylic acid solution was used as a protein fixative. Gels were stained at 60° C in Coomassie Brilliant Blue R-250 (Bio-Rad Laboratories or Gurr) for 10 minutes. Gels were then destained for 24 hours in several baths of 25% ethanol and 8% glacial acetic acid. Ten % glycerol in destaining solution was used to preserve gels. All stained gels were retained for future data analysis between two sheets of GelBond PAG.

Inter-individuals differences in the mobility of individual protein bands were determined by visual inspection. The relative percentage of protein in each individual band was found by scanning each lane with an UltraScan-XL laser densitometer (LKB Model-2222-010). Isoelectric points of all readable bands were determined via comparison to a standard curve obtained from mobility of protein standards with known isoelectric points that were focused on the same gels as all samples. Comparisons were conducted by using a linear regression computer program (Sall, 1982). Protein concentrations of all samples were determined through the use of the Bio-Rad protein assay (Bio-Rad Laboratories). The mean protein concentration for samples in each age group was calculated. The protein concentration in each band was calculated by multiplying the determined sample protein concentration by the percentage of total protein represented by that band.

Protein bands were said to be representatve of an age group if they appeared in at least 50% of the focusing patterns of fish in that age group. Band identification was based on the classification proposed by De Jong (1981). Bands were numbered in sequence from the most acidic to the most basic. Mean values for the isoelectric point, percentage total protein, and absolute protein concentration were calculated for each band.

Results

Stock identification

The reproducible separation and scoring of a maximum of 12 crystallin bands was accomplished by isoelectric focusing on both wide (pH 3.0-9.5) and narrow range gradients (pH 4.0-6.5). The isoelectric points of these bands were determined via comparison to known protein standards run in adjacent lanes on all gels. Based on their isoelectric points, these proteins were divided into the different classes of eye lens proteins based on the categorization proposed by Righettii and Caravaggio (1976). It was determined that two of these bands were gamma crystallins (gamma$_1$ and gamma $_2$); seven were beta crystallins (beta $_6$, beta $_7$, beta $_8$, beta $_A$, beta$_x$, beta $_y$, and beta $_z$); and three were alpha crystallins (alpha $_{A1}$, alpha $_1$, and alpha $_2$). The mean calculated isoelectric point (pI), mean relative percentage of protein (%), and mean concentration of protein (mg/ml) for each band are recorded in Tables 21 and 22 for fish from all sampled locations. Mean standard lengths and eye lens weights (blotted wet weight) for all fish are presented in Table 23. Figure 10 reveals representative banding patterns for all localities examined while Figure 11 shows densitometric tracings of the three different phenotypes observed; (E), (F), and (F$_2$).

In comparing striped bass from the Roanoke River and three Chesapeake tributaries; including the James and Rapahannock rivers in Virgina and the Chesapeake and Delaware Canal, no variation in either primary protein structure (as revealed by isoelectric point) or concentration of individual protein bands (as detected by densitometry) was observed in either pH range gradient. Five Hudson sites were sampled including Cornwall, Claverack, Troy, Westway in lower Manhattan, and 125th St. in upper Manhattan. In narrow range gels, all fish collected from Cornwall, Claverack, and Westway exhibited banding patterns identical to those observed in fish from the Roanoke and the Chesapeake. In addition, three fish from Troy displayed the same phenotype. However, the other nine fish from Troy and all fish from 125th St. shared a phenotype in which protein bands differed in both isoelectric point and individual band concentration from the majority form reported above. The phenotype observed in the Chesapeake Bay, the Roanoke River, and most of the Hudson sites was denoted as (E) while the phenotype observed in a minority of Hudson fish from Troy and 125th St. was denoted as (F).

The (E) phenotype was characterized by nine crystallin bands (gamma $_1$, gamma $_2$, beta $_6$, beta $_7$, beta $_8$, beta $_A$, alpha $_{1A}$, alpha $_1$, and alpha $_2$). The (F) phenotype always contained all the bands present in the (E) phenoype with the addition of three more beta crystallin bands (beta $_x$, beta $_y$, and beta $_z$). Thus, a total of 12 bands were evident. In addition, the (E) phenotype always revealed the beta $_7$ band to be at a lower concentration than the beta $_8$ band, while the (F) phenotype displayed the beta $_7$ band at an equal concentration to beta $_8$ (Tables 21 and 22; Figure 11).

All nine fish sampled from the St. John's River on Florida's Atlantic coast showed an (E) phenotype identical to that observed in the coastal stock. Four of the twelve fish sampled from the Apalachicola River on the Gulf coast showed an (E) phenotype and had a corresponding mean standard length and lens weight of 375.4 mm and 242 mg respectively. The other eight fish shared a phenotype that differed considerably in the concentration of individual protein bands when compared with the (E) phenotype. These eight fish had a mean standard length and lens weight of only 181 mm and 27.5 mg respectively. Their phenotype designated as (F_2), was similar to the (F) phenotype described in some small Hudson River fish collected at Troy and 125th St. However, their phenotype differed from the (F) phenotype in the absence of the three extra beta crystallin bands (beta $_x$, beta $_y$, and beta $_z$). Thus, the (F2) phenotype could be easily distinguished from the (F) phenotype. The Moncks Corner, South Carolina sample exhibited three fish with the (E) phenotype, while the other six fish showed an (F_2) phenotype identical to that observed in some Apalachicola fish.

Age-related changes

The mean values for isoelectric point (pI), percentage total protein, and protein concentration of each band is recorded in Table 24. Thirty-five bands were examined, all were alpha or beta crystallins based on the enumerated criteria. There were 6 alpha crystallins and 29 beta crystallins. Qualitative analysis of banding patterns revealed three major features. When the youngest age group (group-A) was compared to the oldest group (group-C), 10 bands present in group-A were absent in group-C. The crystallin bands that followed this pattern included alpha-1,2, and 4, and beta bands 5,9,18,20,25,26, and 28. Secondly, 5 crystallin bands were present in group-C that were absent in group-A. These included beta-1,2,3,11,and 21. Thirdly, the middle-aged group (group-B) exhibited 2 bands that were absent in both groups A and C. These bands were beta-16 and 29.

Quantitative analysis of banding patterns also showed three patterns. With increasing age (from group-A to group-C), the percentage protein of 6 bands steadily decreased. These bands included alpha-5, beta-5,19,24,25, and 28. In contrast, the percentage of protein in

7 bands steadily increased. These bands were alpha-6, beta-4,6,7,13,15, and 27.

Densitometric data

The densitometric tracing of one representative of each age group is seen in Figure 12. Three striking features are apparent. All three samples were scanned under the same conditions, but the absorbance range for the tracings decrease with increasing age. For group-A the absorbance range is 2.85 units, for group-B it is 2.75 units, and for group -C the range is 1.98 units. An examination of identifiable peaks showed six major peaks for both groups-A and B. These peaks represented alpha-3, alpha-5, beta-9, beta-10, beta-12, and beta-24 crystallin. The tracing for group-C showed only four of these peaks; alpha-5, beta-10, beta-12, and beta-24 crystalllin. The intensity of the alpha-5, beta-12, and beta-24 peaks was lower in group-C that it was in groups A and B. The beta-10 peak showed an increase in intensity.

Protein concentration

The mean protein concentration for samples in each age group are listed below:

Group	Number in group	Mean protein conc. (mg./g of lens)	Std. dev.
A	12	36.22	10.79
B	12	39.05	10.26
C	13	30.74	6.33

Discussion

Stock identification

Isoelectric focusing was effective in generating reproducible banding patterns for the crystallin lens proteins of all the fish examined. Using this technique, striped bass representative of Chesapeake Bay spawning localities and the Roanoke River proved homogeneous in regards to eye lens protein patterns. Likewise, no detectable intraspecific variation was observed when fish from three Hudson River sites; Cornwall, Claverack, and Westway, were compared to the phenotype shared in the Roanoke River and the Chesapeake Bay. This common phenotype was denoted as (E).

When the Hudson River population at Troy, N.Y. was sampled, two phenotypes were observed. One was identical to that previously mentioned (E) while the other showed both protein concentration and mobility differences. This second phenotype, denoted as (F), was also observed at another Hudson site; 125th St., upper Manhattan. Based on this data, it was initially suggested that this intraspecific variation could best be explained by a model in which a Hudson River sub-population of striped bass exists, having undergone evolutionary changes independent of other Hudson River striped bass, as well as striped bass from other spawning systems. However, considering the lack of spatial or temporal barriers to panmixia, it is difficult to envision a mechanism whereby reproductive isolation could be enacted within the Hudson. Female spawning striped bass are believed to enter the Hudson in a single short pulse, with all spawning occuring over a relatively short geographical stetch. In support of this hypothesis, Hitron's (1974) study of transferrin variation within the Hudson was unable to demonstrate any geographically partitioned protein polymorphism.

A comparison of standard lengths and wet weight of blotted eye lenses from fish with phenotypes (E) and (F) was conducted. Those Hudson fish exhibiting the (E) phenotype were on the average larger and concommitantly had heavier lenses (411.3 mm and 240.6 mg) than those Hudson fish exhibiting the (F) phenotype (217.8 mm and 54.5 mg). According to the age-length relationships reported in Westin and Rogers (1978), the Hudson fish with an (E) phenotype, represented fish of three years or older while those with an (F) phenotype, having an average standard length of 217.8mm, corresponded to fish of one year or less in age. Likewise, the fish from Chesapeake Bay and the Roanoke River, all of which exhibited an E-phenotype, had average standard lengths of 372.6 mm and 316 mm, respectively, corresponding to fish of three years of age or more (Manuseti, 1961). It was apparent that age was playing a role in either the expression of crystallins at the transcriptional level, or the post-translational modification of these lens proteins. Based on isoelectric points, all of the protein bands associated with the concentration and mobility differences between the E- and F-phenotype (beta$_7$, beta$_8$, beta$_x$, beta$_y$ and beta$_z$) were beta crystallins.

In expanding this analysis of lens proteins to southeastern populations beyond the range of the coastal migratory stock, several interesting results emerged. All of the fish examined from the St. John's River in Florida had mean standard lengths and eye lens weights comparable to those of fish of the older group from the Chesapeake Bay, Roanoke River, and Hudson River. In addition, they all exhibited the E-phenotype and thus, no intraspecific variation was observed between these southern Atlantic riverine fish and those participating in the mixed coastal fishery. Representative younger fish from the St. John's River were not available for analysis.

The two phenotypes observed in the Apalachicola River on the Gulf Coast were assumed to be the result of age differences. The large difference between their mean standard lengths lent credibility to this assumption. Once again the larger group or older group exhibited the E-phenotype while the younger group shared a phenotype with distinct concentration differences. This phenotype, denoted as F_2, was quite similar to the F-phenotype observed in young striped bass from the Hudson River, particularly at Troy, N.Y. and 125th St. N.Y.C.. It differed from the F-phenotype in that the F-phenotype contained three bands, $beta_x$, $beta_y$ and $beta_z$, which were absent in the F_2-phenotype. Thus, intraspecific variation was shown to exist between young Gulf Coast striped bass and young Atlantic coast striped bass from two Hudson River sites.

Even though all of the Monck's Corner hatchery striped bass were quite small, those exhibiting the E-phenotype were on the average larger (158.6 mm) than those exhibiting the F_2-phenotype (149.3 mm), leading to the assumption that this intrapopulation variation was the result of age. The F_2-phenotype was not restricted to the Apalachicola River on the Gulf Coast. It also appeared in these young South Carolina hatchery fish. If we consider the history of striped bass stocking in the southeast a possible scenario can be envisioned to explain the occurrence of the F_2-phenotype in these two systems. From the mid-1960's to the mid-1970's striped bass from the Monck's Corner hatchery were stocked in the Apalachicola system at irregular intervals. Thus, the F_2-phenotype may represent a fairly rare Atlantic phenotype seen exclusively in southeastern Atlantic riverine systems.

Age-related changes

The age-group analysis of crystallins protein in striped bass has produced interesting information about the response of these proteins to senescence. The crystallins that were examined in this aspect of this study were exclusivey alpha and beta crystallins as defined by their isoelectric points. The banding pattern data depicted in Figure 12 shows very distinct and predictable patterns. The fact that there are ten bands that appear in young-of-the-year fish (group-A), that don't appear in the oldest fish (group-C) may be related to phenomena that occurs in the alpha and beta crystallins of other organisms. Bloemendal (1977) has hypothesized that one consequence of crystallin aging is the deamidization of the primary polypeptide chains including the alpha, beta, and gamma crystallins (Krichevsdaya et al. 1984; De Jong, 1981). Bloemendal has been pointed out that a second effect on the crystallins of the aging process is a defined degradation of polypeptides starting from the COOH-terminal end. In addition, we have observed that a dimunition of protein concentration in peaks from young to older individuals primarily involved the beta crystallins. This observation is consistent with Bours' (1980) results in which he detected a substantial loss of low molecular weight beta crystalluns with aging. This fraction almost disappeared in the lenses of aging rats, mice, and men. Krichevsdaya et al. (1984) explained this selective loss of beta crystallins on their higher amide content in comparison to that observed in the alpha crystallins. Bours (1980) also observed an increase in the insoluble albumenoid fraction, with this protein probably derived mainly from the degradation of the soluble alpha crystallin fraction.

The seven bands that were present in the oldest group (C) but absent in the youngest group (A) may have resulted from added aggregation of crystallin monomers or oligomers. Bloemendal (1977) found that alpha crystallins formed high molecular weight aggregates as a result of aging. He also reported evidence of the formation of alpha-beta crystallin heteromers associated with the aging process. Thus, change in the protein banding pattern and relative concentrations may be caused by either differential gene expression or by post-translational modification. The mechanism(s) of post-translational modification may either by protein aggregation, deamidation, fragmentation, or polypeptide degradation at the COOH-terminal end.

This data clearly shows that there is an alteration of the isoelectric focusing pattern of striped bass crystallin proteins with senescence. In an earlier work, Smith and Gilman (1983) also reported size related polymorphisms in lens protein patterns in ten out of thirteen marine fish species examined. This study is particularly noteworthy, in that it focused on the nuclei of eye lenses to minimize ontogenetic or environmental effects for any observed polymorphisms. It was believed that the nucleus was particularly resistant to developmental variation in banding patterns due to the low presumed turnover number of its protein molecules.

An additional complication should be considered in the interpretation of eye lens banding patterns. While it has been demonstrated that the age related loss of cryatallin fractions is common in many organisms, Leveille et al. 1984 have demonstrated that this process may inhibited by alteration of the diet. Specifically, these workers reported that restricting the intake of carbohydrates and fat in the diet of inbred strains of mice resulted in a retardation in the age-related loss of gamma crystallins. Other workers have also shown that sugars induce conformational changes in the alpha-crystallins (Liang and Chakrabarti, 1981). Conformational changes of bovine lens crystallins have also been reported in a photodynamic system (Andley and Chapman, 1986). The formation of intersubunit crosslinks was observed involving all three classes of crystallins when irradiated in the presence of the photosensitizer methylene blue. The implications of these findings for the use of eye lens proteins in stock identification are profound. They have demonstrated that both environmentally induced chemical and physical factors can change the chemical composition and molecular weight of the lens proteins.

The results of this study bear on the utility of isoelectric focusing as a tool for striped bass stock identification. Certainly, the age-related protein alteration phenomena reported herein must be taken into consideration when designing such studies. This is not to imply that the study of crystallins provides no pertinent information. However, it is clear, to avoid age-related misinterpretation of results, it is imperative that fish of similar ages be compared. An additional method might be to identify those proteins that change with age, and concentrate instead on those fractions with have been shown resistant to developmental change.

VI. Isozyme studies on striped bass spawning populations

Introduction

Protein electrophoresis has served as a valuable tool in the stock discrimination of several valuable commercial and recreational species. It is a procedure which is relatively inexpensive and routinely practiced in many laboratories. The most informative efforts have been those studies which have screened isozyme loci for genetic variability. Due to the known genetic basis of protein variability at these loci, the long term utility of these data sets may be appreciated due to the known genetic basis of protein variability at many of these loci. The Mendelian basis of protein polymorphisms at these loci may be clearly demonstrated. The most successful attempts at stock identification include the efforts by Utter and his group for species of Pacific salmon (see review in Utter et al. 1974) and Phillip et al's (1982) studies on largemouth bass. In general, the magnitude of protein polymorphisms in fishes has been comparable to that observed in other vertebrate species. In reviewing the electrophoretic literature for vertebrate species, Nevo (1978) compared mean heterozygosity levels for all vertebrates groups versus fish (H=0.0494-vertebrates; H=0.0512-fish). Marine and freshwater fish displayed comparable levels of genetic heterozygosity (H= 0.0555-marine species and H=0.0558-freshwater species). Mean heterozygosity approximates 5% in fish species, while the mean percentage of polymorphic loci is about 17% (Nevo, 1978; Kirpichnikov, 1981).

The striped bass has served as the focus for several multi-loci isozymic studies intent on discovering polymorphic markers to aid in management of this species. Otto (1975) and Grove et al. (1976) both unsuccessfully surveyed Hudson, Chesapeake, and Roanoke populations for discriminatory polymorphic markers. Despite screening 52 loci representing 28 different enzyme systems and 45 general proteins, Grove et al (1976) found only rare alleles at two loci; isocitrate dehydrogenase and alpha-glycerophosphate dehydrogenase. Sidell et al. (1980) were unsuccessful in identifying distinct spawning populations among the spawning river systems comprising the upper Chesapeake Bay. These investigators examined multiple serum proteins including transferrin previously reported polymorphic by Morgan et al. (1973). They found that many protein bands provided inconsistent resolution. Those which could be routinely scored, did not exhibit river-specific polymorphisms. They also examined allelic frequencies at two previously reported polymorphic isozymic loci. Variability was observed in alpha-glycerophosphate dehydrogenase, however, river-specific signficant differences in allelic frequencies were not encountered. Consistent resolution of isocitrate dehydrogenase was not achieved, thus, this enzyme system was excluded from the analysis. These workers concluded that due to possible gene flow, striped bass in the upper Chesapeake constitute a single homogeneous population Hitron (1974) screened the transferrin locus for genetic polymorphisms within Hudson River striped bass. He concluded that the Hudson maintained a single panmictic population. Finally, Rogier et al. (1985) scored 56 loci representing 31 enzyme systems in striped bass from two tributaries entering Kerr Reservoir in Virginia-North Carolina; the Dan and Roanoke Rivers. Only three isozymic loci proved polymorphic; muscle creatine kinase, inorganic pyrophosphatase, and inosine triphosphatase. Mean heterozygosity was only 1.6%, with 5% of loci proving polymorphic.

In summary, it may be concluded that despite massive efforts in genetic stock discrimination, the striped bass has proven to be one of the most monomorphic vertebrate species examined in depth to date. In the absence of at least moderate levels of genetic variability, efforts at stock discrimination prove futile. Rogier et al. (1985) provided various scenarios which may be invoked to account for this depravation of genetic variability in striped bass; including extreme environmental heterogeneity, bias in sampling isozymic loci, and recent population bottlenecks.

All of the above surveys of isozymic loci have utilized starch gel electrophoresis as the means to resolve enzyme products of interest. In many laboratories the use of polyacrylamide gel electrophoresis (PAGE) has frequently provided superior resolution (narrower bandwidth) of protein bands than available through use of the starch gel methodology. Thus, small differences in mobility are potentially more readily discernable. In addition, PAGE allows for more precise manipulation of gel pore size than possible with other methodologies. Most studies utilizing PAGE have employed the continuous buffer systems initially proposed by Davis (1964). In our laboratory, considerable success in the purification of Drosophila proteins has been enjoyed by use of "multiphasic zone electrophoresis (MZE) (Bargiello and Grossfield, 1980). MZE permits the systematic use of buffer and pH discontinuities to optimize separation of heterogeneous protein products. We used PAGE in conjunction with multiphasic buffer systems (MBS) to explore for isozymic polymorphisms in striped bass. This study is a test of the null hypothesis that significant allelic differences detectable via PAGE and MBS do not exist among striped bass collected from different spawning systems.

The theoretical rationale and practical advantages of using MZE in contrast to continous buffer systems have been systematically discussed by Chrambach et al. (1976). Briefly, multiple phases, each characterized by both common and separate buffer constituents and unique pH values, are generated during the electrophoretic process. These phases, comprised of homogeneous regions of electrolytes, are separated by a steady state moving boundary. The resolution characteristic of MZE gels is the result of two separate but co-ordinate phases. A "stacking phase" is initially generated in which the protein to be investigated is differentially concentrated between leading and trailing buffer constituents, resulting in a very fine starting zone. The finer the bandwidth of the protein entering the gel, the narrower it will be at the conclusion of the electrophoretic separation. These conditions may be optimized by selecting leading and trailing buffer constituents for the stacking phase whose electrophoretic mobility will be slightly greater and slightly less; respectively, than that of the protein of interest. That is, the sample components develop migration velocities intermediate to that of the leading and trailing constituents. The electrophoretic mobility of the sample protein may be controlled to maintain its central position in this "sandwich" by both the pH of the stacking phase and by the use of non-restrictive gel pore sizes. This "stack" continues to migrate until it encounters a discontinuity in the system either in terms of the buffer environment or the supporting medium.

"Unstacking" will result from one of two mechanisms which disrupt the steady-state conditions which have served to maintain the"stack". First, the mechanical properties of the separating gel due to its pore size will differentially retard the mobility of the protein species under investigation. Second, mobility of the trailing constituent in the "unstacking phase" will exceed that of the protein. As the moving boundary migrates further in the separating gel, new phases are formed, each with their own characteristic velocity, giving rise to banding patterns.

Operationally, implementation of this procedure requires access to the computer output of 4269 MZE buffer systems (Jovin et al. 1970) available on microfiche from the National Technical Information Service (NTIS). Buffer systems described in this output are generated by a computer program based on the MZE theory using 45 different buffer constituents. The output lists the recipes needed for making up the different phases used in each of the buffer systems. This includes buffer constituents and their concentrations for each of the phases in the 4269 systems. It also provides the pH of each phase, plus the mobility of its leading and trailing constituents in relation to sodium. Thus, if one knows the relative mobility of the protein of interest in relation to that of sodium, one may optimize the selection of buffer sytems.

Materials and methods

Striped bass were collected from the various Hudson, Chesapeake, Roanoke, Pacific coast, and coastal sites listed in Table 25. Standard lengths and sex (where possible) were determined for each fish. Approximately one ml of blood was obtained by cardiac puncture with the use of pediatric sized vacutainers (plain-no additives). Blood was centrifuged in the vacutainer tubes in a Sorval SS-34 at 2000 RPM to separate the cellular and serum fractions. Serum was stored at -40^0 C until further use. Livers were excised from all fish and both were immediately placed on ice, then stored at -40^0 C prior to homogenization. Livers were homogenized by a Virtis tissue homogenizer in 0.05 M Tris-HCl, pH 7.4 and then centrifuged in the Sorval SS-34 for one hour at 15,000 RPM. The supernatant was retained. If the supernatant still contained cellular debris, it was recentrifuged at the above conditions until it was free of debris. Homogenates were refrozen at -40^0 C until further use.

Gel Preparation

Three different polyacrylamide gel buffer systems were used in this study. They included Jovin et al. (1970) systems 4062 and 3017; and the Davis (1964) system. The choice of an optimal buffer system for each enzyme was determined empirically with a small subset of the samples. The final concentrations (M) of the leading and trailing constituent in each of the phases of these systems is depicted in Tables 27A and 27B. In addition, phase pH and the mobility of phase constituents in relation to sodium are also shown.

Both 15 cm x 1.6 cm i.d. pyrex tubes and 8.4 cm x 8.4 cm vertical slab gels were used in all separations. Recrystallized (Chrambach et al. 1976; Appendix D) reagent grade acrylamide (Kodak) was used in all procedures. 8% T, 5% C resolving gels were photopolymerized by the addition of 2mM TEMED, 2 mM ammonium persulfate, and 2 mM riboflavin. One-centimeter stacking gels (5% T, 15 $C\%_{DADT}$) were polymerized by the addition of 5.0 mM TEMED and 2.5 mM ammonium persulfate. In both cases polymerization was complete in about 10 minutes.

Samples were diluted in upper buffer or upper gel buffer in 20-50% sucrose and bromphenol blue tracking dye. Samples were diluted 1:1 and 10 ul were applied per lane. Electrophoresis was conducted in Pharmacia Vertical Apparatus PE-4 at room temperature under independently recirculating upper and lower chamber buffers. Electrophoresis was perfomed under constant current at 2-4 mA/cm of gel. Typical run times were 4 hours. Isozymes were visualized by the staining procedures of Shaw and Prasad (1970) or Johnson et al. (1972). R_f values were calculated for all protein bands in all lanes (Chrambach et al. 1976).

Results

We surveyed for polymorphisms at 22 presumptive enzymatic loci among the populations listed in Table 26. Only three loci proved polymorphic. These were Est 2, alpha-GPDH-B, and G6PI Est 2 was fixed for a single allele (R_f= 0.75) in all populations except Coos Bay, Oregon. The Oregon population was fixed for a second slower allele (R_f= 0.74) which was not found in any other population. This polymorphism was detected using Jovin buffer system 3017.

Low levels of polymorphism were detected in both GP6I and alpha-GPDH-B. Two alleles were noted at GP6I with R_f values of 0.193 for the common allele and R_f of 0.213 for the rarer variant. Low levels of the variant allele were found in most populations. These were visualized on Jovin buffer system 4062. Three different alleles were noted at the alpha-GPDH-B locus. The common allele had an R_f value of 0.153, while variant forms had R_f values of 0.115 and 0.173 on Jovin system 3017. All populations were polymorphic at this locus, although significant allelic frequency differences among populations was not observed.

Discussion

Striped bass isozymes systems are probably the most thoroughly characterized for any species of fish. Yet, despite this intensive effort to locate innate genetic tags which could prove valuable in stock management, striped bass proteins have proved exceedingly monomorphic. We report a percentage of polymorphic loci of 14% (3 out of 22 loci), which is slightly above the range (4% -10%) observed in the other striped bass multi-loci surveys conducted to date (Otto, 1975; Grove et al. 1976; and Rogier et al. 1985). Several hypothesis may be invoked to explain this discrepancy: 1) We selected a set of isozymes loci to screen which included a disproportionate percentage of enzyme systems already shown to be polymorphic by other workers. This was done in an effort to enhance the potential possibility of detecting effective stock discriminators, however, it likely inflated our estimate of polymorphism. 2) Our methodology may have been more sensitive in detecting small differences in protein band mobility than the starch gel systems previously employed. Although PAGE probably provides superior resolution of protein bands than starch systems, to our knowledge no study has been conducted on the same set of samples to compare the relative efficiencies of PAGE versus starch in detecting protein polymorphisms. 3) The geographical range of sampling locales in this study exceeded that of other workers, although sample size per site may have been smaller. Otto (1975) did include the California population with multiple Atlantic coast sites including Maine in his survey, however, he did not sample the Oregon population. One of the three loci which proved polymorphic was Est 2; for which the polymorphic allele was exclusively found and in fact fixed in the Coos Bay, Oregon population. Thus, by including the Oregon population, we increased our percentage of polymorphic loci by approximately one-third. To our knowledge, this is the first isozyme study to include the Oregon population in its analysis. If we exclude the Oregon population from our analysis, the percentage of polymorphic loci (9%) falls within the range of earlier reports.

Polymorphism at the Est 2 locus provides the ability to distinguish Oregon striped bass from all other fish analyzed, including those from San Francisco Bay. Otto (1975) also reported polymorphisms for an esterase locus. A word of caution however, must be acknowledged. Esterases are isozymes which are notoriously susceptible to both ontogenetic and environmental influence in their expression. We found the polymorphic allele to be fixed in the Oregon population while the common allele was fixed in the San Francisco Bay population. No heterozygotes were observed in either Pacific coast population. In addition, the Hudson River population was also fixed for the common allele. Considering the historical origin of the Pacific striped bass populations from the Navesink River in New Jersey about 100 years ago, one might expect to find the polymorphic Oregon allele in low frequency in the

Hudson population. In view of the absence of this allele in both our California and Hudson samples two plausible scenarios may be invoked to explain this finding. First, this allele may have arisen in the San Francisco Bay population, remained in very low frequency over the years, and then by a founder effect become the predominant allele in Oregon. Alternatively, this allele may have resulted from a mutation within the Oregon population and have rapidly become fixed in the population. Nonetheless, if this allelic difference in Est 2 is not a result of environmental or ontogenetic influence, it serves to distinguish Oregon from California striped bass.

In regards to Atlantic coast systems, polymorphisms were only observed at two enzymatic loci, alpha-GPDH and G6PI. The low degree of protein diversity at these two loci prohibited the use of these two proteins as markers to aid in stock identification. Both Grove et al. (1976) and Sidell et al. (1980) reported low level polymorphisms at one of the alpha-GPDH loci with two alleles reported. Using frequency differences at the alpha-GPDH locus, Sidell et al (1980) were unable to distinguish separate populations among multiple tributaries in the upper Chesapeake Bay. Grove et al. (1976) concluded that the degree of variation of alpha-GPDH was greater in the Hudson than in Southern spawning stocks, but they were unable to differentiate between hypothesized populations based on this information. Both of these groups of workers reported monomorphism at the two G6PI loci.

VII. Synopsis

We have explored the extent of both congeneric and conspecific mtDNA sequence diversity in the genus Morone through the use of restriction endonuclease analysis. The reported rich levels of intraspecific mtDNA sequence divergence regularly encountered in mammalian comparisons suggested the presence of sufficient interindividual diversity to permit the adoption of this approach to the field of unit stock discrimination, and the reconstruction of the phylogenetic history of recently diverged taxa. We had three primary objectives in conducting this study.
First, we sought to determine if sufficient mtDNA diversity exists between striped bass populations to permit: (1) the identification of discrete Mendelian populations; and (2) to determine if genotypic frequencies between individual populations are sufficiently diverse to identify single fish of unknown ancestry in a test set or mixed fishery. This could be accomplished with the use of a single enzyme or an entire battery of different restriction endonucleases. If this approach is adaptable to fisheries problems, is it necessary to prepare the

highly purified mtDNA needed for radioactive-labeled visualization of small fragments resulting from four-and five-base cutter digests, or is sufficient information obtained through ethidium bromide or hybridization probe visualized products of six-base-cutter digests? In this regard we have examined the distribution of mtDNA genotypes among Atlantic coastal and southeastern U.S. populations of striped bass. Second, several previously conducted studes on mtDNA diversity among lower vertebrates have reported conflicting levels of nucleotide diversity. In addition, accepted hypotheses concerning the dominant form of expression of mtDNA diversity have recently been questioned. Does the rate and mode of mtDNA evolution within and between the Morone species resemble that routinely observed in the mammalian literature? If not, does the life history of the striped bass provide any reasons for this departure? Third, does the data obtained by this molecular approach, restriction endonuclease of mtDNA, confirm the phylogenetic conclusions obtained via the analysis of morphological or protein electrophoresis characters? This was to be confirmed by exploring intrageneric relationships among the six Morone species.

Striped bass revealed the lowest level of base sequence diversity, as determined by cleavage site gain or loss, of any animal species examined to date (max. $p= <0.0004$). However, despite this paucity of conventionally observed base sequence heterogeneity, striped bass did display both major (100-400 bp), and minor (1-5 bp) mtDNA length variation frequency differences which were partioned along geographic bounds. Within the Atlantic coastal stock, five different discrete major length classes of mtDNA were identified, three of which were common. Based exclusively on major length variation, different unit stocks could be identified. Genetic differentiation between the combined Chesapeake versus Hudson and Roanoke stocks was observed. However, no genetic discontinuity was reported between Hudson and Roanoke river striped bass. A class of minor length variants detected by the four-base-cutting enzyme, Taq I, was exclusively observed in the Choptank River and in the Upper Chesapeake Bay. It allowed for subdivision of the Chesapeake stock into two component stocks. In considering both major and minor length variants, individual Hudson River and Chespeake Bay striped bass could be uniquely identified to origin. While the geographic distribution of major length variants frequencies does provide a framework in which to discriminate individual populations, the additional information obtained from stock specific microvariants (length or base substitutions), exclusively revealed by four-base cutters, brings us much closer to the ultimate goal of identifiction of ancestry of all individual fish in a mixed fishery. It seems likely that the further use of additional four-base-cutters will bring us much closer to that objective, albeit with the added inconvenience of multiple steps of mtDNA purification. Considering the length of the spawning season, and the acknowledged arrival of different "pulses" of fish on the breeding grounds, the potential exists for temporal partitioning of individual fish bearing different mtDNA genotypes. Thus, future sampling regimes should collect fish over the entire span of the spawning season, not only in a small window.

In the southeastern U.S., the use of the mtDNA approach enabled us to identify ancestry of individual striped bass (taken from the Apalachicola River) which we believe of Gulf descent based on unique major length genotypes or base substitutions not seen in any of our Atlantic collections in Florida, South Carolina, or the mixed coastal stock. This innate tag will allow for future genetic marking of all progeny of hatchery matings who can be easily monitored over their entire lives. Thus, the relative viabilities, growth, or fecundity of different fish can be followed without the added inconvenience of conventitonal tags. Future research in this region should focus on the identification of the extent of interbreeding between striped bass of Gulf ancestry and the introduced Atlantic fish. This question could be resolved by directly quantifying the extent of nuclear DNA divergence between Gulf and Atlantic striped bass using "DNA fingerprinting" or restriction fragment length polymorphisms.

Our analysis of congoneric Morone relationships via the mtDNA methodology generally confirmed the results previously obtained from morphological approaches. Similar concordance of approaches has been recorded by other mtDNA workers using other groups of fishes.

In summary, our studies on Morone populations were marked by an extreme paucity of expected base substitutions, geographic partitioning of macro-and-micro-length mtDNA genotypes, and an abundance of heteroplasmy. With the exception of sunfish populations, levels of mtDNA diversity in the lower vertebrates are depressed in comparison to that observed in higher taxa. mtDNA length variation was observed within multiple Morone species, and in one interspecific comparison. Substantial levels of heteroplasmy (>20%) were detected in striped bass with lower frequencies observed in other Morone species. Based on their data demonstrating length variation and heteroplasmy in bowfin, Bermingham et al. (1986) cautioned against "overthrowing previous generalizations about the major features of mtDNA evolution." Perhaps these generalizations should be reconsidered on a taxon by taxon basis.

Table 1
Estimated intraspecific divergence in mtDNAs between fishes

Species	Animals surveyed	Geographic locality	Maximum p*	Authors
Lepomis macrochirus bluegill sunfish (two subspecies)	38	Southeastern United States	0.085	Avise et al. (1984)
Katsuwonus pelamis skipjack tuna	13	Hawaii, Brazil, Puerto Rico	0.006	Graves et al. (1984)
Salmo gairdneri rainbow trout and steelhead	38	British Columbia, California	0.0013-0.011	Wilson et al. (1985)
Salmo salar Atlantic salmon (anadromous and non-anadromous)	15	Newfoundland	0.002	Birt et al. (1986)
Anguilla rostrata American eel	109	Maine to Florida, Louisiana	0.006	Avise et al. (1986)
Amia calva bowfin	75	Southeastern United States	0.01	Bermingham and Avise (1986)
Lepomis gulosus warmouth	74	Southeastern United States	0.06-0.07	Bermingham and Avise (1986)
Lepomis microlophus redear sunfish	77	Southeastern United States	0.08	Bermingham and Avise (1986)
Lepomis punctatus spotted sunfish	79	Southeastern United States	0.061	Bermingham and Avise (1986)

* generally calculated by the method of Nei and Li (1979)
p =base substitutions per nucleotide

Table 2

Coastal Sample Characterizations

Sample site	Date	# Individuals	Sex [(a)]	Size range(mm) (SL)
Chesapeake Bay				
Choptank River	5/9/83	1	M(1),F(0)	271
Upper Bay	5/10/83	10	M(10),F(0)	271-417
Choptank River	4/18/84	13	M(13),F(0)	245-319
Rappahannock	4/25/84	13	M(13),F(0)	216-394
James River	4/26/84	12	M(12),F(0)	205-344
C & D Canal	5/8/84	13	M(13),F(0)	230-281
Roanoke River, North Carolina				
Weldon	6/1/83	9	M(9),F(0)	369-479
Weldon	5/17/84	14	M(14),F(0)	279-355
Hudson River				
Croton Bay	5/21/83	2	M(1),F(1)	490-639
Cornwall	6/5/83	4	M(4),F(0)	450-539
Seatrain, NJ	3/3/83	15	M(4),F(0)	114-212
Croton Bay	3/21/83	3	M(2),F(0)	320-330
GW Bridge	3/3/83	5	M(3),F(0)	205-269
125th St, NYC	6/20/83	4	M(0),F(0)	137-220
Danskammer	6/27/83	2	M(0),F(0)	207-210
Tubby Hook	3/17/83	1	M(0),F(1)	306
Westway	12/17/83	15	M(6),F(1)	185-290
Cornwall	5/31/84	6	M(5),F(1)	392-552
Claverack	6/3/84	6	M(6),F(0)	429-525
Troy	7/15/84	9	M(0),F(0)	213-410
125th St, NYC	7/18/84	5	M(1),F(0)	162-255
Connecticut River	6/22/83	12	M(3),F(0)	205-255

Table 2 (continued)

Coastal Collection				(TL)
Montauk, NY	11/8/84	12	unknown	501-850
Montauk, NY	11/11/84	12	unknown	546-838

a- sex was not determined on all fish.

Table 3

Characterization of coastal samples collected at Montauk Point, New York

Collection site	Date	Gear	Size(FL) mm	Major mtDNA length	Taq I Min. length	Heteroplasmy
Gurney's	11/8/84	Haul seine	735*	1	1	no
Gurney's	11/8/84	Haul seine	520*	1	1	no
Gurney's	11/8/84	Haul seine	510*	2	1	no
Gurney's	11/8/84	Haul seine	456*	1	1	1A & 1B
Gurney's	11/8/84	Haul seine	473*	1	1	no
Gurney's	11/8/84	Haul seine	771*	2	1	no
Gurney's	11/8/84	Haul seine	464*	2	1	1A
Gurney's	11/8/84	Haul seine	482*	2	1	no
Gurney's	11/8/84	Haul seine	505*	2	2	1
Gurney's	11/8/84	Haul seine	491*	2	1	3
Gurney's	11/8/84	Haul seine	731*	2	1	3
Gurney's	11/8/84	Haul seine	491*	2	1	no
Elbow	11/11/84	Sport	584	2	1	3
Elbow	11/11/84	Sport	546	2	1	3
Elbow	11/11/84	Sport	584	2	1	3
Elbow	11/11/84	Sport	673	3	1	no
Elbow	11/11/84	Sport	635	2	1	no
Elbow	11/11/84	Sport	629	2	1	no
Elbow	11/11/84	Sport	711	2	1	no
Elbow	11/11/84	Sport	737	2	1	no
Elbow	11/11/84	Sport	572	3	1	no
Elbow	11/11/84	Sport	667	2	1	no
Elbow	11/11/84	Sport	686	1	1	no
Elbow	11/11/84	Sport	838	2	1	1A & 3

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* converted TL to FL by FL= 4.60 +0.902TL (Texas Instruments 1973- found in Westin and Rogers, 1978)

Table 4 A

Mean number and size of mtDNA fragments produced by four-base-cutter digests

Fragment number	Fragment size (bp) Rsa I	Taq I	Mbo I
1	2816	Polymorphic	1861
2	Polymorphic	1702	1604
3	2136	1620	Polymorphic
4	1974	1381	1262
5	1204	1135	1163
6	1104	1085	1092
7	838	924	1023
8	703	840	925
9	498	806	865
10	459	776	809
11	431	735	735
12	405	687	690
13	366	627	642
14	346	597	526
15	330	509	410
16	317	492	381
17	303	452	349
18	254	325	336
19	222	257	321
20	206	213	298
21	172	204	266
22	103	196	213
23	96	187	148
24	71	118	115
25		115	98
26			90
27			70
28			63
29			47
30			32

Table 4 B

Mean number and size of mtDNA fragments produced by five-base-cutter digests

	Fragment size (bp)	
Fragment number	Ava II	Hin FI
1	3715	Polymorphic
2	3126	1900
3	Polymorphic	1643
4	1926	1429
5	1651	1257
6	1184	1068
7	1146	868
8	693	625
9	653	603
10	515	560
11	383	533
12	222	509
13	208	495
14		483
15		460
16		428
17		391
18		361
19		348
20		323
21		222
22		195
23		167
24		117
25		111
26		97
27		92
28		59
29		41

Table 4 C

Mean number and size of mtDNA fragments produced by six-base-cutter digests

Fragment number	Fragment size (bp)						
	Ava I	Bgl II	Eco RI	Hind III	Pvu II	Sac I	Xba I
1	3952	7737	6330	7971	Poly	11277	5635
2	3645	Poly	5104	6033	5608	Poly	3535
3	2329	2792	2791	2296	1328	1276	3456
4	2126	1947	Poly	Poly			3233
5	1396						1703
6	1357						
7	1216						
8	Poly						
9	417						
10	398						
11	261						

Table 5

Frequency of major mtDNA length variants in various breeding populations

mtDNA genotype	Roanoke 1983	Roanoke 1984	James 1984	Rappahannock 1984	Choptank 1983-4	C & D 1984	Upper Bay 1983
1A	-	-	-	1/8%	-	-	-
1	3/33%	1/7%	-	-	1/7%	1/8%	-
2	3/33%	4/29%	6/50%	6/46%	8/57%	8/61%	8/80%
3	3/33%	9/64%	6/50%	6/46%	4/29%	4/31%	2/20%
4	-	-	-	-	1/7%	-	-

mtDNA genotype	Lower Hudson	Cornwall	Troy	Claverack	Connecticut
1A	1/3%	-	-	-	1/8%
1	12/31%	3/25%	4/45%	1/17%	2/17%
2	12/31%	7/58%	2/22%	2/33%	-
3	14/35%	2/17%	3/33%	3/50%	9/75%
4	-	-	-		-

Table 6A

Chi square table of population differences

	Upper Bay, Ches.	Choptank River	C & D Canal	Rappahannock River
Upper Bay, Ches.				
Choptank River	2.57			
C & D Canal	1,797	1,059		
Rappahannock River	2,945	3,654	2,967	
James River	2,121	2,547	1,857	0,962
Chesapeake Total	1,853	2,188	1,103	2,816
James & Rappahannock	3.08	5,103	3,613	0,234
Upper Bay & C & D	0,572	2,158	0,247	4,378
James, Rappahannock, Chop	2,876	1,994	2,032	1,445
Upper Bay, C & D, Chop	1,148	0,744	0,454	5,779
Roanoke 1983	5,435	3,494	3,207	5,453
Roanoke 1984	6,296	4,256	3,846	2,967
Roanoke Total	7,207	4,972	4,972	4,659
Troy, NY	7,769	5,727	5,574	7,521
Claverack, NY	4,053	1,837	1,664	2,801
Troy & Claverack	7,639	5,372	4,961	6,289
Cornwall, NY	2,909	2,595	1,773	6.047
Lower Hudson	* 9,787	8,341	7,075	5,806
Hudson Total	8,556	9,014	5,803	5,759
Connecticut River, Mass	15.4	** 12.175	** 12.175	8.574

Table 6B

Chi square table of population differences

James River	Chesapeake Total	James & Rappahannock	Upper Bay & C & D	James,Rappahannock,Chop
1,511				
0,493	2,811			
2,543	1,753	4,844		
1,097	0.745	1,585	3.411	
2,913	1,741	6,337	0,733	3,261
4,667	** 11,52	9,339	6,279	9.37
1.857	5.317	4.645	6,464	3,645
2.907	* 9.516	6.243	7,949	6,966
6,708	**** 18.324	** 12.862	* 9.861	*** 14.231
2,251	3,453	4.641	3,191	3,076
5,301	**** 15.212	* 10.133	9,123	** 11.48
5.077	8.633	9,002	3,562	8,134
6,738	**** 20.945	* 10.909	**13,173	*** 14,365
5,937	**** 20.324	* 10.238	**11,974	*** 13,964
9.6	**** 16.316	** 11.250	****16.8	** 12.504

Chi square table of population differences

Table 6C

Upper Bay, C & D, Chop	Roanoke 1983	Roanoke 1984	Roanoke Total	Troy, NY
7.043				
7.009	3.207			
8.818	1.259	0.911		
** 11.521	0.343	4.597	2.539	
3.02	0.625	0.556	0.019	1.25
* 10.486	0.152	3.236	1.303	0.296
4.301	1.4	6.155	4.248	2.748
**** 16.538	0.636	5.708	3.899	0.859
**** 15.977	0.314	6.264	3.904	0.993
**** 18.548	6.912	5.21	6.256	6.368

Table 6D

Chi square table of population differences

Claverack, NY	Troy & Claverack	Cornwall, NY	Lower Hudson	Hudson Total
0.583				
2.225	3.022			
1.281	1.04	4.158		
1.155	0.927	2.984	0.656	
4.875	6.634	** 12.655	7.625	7.625

Table 7

Frequencies of Taq I Microvariants at Chesapeake Spawning Sites

	Upper Bay	Choptank	C & D	Rappahannock	James
Common Genotype	7	4	10	12	10
Rare Genotype	4	6	0	0	0

Table 8

Frequency of heteroplasmy in coastal migratory striped bass

Spawning system	# of heteroplasmic individuals	% of sample from that system
Hudson River	10	17
Chesapeake Bay	10	16
Lower Bay	6	24
Upper Bay	4	10
Roanoke River	8	38
Connecticut River	2	17
Coastal Collection (Montauk Pt, NY)	8	33

Table 9

Stocking history of striped bass in the St. Johns River, Florida system.

Year	Total no. of striped bass released	Sources of parents for striped bass released	%
1972	184,000	St. Mary's River, Ga. Nassau River, Ga. St. Johns River, Fla	Unknown
1973	24,300	Nassau River, Ga.	100%
1974	180,550	Monck's Corner Hatchery, S.C. Nassau River, Ga. St. Mary's River, Ga.	51.9% 48.1%
1975	577,750	St. Johns River, Fla Nassau River, Ga.	86.8% 13.2%
1976	499,760	St. Johns River, Fla Monck's Corner Hatchery, S.C.	Unknown
1977	679,400	St. Johns River, Fla Monck's Corner Hatchery, S.C.	71.8% 28.2%
1978	None		
1979	372,625	Monck's Corner Hatchery, S.C. St. Johns River (Lake Talquin) Fla.	80.9% 19.1%
1980	846,660	Monck's Corner Hatchery, S.C. St. Johns River (Lake Talquin) Fla.	54.2% 45.8%
1981	None		
1982	787,800	Monck's Corner Hatchery, S.C. St. Johns River (Lake Talquin) Fla.	59.2% 40.8%
1983	1,193,960	Monck's Corner Hatchery, S.C. St. Johns River (Lake Talquin) Fla.	54.8% 45.2%

Table 9 (continued)

1984	500,825	Monck's Corner Hatchery, S.C.	65.1%
		St. Johns River (Lake Talquin) Fla.	34.9%
1985	None		

Table 10

Stocking history of striped bass in the Apalachicola River system.

Year of stocking	No. of striped bass stocked	Source of stripers	Origin	Where released
1966	60,157	Monck's Corner Hatchery, S.C.	Atlantic	Lake Seminole
1968	1,780	Monck's Corner Hatchery, S.C	Atlantic	Lake Seminole
1974	26,600	Monck's Corner Hatchery, S.C	Atlantic	Lake Seminole
1976	33,600	Monck's Corner Hatchery, S.C	Atlantic	Apalachicola River
1980	100,800	Welaka Hatchery, Fla.	Gulf	Lake Seminole
1980	12,500	Welaka Hatchery, Fla.	Gulf	Apalachicola River
1982	37,600	Welaka Hatchery, Fla.	Gulf	Lake Seminole
1983	11,025	Welaka Hatchery, Fla.	Gulf	Apalachicola River
1983	20,000	Welaka Hatchery, Fla.	Gulf	Apalachicola River
1983	80,000	Welaka Hatchery, Fla.	Gulf	Lake Seminole
1983	32,400	Welaka Hatchery, Fla.	Gulf	Lake Wimico
1984	21,000	Welaka Hatchery, Fla.	Gulf	Lake Seminole
1984	20,000	Welaka Hatchery, Fla.	Gulf	Apalachicola River

Table 11

Sample origin and characterization.

Collection site	Size of mtDNA molecule	Xba I Genotype	Standard length (mm)	Lateral scale ct.
Monck's Corner Hatchery, S.C.	C	1	158	61
Monck's Corner Hatchery, S.C.	C	1	150	53
Monck's Corner Hatchery, S.C.	C	1	149	63
Monck's Corner Hatchery, S.C.	C	1	149	61
Monck's Corner Hatchery, S.C.	C	1	151	65
Monck's Corner Hatchery, S.C.	C	1	167	65
St. Johns River, Fla.	C	1	345	58
St. Johns River, Fla.	C	1	275	55
St. Johns River, Fla.	B	1	302	65
St. Johns River, Fla.	C	1	316	53
St. Johns River, Fla.	C	1	380	55
St. Johns River, Fla.	C	1	460	68
St. Johns River, Fla.	B	1	459	60
St. Johns River, Fla.	C	1	362	54
St. Johns River, Fla.	B	1	460	59
Warm Springs Hatchery, Ga. (1984)	C	2	194	64*
Warm Springs Hatchery, Ga. (1984)	C	2	168	64*
Warm Springs Hatchery, Ga. (1984)	C	2	183	64*
Warm Springs Hatchery, Ga. (1984)	C	2	193	64*
Warm Springs Hatchery, Ga. (1984)	C	2	198	64*
Warm Springs Hatchery, Ga. (1984)	C	2	191	64*
Woodruff Dam, Apalachicola River	A	2	242	67
Woodruff Dam, Apalachicola River.	A	2	227	69
Warm Springs Hatchery, Ga. (1983)	D	1	452	63
Warm Springs Hatchery, Ga. (1983)	D	1	465	65
Warm Springs Hatchery, Ga. (1983)	D	1	460	66
Warm Springs Hatchery, Ga. (1983)	D	1	472	67
Woodruff Dam, Apalachicola River	D	2	908	66
Chattahoochee River-Apalachicola System	C	1	1303	66
Chattahoochee River-Apalachicola System	D	1	1203	66

*Mean count

Table 12

1985 Apalachicola year class: sample origin and characterization

Collection site	Size of mtDNA molecule	Xba I Genotype	Standard length (mm)	Lateral line scale count
Lower Apalachicola River, Apalachicola, Fla.	A	2	153	65
Lower Apalachicola River, Apalachicola, Fla.	D	1	125	59
Lower Apalachicola River, Apalachicola, Fla.	D	1	122	62
Lower Apalachicola River, Apalachicola, Fla.	C	1	173	63
Lower Apalachicola River, Apalachicola, Fla.	C	2	141	64
Lower Apalachicola River, Apalachicola, Fla.	D	1	143	60
Lower Apalachicola River, Apalachicola, Fla.	D	2	135	59
Lake Seminole, Apalachicola System	C	2	117	63
Lake Seminole, Apalachicola System	C	2	118	60
Lake Seminole, Apalachicola System	C	2	133	56
Lake Seminole, Apalachicola System	C	2	134	58
Lake Seminole, Apalachicola System	D	1	127	64
Lake Seminole, Apalachicola System	D	1	150	62

Table 13

Mean size and number of mtDNA fragments in southeast striped bass

Aha III	Ava I	Ava II	HinF I	Hind III	Mbo I	Msp I	Rsa I	Taq I	Xba I
11208	3761	3766	2699*	8814	1681	1843	2810	1934*	5524
3234*	3488	3174	1861	6387	1604	1640	2469	1679	3478
1636	2270	2920*	1604	2248	1358*	1499	2129*	1608	3405
1036	2090	1930	1409	1609*	1262	1459	1969	1354	3165
	1392	1634	1230		1163	1222*	1200	1124	1714
	1352	1118	1054		1092	839	1104	1075	121
	1223	1070	848		1023	816	822	914	
	826*	663	612		925	745	677	845	
	375	621	567		865	622	589	793	
	367	356	544		809	564	477	758	
	263	217	515		735	538	432	705	
		201	494		690	497	397	641	
			457		642	469	346	590	
			409		526	442	324	510	
			358		410	386	307	459	
			341		381	367	291	362	
			312		349	351	240	303	
			289		336	342	209	255	
			191		321	290	191	211	
			164		298	270	104	202	
			105		255	259	98	194	
			90		213	247	66	188	
			84		148	232		108	
			60		115	181		105	
			57		98	150			
			44		90	138			
					70	128			
					63	97			
					47	86			
					32				
4	11	12	26	4	30	29	22	24	6

Number of fragments generated

*** Fragments which display length polymorphisms**
(Where individual fragments display length polymorphisms, the size depicted is that of a Monck's Corner striped bass.)

Table 14

The fragment number which shows length polymorphisms and the alternate sized fragments observed

	Aha III	Ava I	Ava II	Hin FI	Hind III	Mbo I	Msp I	Rsa I	Taq I
Fragment number which shows length polymorphisms	2	8	3	1	4	3	5	2	1
Alternate sized	3716	931	3196	3058	1927	1450	1219	2810	2248
	3402	810	3058	2837	1690	1262	970	2630	2049
fragments (bp)	3234	676	2920	2699	1609	1163		2469	1934
	3153		2791	2526	1506	1092		2369	1835

Table 15

Comparative characteristics of species in the fish genus Morone

Scientific name	Common name	Endemic Distribution	Salinity Preference	Commercial val.
Morone americana	white perch	South Carolina to Nova Scotia	anadromous	moderate
Morone chrysops	white bass	Mississippi and Ohio Valleys Great Lakes	freshwater	small
Morone labrax	European sea bass	Europe, Mediterranean, northwest Africa	marine	moderate
Morone mississippiensis	yellow bass	Mississippi valley	freshwater	minor
Morone punctata	spotted sea bass	southwest Europe, western Africa, Mediterranean	anadromous	moderate
Morone saxatilis	striped bass	St Johns River, Florida to St Lawrence River, Canada; Gulf of Mexico	anadromous	high

Table 16

Estimated genetic distance in mtDNA (p) in interspecific comparisons of fishes

Organisms	p Value*	Authors
Salmo gairdneri and Onchorynchus tshawytscha Rainbow trout and chinook salmon	0.034	Berg and Ferris (1984)
Salmo trutta and Onchorynchus tshawytscha Brown trout and chinook salmon	0.072	Berg and Ferris (1984)
Salmo trutta and Salvelinus fontinalis Brown trout and brook trout	0.1	Berg and Ferris (1984)
Lepomis (nine species) Sunfishes L. auritus, L. cyanellus, L. gibbosus, L. gulosus, L. marginatus, L. megalotis, L. microlophus, L. punctatus, L. macrochirus	0.07-1.0 median= .206	Avise and Saunders (1984)
Salmo gairdneri and Salmo clarki Rainbow trout and cutthroat trout	0.02-0.035	Wilson et al. (1985)
Oncorhynchus (five species) Pacific salmon O. kisutch, O. tschawytscha, O. nerka, O. gorbuscha, O. keta	0.025-0.069	Thomas et al. (1986)
Anguilla anguilla and Anguilla rostrata European eel and American eel	0.037	Avise et al. (1986)

* p was generally calculated by the method of Nei and Li (1979)
p=base substitutions per nucleotide

Table 17

Congeneric Morone sample characterizations

Species	Collection site(s) (number of individuals)	Collection date(s)	Gear
Morone americana	Hudson River (4); Hook Creek, N.Y. (4); James River, Va. (2)	12/84; 2/83; 4/84	hook & line; hook & line; trap net
Morone chrysops	Lake Burlington, N.C. (8); Hulston River, Va. (11)	5/84; 5/85	electroshock electroshock
Morone labrax	Casablanca, Morocco (10)	2/85	city aquarium
Morone mississippiensis	Reelfoot Lake, Tenn. (9)	4/84	electroshock
Morone punctata	Senegal River, Senegal (8) Dakar Harbor, Senegal (2)	8/85	beach seine commercial
Morone saxatilis	Hudson River; Chesapeake Bay; Roanoke River, N.C.	4/84 4/85	hook & line; trap net; gill net

Table 18

Restriction endonucleases used, their recognition sequences, and the number of mtDNA fragments produced in each species.

	Morone					
	americana	chrysops	labrax	mississippi	punctatus	saxatilis
6-base enzymes						
Ava I (CPyCGPuG)	9	8	12	10	8	11
Eco RI (GAATTC)	3	2	3	2	4	3
Hind II (GTPyPuAC)	8	12	6	8	11	10
Hind III (AAGCTT)	6	4	6	7	4	4
Pvu II (CAGCTG)	5	5	5	5	8	5
Sac I (GAGCTC)	3	1	2	2	0-1	4
Xba I (TCTAGA)	3	4	3	0-1	2	7
5-base enzymes						
Ava II (GG $\overset{A}{\underset{T}{\,}}$ CC)	14	11	15	13	17	12
Hin FI (GANTC)	24	28	21	22	23	26
4-base enzymes						
Taq I (TCGA)	27	26	-	28	-	23
Total	102	101	73	97	77	105

Total number of fragments examined-555

Table 19-A

Estimates of mtDNA differentiation among the six species in the genus Morone based on restriction profiles obtained exclusively from five-base cutters

	Morone					
	1 americana	2 chrysops	3 labrax	4 mississi.	5 punctata	6 saxatilis
1.M. americana		0.077	0.132	0.045	0.165	0.082
2.M. chrysops	0.338		0.102	0.093	0.091	0.083
3.M. labrax	0.162	0.240		0.128	0.071	0.117
4.M. mississippiensis	0.521	0.270	0.169		0.146	0.079
5.M. punctata	0.103	0.279	0.368	0.133		0.124
6.M. saxatilis	0.316	0.312	0.197	0.329	0.180	

Results are based on restriction profiles obtained from two enzymes each of which recognize five-base sequences. Values above the diagonal represent mean estimates of base sequence divergence (p). Values below the diagonal are estimates of the proportion of shared fragments (F).

Table 19-B

Estimates of mtDNA differentiation among the six species in the genus Morone based on restriction profiles obtained exclusively from six-cutters

	Morone 1 american	 2 chrysop	 3 labrax	 4 ississ	 5 punctata	 6 saxatilis
1.M. americana		0.0699	0.131	0.043	0.119	0.061
2.M. chrysops	0.310		0.148	0.074	0.105	0.055
3.M. labrax	0.119	0.090		0.131	0.101	0.110
4.M. mississippiensis	0.479	0.290	0.119		0.096	0.081
5.M. punctata	0.143	0.177	0.188	0.203		0.124
6.M. saxatilis	0.359	0.395	0.164	0.260	0.132	

The above results are based on restriction profiles obtained from seven endonculeases which all recognize six-base sequences. Values above the diagonal are mean base sequence divergence estimates (p). Below the diagonal are estimates of the total proportion of shared restriction fragments (F).

Table 19-C

Estimates of mtDNA differentiation among the six species in the genus Morone

	Morone					
	1 americana	2 chrysops	3 labrax	4 mississip.	5 punctata	6 saxatilis
1. *Morone americana*		0.080	0.132	0.043	0.140	0.073
2. *M. chrysops*	0.325		0.127	0.080	0.098	0.083
3. *M. labrax*	0.142	0.169		0.130	0.087	0.113
4. *M. mississippiensis*	0.533	0.325	0.145		0.119	0.089
5. *M. punctata*	0.122	0.231	0.286	0.167		0.124
6. *M. saxatilis*	0.353	0.327	0.181	0.289	0.156	

The above results are based on restriction profiles obtained from ten restriction endonculease digests per sample, including four, five, and six-base-cutters. Values above the upper-left-to-lower right diagonal represent base sequence divergence values (p). These p values have been weighted according to the number of base pairs in the various restrictions sites. Values below the diagonal are the total proportion of shared fragments (F).

Table 20

Collection Sites for Striped Bass for Isoelectrofocusing of Eye Lens Proteins

SITE	NUMBER OF FISH	DATE
HUDSON		
CORNWALL	6	5/31/84
CLAVERACK	6	6/3/84
TROY	9	7/15/84
WESTWAY	3	12/17/83
CHESAPEAKE BAY		
JAMES R.	11	4/24/84
RAPPAHANNOCK R	13	4/25/84
CHOPTANK R.	13	4/8/84
C. AND D. CANAL	14	5/8/84
ROANOKE R. (N.C.)	14	5/17/84
SOUTH CAROLINA	6	11/6/84
FLORIDA		
ST. JOHNS R.	9	11/14/84
APALACHICOLA R.		
WILD	2	4/26/85
WARM SPRINGS-1984	6	5/9/85
WARM SPRINGS-1983	3	12/1/85

Table 21

Chesapeake			Roanoke			Hudson E			Hudson F			
%	pI	mg/ml	%	pI	mg/ml	%	pI	mg/ml	%	pI	mg/ml	BAND
13.43	6.546	0.031	?	6.522	?	8.62	6.503	0.033	5.897	6.522	0.004	GAMMA 1
16.15	6.433	0.035	?	6.387	?	8.62	6.404	0.04	5.897	6.41	0.006	GAMMA 2
2.04	5.77	0.003	6.39	5.76	0.016	6.65	5.797	0.015	2.81	5.79	0.003	BETA 6
5.26	5.74	0.006	4.44	5.71	0.013	5.09	5.75	0.02	6.84	5.74	0.008	BETA 7
9.26	5.70	0.018	10.06	5.68	0.033	10.3	5.70	0.04	7.88	5.68	0.007	BETA 8
2.11	?	0.017	?	5.68	?	2.49	5.69	0.01	2.49	5.64	0.004	BETA a
np	np	np	np	np	np	np	np	np	2.59	5.58	0.004	BETA X
np	np	np	np	np	np	np	np	np	2.41	5.50	0.004	BETA Y
np	np	np	np	np	np	np	np	np	1.33	5.38	0.002	BETA Z
1.492	5.073	?	4.12	?	0.015	7.025	5.059	0.028	1.95	5.055	0.003	ALPHA 1a
2.235	5.029	?	2.43	5.018	0.008	3.28	5.021	0.012	1.431	5.002	0.003	ALPHA 1
2.27	5.008	?	2.475	5.004	0.008	1.53	5.003	0.006	1.708	4.976	0.004	ALPHA 2

MEAN PERCENTAGES OF PROTEIN, CONCENTRATIONS OF PROTEIN, AND ISOELECTRIC POINTS FOR MAJOR SPAWNING LOCALITIES

Table 22

Apalachicola E		Apalachicola F 2		St. John's		S. Carolina-E		S. Carolina-F_2		
%	pI	%	pI	%	pI	%	pI	%	pI	BAND
?	6.502	10.55	6.520	5.56	6.506	6.87	6.54	?	6.54	GAMMA 1
?	6.41	6.86	5.897	4.91	6.413	3.75	6.43	?	6.43	GAMMA 2
?	5.769	3.46	5.77	4.08	5.84	3.75	5.79	2.04	5.79	BETA 6
2.24	5.74	7.97	5.71	4.37	5.78	5.63	5.74	5.61	5.74	BETA 7
8.23	5.68	7.30	5.68	11.35	5.71	8.75	5.70	6.12	5.70	BETA 8
3.23	5.69	1.41	5.64	5.70	5.560	3.13	5.69	3.12	5.68	BETA a
np	np	np	np	np	np	np	np	np	np	BETA X
np	np	np	np	np	np	np	np	np	np	BETA Y
np	np	np	np	np	np	np	np	np	np	BETA Z
0.543	5.104	1.76	5 104	?	?	?	?	?	?	ALPHA 1a
1.32	5.046	4.12	5.046	1.48	5.075	?	?	?	?	ALPHA 1
3.21	5.004	2.94	5.004	1.51	5.027	?	?	?	?	ALPHA 2

MEAN PERCENTAGES OF PROTEIN AND ISOELECTRIC POINTS FOR SOUTHEAST LOCALITES.

Table 23

CHESAPEAKE		ROANOKE		HUDSON E		HUDSON F	
LW	SL	LW	SL	LW	SL	LW	SL
125mg	372.6mm	132.5mg	316.9mm	240.6mg	411.3mm	54.5mg	217.8mm

ST. JOHN'S		APALACH.-E		APALACH.-F_2	
LW	SL	LW	SL	LW	SL
216.6mg	372.7mm	242mg	375.4mm	27.5mg	181mm

S. CAROLINA - E		S. CAROLINA - F_2	
LW	SL	LW	SL
36.6mg	158.6mm	20.0mg	149.3mg

MEAN EYE LENS WEIGHT (LW)

AND STANDARD LENGTHS (SL) FOR ALL SPAWNING LOCALITIES

Table 24

Band Designation	GROUP-A			GROUP-B			GROUP-C		
	pI	%	ug/uL	pI	%	ug/uL	pI	%	ug/uL
ALPHA-1	4.82	0.74	0.0094	*	*	*	*	*	*
ALPHA-2	4.88	1.00	0.0047	*	*	*	*	*	*
ALPHA-3	4.91	0.70	0.0124	4.89	1.20	0.0267	4.90	0.90	0.0797
ALPHA-4	4.94	1.68	0.0325	*	*	*	*	*	*
ALPHA-5	4.97	1.40	0.0120	4.97	1.35	0.0391	4.96	1.33	0.1020
ALPHA-6	4.99	0.87	0.0224	5.00	1.22	0.0346	4.99	1.23	0.1064
BETA-1	*	*	*	*	*	*	5.02	3.48	0.3066
BETA-2	*	*	*	*	*	*	5.11	0.62	0.0527
BETA-3	*	*	*	*	*	*	5.14	1.13	0.0992
BETA-4	5.20	0.60	0.0057	*	*	*	5.20	0.62	0.0535
BETA-5	5.24	1.30	0.0326	5.24	1.26	0.0388	*	*	*
BETA-6	5.27	1.03	0.0050	*	*	*	5.26	1.33	0.1028
BETA-7	5.30	0.91	0.0091	5.28	1.32	0.0328	5.29	1.82	0.1457
BETA-8	5.33	1.79	0.0192	5.33	1.94	0.0464	5.32	2.09	0.1907
BETA-9	5.36	1.29	0.0281	5.35	1.40	0.0405	*	*	*
BETA-10	5.39	2.50	0.0422	5.39	1.67	0.0455	5.38	2.20	0.1099
BETA-11	*	*	*	5.43	0.82	0.0215	5.41	1.52	0.1410
BETA-12	5.45	1.64	0.0273	5.45	1.80	0.0473	5.44	1.57	0.1032
BETA-13	5.48	2.20	0.0258	*	*	*	5.47	3.08	0.2537
BETA-14	5.51	1.73	0.0533	*	*	*	5.50	1.68	0.1314
BETA-15	5.54	1.81	0.0352	5.54	2.20	0.0568	5.54	3.80	0.3459
BETA-16	*	*	*	5.57	2.10	0.0642	*	*	*
BETA-17	5.60	1.93	0.0623	*	*	*	5.60	2.61	0.2059
BETA-18	6.10	1.98	0.0095	*	*	*	*	*	*
BETA-19	6.14	8.83	0.0612	6.15	8.43	0.1750	6.15	6.07	0.4965
BETA-20	6.17	4.75	0.0401	*	*	*	*	*	*
BETA-21	*	*	*	*	*	*	6.21	6.54	0.5633
BETA-22	6.23	4.81	0.0491	6.23	5.03	0.1238	6.24	5.70	0.4782
BETA-23	6.26	5.07	0.0331	6.26	3.07	0.0889	6.26	4.56	0.3996
BETA-24	6.30	10.16	0.0652	6.29	5.92	0.1219	6.30	5.76	0.5271
BETA-25	6.32	11.40	0.1283	6.32	3.40	0.1011	*	*	*
BETA-26	6.35	5.35	0.0945	6.35	6.03	0.1639	*	*	*
BETA-27	6.38	6.04	0.0992	6.37	3.75	0.1104	6.37	6.51	0.5743
BETA-28	6.41	9.20	0.0846	6.41	3.35	0.1066	*	*	*
BETA-29	*	*	*	6.45	7.67	0.1695	*	*	*

Mean Isoelectric Point, Mean Percentage of Total Protein, and Mean Protein Concentration for each Band.

Note : Protein Concentrations are expressed as ug. of protein per uL. of sample.

Table 25

Sampling locales for striped bass examined in isozymic study

Site	Number of fish	Date of Collection
Hudson River, New York	25	4/79
Hudson River, New York	32	4/79
Suisun Bay, California	50	6/79
Little Neck Bay, LI, New York	44	8/79
Lake Moultrie, South Carolina	40	9/79
Montauk Point, LI, New York	52	10/79
Albermarle Sound, North Carolina	46	11/79
York River, Virginia	28	11/79
Rapahannock River, Virginia	24	11/79
Huntington, LI, New York	28	11/79
Coos Bay, Oregon	30	season 1979
Pamlico Sound, North Carolina	30	11/79
Hudson River, New York	51	4/80
Lake Moultrie, South Carolina	38	5/80

Table 26

Enzyme loci screened in striped bass from different spawning populations

Enzyme system	Enzyme Council Number	Abbreviation	No. of Loci
Alcohol dehydrogenase	1.1.1.1	ADH	1
alpha-glycerophosphate dehydrogenase	1.1.1.8	AGP	2
Esterase (serum)	3.1.1.1	EST	1
Esterase (liver)	3.1.1.1	EST	5
Glucose-6-phosphate dehydrogenase	1.1.1.49	G6PD	1
Glucose phosphate isomerase	5.3.1.9	GPI	2
Glutamate dehydrogenase	1.4.12	GDH	1
3-Hydroxybutyrate dehydrogenase	1.1.1.30	3HBDH	1
Isocitrate dehydrogenase	1.1.1.42	IDH	1
Lactate dehydrogenase	1.1.1.27	LDH	2
Malate dehydrogenase	1.1.1.37	MDH	1
Phosphoglucomutase	2.7.5.1	PGM	1
Sorbitol dehydrogenase	1.1.1.14	SDH	2
Xanthine dehydrogenase	1.2.1.37	XDH	1
Total systems=14			Total loci=22

Table 27-A

Final Phase Concentrations (M) in System 4062

Constituent	Upper electrophoretic buffer	Operative stacking phase	Stacking gel prior to electrophoresis	Lower electrophoretic buffer
C_1 Glycine	0.0400	0.0400		
C_2 Phosphate			0.0304	0.0564
C_3 Chloride				
C_6 Tris	0.0380	0.0380	0.0384	0.1367
pH	8.89	8.89	6.44	7.73
Rm (1)	-0.090	-0.090		
Rm (2)			-0.685	-0.909
Rm (3)				
Rm (6)	0.065	0.065	0.489	0.383

Table 27-B

Final Phase Concentrations (M) in System 3017

Constituent	Upper electrophoretic buffer	Operative stacking phase	Stacking gel prior to electrophoresis	Lower electrophoretic buffer
C_1 Cacodylic acid	0.0400	0.0400		
C_2 Acetic Acid			0.0509	0.0500
C_3 Acetic Acid				
C_6 Triethanolamine	0.0008	0.0008	0.0116	0.0259
pH	4.50	4.50	4.19	4.75
Rm (1)	-0.009	-0.009		
Rm (2)			-0.183	-4.14
Rm (3)				
Rm (6)	0.550	0.550	0.550	0.550

Figure 1
mtDNA genetic map

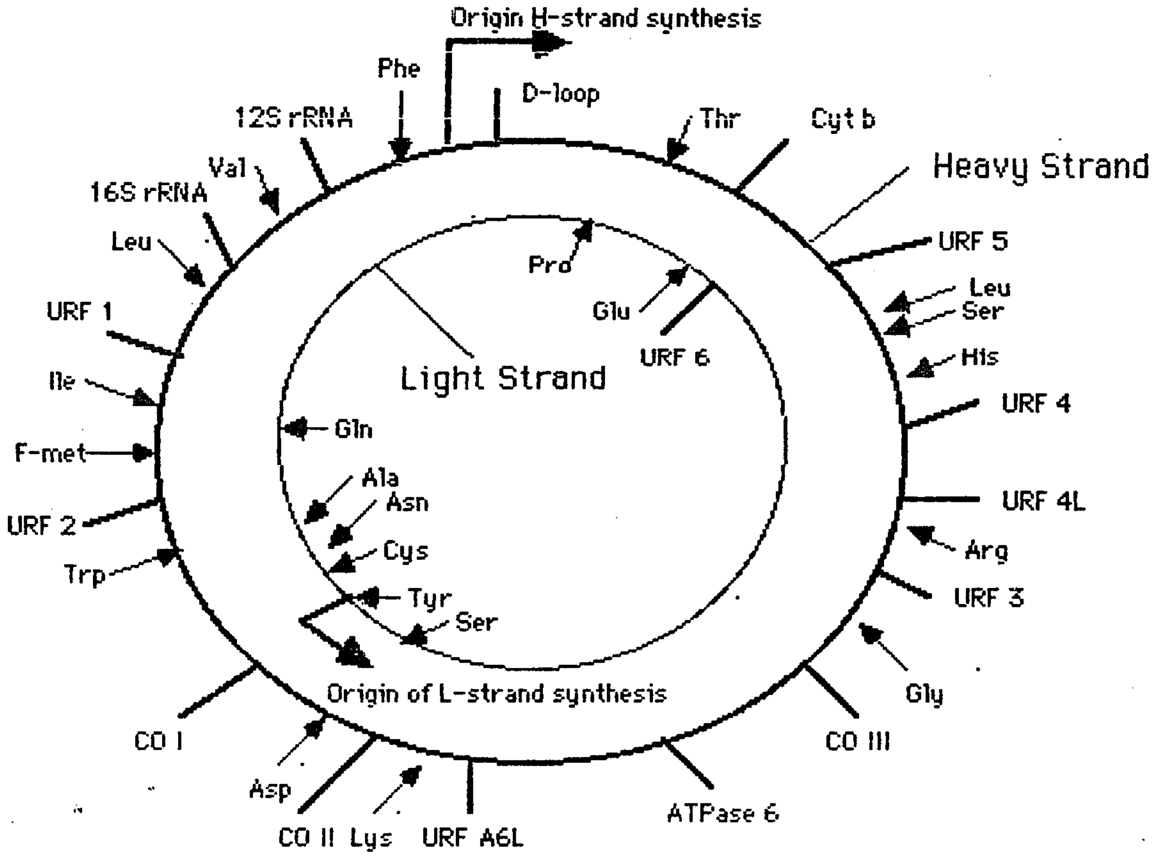
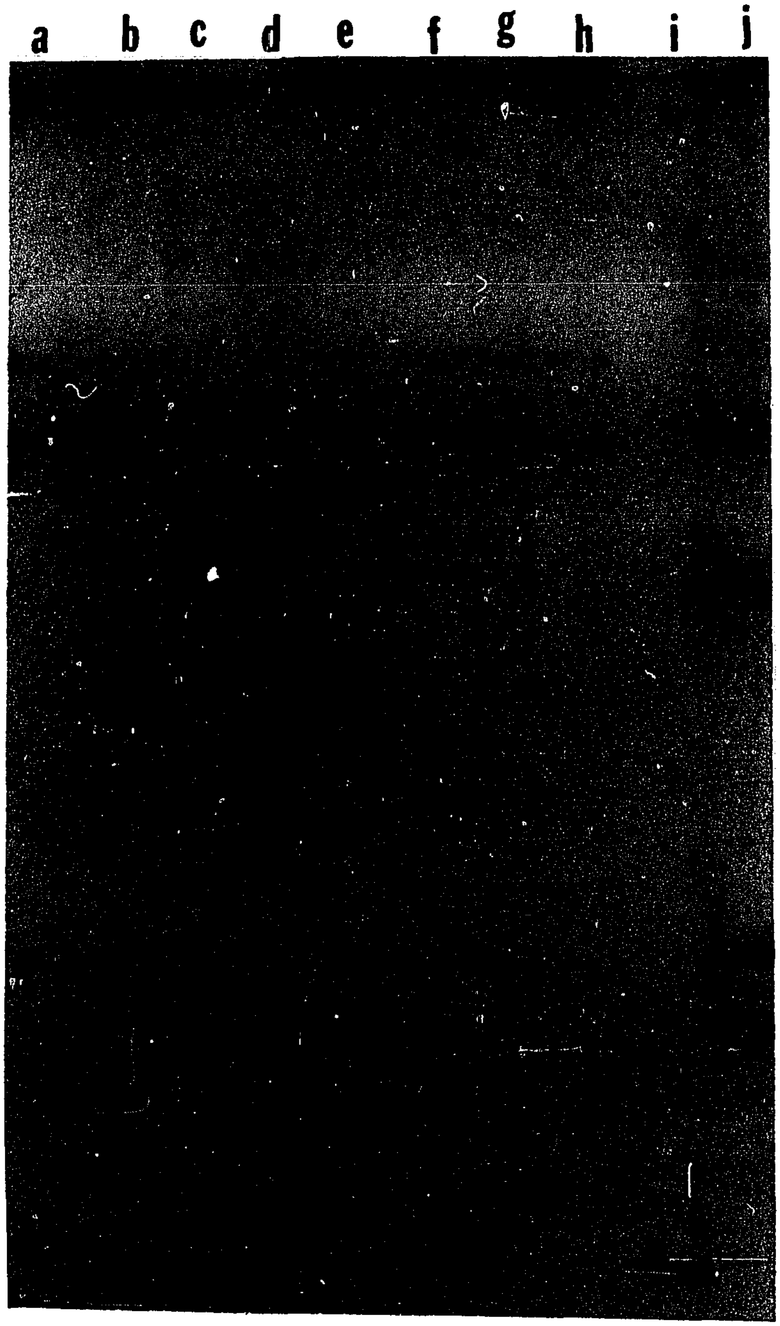


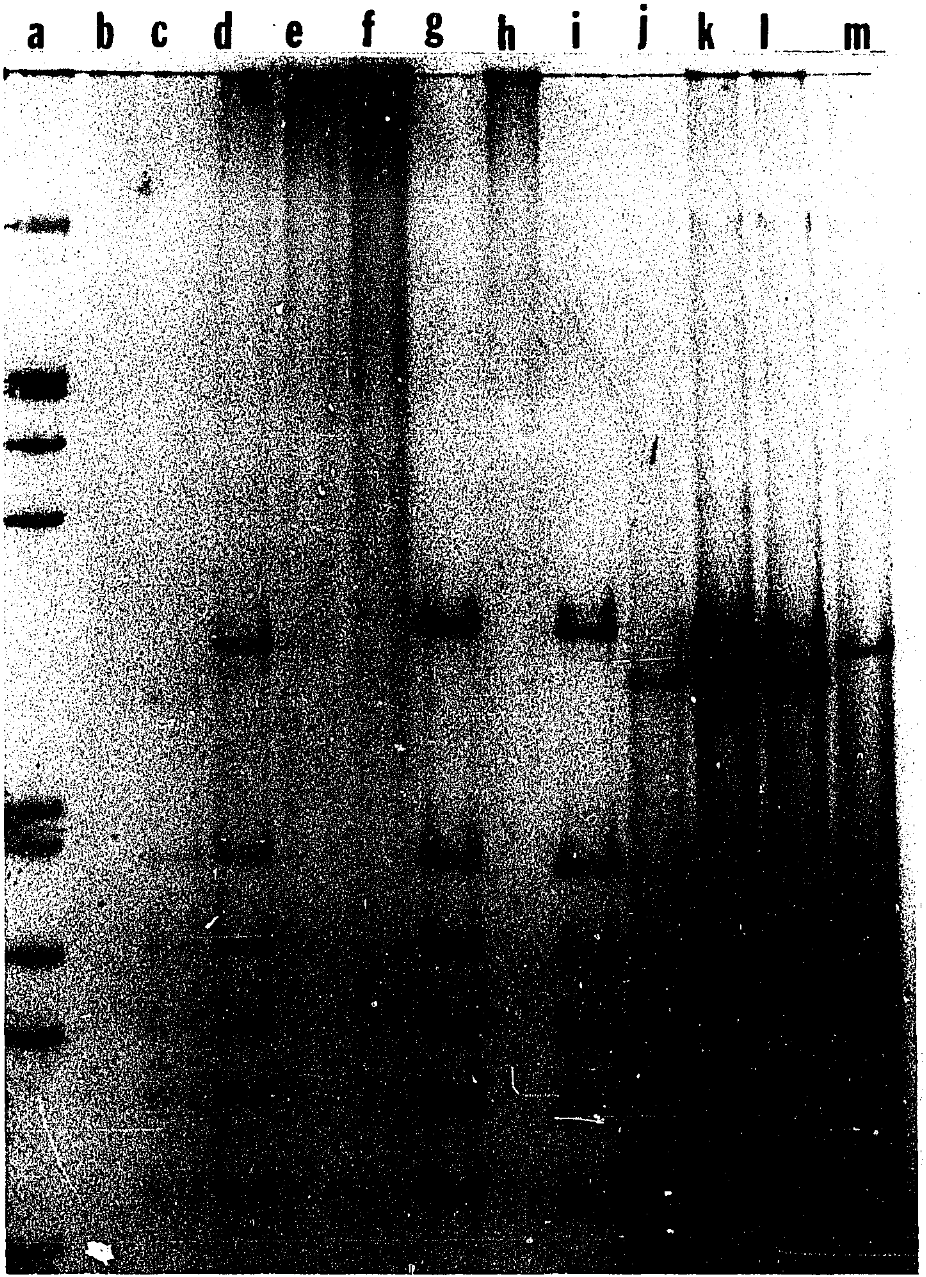
Figure 1. Generalized genetic map of the vertebrate mitochondrial genome. Map positions of the twenty-two tRNA genes are indicated by solid lines with arrows. CO I, CO II, and CO III are genes for cytochrome oxidase subunits. URF 1-6 refer to unidentified reading frames. Cyt B is cytochrome B, while ATPase 6 is the subunit 6 of that enzyme. (Modified from Avise and Lansman, 1983; Attardi, 1985)

Figure 2



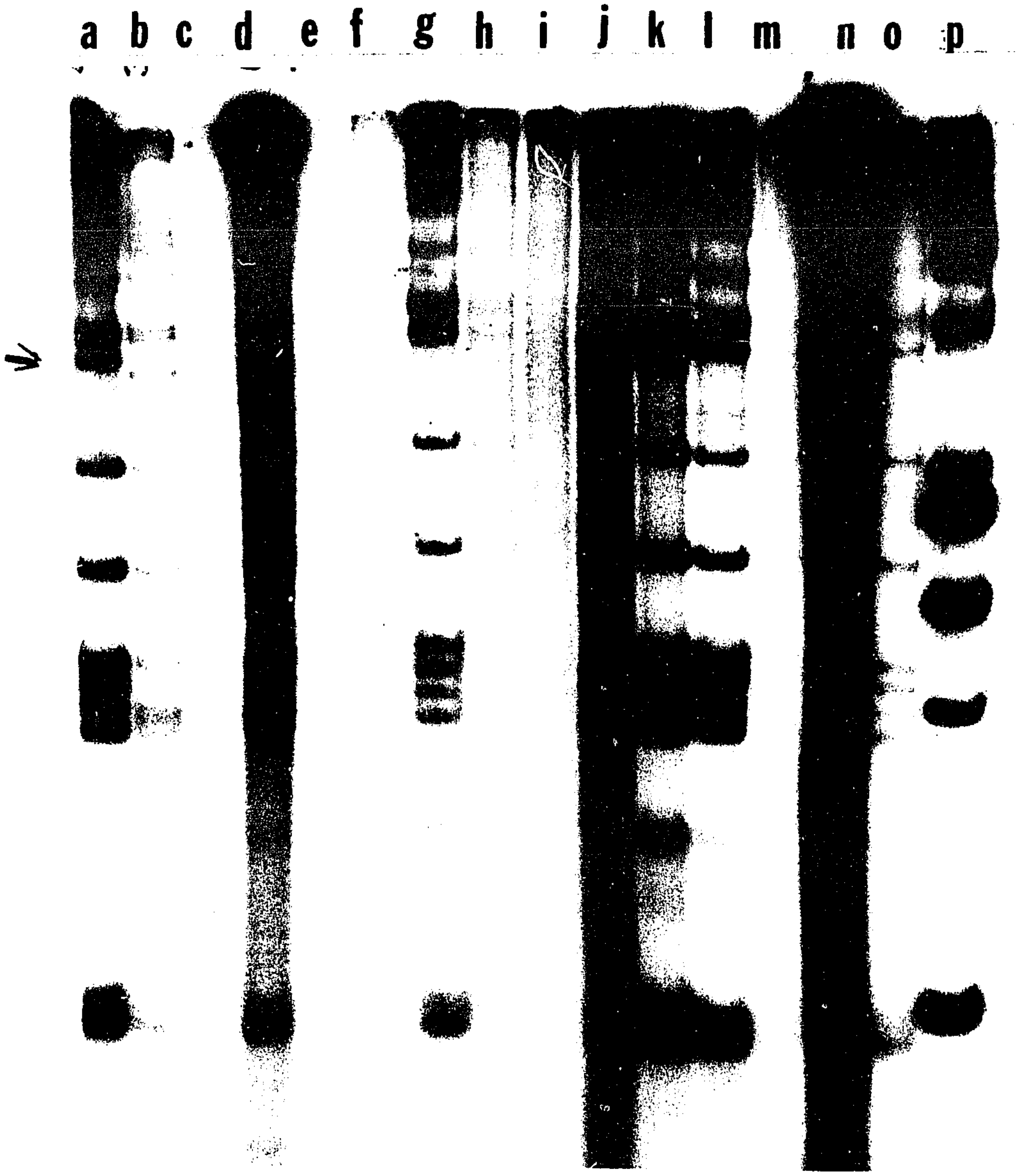
1.0 % agarose gel of Hind III digested striped bass mtDNA. DNA fragments were end-labeled with 32P. All fish included are representative of the Atlantic coastal migratory stock. The three common major length genotypes may be observed; 1, 2, and 3. In addition, one white perch's mtDNA also digested with Hind III is shown. Heteroplasmy for a length variant may clearly be seen in Lane e. Lane a-3; Lane b-1, Lane c-white perch-Hind III, Lane d-3; Lane e-1; Lane f-3; Lane g-2; Lane h-2; Lane J-lambda-Hind III.

Figure 3



1.5% agarose gel of Hin FI digested striped bass mtDNA from the coastal migratory stock. All mtDNA fragments were end-labeled with 32P. The three common length genotypes may be observed; 1, 2, and 3. In addition genotype 4 is exhibited by one fish. Several of the samples are displaying heteroplasmy. Particularly interesting is the sample in lane K which is exhibiting four different lengths (1,2,3,4) of the mtDNA molecule. Lane a-Lambda-Hind III-Eco RI; Lane c-4; Lane d-2; Lane e-2; Lane g-1; Lane i-1; Lane j-3; Lane k-2; Lane l-3; Lane m-2.

Figure 4



6.0% polyacrylamide gel of Taq I digested striped bass mtDNA. Minor length variant may be observed in fragment #17. All Hudson and Roanoke striped bass are monomorphic for this fragment. Only upper and mid- Chesapeake striped bass exhibited minor length variant for this marker. This rare fragment was 16 bp smaller than the common fragment we have observed to be 452 bp. The rare variant may be observed in lanes B and F.

Figure 5

Restriction map of striped bass mtDNA

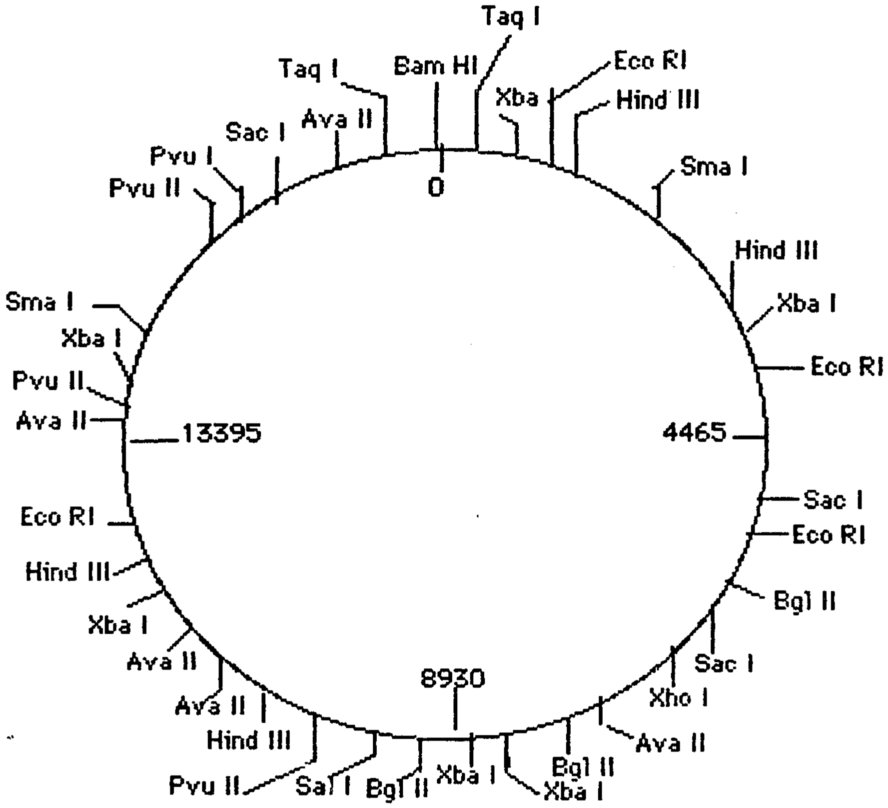
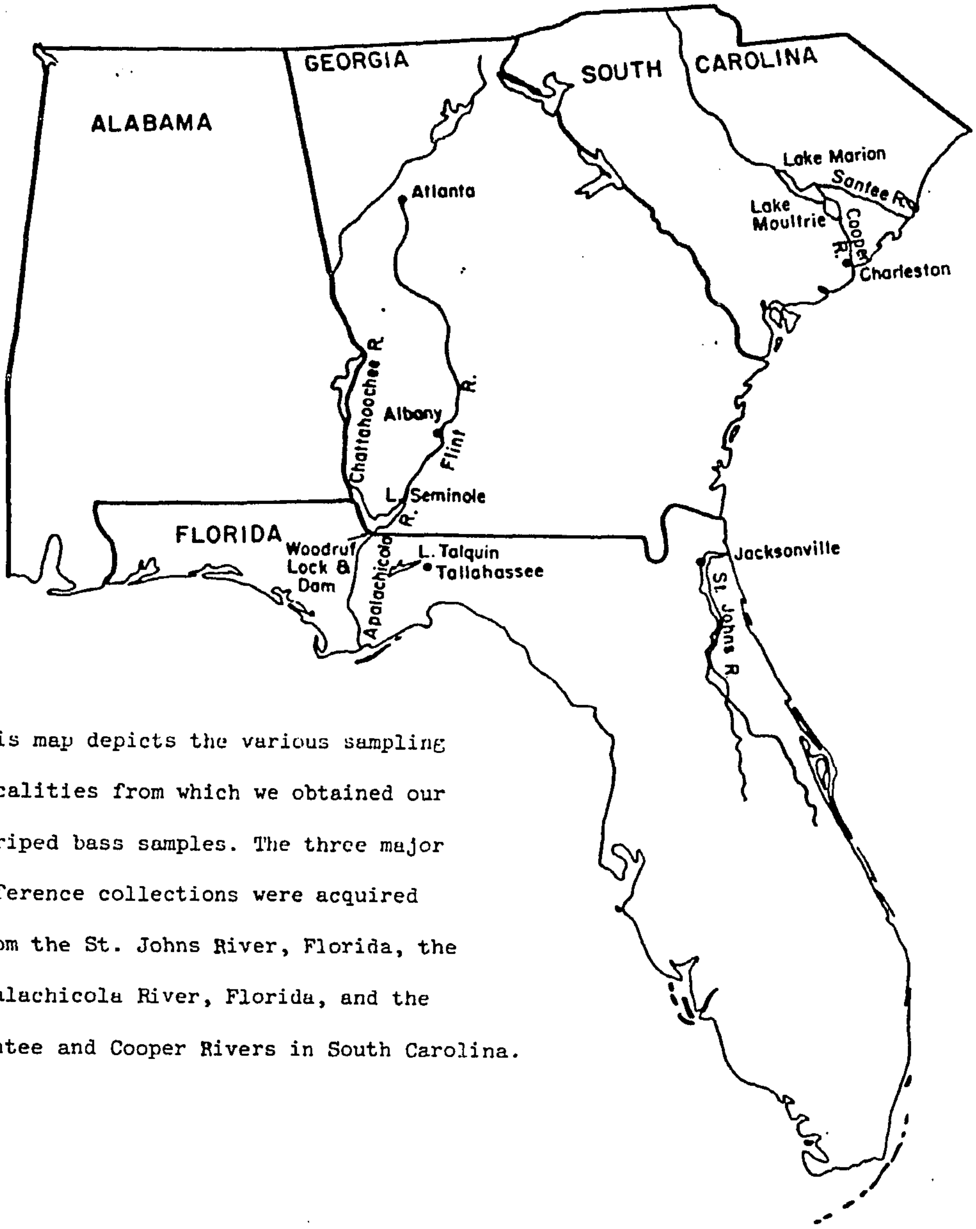


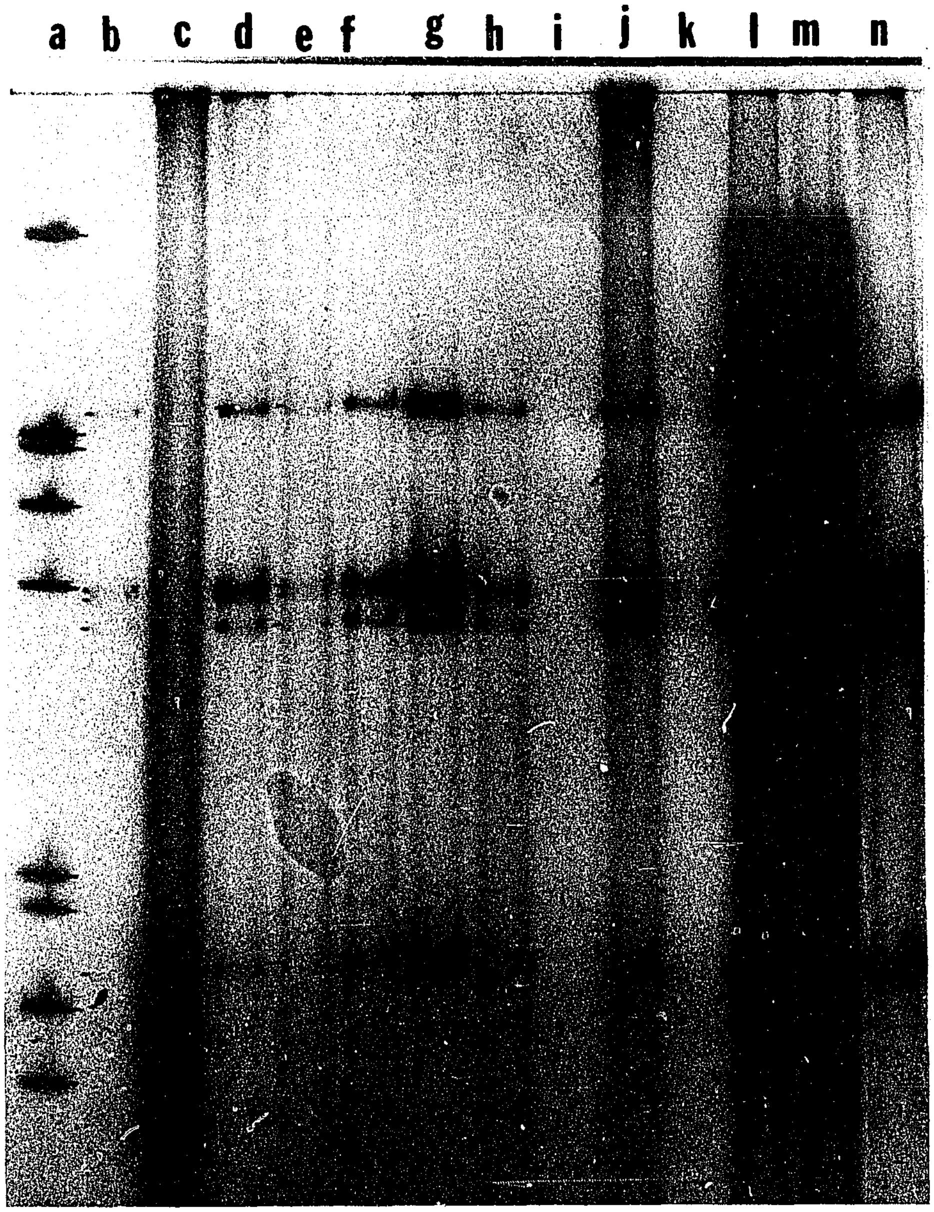
Figure 5. Restriction map of the circular striped bass mitochondrial DNA molecule. The mean size of this genome was determined to be 17,860 kb based on all digests using all the restriction enzymes described in this study. The single Bam HI site was arbitrarily assigned map position zero. All other restriction sites were assigned in relationship to this Bam HI reference point. The actual map distance between individual restriction sites is not drawn to scale.

Figure 6



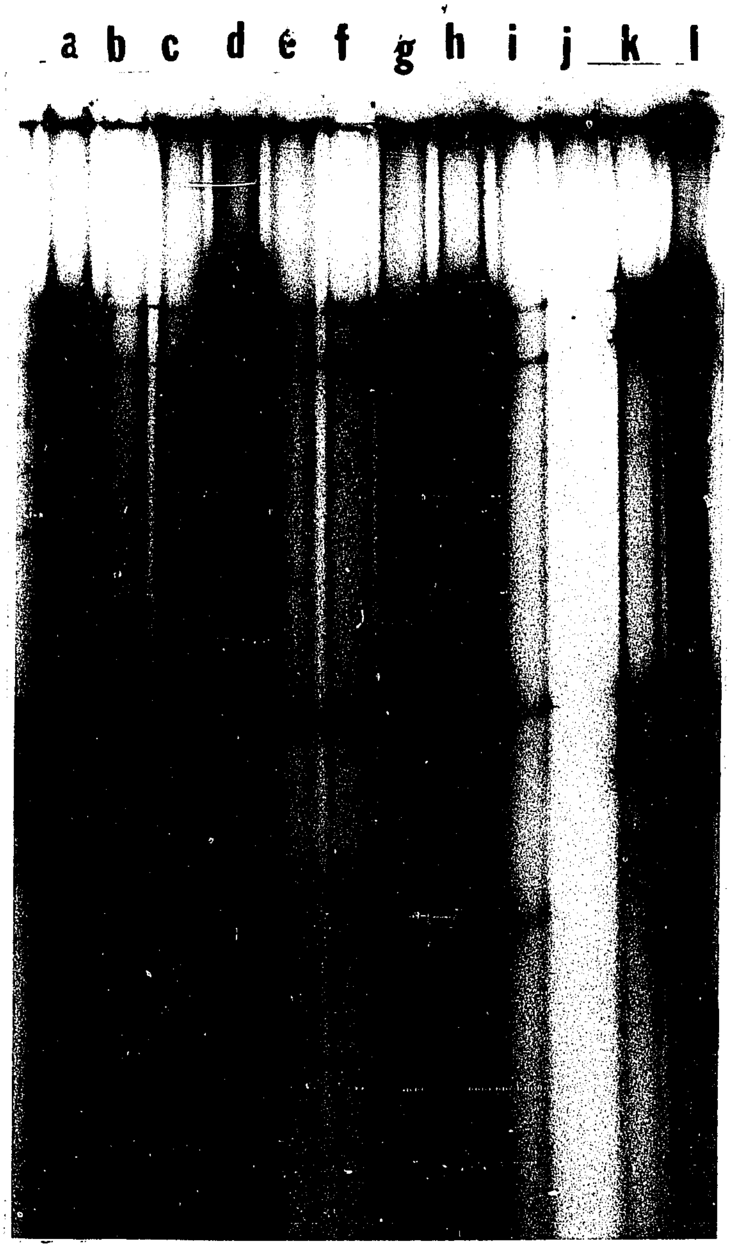
This map depicts the various sampling localities from which we obtained our striped bass samples. The three major reference collections were acquired from the St. Johns River, Florida, the Apalachicola River, Florida, and the Santee and Cooper Rivers in South Carolina.

Figure 7



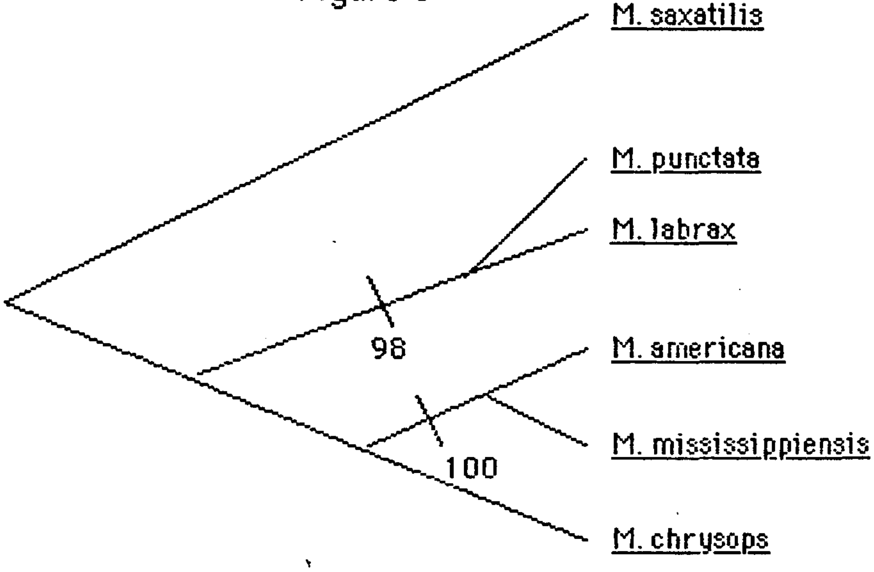
1.1% agarose gel of Xba I digested striped bass mtDNA. All fish were collected from the Atlantic stock and display Xba I genotype-1. This is the common Atlantic genotype. It is represented by a five fragment pattern on agarose gels. Molecular sizes of these fragments are 5524 bp, 3478 bp, 3405 bp, 3165 bp, and 1714 bp. The 121 bp fragment can only be visualized on a polyacrylamide gel. Xba I genotype-2 was only seen in some fish from the Apalachicola system. This Xba I marker serves to uniquely identify striped bass of Gulf lineage. This genotype is represented by only a four fragment pattern on agarose gels.

Figure 8



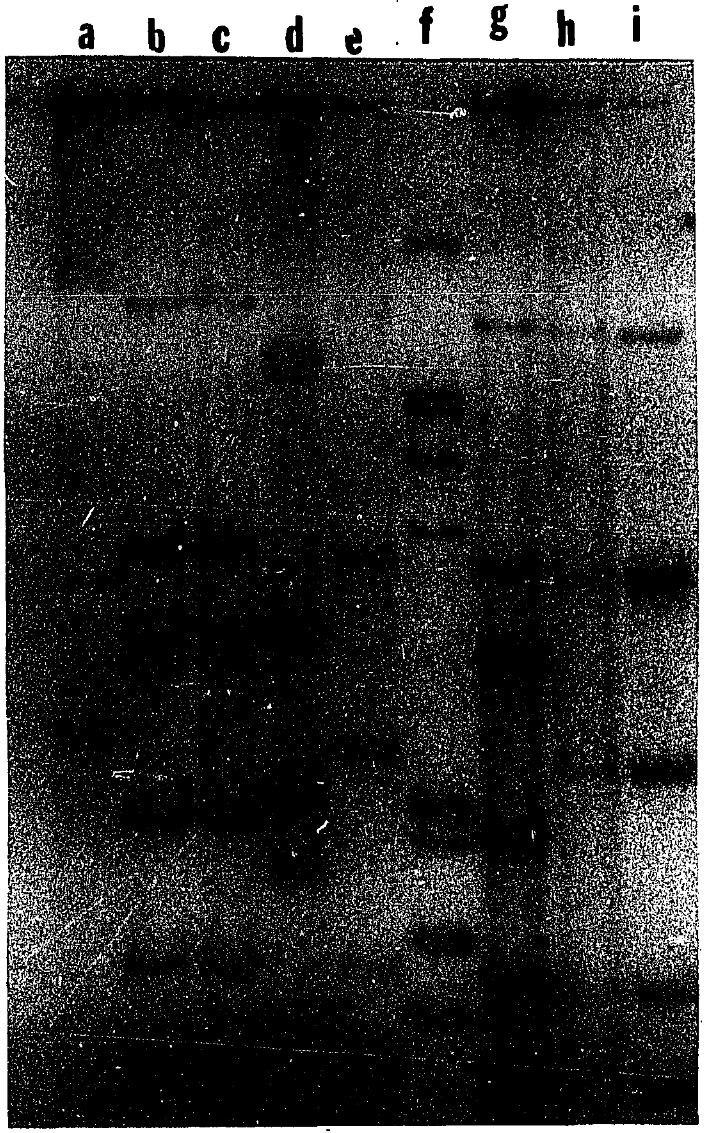
1.0% agarose gel of Hind III digested mtDNA from southeastern U.S. striped bass. Four different length mtDNA genotypes may be observed in the smallest Hind III fragment. The fish in Lanes k and L display the largest length genotype; A, unique to the Apalachicola system.

Figure 9



Wagner parsimony network of mtDNA genotypes generated from the presence-absence data matrix by the METRO program. Numbers along branches indicate the proportion of times a grouping was supported in the bootstrap analysis (BOOTM).
(note-Hin FI and Taq I data were not included)

Figure 10



Hind III digest of mtDNA from individuals representative of several different species in the genus Morone. mtDNA fragments were end-labeled and run on a 1.1% agarose gel. Lane a-M. chrysops; Lane b-M. mississippiensis; Lane c-M. mississippiensis; Lane d-M. labrax; Lane e-M. americana; Lane f-Lambda-Hind III-Eco RI; Lane g-M. mississippiensis; Lane h-M. americana; Lane I-M. americana. Length variation between M. americanus and M. mississippiensis can be easily seen in comparing Lane G to Lane H. All fragments show identical mobility (and thus equivalent molecular size) except for the the third and fourth largest fragments in M. mississippiensis (Lane g) when compared to the third largest fragment in M. americana. Together the size of these two fragments is approximately 2000 bp larger than the size of fragment three in M. americanus. All three yellow bass depicted on this gel are monomophic for Hind III digest patterns.

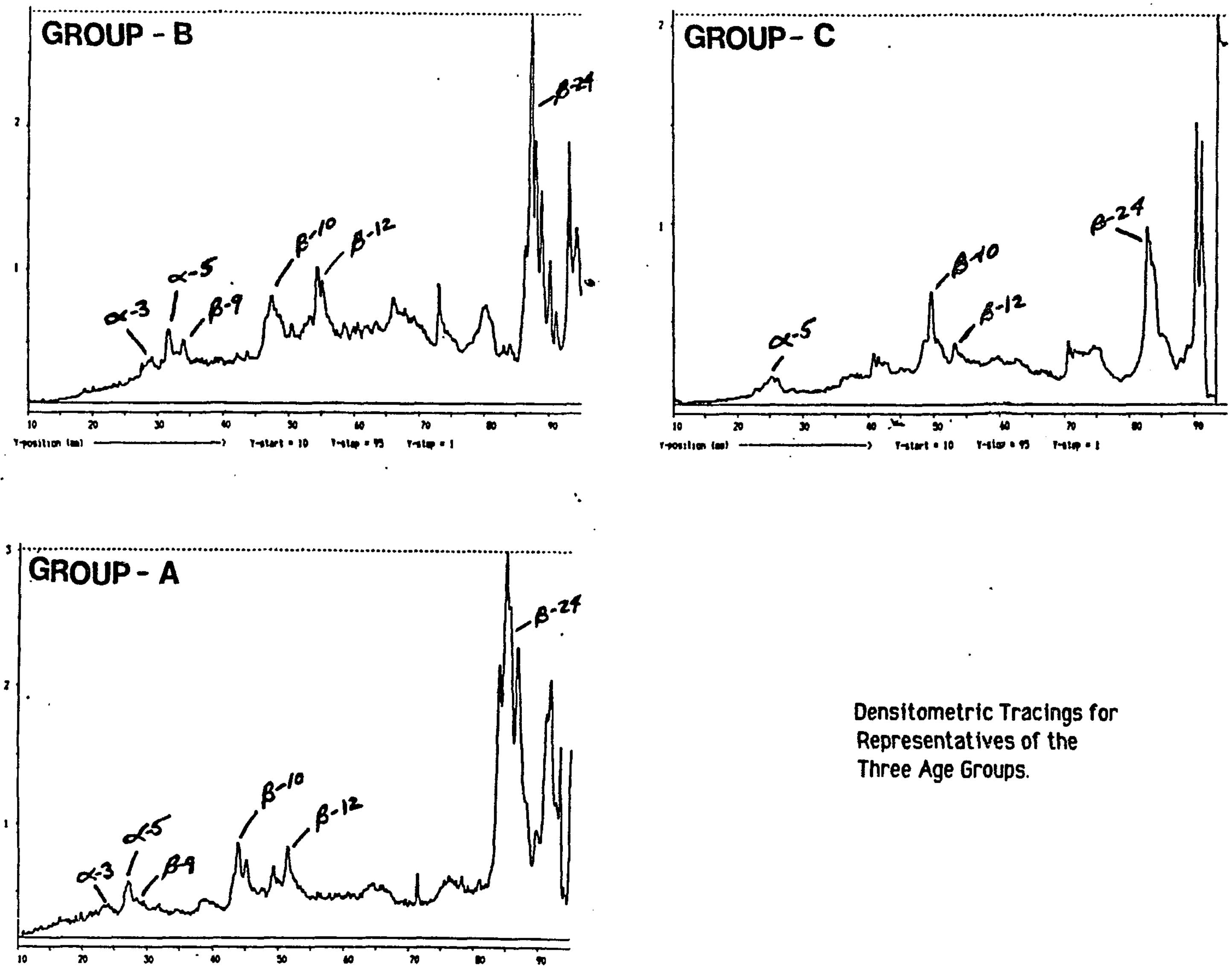


Figure 11

Densitometric Tracings for Representatives of the Three Age Groups.

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