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An electrophoretic analysis of Texas Gulf Coast red drum (*Sciaenops ocellata*): Identification of possible stocks and implications for fisheries management

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AN ELECTROPHORETIC ANALYSIS OF TEXAS GULF COAST RED DRUM (*SCIAENOPS OCELLATA*): IDENTIFICATION OF POSSIBLE STOCKS AND IMPLICATIONS FOR FISHERIES MANAGEMENT

by

WILLIAM RAY WILDER

A THESIS SUBMITTED
IN PARTIAL FULFILLMENT OF THE
REQUIREMENTS FOR THE DEGREE

Doctor of Philosophy

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HOUSTON, TEXAS

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ABSTRACT

AN ELECTROPHORETIC ANALYSIS OF TEXAS GULF COAST RED DRUM (SCIAENOPS OCELLATA): IDENTIFICATION OF POSSIBLE STOCKS AND IMPLICATIONS FOR FISHERIES MANAGEMENT

by

William Ray Wilder

Red drum (Sciaenops ocellata) from seven of the nine major embayments of the Texas Gulf Coast were electrophoretically analysed for genetic variability. Indices of genetic similarity and distance were derived, as well as estimates of genetic divergence between bays. Cluster analysis phenograms were developed, and possible causes for population structure were addressed.

Forty presumptive loci were screened, of which 30 proved to be of value for genetic population analysis. Percent polymorphic loci and heterozygosity/locus/individual ranged from 6.7% - 13.3% and 0.025 - 0.042 respectively. These values were lower than those reported in similar studies, including some dealing specifically with Sciaenidae, but remained within the range reported for teleosts in general.

A total of thirteen tests of genetic similarity/distance were performed. Of these tests, no segregation below a genetic identity of 0.95 was detected in those samples large enough to statistically satisfy the analysis. Upon exclusion of the small samples, no differentiation below 0.97 was demonstrated.

Contingency chi-square tests and F-Statistics found only extremely low levels of divergence. Indeed, none of the divergence indicated was significant in terms of distinction of subpopulations among bays. This lack of differentiation in spite of apparently low levels of interbay migration was explained as a function of the dichotomus life stages. While juveniles are geographically isolated, adults occupy the open waters of the Gulf of Mexico, and have yet to be thouroughly described from either a life history or reproductive strategy viewpoint.

The indicated single stock of reproductively active red drum in the Gulf requires further investigation, in order to accurately determine migrational habits as well as breeding success. The implications for management of the fishery at present are to regulate the resource as a single stock; as well as institute a comprehensive physical and biochemical study for future policy formulation.

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I would like to thank the Texas Parks and Wildlife Department for their assistance and support in this project. Dr. Gary Matlock and his entire staff of exceptional field biologists deserve special recognition. Their aid in collecting specimens, frequent comments concerning this work, and extraordinary concern with the welfare of natural resources were both helpful and inspirational. Thanks are also extended to the Ralph Leggett family and the Brown Foundation for access to the H&G Ranch, Chambers County, Texas, where a substantial portion of the initial experimentation was performed.

Finally, I gratefully extend thanks for the encouragement and generous financial support by Mr. Perry R. Bass through the Sid W. Richardson Memorial Fund and Foundation. This project was made possible by their exceptional generosity.

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Fishery biologists must be prepared to accept variation, dynamic populations, an environment largely uncontrolable, the need for compromise, optimum rather than maximum results in the resolution of many harvest problems, and the conflicting desires of the people we work for. More likely than not there will be no formulas, no handbooks, no set rules to follow in making the numerous decisions fishery management demands whether it be in the writing of an international treaty or deciding what size fish to stock in the local sportsmen's favorite stream. The fishery biologist lives in the demanding world of gray where black and white in decision making are an exception. The challenges are obvious, and for those who enjoy such contests, fishery biology is an exciting and satisfying profession.

W. HARRY EVERHART
in Principles of
Fishery Science 1975
Cornell Univ. Press

INTRODUCTION

The value of fish as a harvestable crop, in conjunction with their abundance and variety, has made them the subject of scientific investigation for many years. Likewise, management of commercially important species as well as programs utilizing artificial propagation of captive fish have been frequently implemented (Rounsefell, 1975). It was the goal of this study to provide fisheries managers with an understanding of stock composition and population dynamics of red drum (Sciaenops ocellata) in Texas' waters. As indicated by Everhart et al. (1975), this information is vital to creation of a comprehensive utilization schedule for any fishery, specifically those with a possible multiplicity of genetically variant stocks.

Recently, Gulland (1983) pointed out that conservation and management of fish stocks have been experiencing increased attention from both the general public and administrators of natural resource agencies. The general public has become more sensitive to environmental issues as a whole, with a widespread sense of responsibility to natural resources. Fisheries administrators have become more aware of the need for well planned utilization schedules, in order to avoid over-exploitation of fish stocks and insure reasonable yields per season. In addition, the 3rd United Nations Conference of the Law of the Sea began giving coastal states (or nations) greater jurisdiction over the fish stocks occurring in their respective coastal waters. The extension of the Exclusive Economic Zone

(EEZ) to 200 miles has allowed government officials to actively regulate the exploitation of fish stocks in nearshore waters.

The advent of research scientists leading decision making processes for resource utilization dates back to the first half of the twentieth century. The use of scientific, quantifiable information for resource management was initiated by Graham (1939), when he graphically demonstrated overfishing with sigmoidal functions. It was not immediately obvious however, that basic scientific research conducted for reasons other than economic maximization, was the ultimate tool for optimizing and streamlining a fishing industry. Currently, it is still difficult to convince the citizenery that the inherent value of scientific endeavor is greater than the economic/monetary benefit of a fishery. Fortunately, however, satisfactory fulfillment of the end (ie. a financially dependable and profitable fishery) is crucially linked to scientific means (ie. a well studied and biologically interpretable fishery).

In his classic and frequently referenced college textbook, Rounsefell (1953) stated that: "Fishery science is the application of scientific knowledge concerning fish populations to the problems of obtaining the optimum production of fishery products, whether stated in tons of factory material or in hours of angling pleasure." The financial aspects of the fishing industry have obviously changed since 1953, but this definition still holds true today. In order to achieve this optimum production level in a mixed fishery, that is a fishery consisting

of multiple stocks, all stocks involved in the system must be identified and delineated (Gulland 1983). It should be noted here that the term 'stock' is used according to Matlock (1984): "stock" refers to an intraspecific group of individual fish, randomly interbreeding within the group, having free gene flow, and continuous spatial and temporal integrity; furthermore, biological characteristics are ubiquitous to all members, and impacts of fishing and environment are uniform throughout the group.(see Lackey and Hubert 1977; Tyler and Gallucci 1980; Ihssen et al. 1981)

The most recent and comprehensive review of research pertaining to red drum was presented by Matlock (1984). His proposed management plan detailed analyses completed through 1984 (meristic, morphometric, tag/recapture,etc.), as well as outlined future research needs. Red drum have been cause for conflicting interests for as long as 100 years (Matlock 1980), with allocation schedules being the predominant problem. Historically, red drum have been fished commercially in the Gulf of Mexico since the 1700's (Galtsoff 1954), with intermittent landing statistics available through 1880 (Matlock 1980). Commercial landings reached a maximum in 1976 (approx. 5 million pounds) and subsequently declined to half that value by 1980 (Swingle et al. 1984). Quite recently, exploitation of offshore red drum in Florida, Mississippi, Alabama and Louisiana have prompted federal agencies to declare emergency guidelines and request increased research efforts (Matlock, pers. comm.)

This decline in landings remains difficult to assess, as a result of many possible causes. Lack of precise records of commercial fishing effort prevents direct assessment, while naturally occuring phenomenon further confuse the issue. Fluctuations in population number can be drastic from season to season as a result of winter 'freezes' (Gunter 1941; Simmons and Breuer 1962) or 'red tides' (Gunter 1952), a rapid blooming of toxic phytoplanktonic organisms. Further fluctuations can be the result of temporary harvest restrictions (Heffernan and Kemp 1980) or even due to a decrease in the fishery labor force as a result of enlistment in an armed service during a world war (Simmons and Breuer 1962). Simple supply/demand economics also affect landings, by varying the economic benefit of a given unit of fishing effort (Simmons and Breuer 1962; Heffernan and Kemp 1980). Potential long term effects can be expected from changing trends in gear type and fluctuations in estuarine surface area (Yokel 1966), as well as climatic cycles and build-up of pollutants from industry and agriculture (Joseph 1972).

Most recent estimates of the potential value of the Texas red drum fishery are approximately $125,000,000.00 per year, including recreational, commercial, direct and indirect benefits (Matlock 1984). Since complete cessation of such a valuable industry is as unsatisfactory as destructive over-fishing, state administrators are seeking solutions which will enhance the fishery, thereby sustaining the industry. Early efforts to protect the red drum fishery were size limitations and

spatio/temporal gear restrictions enacted in the 1920's to aid both juveniles and spawning adults (Pearson 1929; Heffernan and Kemp 1980). The sale of native red drum was prohibited in September 1981, the result of investigations that indicated overfishing (Matlock 1982). In September of 1982, the Commissioners of the Texas Parks and Wildlife Department (TPWD) enacted a regulation prohibiting the retention of red drum taken by nets, seines or trotlines; exceptions being made for dipnets and sail lines (Swingle et al. 1984). The gear restriction regulation was intended to alleviate problems concerning enforcement of red drum conservation measures; state wardens were faced with truckloads of red drum accompanied by falsified invoices, declaring the fish as having been taken in Mexico or Louisiana. With the restriction on gear however, officials could confiscate illegal nets and arrest or detain violators, increasing the economic and legal risks of those involved in the black market fishery.

In conjunction with the increased legislative activity, TPWD administrators began to expand their data collection activities as well. The management goal was modified from 'reduction of conflict' to attainment of 'optimum yield' for the red drum fishery (Anonymus 1982). To achieve this new objective, Matlock (1984) stated the need for critical evaluation of the current understanding of red drum life history and population dynamics, as well as the sociology and economics of the fishery. Furthermore, he recommended the scrutiny of management

structures, strategies and policies to aid in clearly defining future research needs and realistic expectations for optimum yield. A final effort to improve population numbers of red drum in Texas' bays and estuaries was the initiation of intense fish stocking in these waters in 1975. Matlock (1984a) summarized the stocking program, which spanned 7 years and involved an estimated 56,000,000 red drum (eggs, fry and fingerlings). An assessment of stocking success has not been completed however, and will be considered in the Discussion section.

Recently, Ihssen et al. (1981) compiled an extensive review of techniques available to researchers attempting to elucidate stucture and dynamics of fish populations. He identified eight categories of techniques, ranging from simple mark-recapture studies to advanced cytogenetic and immunochemical methods for stock identification. Cooperative efforts between the varied disciplines is stressed throughout the review, with composite studies yielding higher quality and quantity than single method investigations. Of the techniques listed, some, but certainly not all, have been applied to red drum. The TPWD has compiled an extensive collection of population census data, with detailed meristic, morphometric and mark/recapture information, but lacks biochemical genetic data. This study was designed to take advantage of the existing data base, adding new data and clarifying previously incomplete bodies of information.

Pearson (1929) was first to publish a reasonably complete natural history for red drum in the Texas Gulf Coast region. His

study, as concise and elaborate as available techniques would allow, was based predominantly upon visual observations of red drum in their native habitat. The life history of red drum, however, separates juvenile and sexually mature life stages spatially, rendering information based solely upon visual observations inadequate. Questions concerning red drum migration and breeding strategy went unanswered by Pearson, and are indeed only partially understood today.

Pearson (1929) reported that red drum participate in a seasonal migration from the bays into the Gulf in the fall months, returning to the bays again in spring. This view was also held by Gunter (1945) and Miles (1950), explaining the movements as evasions of fluctuating water temperature. The deeper waters of the Gulf are far less susceptible to rapid temperature drops than the shallow waters of Texas' bays, thereby offering greater protection from rapidly appearing cold fronts out of the north. Simmons and Breuer (1962) reported that although records of fish landings indicated a greater number of red drum in the bays in spring and fall, substantial numbers were also captured in the winter and summer months. They concluded that seasonal migration was occurring, but to a lesser extent than assumed by the previous studies.

Matlock (1984) stated that sub-adult red drum "apparently remain in estuaries throughout the year and do not migrate seasonally between estuaries and Gulf." He supported his statement with reports of frequent recapture of tagged fish in

estuaries during winter (Osburn et al. 1982) as well as historical and current catch statistics, which show availability of red drum throughout the year (Gunter, 1945; Matlock et al. 1978; Hegen 1981). Matlock et al. (1984) concluded their report on the tagging studies of red drum from Matagorda Bay by stating that stocked fish remained in the bay where released; going on to point out the inability of their study to describe intra-bay migration patterns, as a result of low returns (0.2%) and lack of fishing effort distribution adjustments.

While seasonal migrations are currently refuted, it is widely accepted that red drum permanently emigrate from the estuaries into the Gulf upon sexual maturation. Sexual maturity is as yet only partially correlated with age and size, with reported values ranging from 3 - 5 years and 500 - 700mm total length respectively. Sexually mature red drum of 300 - 400mm total length have been reported in Alabama, indicating the wide range of biological parameters exhibited by this species (Matlock 1984). While the occurrence of large offshore schools of red drum is well documented in the literature, very little is known about the population parameters and dynamics of these fish. The fish might be recruited from the estuaries into specific 'sub-groups' of Gulf fish, creating distinct lineages; or they might be recruited into one large, effectively singular breeding aggregate, with no apparent sub-categorization. A study involving red drum and spotted seatrout in Louisiana (Ramsey and Wakeman 1983) indicated little differentiation in red drum, but

concluded with a call for further research.

Given the inability of standard methods to solve problems concerning migrational patterns and reproductive strategies, alternative techniques were sought by administrative and scientific personnel. Initial efforts in this study focused on the successful use of trace metals in scales as indicators of native origin of salmon in British Columbia (Calaprice 1971). Lapi and Mulligan (1981) had developed special methods to allow scanning electron microprobe analysis of whole scales, resulting in partial assignment of mixed fishery salmon to native streams in western Canada. Differences in scales and available equipment prohibited equally rewarding results in analysis of red drum scales. Beam energies were too high, causing sample volatilization, and irregularities in scale topography negated any quantitative assessment of elemental content (Goldstein et al. 1981). Presently, Mulligan (1985) is engaged in microprobe analysis of otoliths. Using the much denser and less environmentally exposed otoliths, he has had some measure of success with white perch. The technique was more effective in cases of greater geographic separation (77.8% correctly identified to riverine origin) than in cases with little spatial separation (44.4% correct). Future improvements in this technique should make it extremely valuable for physiochemical stock discrimination.

In a second attempt to determine trace metal signatures for red drum scales, an inductively coupled plasma emission

spectrophotometer (ICPS) was employed to assess metal content. While this technique proved extremely useful in the determination of heavy metals in avian tissues (Hall and Fisher 1985), it was far too variable when used for fish scales. The fish study was not predetermined, i.e., no 'target' elements had been selected prior to analysis; as a result, the standard deviations between replicate samples were greater or equal to the deviations between individuals, invalidating any conclusions drawn from the results.

Based upon reports of no significant interbay movement of red drum (Matlock and Weaver 1979), it was hypothesized that geographic isolation was occurring. This being the case, allopatric populations of red drum could be diverging genetically from one another. If indeed divergent, the various 'sub-populations', being genetically distinguishable, would have to be treated as separate stocks in the proposed management plan. An accurate assessment of the number and identity of stocks in a fishery is mandatory information for fishery administrators (Rounsefell 1975; Gulland 1983).

The use of electrophoretic analysis for detection of the aforementioned genetic divergence is commonplace in modern population biology investigations. As a technique for taxonomic value, it may be traced back to the experiments of Tesilius (1937), who established moving boundary separation for protein fractions for comparative tissue analysis.

Deutch and Goodloe (1945) applied this technique to plasmas from a variety of animals, discovering that similarities exist

between some of the vertebrates tested. In extending their previous work, Deutch and McShan (1949) electrophoretically tested many "lower invertebrates" and vertebrates, finding a particular pattern for all species examined. Many cases of easily recognizable differences within families, such as Salmonidae and Ictaluridae, were reported. Moore (1945), working with rats, reported similar results, with highly reproducible patterns even allowing the identification of certain strains of the rat population.

In the 1950's, finer and more highly resolute methods began to be developed, all of which furthered the concepts of species specific patterns of protein systems. Researchers were separating proteins on substrates such as filter paper, a variety of gels including hydrolyzed starch, and cellulose acetate. The gels seemed to show the most promise, as a result of their versatility and more effective separation and resolution of biochemical components. Starch gels in particular were found to be excellent, due to applicability to processing of large samples for a variety of proteins. Woods and Engle (1957), and Engle et al. (1958) both concluded from their work with marine invertebrates and elasmobranchs that starch gels provided maximum resolution and might provide valuable information for both taxonomic and populational work.

Much of the work done in the 1950's was merely initial characterization of protein patterns, such as Irisawa and Irisawa (1954), with the authors concentrating on the differences among

the proteins studied, and not the taxonomic implications which might have been inferred. Drilhon et al. (1958) and Drilhon (1960) however, produced papers concerning the complexities of fish serum protein patterns, and noted that the patterns attained were species specific, with only minor variations due to age, seasonal feeding stages and sex. They also noted that variability within a species was generally quite small, while variations between species were larger.

The early 1960's witnessed another breakthrough in terms of more highly resolving protein separation techniques. Davis (1964) and Ornstein (1964) developed the method of discontinuous electrophoresis (disc electrophoresis), using a series of variable concentration polyacrylamide gels to drastically increase resolution of protein fractions. With this refinement, many researchers began to analyze all major groups of protein, with liver, skeletal muscle, brain or other neural tissue, gonads and blood all being analyzed for basic proteins as well as specific enzymes.

Myogen was of primary interest to many researchers, with Tsuyuki et al. (1965a) applying his work primarily to fish muscle. He analyzed 659 adults from 27 races of sockeye salmon (Oncorhynchus nerka) from the Pacific Northwest and found a very significant degree of specificity in the electrophoretic patterning. In subsequent work, Tsuyuki et al. (1965b) investigated 50 species of fish and found that a very high specificity was again easily discernible. Furthermore,

conclusions drawn concerning taxonomic relationships of Salmonidae and Scorpaenidae were compatible with existing classical, morphological assumptions.

Huntsman (1970) reviewed catastomid taxonomy with serum protein analysis, and found himself unable to use serum effectively (except in one case). He therefore suggested muscle tissue as a more suitable source of protein, particularly myogen. Various proteins will undoubtedly differ in the importance of their functions, as Florkin (1964) pointed out when he grouped proteins into categories of 'closely tailored to an essential functional requirement', 'those whose function is relatively dispensable', and those that fall in between the other two groups. Genetic conservativeness would obviously be suspected more in tissues such as nerves, where regeneration occurs less frequently, if at all, and where functioning is not only essential, but highly standardized; in other words, the serum encounters a variety of physiological conditions in its functioning, while the nervous system is quite homeostatic in terms of existing chemical conditions or environments. Gray and McKenzie (1970) also worked with fish muscle protein, and noted polymorphism within the myogen patterns of rainbow trout (Salmo gairdneri). Nyman (1967) had noted similar polymorphisms in Atlantic salmon (Salmo salar), but in both studies, the intraspecific polymorphism occurred at much lower frequencies in muscle extracts than in blood samples.

Other than general proteins, specific enzymes have proven to

be an effective analytical tool. Page and Whitt (1973) used malate dehydrogenase (MDH), lactate dehydrogenase (LDH) and tetrazolium oxidase (TO) in an extensive analysis of darters (Etheostomatini). Their work supported many of the classical relationships, and resulted in proposal of new ideas concerning these relationships. They used the data to show genetic discontinuity between Percina and Etheostoma, and to support a monophyletic origin of the two genera, based on a virtual lack of intraspecific polymorphism in LDH and TO (renamed superoxide dismutase,SOD) and a total lack of polymorphism in MDH.

In the late 1960's and early 1970's other research teams also began to make inferences concerning population dynamics using electrophoretically attained genetic data. Scholl and Eppenberger (1972) found significant differences in creatine kinase (CPK) from fishes and higher vertebrates. They concluded that this complexity of enzyme patterns is a result of polyploidy in these organisms. Massaro and Markert (1968) working with LDH, and Bailey et al. (1969) using MDH also believed polyploidy to be responsible for the complex patterns found.

Similarly, Nyman (1970) showed evidence for the advantage of biochemical data over the classic, meristic data in identifying F1 hybrids in fish. He believed it to be superior because meristic traits are generally the result of multigene systems, whereas proteins (or at least their subunits) are generally coded for by codominant allel single genes.

Wright and Hassler (1967) executed some of the earlier work

when they analyzed populations of white bass (Morone chrysops) from several Wisconsin lakes. Using the Sokal and Sneath (1963) method of taxonomic distance determination between populations, they verified the existence of separate populations based on electrophoretic data. Nyman and Pippy (1972) identified the continental origins of the atlantic salmon (Salmo salar) which were captured at sea (western coast of Greenland and the Labrador Sea) using analyses of blood sera and liver tissue extracts separated with horizontal starch gel electrophoresis. Qualitative differences in the gene pools of the fish were large enough to cause the authors to recommend the names S.s. americanus and S.s. europeus for the two forms. Similarly, Fujino and Kang (1968) demonstrated the existence of some sub-populations of Pacific and Atlantic tunas using serum esterase groups for their electrophoretic investigation.

Morziot and Siciliano (1982) performed extensive analysis of 24 genetic loci in 14 groups of Xiphophorus, representing 3 species, including 12 sub-populations of maculatus from 5 river systems in Central America. Polymorphism was found in 16 of the 24 loci examined, with interspecific dissimilarity indices always being higher than intraspecific ones. Furthermore, they found that dissimilarity indices were also always lower between groups of the same species in the same drainage area as opposed to groups of the same species from different drainage areas. A final comment referred to the existence of mutually exclusive alleles at certain loci in fish of the same species from adjacent

drainages, suggesting that random fixation of selectively neutral mutants may contribute significantly to molecular evolution.

Utter et al. (1974) surveyed the available techniques with respect to their usefulness in fishery biology. Although the authors cautioned against acceptance of the techniques in rare instances, they endorse electrophoretic analysis of fish populations completely, citing the technique's ability to circumvent problems endemic to fisheries research.

These problems, such as poorly defined habitat boundaries, highly transient groups of individuals mixing with extremely localized groups, and even the unpredictable environmental conditions of marine and estuarine waters, all contribute to uncertainty of results. Obstacles presented by unknown ranges and boundaries can be easily overcome however, since the genetic data resolved with electrophoresis are independant of these variables.

Aquatic populations are difficult to delineate as a result of the inability to differentiate between spatial and temporal aggregations and real populations. Sprague (1970) stated that electrophoretic techniques and their subsequent refinement would be a most useful approach to what they termed the "sub-population problem and the related problem of defining the relationships between biological populations of marine animals and the physical/chemical nature of their environment." Although the work was done with skipjack tuna (Katsuwonus pelamis), Sprague indicated the usefulness of this technique in studies of marine

invertebrates as well as all fishes and other vetebrates.

That is not to say however, that environmental conditions do not affect allele frequncies, as is noticed when analysing clinal variation (Oakeshott 1982; Phillip et al. 1985) or simply correlating thermal variations and varying habitat types with polymorphisms (Graves and Somero 1982; Manly 1983).

Gartner-Kepkay et al. (1983) found significant genetic differences between populations of mussels from the same Nova Scotian embayment. They found however, that the difference reflected was due to the dissimilarity of the environments from which the samples were taken. One location experienced large fluctuations of temperature and salinity, while the other site was maintained at rather stable, non-fluctuating conditions. Allelic populations were found to cluster according to their environment, and not their geographical proximity. They also reported that this sensitivity to environmental conditions varied from locus to locus.

Much of the recent work in stock identification has centered on the salmon fishery, an obvious choice considering its global importance. May (1980) reported in detail on the applicability of electrophoretic analysis to various aspects of the genus Oncorhynchus. In addition to finding the technique highly valuable, polyploidy was discussed as a genetic basis for the complex findings of breeding experiments performed with members of this genus. This work also established a foundation of techniques and methods largely adapted for the current study.

Allendorf and Phelps (1981) used variance of allelic frequencies to estimate the degree of isolation among localized geographic units, as well as describe patterns of genetic exchange between groups. Specifically, they wished to determine how much genetic exchange must occur between sub-populations to account for the observed patterns of allelic divergence. The amount of divergence was proportional to the absolute number of migrant individuals, not the percentage of individuals exchanged, implying that estimation of true genetic divergence requires knowledge of population size. Furthermore, their simulations showed significant allelic divergence even with substantial exchange between groups. In conclusion, the authors cautioned against the use of allelic frequencies from juveniles to make inferences concerning reproducing adults.

Yet another caution was issued by Kobayashi et al. (1984) in their study of ADA in salmon. The researchers found that improper handling of samples could easily lead to a misinterpretation of gel scores. Working with known familial lots of chinook salmon (Oncorhynchus tshwaytscha), they determined absolute origins for banding patterns, and subsequently found that improper sample handling and storage could cause appearance of artifactual bands, as well as prompt the disappearance of real bands.

Turner (1983) reported the possible overestimation of geographical isolation as a differentiating force. In his study of desert pupfish (Cyprinodontidae), he detected quite low levels

of divergence between unquestionably separate localities. Although the habitats of the various populations (ponds, springs and river drainage systems) were isolated from one another spatially, morphologically distinct groups failed to exhibit divergence below an average similarity index of 0.959; a value compatible with within-drainage comparisons for other teleosts.

Grant and Utter (1984) used geographical distributions of inherited biochemical markers to evaluate the genetic component of the stock structure of Pacific herring (Clupea pallasi). Although regional differentiation had been noted morphologically and with tagging studies, little genetic differentiation was detected. The lack of differentiation is not expected under the stepping-stone model of migration, and demonstrates the small amount of migrational exchange needed to support homogeneity in large populations. The study did detect two distinct genetic races of herring however, not coinciding with demarkations previously assumed through morphological data. (see Okazaki 1982a, 1982b and 1983 for equivalent study with Japanese stocks)

In a similar study, involving Atlantic herring (Clupea harengus), Grant (1984) demonstrated low levels of genetic variability throughout the northern Atlantic Ocean. Analysis of North American and European populations of C. harengus showed intra-regional differentiation to be of the same magnitude as inter-regional. The author explained the data with the radiation model of colonization, where a single population radiates into several populations that eventually cease exchanging genes. This

concept is also refered to as 'recent divergence' in some of the literature.(see Anderson et al. 1983; Ryman 1983)

Parkinson (1984) found generally little differentiation in populations of steelhead trout (Salmo gairdneri), the anadromous form of rainbow trout. While the previous studies had indicated the minor correlation of geographic or temporal segregation with genetic divergence, data from this study introduced yet another permutation. On large and intermediate geographic analyses, little divergence was found, but on a micro-geographic scale, significant differentiation was found; even between populations from adjacent streams. This phenomenon was explained as individual populations adapting themselves to local environmental conditions (which can vary drastically), in the face of low gene flow over short distances.

Shaklee et al. (1983) undertook a preliminary investigation of Pacific blue marlin (Makaira nigricans) to determine the feasibility of an electrophoretic analysis of this fish. Values of heterozygosity were surprisingly high for large marine teleosts, but within the range for other scombroid fish. ADH alone showed a deviation from Hardy-Weinberg equlibrium, apparently not for reasons of sex linkage or size distribution. The authors indicated the possibility of mixing of two divergent stocks to maintain variabilty without normal levels of heterozygosity (the Wahlund effect); not surprising in an animal as far ranging and migratory as Pacific blue marlin.

Differences in intraspecific distribution of genetic

variation was discussed by Gyllensten (1985) with respect to groups of different fish. He compared genetic variabilty ranges from anadromous, marine and freshwater teleosts, and noted distinct differences. Most importantly, the levels of heterozygosity were found to be highest in marine fishes. The variability present is correlated with migration. Marine fishes, the least confined group, have only 1% of their variability between localities, while some land-locked freshwater species have up to 25% of their variability between locales.

In an effort to categorize a mixed stock fishery in southern British Columbia, Beacham et al. (1985) employed electrophoresis to analyse geographic groups of salmon. Significant differences in allele frequency were found between some stocks, but some loci also demonstrated high levels of variability within regions. Allele frequencies were stable over a two year sampling program, indicating the long term usefulness of the technique. Although only some of the stocks could be accurately distinguished, the data obtained was vital for fisheries managers in the area.

Information on the existence and configuration of subpopulations is of basic importance to fisheries management (Berst and Simon 1981). Shaklee et al. (1983) indicates the power of electrophoresis as a tool to analyse genetic aspects of sexually reproducing populations, and Ayala (1976) summarizes a series of analyses on vetebrates and invertebrates. Population genetic studies of fishes have been reviewed by de Ligny (1969) as well as Allendorf and Utter (1979).

In summary, the objectives of this study are 1) to analyse allelic variation in red drum from various major embayments of the Texas Gulf coast and 2) to delineate genetically distinguishable stocks, if they exist, in order to develop more precise management plans for the red drum fishery in Texas. In order to achieve these objectives, a series of questions were addressed: Are values of allelic frequency within the ranges reported for other nearshore and estuarine teleosts? Are the variable loci in the Texas fish similar or identical to those found in studies conducted in other localities? Is the apparent geographic isolation real, and if so, sufficient to establish genetically divergent subpopulations? Are the reported migrational habits universally applicable, and do they represent a plausible source of gene flow/exchange?

It was hypothesized that the geographic isolation of juveniles will lead to genetically divergent subpopulations, unless reproductive aggregates of red drum are assembled from geographically indiscriminant origins. In addition, the lack of discrimination in the breeding component of red drum could necessitate the consideration of a single stock in management plan formulation, wherein recruitment from all juvenile groups is directed into one reproductively active population.

MATERIALS AND METHODS

I Sampling Procedure

Red drum were collected from gill net sets in seven (7) major bays of the Texas Coastal Zone (TCZ) from April, 1984 through June, 1985. Locations, dates, number of fish per collection are listed in Table # 1. The collections were made with the assistance of Texas Parks and Wildlife Department (TPWD) regional biologists, who were engaged in bi-annual population census netting of the state's bays and estuaries. These census efforts are seasonally executed, with two ten week sampling periods per year, coinciding with the historically determinable, maximally productive periods of early fall and spring.

The TPWD gill net gear is standardized throughout the TCZ. The nets are 183 meters (m) long and 1.2m deep, with four separate 46m panels, representing 7.6, 10.2, 12.7 and 15.3cm stretched monofilament mesh sizes. Thread sizes are #12 for the 7.6 and 10.2cm mesh, #6 for the 12.7cm mesh and #7 for the 15.2cm mesh. The webbing was hung from the float and lead line on a 1:2 basis, indicating the finished net was 1:2 or 50% as deep as the untied webbing. The four panels were tied to one another in order of size, small to large, with nets deployed perpendicular to the coastline, smallest mesh nearest the shore.

Gill net sampling was conducted in Galveston Bay, East Matagorda Bay, Matagorda Bay, San Antonio Bay, Aransas Bay, Corpus Christi Bay, the Upper Laguna Madre and the Lower Laguna

Madre (Fig. # 1). For detailed descriptions of the Texas Gulf coast region, see Diener (1975). (also see Hegen and Matlock 1980; Matlock and Weaver 1979) It should be noted that red drum used in this study were taken in different quantities from each bay, depending on the availability of red drum in the nets. Matagorda Bay was not sampled due to the deteriorated condition of the fish in the nets (crab damage, etc.) as well as a general lack of red drum in the net sets made during the collection periods. East Matagorda Bay and the Upper Laguna Madre were poorly represented for similar reasons. Stations were selected at random from previously compiled lists of sample station locations for each bay. In 1984 TPWD adopted a new sampling plan, based on the superimposition of grids onto a map of the bay. The gridsquares containing shoreline were then further subdivided with a "gridlet" overlay, and points in this subgrid sampled randomly.

The red drum used in this study were all alive when removed from the gill net. Dead fish were excluded to preclude possibly inconsistent electrophoretic patterns due to varying post-mortem conditions. The fish were placed on wet ice for transport back to the laboratory, where they were stored frozen at -10^C. All fish were processed completely within seven days of their original capture. Approximately half of the fish taken from the Lower Laguna Madre were biopsied immediately after collection and stored on dry ice. This was done to avoid storage of whole fish on wet ice for periods in excess of 24 hours, as the Lower Laguna

Madre trip spanned three days. The biopsies were frozen in homogenization media prior to storage on dry ice in order to minimize handling artifacts.

II Tissue Processing and Data Collection

Complete processing within seven days of capture included determination of reproductive status, length to the nearest millimeter, tissue biopsy, tissue homogenization and, in some cases, collection of scale samples for analysis of heavy metal content. Scale samples were collected from fish that were not included in the isozyme study because of questionable collection and storage history. This allowed faster processing of those fish suitable for enzyme analysis while also utilizing fish that were collected but had a questionable storage history.

The stored fish were partially thawed to facilitate removal of biopsies, as well as to expose the reproductive organs to inspection. After the liver had been sampled, the head was severed from the body and a skeletal muscle biopsy was removed from the anterior dorsal region, between the skull and the first dorsal ray. The head was then split in half using the apparatus detailed in Figure # 2; with the knife being applied slightly off-center, to avoid excess distortion of the central nervous system. Whenever possible, the brain sample was augmented with spinal material or sections of the cranial nerve trunks. This was specifically helpful in smaller red drum, where the wet weight of the whole brain was frequently less than 0.35 grams.

After the biopsies had been weighed, they were placed in thick walled glass tubes (Tri-R Industries,New York) and homogenization media (0.001M Tris-0.001M EDTA-0.001M 2-Mercaptoethanol) was added in a 1:3 w/v ratio. The samples were then homogenized, with a loose fitting motorized teflon pestal matched to the glass tubes, while being maintained at 0^C to prevent heat denaturation during homogenization. The resulting homogenate was decanted into 50ml plastic centrifuge tubes. In instances where the homogenate was too viscous to be decanted easily, a small quantity of homogenization media (10-20% total volume) was forcefully pipetted into the glass tubes to resuspend the sample and make it fluid enough for decantation. Only skeletal muscle samples required this special measure, and staining intensities were not reduced by the additional dilution.

The homogenates were clarified by centrifugation in a refrigerated centrifuge (Sorval RC2-B) at 12,000x G for twenty minutes. The supernatant from the first centrifugation was pipetted into 1.5ml micro-centrifuge tubes and re-centrifuged at 20,000x G for another twenty minutes. If the sample appeared to have a high lipid content, 2-3 drops of toluene were added to the 1.5ml tube in order to separate the lipid from the proteinacious fraction. After the second centrifugation, the supernatant was glass pipetted into a labeled 1.5ml tube and placed in a supercold freezer at -70^C, until it was analysed by gel electrophoresis.

III Electrophoresis and Enzyme Staining

The gels used in this study were 12.5% mixtures of hydrolyzed potato starch (Connaught Laboratories, Pennsylvania) solubilized in the various buffer systems. Recipes of solutions used and explanations of the abbreviations used for each buffer are listed in Table # 2. The majority of the following techniques are modifications of methods reported by Siciliano and Shaw (orig. manu.).

Seventy (70) grams of hydrolyzed starch were placed into a 2000ml Erlenmeyer flask, and suspended in 450ml of glass distilled water. After swirling the flask for 10 to 15 seconds, 50ml of the appropriate buffer was added, and the heating cycle begun. It should be noted here that not all buffers were mixed in a 1:10 ratio (50ml plus 450ml). The recipes detail exact mixture ratios for each buffer. The flask was then held by the neck and swirled constantly over a broadtip Bunsen burner until the solution boiled or the flask became too hot to hold comfortably. This technique consistently yielded uniform, unburned gels of high quality. Boiling of the buffer or water prior to mixing with the starch was discounted as a usable technique due to inconsistencies in gel structure. As the starch solution heats, it progresses through a series of increasingly denser viscosities. Immediately prior to attaining proper temperature the solution becomes much less viscous and bubbles begin to appear as a result of boiling.

Upon completion of the heating phase three drops of 2-

mercaptoethanol were added to the flask, as well as any cofactors required in the particular gel being run. The addition of cofactors (NAD or TPN) was necessary in some cases to attain scorable bands. Addition of cofactors is indicated in the listing of methods for each individual enzyme/protein system. The boiling mixture was then placed in a protective enclosure and subjected to a water aspirator vacuum for approximately 60 seconds, or until only large bubbles remained visible in the solution. The degassed starch mixture was then ready to be poured into the gel form described below.

Gel forms, shown in Figure # 3, were constructed from plexiglass (lucite). The forms used in this study were 123mm wide, 305mm long and either 6mm or 10mm deep. The 10mm deep forms were used when 6 to 8 different enzymes were to be analyzed from the same buffer system, and the 6mm gels were used in cases where fewer than 5 enzymes where examined per buffer system. Combs were made from 6 inch plastic rulers (Arthur Thomas Co.), allowing wells of uniform size and required numbers to be formed; the combs used for this study were mostly 17 well combs, 16 sample wells and one well for the dye standard. Other arrangements of well size and number could be created for special purposes; one or two very large wells for a weakly staining enzyme for instance, where large amounts of sample are required for scorable banding. The combs are approximately 0.5mm in thickness, so the standard well volume used in this study was $12mm^3$ for the 6mm thick gels and $20mm^3$ for the 10mm thick gels.

After the solution has been poured into the form, the lid/comb assembly is carefully placed onto the form and cylindrical lead weights approximately 50mm tall and 35mm in diameter are placed on top of the gel mold lid to keep it in place as the gel solidifies. The gels were allowed to cool for 30 minutes at room temperature, and another 45 minutes at the temperature at which the gel would be run; in this case 4°C. Upon completion of the cooling phase, the lids were carefully removed in the following sequence: The screws holding the well comb in place were removed; the lids pried up from one corner very slowly, with care being taken not to lift the entire gel out of the form with the lid; the endplates and comb were removed, again very carefully; the gel was now ready to be loaded.

The wells formed by the combs are separated by 2-4 mm of starch gel, depending upon the total number of wells. In order to prevent sample cross-contamination, molten petroleum jelly was topically pipetted between the wells. Once solidified, the petroleum jelly acted as a physical barrier, preventing overflow from one well to another.

The small size of the well openings necessitated the use of fine-tipped glass pipettes for sample loading. Pasteur pipettes (9" long) can be heated over a bunsen burner and 'pulled' into tapered tip pipettes quite easily; the narrow aperature of the 'pulled' pipettes also prevent rapid release of sample, which can cause cross-contamination and loss of sample.

Once all of the samples were in the wells, the marker dye was injected into the marker well, and the entire row of wells was capped with molten petroleum jelly. This prevented the sample from pouring out of the well when the gel is placed into the vertical support rack. A plastic film such as Saran Wrap was also placed around the gel form to provide support; care was taken to leave the ends of the gel unwrapped, to insure adequate contact between the gel surface and the electrode buffer wick.

Gels were run either overnight or during the day, depending upon available time. The overnight gels were run at lower voltages (100-180 V.), since they ran for 12 - 14 hours, as opposed to the 5 - 6 hour daytime runs (200-350 V.). The power never exceeded 350 volts or 35 milliamps (10-15 Volts/cm), with some systems showing a tendency to sub-band if run at higher levels. Generally, to aid in comparison of gels, the run was stopped after the marker dye had migrated 5 - 8 cm from the origin. Systems requiring special run conditions are indicated in the individual descriptions for each enzyme, following the explanation of gel slicing.

Upon completion of the electrophoretic run, the gel form was removed from the rack, and the gel was cut from the mold, leaving the end pieces behind. Usually, the gel was cut just below the marker dye (approx. 5 - 8 cm anodally) and about 5 cm above the origin (cathodally), enzymes screened in this study were never shown to migrate further than these limits. This section of the gel was then horizontally sliced into identical slices, allowing

for staining of various enzymes from one electrophoretic separation. A sled-type gel slicer (Buchler Instruments) was used, strung with 0.254 mm diameter stainless steel wire (GHS guitar strings, GHS Strings). It was advantageous to place one or more thin glass plates on top of the gel prior to slicing, to help prevent gel buckling and uneven thickness of replicate slices. The individual slices were then placed in clear plastic trays (Cat. # T79C, Tri State Molded Plastics, Kentucky) and stained according to the protocols below. When an agar overlay was specified, the staining solution was mixed in 5 - 10ml of buffer and added to 10ml of 2% agar solution (no warmer than 60^C) immediately prior to being poured onto the gel slice. This allowed weakly staining systems to be enhanced, by providing a stable matrix for enzyme-stain reaction. Unless otherwise noted, the gels were all incubated in the staining solution, in covered trays, at 37^C, for 30 to 60 minutes. Quickly fading or slowly developing gels are indicated in the recipes.

INDIVIDUAL PROTOCOLS FOR EACH ENZYME STUDIED:

a.) Acid phosphatase: separated on C buffer, addition of co-factors not required; staining solution: 50ml 0.02M sodium acetate (pH 5.0), 100mg a-napthyl acid phosphate and 50mg fast garnet GBC salt.

b.) Adenosine deaminase: separated on TVB or R buffer, addition of co-factors not required; staining solution: 5ml of 0.5M phosphate (pH 7) and R buffer (in a 4:1 ratio), 80mg adenosine, 5

units nucleoside phosphorylase, 1.6 units xanthine oxidase, MTT and PMS; AGAR OVERLAY.

c.) Adenylate kinase: separated on R buffer, 20mg of TPN added to gel before degassing; staining solution: 10ml R buffer (Sol. B), 200mg glucose, 100mg ADP, 60 units G6PDH, 10mg TPN, 100 units hexokinase (immediately prior to pouring onto gel), MTT, PMS. AGAR OVERLAY.

d.) Alcohol dehydrogenase: separated on TVB or M buffer, 40mg of NAD added to gel before degassing; staining solution: 50ml Tris/HCl buffer (pH 8), 2ml 100% ethanol, 20mg NAD, MTT, PMS.

e.) Aldolase: separated on M buffer, 40mg of NAD added to gel prior to degassing (to avoid sub-banding); staining solution: 50ml Tris/HCl buffer (pH 8), 100mg fructose-1,6-diphosphate, 100mg arsenic acid, 100 units GAPDH, 20mg NAD, MTT, PMS.

f.) Aspartate aminotransferase: separated on TC buffer, addition of co-factors not required; staining solution: 50ml AAT buffer, 1g fast garnet GBC salt, 200mg fast blue BB salt. Pour solution over gel, wait 1 minute, carefully pour off solution, cover and incubate as usual.

g.) Creatine phosphokinase: separated on R buffer, 20mg of TPN added to gel prior to degassing; staining solution: 10ml R buffer (Sol. B), 150mg phosphocreatine, 20mg glucose, 15mg ADP, 60 units G6PDH, 100 units hexokinase, 10mg TPN, MTT, PMS. AGAR OVERLAY.

h.) Diaphorase: separated on C buffer, addition of co-factors not required; staining solution: 50ml Tris/HCl buffer (pH 8), 25mg

NADH, 10mg MTT, 2mg 2,6-dichlorophenol - indophenol. Can take up to 2 hours to stain completely.

i.) Esterase: separated on M buffer, addition of co-factors not required; staining solution: 50ml 0.01M phosphate buffer (pH 6), 3ml 1% a-napthyl acetate solution, 3ml 1% a-napthyl butyrate solution, 1.5ml 1% a-napthyl propionate, 100mg fast blue RR salt. Incubate in covered glass dish (plastic will be etched by acetone in substrate solutions), in dark at room temperature.

j.) Fumarase: separated on TVB buffer, 40mg of NAD added to gel prior to degassing; staining solution: 50ml Tris/HCl buffer (pH 8), 400mg fumaric acid, 100 units MDH, 20mg NAD, MTT, PMS.

k.) General proteins: separable on all buffers tested, addition of co-factors not required; staining solution: 0.1% Brilliant Blue R-250 (Coomassie) in 17% acetic acid and 41.5% methanol. Pour over gel and let stand at room temperature for 4 - 6 hours; destain with acetic/methanol destaining solution for 24 hours.

l.) Glucosephosphate isomerase: separated on R buffer, 20mg of TPN added to gel prior to degassing; staining solution: 10ml R buffer (Sol. B), 50mg fructose-6-phosphate, 60 units G6PDH, 10mg TPN, MTTx2,PMS. AGAR OVERLAY. Incubate at room temperature in dark; observe carefully after 10 - 15 minutes, as bands show up rapidly, followed by darkening of the backround and overstaining.

m.) Glucose-6-phosphate dehydrogenase: separated on R buffer, 20mg TPN added to gel prior to degassing (to prevent sub-banding); staining solution: 50ml R buffer (Sol. B), 100mg glucose-6-phosphate, 10mg TPN, MTT, PMS.

n.) Glutamate dehydrogenase: separated on R buffer, 20mg TPN and 20mg NAD added to gel prior to degassing; staining solution: 2g glutamic acid, 20mg NAD or 10mg TPN, MTT, PMS. Inspect frequently for first 30 - 60 minutes, stains weakly and radiply fades.

o.) Glycerol-3-phosphate dehydrogenase: separated on C buffer, 40mg NAD added to gel and an additional 40mg added to cathodal buffer tank; staining solution: 50ml Tris/HCl buffer (pH 8), 300mg a-glycerophosphate, 20mg NAD, MTT, PMS.

p.) Glyceraldehyde-3-phosphate dehydrogenase: separated on C buffer, addition of 40mg of NAD not required but recommended; staining solution: 50ml Tris/HCl buffer (pH 8), 55mg fructose-1,6-diphosphate, 100 units aldolase, 150mg arsenic acid, 20mg NAD, MTT, PMS. If banding too weak, incubate F-1,6-DP and aldolase for 30 - 60 minutes at 37°C prior to mixing with other components (Siciliano, pers. comm.).

q.) Isocitrate dehydrogenase: separated on C buffer, addition of 20mg of TPN to gel prior to degassing increases visibilty of rare bands; staining solution: 50ml Tris/HCl buffer (pH 8), 75mg isocitric acid, 10mg TPN, 50mg magnesium chloride, MTT, PMS.

r.) Lactate dehydrogenase: separated on TVB or M buffers, additional co-factors not required; staining solution: 50ml Tris/HCl buffer (pH 8), 10ml 0.5M lactate (pH 7), 20mg NAD, MTT, PMS.

s.) Malate dehydrogenase: separated on TVB or M buffers, addition of 40mg of NAD gave clearer bands; staining solution: 50ml

Tris/HCl buffer (pH 8), 5ml 0.5M malate (pH 7), 20mg NAD, MTT, PMS.

t.) Malic enzyme: separated on C buffer, addition of 20mg TPN required for well defined bands; staining solution: 50ml Tris/HCl buffer (pH 8), 10 ml 0.5M malate (pH 7), 10mg TPN, MTT, PMS.

u.) Mannosephosphate isomerase: seperated on TVB buffer, 20mg of TPN added to gel prior to degassing; staining solution: 10ml Tris/HCl buffer (pH 8), 35mg mannose-6-phosphate, 60 units G6PDH, 100 units GPI, 10mg TPN, MTT, PMS. AGAR OVERLAY.

v.) Octanol dehydrogenase: separated on M buffer, 40mg of NAD added to gel prior to degassing; staining solution: 50ml Tris/HCl buffer (pH 8), 1ml 2-octanol/ethanol mixture, 20mg NAD, MTT, PMS. Mix one part 2-octanol with three parts ethanol and warm to 37^C prior to addition to staining solution.

w.) Phosphoglucomutase: separated on C buffer, additional co-factors not required; staining solution: 50ml Tris buffer (pH 8), 100mg glucose-1-phosphate, 60 units G6PDH, 0.2mg glucose-1,6-diphosphate, 10mg TPN, MTT, PMS.

x.) Phosphogluconate dehydrogenase: separated on C buffer, addition of 20mg TPN to gel prior to degassing recommended; staining solution: 50ml Tris/HCl buffer (pH 8), 80mg 6-phosphogluconate, 50mg magnesium chloride, 10mg TPN, MTT, PMS.

y.) Sorbitol dehydrogenase: separated on TC buffer, addition of 40mg of NAD recommended; staining solution: 50ml Tris/HCl buffer (pH 8), 250mg sorbitol, 20mg NAD, MTT, PMS.

z.) Superoxide dismutase: separated on M, TVB, and TC buffers, addition of co-factors not required; staining solution: 10ml Tris/HCl buffer (pH 8), MTTx4, PMSx4. AGAR OVERLAY. Incubate at room temperature in light; areas showing lack of staining (ie clear zones) are bands of SOD.

aa.) Triosephosphate isomerase: separated on C buffer, addition of co-factors not required; staining solution: 10ml Tris/HCl buffer (pH 8), 20mg dihydroxyacetone phosphate, 100 units GAPDH, 100mg arsenic acid, 20mg NAD MTT, PMS. AGAR OVERLAY. The dihydroxyacetone phosphate is prepared according to the directions from Sigma Chemicals, and stored frozen in 20mg aliquots. If banding is too weak, 4x GAPDH and 2x MTT can be added to the staining solution to increase banding intensity.

Table # 3 summarizes all enzymes and proteins selected for analysis in this investigation. In addition to the full name of the enzyme or protein, Enzyme Commission number (E.C.#), number of loci and tissue sources are listed.

[these recipes were taken or modified from Siciliano and Shaw (orig. manu.) and May (1980)]

IV Data Interpretation and Analysis

Banding patterns were labeled according to a numerical system described by Ramsey and Wakeman (1983), in which the most anodal band is designated as the 100 band. This modification eliminates the calculation of which band occurs most frequently, as in the method reported by Allendorf and Utter (1979). Bands other than the most anodal one (100) are assigned numerical values based upon ratios of their migrational distance relative to the migrational distance of the 100 band. A band that migrates only 10mm compared to a 100 band that migrates 40mm would therefore be labeled 25 and so forth.

Interpretation of banding patterns followed the reasoning of Allendorf et al (1977). Information concerning enzyme structure and composition was taken from Dixon and Webb (1979) as well as Bergmeyer (1983) and Fersht (1985). Conservative interpretations were used throughout the study, and will be discussed in the Results and Discussion section.

Statistical analyses of the allele frequency data was accomplished with the BIOSYS I software package (Swofford and Selander 1981) and the Statistical Analysis System (SAS). The programs were executed on the NAS Advanced System/9000-II computer at the Rice University Institute for Computer Services and Applications. Selection of appropriate statistical analyses are discussed in the Results and Discussion section.

RESULTS AND DISCUSSION

The data presented here are the culmination of an exhaustive experimental regimen designed to determine most effective methods of separation and visualization. Tissue sources are given for all samples in order of usefulness. Several systems are expressed in some or all of the tissue examined, but will only be listed for those tissues in which they showed either variability or most reliable staining. Estimates for number of loci responsible for a given banding pattern are conservative throughout the analysis. In a system exhibiting a single invariant band only a single locus is presumed, although several loci could be encoding identical alleles. While this might underestimate the number of loci screened for variability, it prevents erroneous assignment of multi-locus structure to any questionable system.

I. Electrophoretic Patterns

a) Acid phosphatase: 2 anodal zones of banding in liver samples, with strong streaking between zones; no variance detected in either banding zone, although intensities of streaking did vary; indicates possibility of 2 loci, but too ambiguous for positive interpretation.

b) Adenosine deaminase: single anodal band in liver, invariant and quite faint.

c) Adenylate kinase: single anodal band in liver, invariant with occasional sub-banding at high voltages; appears on gels stained for creatine phosphokinase as well.

d) Alcohol dehydrogenase: presumptive single locus migrating cathodally in liver samples; extremely variable, with 3 alleles (100/66/37) exhibited; all possible heterozygote patterns seen, but no 40/40 homozygotes found.

e) Aldolase: single anodal band in liver, invariant; prone to inconsistent staining intensities and some sub-banding.

f) Aspartate aminotransferase: single cathodal band in muscle (also appears faintly in liver), invariant; presumptive single locus migrating anodally in liver, exhibiting 4 alleles (100/92/84/65); 84 and 65 are extremely rare variants.

g) Creatine phosphokinase: presumptive single locus migrating anodally in brain and in liver; brain samples invariant, liver samples too inconsistent to be scored confidently; NOTE- the adenylate kinase bands are present on this gel, so caution must be exercised to avoid scoring AK bands as CPK variants.

h) Diaphorase: single anodal band in liver, invariant; weakly staining even under long incubation times, difficult to distinguish from background staining.

i) Esterase: 3 anodal zones detected in liver; least anodal too faint to score accurately (EST-1); EST-2 and EST-3 invariant, with EST-2 exhibiting double banding in all liver samples, but single bands in brain tissue; since migrational distances were the same, incomplete knowledge of loci number and expression must

be assumed, and care must be exercised if these loci are to be included in the data catalog.

j) Fumarase: single anodal band in liver, invariant; frequently very faintly staining, causing difficulty in detection of bands.

k) General proteins: 4 distinct anodal zones exhibited in muscle samples; lack of certainty concerning genetic character of these banding zones prevents inclusion of this information into the genetic analysis.

l) Glucosephosphate isomerase: single cathodal band in muscle, invariant (NOTE- this system showed variability in another study, Paul Ramsey, pers. comm.); single anodal band in liver, invariant; cathodal form also stained weakly in liver, again showing no variance.

m) Glucose-6-phosphate dehydrogenase: single anodal zone of banding in liver, invariant; quite prone to spurious sub-bands, varying from replication to replication; tetrameric structure could lead to frequent polymerization of the various subunits of the active enzyme.

n) Glutamate dehydrogenase: single anodal band in liver, invariant; reasonably inconsistent in staining intensity, possibly due to difficulties encountered with solubility of glutamic acid in staining buffer solution.

o) Glucose-3-phosphate dehydrogenase: single anodal band in liver, invariant and often faint; presumptive single locus migrating cathodally in muscle, exhibiting 2 alleles (100/70).

p) Glyceraldehyde-3-phosphate dehydrogenase:single anodal band in liver and brain, invariant; single cathodal band in liver, invariant.

q) Isocitrate dehydrogenase: anodally migrating system in liver, exhibiting rare variant (100/57); extremely sensitive to high voltage, to avoid excessive sub-banding run only at low voltage.

r) Lactate dehydrogenase: single anodal band in brain, invariant; single anodal band in muscle, invariant (migration rate different than liver sample).

s) Malate dehydrogenase: single anodal band in liver, invariant; single anodal band in muscle, invariant (migration rate different than liver sample).

t) Malic enzyme: single anodal band in liver, invariant; this enzyme was never resolved as highly as other systems, bands were generally diffuse with poor definition, but no apparent variance.

u) Mannosephosphate isomerase: single anodal band in liver, invariant; occasional forward sub-banding, but easily distinguished from real band when present.

v) Octanol dehydrogenase: single anodal band in liver, invariant; far too faint and erratic to score effectively, Sciaenidae obviously do not encode for ODH as extensively as Salmonidae.

w) Phosphoglucomutase: single cathodal band in liver, invariant; anodal banding in liver was always a double banding pattern, suggesting possible polymerization between loci.

x) Phosphogluconate dehydrogenase: single anodal band in liver; variance detected at this presumptive locus was generally too

inconsistent to score confidently, salt effects or even storage artifacts could be responsible for the occasional 'variant band'.

y) Sorbitol dehydrogenase: single anodal band in liver, invariant; some sub-banding occurred, possibly due to polymerization of the tetrameric subunits.

z) Superoxide dismutase: single anodal band in liver and muscle, invariant; this system tended to be scorable in most cases where an agar overlay had been employed, and required no special staining procedure.

aa) Triosephosphate isomerase: single cathodal band in liver (almost no migration, stayed close to origin), as well as 2 anodal (near origin) bands and 2 rapidly migrating anodal bands in liver; rapid forms also encoded in brain samples; lack of further information concerning distribution of banding phenotypes (ie breeding data) necessitates the exclusion of this enzyme from the data catalog.

II Intrabay Variability

Detailed listings of locus by locus variability are given in Table # 4. Not all loci mentioned in the 'Electrophoretic Pattern' section have been included in the population analysis. Some loci were omitted from the analysis because they could not be satisfactorily separated (ACP,CPK [brain],ODH). The general protein phenotypes were not included in the analysis because of their pseudo-genetic nature. It is impossible to assign specific loci to GPx patterns, thus negating their informative value as indicators of true 'genetic' variability. Triosephosphate

isomerase was excluded because of its multiple banding pattern. Without extensive breeding data, little can be said about number of loci and alleles present in the TPI system.

As a result of possible genetic divergence, driven by geographical isolation, the individual bay systems sampled in this study are each treated as a 'subpopulation'. In order to accurately assess the significance of genetic variation between these 'subpopulations' (bays), the variability of allele frequencies within each embayment must first be addressed. Possible causes for allele frequency variation and genetic diversity must be examined, and overall congruence with existing data should be determined if interbay analyses are to be meaningful.

Values of p and H (Table # 5) averaged 10.0% and 0.031 respectively. While these values are within ranges reported for teleosts prior to this study, they are below average (expected) values. Surprisingly, these values also differ somewhat from those reported by Ramsey and Wakeman (1983) in their study of red drum from Louisiana. Given the similarity of the two studies, and their geographic proximity, one would not expect significant differences.

Percent polymorphism is difficult to evaluate as a result of the differing definitions existing to date. The three most common criteria for determining whether or not a locus is polymorphic are as follows: 1) 0.99 criterion - any locus exhibiting a dominant allele less than 99% of the time is

polymorphic; 2) 0.95 criterion - any locus exhibiting a dominant allele less than 95% of the time is polymorphic; 3) no criterion- any locus exhibiting more than one allele is polymorphic. The values in Table # 5 are based upon the 'no criterion' test, and as such tend to over-estimate significant polymorphism (ie. polymorphism which is frequent enough to yield statistically relevant data). Table # 6 lists the allele frequency data on a per bay basis, as well as document values of p for all three criteria and H for biased, unbiased and direct-count methods.

Nevo (1978) compiled an extensive overview of genetic variation in natural populations, covering 243 species reported on in publications prior to 1976. Using 51 species of fish (Osteichthyes), he gave average values of 15.2% and 0.0513 for p and H respectively. While the values found in this study fall within the ranges reported by Nevo, they are well below his average values. Various explanations for variances in allele frequencies exist, such as selection, gene flow, mutation pressure and combinations of these factors. While the testing of these evolutionary forces lies beyond the scope of this study, the possibility of environmental heterogeneity creating genetic variability must be considered. Some studies dealing with the correlation of ecological amplitude and allelic variability are strikingly contradictory.

A study involving another sciaenid species (H=0.018) was referenced by Smith and Fujio (1982) in their review of 10 marine teleosts (average H = 0.055). They concluded that habitat

specialists from temperate coastal zones demonstrate high levels of genetic diversity. Nevo (1978) however, in the previously mentioned report on genetic variation, concluded that p and H are correlated with ecological heterogeneity and niche breadth, which would assign maximum genetic variation to habitat generalists.

Phillip et al. (1985) demonstrated correlations between environmental variables and allele frequencies of some loci in largemouth bass (Micropterus salmoides). While some thermal adaption was shown, the authors concluded that further research was necessary to determine the extent of the advantage conferred by certain allelic variants. It seems apparent that the relationship between environmental conditions and genetic variability is still mostly undescribed, and that further research is required for a complete understanding to exist.

The range of values reported for teleosts makes any general comparison with red drum inconsequential. While it is reassuring to know that values for p and H derived by this study fall within acceptable limits, only comparisons with other sciaenids are meaningful. Furthermore, the differential allele frequency characteristics between closely related sciaenids (Ramsey and Wakeman 1983) indicate that only comparisons with studies done on red drum are useful.

Beckwitt (1983) reported p and H values for two Pacific sciaenids; white croaker (Genyonemus lineatus) and queenfish (Seriphus politus) had p / H values of 17.6% / 0.029 and 22.2% / 0.043 respectively. His estimates of p are much higher than

values attained in this study, while H was similar in both studies. The lower amount of polymorphism is not diagnostic of genetic restriction in this case however, since levels of heterozygosity appear to be equivalent. Variable rates and mechanisms of allele fixation could be operating to create differing amounts of polymorphic loci, but the acceptable and indeed comparable values of heterozygosity indicate genetically undisturbed populations.

Ramsey and Wakeman (1983) analysed 402 red drum from 6 estuaries in Louisiana and 1 in Texas, and reported p and H of 15% and 0.044 respectively. The variance in the transferrin system was shown only in the few fish from which they collected blood samples, causing the authors to exclude it from their heterozygosity analysis and recommend future examination of this protein. These values agree nicely with those reported in this study, with the higher value of p resulting from inclusion of the transferrin locus as well as variability in the GPI-2 locus in the Louisiana study. No variant GPI-2 alleles were detected in the fish collected for this study, even upon re-examination as suggested by Ramsey (pers. comm.).

Deviations in allele frequency from values expected under Hardy-Weinberg equilibrium are indicators of differential genotype survival, or conversely, as indicators of selected exploitation. Table # 7 lists chi-square test results for deviation from Hardy-Weinberg equilibrium. In cases where significant deviation occurred ($P<0.05$), the table includes data

from statistical tests designed to compensate for low expected frequencies (the pooled chi-square test; see Sokal and Rohlf 1969, as well as the BIOSYS I User's Manual). Furthermore, values of heterozygote deficiency or excess are included, from which degrees of fixation (F) for any given locus can be determined.

Only two cases of deviation from Hardy-Weinberg equilibrium were found. The AAT-1 locus in the Aransas Bay sample showed highly significant deviation (P=0.004), the result of the occurrence of an extremely rare heterozygote (100/84). This heterozygote occurred only once in the entire investigation, and created an artificially high expectation of heterozygotes. The result was an indicated deficiency of heterozygotes (D= -0.028). An apparently less significant deviation (P=0.043) occurred in the ADH locus of the Corpus Christi sample, which proved to be more significant (P=0.017) upon pooling of the genotypes. In this case, there appeared to be a real excess of heterozygotes (D=0.366), resulting from the occurrence of 31 100/66 heterozygotes while only 22.42 were expected. Here again however, the statistical probability of sampling error (drift) easily compensates for the singular deviation.

Table # 8 lists results from an exact probabilities test (see Fisher's exact test for 2x2 contingency tables, Sokal and Rohlf 1969), which circumvents difficulties encountered when testing small sample sizes with chi-square analyses (Haldane 1954). Using this procedure, only the ADH locus in the Corpus

Christi Bay sample exhibits significant deviation (P=0.020). Drift is implicated as the probable cause for the deviation, since selection and gene flow would tend to affect more than one of the locales sampled.

In summary, it can be stated that the red drum in this study did not show significant deviation from previously reported values of p and H on an intrabay basis, although some minor differences were detected. Likewise, deviations from Hardy-Weinberg equilibrium were also infrequent, being limited to two isolated instances. The differences that were shown are not unexpected in a study sampling a finite number of individuals from an extremely large population of fish. Indeed no accurate population numbers for red drum in Texas' waters exist to date.

The red drum examined exhibited below average amounts of polymorphic loci and heterozygosity, although the values of heterozygosity per locus per individual were essentially high enough to dismiss the possibility of genetic restriction. Levels that were demonstrated however, do indicate the potential susceptibility of red drum to reduction of genetic variability through overfishing. Further reference to the fact that red drum do not appear to be overexploited (genetically) to date will be made in the 'Management Implications' section, when suggestions for maintenance of genetic diversity will be discussed.

III Genetic Relatedness Indices (Interbay Comparisons)

Mean values of both percent polymorphic loci (p) and heterozygosity per locus per individual (H) were similar from bay to bay (Table # 5). Heterozygosity ranged from 0.025 to 0.042, while percent polymorphic loci ranged from 6.7 to 13.3%. The H values all fell within 1 standard deviation (0.013) of the mean (0.031), indicating a lack of significant allele frequency variance between bays (or, by definition, between subpopulations). Variation is even further reduced when the small samples from East Matagorda and Upper Laguna Madre are excluded.

Use of a comprehensive data analysis package for population genetic investigations, BIOSYS-I (Swofford and Selander 1981), allowed me to execute a total of twelve different tests for genetic relatedness. While some of these tests are merely slight alterations of each other, the application of a variety of tests insures against distortions resulting from anomalies in the statistical procedures applied to the data. Table # 9 lists 6 genetic similarity and or distance measures for thirty loci examined. Excluding East Matagorda Bay and Upper Laguna Madre localities as a result of the relatively small samples representing those bay systems, there was no evidence for population divergence below a Nei Genetic Identity value of 0.99. (see Nei 1972 and 1978) Similarly, both biased and unbiased minimum distances (Nei 1972; Nei 1978) never exceeded 0.005; indeed, when samples from East Matagorda Bay and Upper Laguna

Madre were omitted, the genetic distance was never greater than 0.001.

Table # 10 shows coefficients of similarity and distance based upon methods described by Rogers (1972), as well as modified methods described by Wright (1978), and Cavalli-Sforza and Edwards (1967) Chord and Arc Distances. Standard Rogers genetic similarity evaluation yielded results similar to those of the Nei index, with none of the bays (excluding East Matagorda Bay and Upper Laguna Madre) exhibiting coefficients of similarity below the 0.99 mark. The modified Rogers distance shows an increase in genetic distance, although coefficients were still all above 0.975. Wright (1978) explains his modification as a method of giving less weight to loci in which allele frequency variations are small. Table # 11 shows why the modified Rogers distance would be greater. The Nei Unbiased Genetic Identity coefficients are listed by single locus in this table, allowing a comparison of relative variability to be made. If East Matagorda and Upper Laguna Madre bays are again excluded, then divergence is evident only in ADH and AAT-1; the effect of equal weighting of G3P-2 and IDH in the standard Rogers method is to increase the apparent genetic divergence among bays. Results from the Cavalli-Sforza and Edwards analysis will be discussed further in the Cluster Analysis section, in an effort to clarify the slightly different patterns indicated by the various procedures.

IV. F-Statistics (Subpopulational Differentiation)

Table # 12 summarizes contingency chi-square analysis and F-statistics at all polymorphic loci. Contingency chi-square tests evaluate the significance of interpopulational heterogeneity in allele frequencies (Workman and Niswander 1970), while F-statistics are the classic tests of population structure using standardized genetic variances for polymorphic loci (Wright 1969;1978).

The contingency table analysis employs the Pearson chi-square statistic for an M by N matrix with (M-1)(N-1) degrees of freedom. Swofford recommends caution in the use of this test in cases where several expected frequencies for a number of classes are low (BIOSYS-I User's Manual). None of the loci showed a statistically significant level of heterogeneity between populations. Given the previous evidence of homogeneity among bay systems with Nei and Rogers indices, the lack of demonstrable heterogeneity using a contingency table approach is not unexpected.

The F-statistics fell within previously reported ranges, with some differences. Few studies on Sciaenids exist with which to compare data, but of those extant, ranges of F_{st} values are 0.014 (Beckwitt 1983) to 0.034 (Ramsey and Wakeman 1983). While the value attained by this study (0.044 averaged over all 7 sample sites) is higher than previously reported values, it is not statistically significant. An exclusion of the two poorly represented bays (East Matagorda and Upper Laguna Madre) resulted

in an extremely low F_{st} (0.008), indicating almost no populational subdivision of red drum in Texas' coastal waters. (see Table # 14 for a summary of the statistics presented so far, but excluding the two poorly sampled embayments)

Wright (1978) indicated the maximum usefulness of F-Statistics in the presence of minimal differentiation. In cases where there are major shifts in allelic predominance at some loci, the absolute variance becomes more informative. Table # 13, labeled non-hierarchical F-Statistics, lists values of actual and limiting variance, as they were employed for calculation of F_{st}. Levels of F_{st} are based upon absolute variance as opposed to limited variance (F_{dt}). Wright indicates that determination of which F-Statistic is most informative is a complicated task, and instead advocates the application of both parameters to population studies. As was the case with values of F_{st}, levels of F_{dt} are not indicative of significant differentiation into subpopulations.

V. Cluster Analyses

Cluster analyses were performed with different algorithms as well as a variety of genetic indices. Comparisons of the various cluster analyses showed that Aransas and San Antonio bays group together in all tests. Galveston, Corpus Christi and Lower Laguna Madre bays also group together, but there is minor of variability within this sub-group. It can be stated here however, that levels of divergence were extremely low, and that cluster analyses of

all types yielded very little indication of genetic subpopulational structure in red drum sampled for this study.

Table # 15 lists values of 4 different cluster analyses: Nei's Unbiased Genetic Identity (Nei 1978; the same criteria was used for the single locus genetic similarity matrices in Table # 11), Modified Rogers Distance (Wright 1978), Cavalli-Sforza and Edwards (1967) Chord Distance, and the analog to this last test, Arc Distance (Cavalli-Sforza 1967). Cluster level values and various goodness of fit statistics are also included for each test. All cluster analyses were undertaken with the unweighted pair group method (UPGMA, see Sokal and Sneath 1973), after initial tests with weighted pair group methods provided almost identical results.

The Nei Unbiased Genetic Identity cluster is widely accepted and applied, and shows excellent goodness of fit in this study ("F"= 0.005, Farris 1972). As is indicated by all other analyses performed in this investigation, the cluster diagram shows no significant division into subpopulations. Table # 16 demonstrates the complete lack of divergence upon exclusion of East Matagorda Bay and Upper Laguna Madre samples from the matrix.

As mentioned before, the modifications Wright (1978) made to the Rogers Distance procedure (Rogers 1972) unweight those loci which exhibit low frequency allele variation. The resulting cluster diagram groups San Antonio and Aransas bays together, and Galveston, Corpus Christi and Lower Laguna Madre bays in the

other major branch. The Upper Laguna Madre and East Matagorda bays shall be excluded from this discussion, as their contributions have been shown to detract from the validity of the overall analysis. Cluster diagrams excluding these two systems from the analysis are shown in Table # 16. The removal of the two bays from the data base does not alter the sequence of cluster levels, it merely clarifies the diagram.

Exclusive of these two bays, the Modified Rogers Distance only diverges slightly below the 0.98 mark, indicating little or no significant divergence. It is generally accepted that cluster levels above the 0.95 mark are not significant (Chakraborty et al. 1980). Therefore, neither the Nei indices nor the Rogers indices (standard and modified) indicate any significant subpopulational structure in red drum from the bays of Texas' Gulf Coast.

The Cavalli-Sforza and Edwards methods incorporate the projection of coordinates onto the positive portion of the surface of a hypersphere. This allows symmetrical stretching of the distance scale at the extremes, but causes symmetrical condensation of the middle portion of the scale. Wright (1978) points out that this symmetrical deformation is in accordance with the behaviour of factors on a percentage scale, and thus superior to techniques that do not compensate for scale distortion at all (ie. Prevosti distances). Even in light of the distension of the distance scale's extremes, the clustering levels of the Cavalli-Sforza and Edwards matrix are still all

above 0.95, therefore indicating no significant divergence.

The variability of grouping within the Galveston, Lower Laguna and Corpus Christi cluster would appear to be the result of slight differences in test procedures, and not statistically significant variance of allele frequencies. The absence of significant divergence negates any conclusions about the arrangement of hierarchy within the cluster diagram. Furthermore, the derived cluster patterns do not follow from geographical proximity of the bays. The differentiation that has been shown is most likely an artifact of random fluctuations in allele frequency due to sampling error, or genetic drift.

VI. Management Implications, Past and Present

Reasons for the low variability of allele frequencies in red drum from the bays of Texas are not easily postulated. While the results of similar studies in Louisiana indicated that levels of heterozygosity and polymorphism would be low, the values derived by this study were even lower. A possible factor, and one that will certainly play an extremely important role in fisheries management in the future, is the amount of artificial stocking performed by TPWD personnel in Texas bays.

Matlock (1984a) reports that stocking efforts for red drum in Texas between 1975 to 1982 have resulted in the addition of over 56,000,000 red drum to the bays of Texas. Eggs, fry and fingerling were all stocked (8.5, 45.1, and 2.8 million respectively), into all the bays except the Lower Laguna Madre.

Since mortality and survival rates are not known for thease stocked fish, little can be said concerning their true impact on the native population.

Matlock et al. (1984), reporting on the stocking of tagged red drum into Matagorda Bay, state that results verified the intrabay confinement of juvenile red drum. They also stated that the number of recoveries were insufficient to assess success of the stocking effort, and that optimum stocking rates could not be identified with this study. As a result of low returns (0.2%) and lack of compensation for fishing effort distribution, no assumptions concerning intrabay migration were made.

Stahl (1983) compared electrophoretic data from salmon occurring both naturally and in hatcheries, and discovered significant differences in allele frequencies. The fish sampled from the hatcheries had average H values of 0.022 as opposed to 0.028 for native salmon. In lieu of the low values reported for red drum this might not seem important, but it does represent a 20% reduction of heterogeneity in the hatchery stocks.

While this might indict hatchery operations as being inefficient, Ryman and Stahl (1981) summarized the genetic structure of various fishes in Scandinavia, and declared hatchery applications as the only solution. Careful planning of matings and attention to requirements of the native stocks to be fortified can result in a well tailored, diversity restoring, stocking program.

The advances which have been made in artificial propagation of red drum (Arnold et al. 1977), along with the excellent facilities operating in Texas currently (ie. the Wilson Hatchery in Corpus Christi, Texas), have set the stage for carefully planned genetic enhancement of the native red drum in the bays of Texas. With the data base developed in this study, as well as data from the studies of Ramsey and Wakeman (1983; 1986 [in prep.]), the development of objectives and subsequent implication of 'genetically oriented' stocking plans is possible.

With respect to implications of this study for fisheries management personnel in Texas, it should be pointed out that the data contained in this study are not absolutely conclusive. A need for data concerning the population structure and migratory behaviour of offshore stocks of red drum is mandatory for complete understanding of fishery structure. Without corroborating evidence for implied stock structure, this study can merely strongly urge the investigation of these offshore red drum, underline the need for a genetic analysis of broodstock involved in production of fish used in stocking efforts and request future cooperation among all agencies involved in management of red drum in the Gulf of Mexico.

This last suggestion stems from the indication that the red drum resident in the bays of Texas originate from one large, geographically indiscriminant breeding aggregate. If red drum in Texas bays do stem from (and therefore obviously recruit to) the same offshore breeding assemblage as fish in the embayments of

Louisiana, Mississippi,etc., it has drastic implications for fisheries managers. The administrators in charge of a state's natural resources are obligated to regulate these resources to the benefit of their state, as well as to avoid detriment to (residents of) other states. If the well-being and diversity of red drum is contingent upon reproductive success of one panmictic offshore stock in the Gulf of Mexico, the regulation of this stock must involve the participation, consent and adherence to guidelines by all involved parties, public or private.

VII. Summary

1) Values of percent polymorphic loci (all variants), p, and heterozygosity per locus per individual, H, averaged 10.0% and 0.031 respectively. While these values were below averages reported for other teleosts, as well as other sciaenids and even red drum, they were not significantly deviant.

2) Deviation from Hardy-Weinberg equilibrium was detected in two cases. In the AAT-1 locus of the Aransas Bay sample, occurrence of a rare heterozygote genotype created unrealistic expected values. The deviation of the ADH locus in the Corpus Christi Bay sample was due to a real excess of heterozygotes in the sample. Given the relatively low sampling rate for a large fish population however, this result was not entirely unexpected.

3) Variability of allele frequencies between bay systems (subpopulations) sampled was non-significant. Values of H fell within 1 standard deviation of the average value. Despite low levels of polymorphism, heterozygosities were common enough to preclude severe genetic restriction of red drum. Chi-square tests of contingency tables for heterozygosities between bays were not significant for any of the variable loci.

4) Twelve separate indices of genetic similarity/distance all indicated a virtual lack of differentiation among bay systems. No values of genetic similarity below 0.95 were detected, and levels were all above 0.98 upon exclusion of the two small samples from East Matagorda Bay and the Upper Laguna Madre.

5) F-Statistics reveal further lack of differentiation into subpopulations. F_{st} was equal to 0.044 across all seven bays, and dropped to 0.008 when the small samples were excluded. Both of these F_{st} values are insignificant in terms of genetic population divergence.

6) Similarly to the similarity/distance indices, cluster analyses failed to detect any significant subpopulational structure of red drum in the bays of Texas. All clusters had acceptable 'goodness of fit' ratings, but indicated no clustering below a level of 0.97.

7) Implications for fisheries management are contingent upon further studies on the offshore population's migrational habits and breeding strategies. As per this study, a single, panmictic assemblage of red drum is indicated in the Gulf.

8) The artificial stocking program is implicated as a possible factor in the low levels of p and H detected in this study; and the future use of stocked fish to carefully increase genetic diversity is briefly discussed.

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FIGURE # 1

Detail of the Texas Gulf Coast area. Scale is approximately 40 miles per inch. (from Hegen and Matlock 1980)

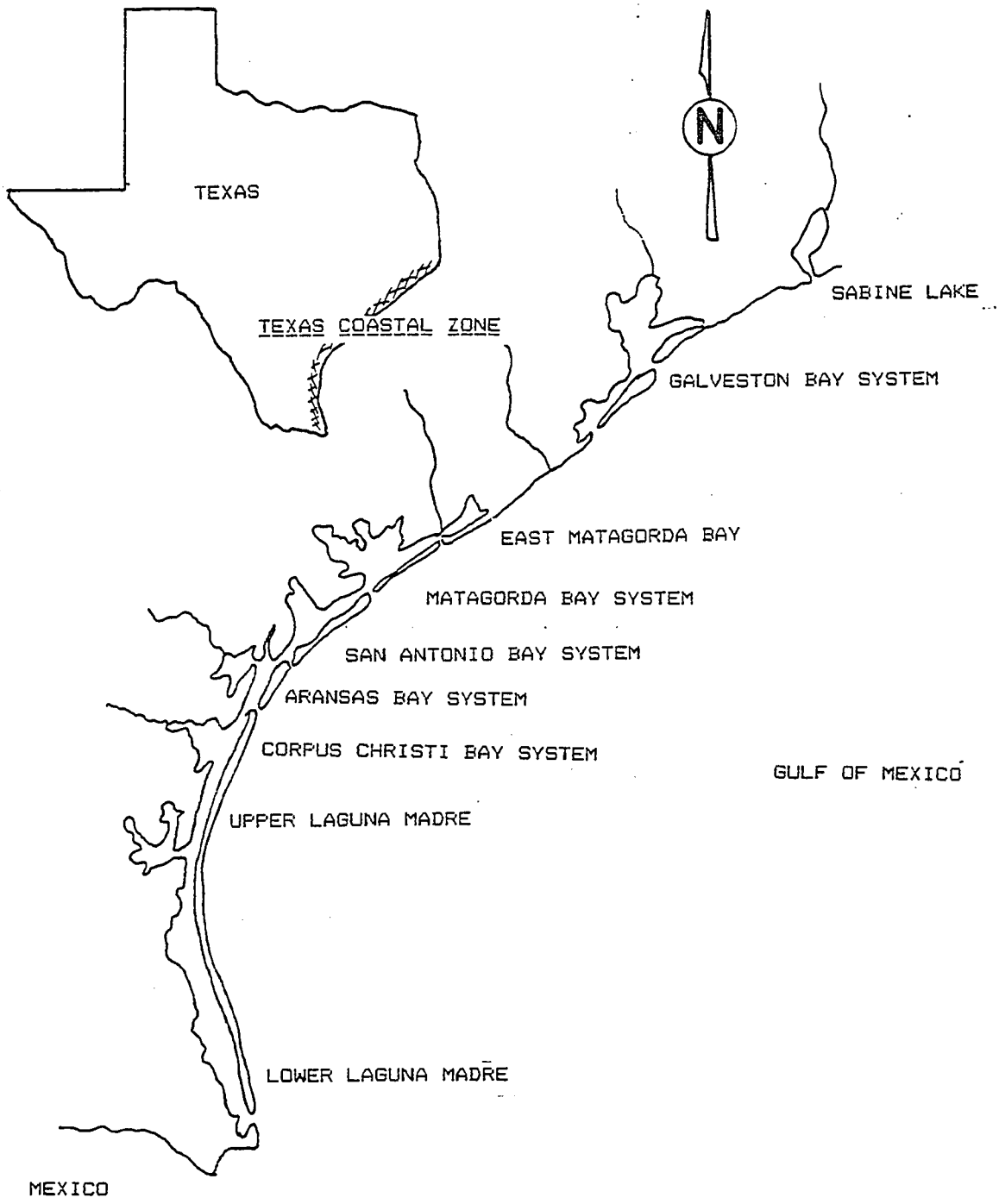

TEXAS
N
SABINE LAKE
TEXAS COASTAL ZONE
GALVESTON BAY SYSTEM
EAST MATAGORDA BAY
MATAGORDA BAY SYSTEM
SAN ANTONIO BAY SYSTEM
ARANSAS BAY SYSTEM
CORPUS CHRISTI BAY SYSTEM
GULF OF MEXICO
UPPER LAGUNA MADRE
LOWER LAGUNA MADRE
MEXICO

FIGURE # 2

Apparatus used to split heads of red drum to facilitate removal of brain and spinal tissues. The base is a 4'x 2" x 6" board with a U-bolt attached to one end. A large butcher knife can be wedged under the U-bolt, allowing development of sufficient force to cleanly slice through the cranial structure.

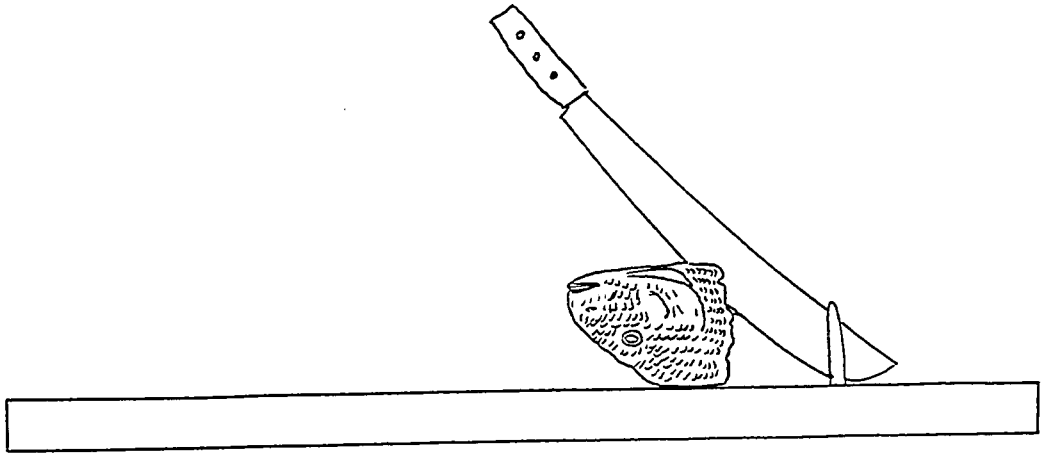


FIGURE # 3

Diagram of typical vertical starch gel molding tray (bottom), with lid assembly (center) and well forming comb (top).

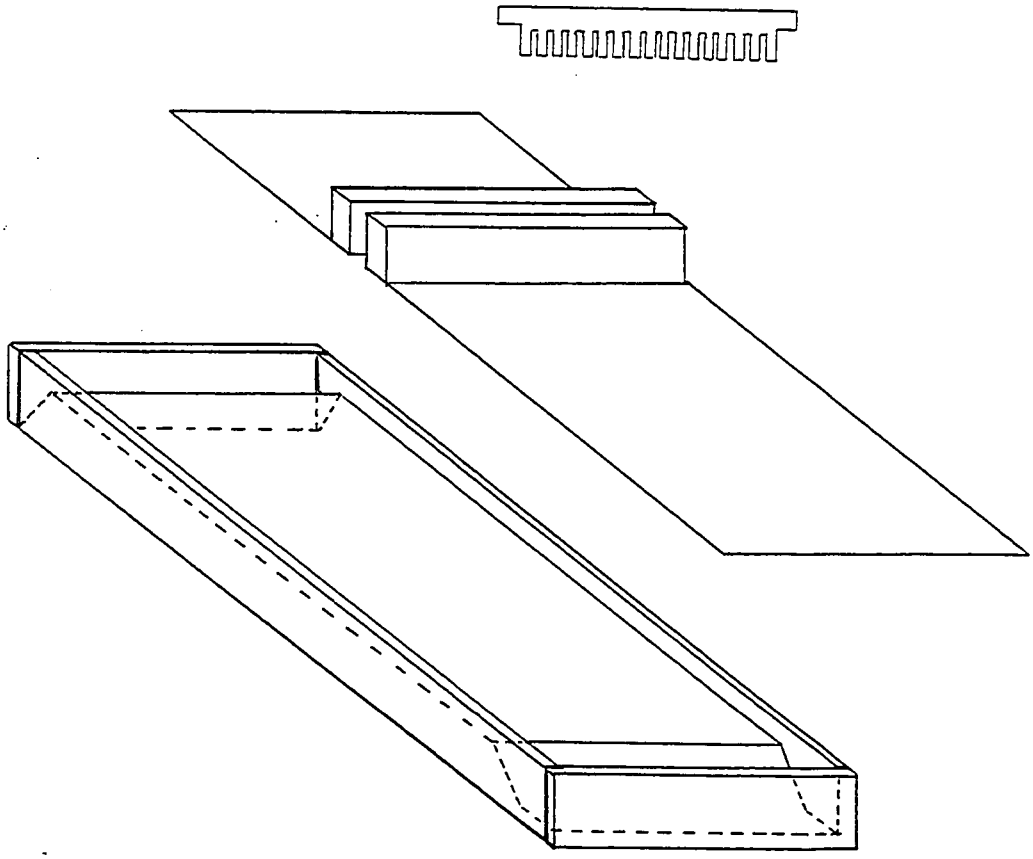


TABLE # 1: Record of sample collection localities, dates and numbers of samples collected per period.

Bay system	Date	Number in Sample	
Galveston	4/84	27	
	6/84	4	
	8/85	3	
	10/85	9	
	11/85	14	TOTAL = 57
East Matagorda	10/84	10	TOTAL = 10
San Antonio	10/84	13	
	11/84	22	TOTAL = 35
Aransas	5/84	23	
	6/84	4	
	10/84	32	
	11/84	12	TOTAL = 71
Corpus Christi	6/84	14	
	10/84	7	
	11/84	23	
	6/85	2	TOTAL = 51
Upper Laguna	11/84	8	TOTAL = 8
Lower Laguna	5/84	42	TOTAL = 42
		GRAND TOTAL	274

TABLE # 2: Buffers used for electrophoretic separations of proteins and enzymes. Parentheses indicate original references.

BUFFER	CODE LETTER	RECIPE
pH 6.1 Citrate	C	0.04M Citric acid, adjusted to pH 6.1 with N-(3 Aminopropyl) -morpholine. Dilute 1:10 for gel, full strength in electrode tanks. (Clayton and Tretiak, 1972)
pH 7.0 Tris-Citrate	TC	0.135M Tris-0.043M Citric acid. Dilute 1:10 for gel, full strength in electrode tanks. (Siciliano, pers. comm.)
pH 8.0 Tris-Versene-Borate	TVB	0.5M Tris-0.65M Boric acid-0.016M EDTA. Dilute 1:10 for gel, full strength for electrode tanks. (Siciliano, pers. comm.)
pH 8.7 Tris-Versene-Borate	M	0.18M Tris-0.1M Boric acid-0.0004M EDTA. Dilute 1:4 for gel, full strength for electrode tanks. (Markert and Faulhaber, 1965)
pH 8.5 Discontinuous Lithium Hydroxide	R	Solution A: (Electrode Buffer) 0.06M Lithium hydroxide-0.3M Boric acid, pH 8.1. Solution B: (Gel Buffer) 0.03M Tris-0.005M Citric acid, pH 8.5. Mix 1 part A to 99 parts B for gel, use B full strength for electrode tanks. (Ridgeway et al., 1970)

TABLE # 2 (CONT.): Recipes for substrate solutions and cofactors. The cofactors can be weighed out individually, but premixed solutions keep well in the refrigerator and greatly simplify processing of many samples.

ENZYME SUBSTRATES (store at 4^C)

1.) AAT Substrate: 0.75g a-ketoglutarate, 2.75g L-aspartate, 1.00g EDTA, 10.00g PVP40, 15.00g NaH2 PO4, 15.00g Na2H PO4; dilute to 1.0 l and store cold.

2.) EST Substrate: Sol. A: 1.00g Na-Acetate/50ml acetone/50ml H2O
Sol. B: 1.00g Na-Butyrate/15ml acetone/85ml H2O
Sol. C: 1.00g Na-Proprionate/100ml H2O

3.) IDH Substrate: 2.58g DL-Isocitric acid Na3 salt, 50ml H2O; adjust to pH 7.0 with conc. HCl and dilute to 100ml with H2O.

4.) LDH Substrate: 6.07g Na2CO3 monohydrate in 50ml H2O; with mixing vessel in ice bath, add 10.6ml 85% DL-Lactic acid, adjust to pH 7.0 with conc. NaOH and dilute to 100ml with H2O.

5.) MDH Substrate: (ME Subst.) 12.15g NaCO3 monohydrate in 50ml H2O; with mixing vessel in ice bath, add 13.4g L-Malic acid, adjust to pH 7.0 with conc. NaOH and dilute to 100ml with H2O.

6.) Dihydroxy-acetone phosphate : prepare according to manufacturer's directions and store frozen in 2ml aliquots.

COFACTORS

a.) Magnesium chloride: 10.00g MgCl2/100ml H2O
b.) MTT: 0.5g [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide]/100ml H2O
c.) NAD: 2.00g Nicotinamide Adenine Dinucleotide/100ml H2O
d.) NADP: 1.00g Nicotinamide Adenine Dinucleotide Phosphate/100ml H2O (to avoid confusion, NADP is refered to as TPN in the text)
e.) NBT: 1.00g Nitroblue Tetrazolium/100ml H2O
f.) PMS: 0.30g Phenazine Methosulfate/100ml H2O
g.) Sodium arsenate: 10.00g Na2HAsO4/100ml H2O

ENZYME DILUTIONS

h.) GPI: 1000 units plus an additional 2.5ml H2O; 4 drops=100 units
i.) G6PDH: dissolve 2000 units in 10.0ml; 6 drops=60 units

NOTE: All dilutions with H2O were made with glass distilled H2O. All cofactors except MgCl and Na-Arsenate must be stored at 4^C. The diluted enzymes must be stored at 4^C, not below 0^C

TABLE # 3: Proteins screened, abbreviations used, Enzyme Commission number, number of presumptive loci, and best tissue source.

Protein (Abbreviation)	E.C.#	Loci #	Tissue
Acid phosphatase (ACP)	3.1.3.2	1	liver
Adenosine deaminase (ADA)	3.5.4.4	1	liver
Adenylate kinase (AK)	2.7.4.3	1	liver
Alcohol dehydrogenase (ADH)	1.1.1.1	1	liver
Aldolase (ALD)	4.1.2.13	1	liver
Aspartate aminotransferase (AAT)	2.6.1.1	2	liver, muscle
Creatine kinase (CK)	2.7.3.2	2	liver, brain
Diaphorase (DIA)	1.6.4.3	1	liver
Esterase (EST)	3.1.1.1	2	2x liver, brain
Fumerase (FUM)	4.2.1.2	1	liver
General proteins (GPx)	-------	4	muscle
Glucosephosphate isomerase (GPI)	5.3.1.9	2	liver, muscle
Glucose-6-phosphate dehydrogenase (G6PDH)	1.1.1.49	1	liver
Glutamate dehydrogenase (GDH)	1.4.1.2	1	liver
Glycerol-3-phosphate dehydrogenase (G3P)	1.1.1.8	2	liver, muscle
Glyceraldehyde-3-phosphate dehydrogenase (GAPDH)	1.2.1.12	1	liver
Isocitrate dehydrogenase (IDH)	1.1.1.42	1	liver
Lactate dehydrogenase (LDH)	1.1.1.27	2	brain, muscle
Malate dehydrogenase (MDH)	1.1.1.37	2	liver, muscle
Malic enzyme (ME)	1.1.1.40	1	liver
Mannosephosphate isomerase (MPI)	5.3.1.8	1	liver
Phosphoglucomutase (PGM)	2.7.5.1	2	liver, muscle
Phosphogluconate dehydrogenase (PGD)	1.1.1.43	1	liver
Sorbitol dehydrogenase (SDH)	1.1.1.14	1	liver
Superoxide dismutase (SOD)	1.15.1.1	1	liver/muscle
Triosephosphate isomerase (TPI)	5.3.1.1	2	liver/muscle, brain

TABLE # 4
(2 pages)

Allele frequencies for all loci in all populations. Alphabetic allele designations corresdond to the numerical codes as follows: A = 100, and so forth through out the record. The population numbers in this table are assigned as follows:

Population #	Population Name (ie bay of collection)
1	Galveston Bay
2	East Matagorda Bay
3	San Antonio Bay
4	Aransas Bay
5	Corpus Christi Bay
6	Upper Laguna Madre
7	Lower Laguna Madre

	POPULATION						
LOCUS	1	2	3	4	5	6	7
ADH-1							
(N)	53	10	34	71	46	8	42
A	0.472	0.450	0.515	0.493	0.554	0.750	0.571
B	0.500	0.550	0.441	0.479	0.435	0.250	0.369
C	0.028	0.000	0.044	0.028	0.011	0.000	0.060
AAT-1							
(N)	46	10	33	71	47	8	42
A	0.163	0.000	0.045	0.070	0.149	0.125	0.143
B	0.837	1.000	0.955	0.923	0.840	0.875	0.857
C	0.000	0.000	0.000	0.007	0.000	0.000	0.000
D	0.000	0.000	0.000	0.000	0.011	0.000	0.000
G3P-2							
(N)	55	10	34	71	47	8	42
A	0.764	0.950	0.721	0.782	0.777	1.000	0.750
B	0.236	0.050	0.279	0.218	0.223	0.000	0.250
IDH-1							
(N)	55	10	35	71	47	8	42
A	1.000	1.000	1.000	0.986	0.989	1.000	1.000
B	0.000	0.000	0.000	0.014	0.011	0.000	0.000
ADA-1							
(N)	55	10	35	71	47	8	42
A	1.000	1.000	1.000	1.000	1.000	1.000	1.000
AK-1							
(N)	55	10	35	71	47	8	42
A	1.000	1.000	1.000	1.000	1.000	1.000	1.000
ALD-1							
(N)	55	10	35	71	47	8	42
A	1.000	1.000	1.000	1.000	1.000	1.000	1.000
AAT-2							
(N)	55	10	35	71	47	8	42
A	1.000	1.000	1.000	1.000	1.000	1.000	1.000
CK-1							
(N)	55	10	35	71	47	8	42
A	1.000	1.000	1.000	1.000	1.000	1.000	1.000
DIA-1							
(N)	55	10	35	71	47	8	42
A	1.000	1.000	1.000	1.000	1.000	1.000	1.000
EST-1							
(N)	55	10	35	71	47	8	42
A	1.000	1.000	1.000	1.000	1.000	1.000	1.000
EST-2							
(N)	55	10	35	71	47	8	42
A	1.000	1.000	1.000	1.000	1.000	1.000	1.000
FUM-1							
(N)	55	10	35	71	47	8	42
A	1.000	1.000	1.000	1.000	1.000	1.000	1.000
GPI-1							
(N)	55	10	35	71	47	8	42
A	1.000	1.000	1.000	1.000	1.000	1.000	1.000
GPI-2							
(N)	55	10	35	71	47	8	42
A	1.000	1.000	1.000	1.000	1.000	1.000	1.000

G6PDH							
(N)	55	10	35	71	47	8	42
A	1.000	1.000	1.000	1.000	1.000	1.000	1.000
GDH-1							
(N)	55	10	35	71	47	8	42
A	1.000	1.000	1.000	1.000	1.000	1.000	1.000
G3P-1							
(N)	55	10	35	71	47	8	42
A	1.000	1.000	1.000	1.000	1.000	1.000	1.000
GAPDH							
(N)	55	10	35	71	47	8	42
A	1.000	1.000	1.000	1.000	1.000	1.000	1.000
LDH-1							
(N)	55	10	35	71	47	8	42
A	1.000	1.000	1.000	1.000	1.000	1.000	1.000
LDH-2							
(N)	55	10	35	71	47	8	42
A	1.000	1.000	1.000	1.000	1.000	1.000	1.000
MDH-1							
(N)	55	10	35	71	47	8	42
A	1.000	1.000	1.000	1.000	1.000	1.000	1.000
MDH-2							
(N)	55	10	35	71	47	8	42
A	1.000	1.000	1.000	1.000	1.000	1.000	1.000
ME-1							
(N)	55	10	35	71	47	8	42
A	1.000	1.000	1.000	1.000	1.000	1.000	1.000
MPI-1							
(N)	55	10	35	71	47	8	42
A	1.000	1.000	1.000	1.000	1.000	1.000	1.000
PGM-1							
(N)	55	10	35	71	47	8	42
A	1.000	1.000	1.000	1.000	1.000	1.000	1.000
PGM-2							
(N)	55	10	35	71	47	8	42
A	1.000	1.000	1.000	1.000	1.000	1.000	1.000
PGD-1							
(N)	55	10	35	71	47	8	42
A	1.000	1.000	1.000	1.000	1.000	1.000	1.000
SDH-1							
(N)	55	10	35	71	47	8	42
A	1.000	1.000	1.000	1.000	1.000	1.000	1.000
SOD-1							
(N)	55	10	35	71	47	8	42
A	1.000	1.000	1.000	1.000	1.000	1.000	1.000

TABLE # 5

Summation of genetic variability at all 30 loci.

GENETIC VARIABILITY AT 30 LOCI IN ALL POPULATIONS
****

(STANDARD ERRORS IN PARENTHESES)

POPULATION	MEAN SAMPLE SIZE PER LOCUS	MEAN NO. OF ALLELES PER LOCUS	PERCENTAGE OF LOCI POLYMORPHIC*	MEAN HETEROZYGOSITY	
				DIRECT-COUNT	HDYWBG EXPECTED**
1. GALVESTON	54.6 (0.3)	1.1 (0.1)	10.0	0.037 (0.021)	0.039 (0.023)
2. EAST MATAGORDA	10.0 (0.0)	1.1 (0.0)	6.7	0.027 (0.023)	0.021 (0.018)
3. SAN ANTONIO	34.9 (0.1)	1.1 (0.1)	10.0	0.034 (0.022)	0.035 (0.022)
4. ARANSAS	71.0 (0.0)	1.2 (0.1)	13.3	0.032 (0.019)	0.035 (0.021)
5. CORPUS CHRISTI	47.0 (0.0)	1.2 (0.1)	13.3	0.042 (0.026)	0.039 (0.022)
6. U. LAGUNA	8.0 (0.0)	1.1 (0.0)	6.7	0.025 (0.018)	0.021 (0.015)
7. L. LAGUNA	42.0 (0.0)	1.1 (0.1)	10.0	0.039 (0.023)	0.039 (0.023)

* A LOCUS IS CONSIDERED POLYMORPHIC IF MORE THAN ONE ALLELE WAS DETECTED

** UNBIASED ESTIMATE (SEE NEI, 1978)

TABLE # 6
(7 pages)

Allele frequencies and genetic variability per bay.

ALLELE FREQUENCIES AND GENETIC VARIABILITY MEASURES

POPULATION: GALVESTON (BS1)

	LOCUS AND SAMPLE SIZE														
ALLELE	ADH-1 53	AAT-1 46	G3P-2 55	IDH-1 55	ADA-1 55	AK-1 55	ALD-1 55	AAT-2 55	CK-1 55	DIA-1 55	EST-1 55	EST-2 55	FUM-1 55	GPI-1 55	GPI-2 55
A	0.472	0.163	0.764	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
B	0.500	0.837	0.236	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
C	0.028	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
H	0.527	0.273	0.361	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
H(UNB)	0.532	0.276	0.364	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
H(D.C.)	0.491	0.283	0.327	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000

	LOCUS AND SAMPLE SIZE														
ALLELE	G6PDH 55	GDH-1 55	G3P-1 55	GAPDH 55	LDH-1 55	LDH-2 55	MDH-1 55	MDH-2 55	ME-1 55	MPI-1 55	PGM-1 55	PGM-2 55	PGD-1 55	SDH-1 55	SOD-1 55
A	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
B	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
C	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
H	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
H(UNB)	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
H(D.C.)	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000

MEAN HETEROZYGOSITY PER LOCUS (BIASED ESTIMATE) = 0.039 (S.E. 0.022)

MEAN HETEROZYGOSITY PER LOCUS (UNBIASED ESTIMATE) = 0.039 (S.E. 0.023)

MEAN HETEROZYGOSITY PER LOCUS (DIRECT-COUNT ESTIMATE) = 0.037 (S.E. 0.021)

MEAN NUMBER OF ALLELES PER LOCUS = 1.13 (S.E. 0.08)

PERCENTAGE OF LOCI POLYMORPHIC (0.95 CRITERION) = 10.00

PERCENTAGE OF LOCI POLYMORPHIC (0.99 CRITERION) = 10.00

PERCENTAGE OF LOCI POLYMORPHIC (NO CRITERION) = 10.00

ALLELE FREQUENCIES AND GENETIC VARIABILITY MEASURES
****

POPULATION: EAST MATAGORDA (BS2)

	LOCUS AND SAMPLE SIZE														
ALLELE	ADH-1 10	AAT-1 10	G3P-2 10	IDH-1 10	ADA-1 10	AK-1 10	ALD-1 10	AAT-2 10	CK-1 10	DIA-1 10	EST-1 10	EST-2 10	FUM-1 10	GPI-1 10	GPI-2 10
A	0.450	0.000	0.950	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
B	0.550	1.000	0.050	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
H	0.495	0.000	0.095	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
H(UNB)	0.521	0.000	0.100	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
H(D.C.)	0.700	0.000	0.100	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000

	LOCUS AND SAMPLE SIZE														
ALLELE	G6PDH 10	GDH-1 10	G3P-1 10	GAPDH 10	LDH-1 10	LDH-2 10	MDH-1 10	MDH-2 10	ME-1 10	MPI-1 10	PGM-1 10	PGM-2 10	PGD-1 10	SDH-1 10	SOD-1 10
A	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
B	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
H	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
H(UNB)	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
H(D.C.)	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000

MEAN HETEROZYGOSITY PER LOCUS (BIASED ESTIMATE) = 0.020 (S.E. 0.017)

MEAN HETEROZYGOSITY PER LOCUS (UNBIASED ESTIMATE) = 0.021 (S.E. 0.018)

MEAN HETEROZYGOSITY PER LOCUS (DIRECT-COUNT ESTIMATE) = 0.027 (S.E. 0.023)

MEAN NUMBER OF ALLELES PER LOCUS = 1.07 (S.E. 0.05)

PERCENTAGE OF LOCI POLYMORPHIC (0.95 CRITERION) = 6.67

PERCENTAGE OF LOCI POLYMORPHIC (0.99 CRITERION) = 6.67

PERCENTAGE OF LOCI POLYMORPHIC (NO CRITERION) = 6.67

ALLELE FREQUENCIES AND GENETIC VARIABILITY MEASURES

POPULATION: SAN ANTONIO (BS3)

	LOCUS AND SAMPLE SIZE														
ALLELE	ADH-1 34	AAT-1 33	G3P-2 34	IDH-1 35	ADA-1 35	AK-1 35	ALD-1 35	AAT-2 35	CK-1 35	DIA-1 35	EST-1 35	EST-2 35	FUM-1 35	GPI-1 35	GPI-2 35
A	0.515	0.045	0.721	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
B	0.441	0.955	0.279	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
C	0.044	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
H	0.538	0.087	0.403	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
H(UNB)	0.547	0.088	0.409	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
H(D.C.)	0.559	0.091	0.382	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000

	LOCUS AND SAMPLE SIZE														
ALLELE	G6PDH 35	GDH-1 35	G3P-1 35	GAPDH 35	LDH-1 35	LDH-2 35	MDH-1 35	MDH-2 35	ME-1 35	MPI-1 35	PGM-1 35	PGM-2 35	PGD-1 35	SDH-1 35	SOD-1 35
A	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
B	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
C	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
H	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
H(UNB)	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
H(D.C.)	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000

MEAN HETEROZYGOSITY PER LOCUS (BIASED ESTIMATE) = 0.034 (S.E. 0.022)

MEAN HETEROZYGOSITY PER LOCUS (UNBIASED ESTIMATE) = 0.035 (S.E. 0.022)

MEAN HETEROZYGOSITY PER LOCUS (DIRECT-COUNT ESTIMATE) = 0.034 (S.E. 0.022)

MEAN NUMBER OF ALLELES PER LOCUS = 1.13 (S.E. 0.08)

PERCENTAGE OF LOCI POLYMORPHIC (0.95 CRITERION) = 6.67

PERCENTAGE OF LOCI POLYMORPHIC (0.99 CRITERION) = 10.00

PERCENTAGE OF LOCI POLYMORPHIC (NO CRITERION) = 10.00

ALLELE FREQUENCIES AND GENETIC VARIABILITY MEASURES

POPULATION: ARANSAS (BS4)

	LOCUS AND SAMPLE SIZE														
ALLELE	ADH-1 71	AAT-1 71	G3P-2 71	IDH-1 71	ADA-1 71	AK-1 71	ALD-1 71	AAT-2 71	CK-1 71	DIA-1 71	EST-1 71	EST-2 71	FUM-1 71	GPI-1 71	GPI-2 71
A	0.493	0.070	0.782	0.986	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
B	0.479	0.923	0.218	0.014	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
C	0.028	0.007	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
H	0.527	0.144	0.341	0.028	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
H(UNB)	0.531	0.145	0.344	0.028	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
H(D.C.)	0.451	0.141	0.352	0.028	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000

	LOCUS AND SAMPLE SIZE														
ALLELE	G6PDH 71	GDH-1 71	G3P-1 71	GAPDH 71	LDH-1 71	LDH-2 71	MDH-1 71	MDH-2 71	ME-1 71	MPI-1 71	PGM-1 71	PGM-2 71	PGD-1 71	SDH-1 71	SOD-1 71
A	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
B	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
C	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
H	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
H(UNB)	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
H(D.C.)	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000

MEAN HETEROZYGOSITY PER LOCUS (BIASED ESTIMATE) = 0.035 (S.E. 0.021)

MEAN HETEROZYGOSITY PER LOCUS (UNBIASED ESTIMATE) = 0.035 (S.E. 0.021)

MEAN HETEROZYGOSITY PER LOCUS (DIRECT-COUNT ESTIMATE) = 0.032 (S.E. 0.019)

MEAN NUMBER OF ALLELES PER LOCUS = 1.20 (S.E. 0.10)

PERCENTAGE OF LOCI POLYMORPHIC (0.95 CRITERION) = 10.00

PERCENTAGE OF LOCI POLYMORPHIC (0.99 CRITERION) = 13.33

PERCENTAGE OF LOCI POLYMORPHIC (NO CRITERION) = 13.33

ALLELE FREQUENCIES AND GENETIC VARIABILITY MEASURES
****

POPULATION: CORPUS CHRISTI (BS5)

	LOCUS AND SAMPLE SIZE														
ALLELE	ADH-1 46	AAT-1 47	G3P-2 47	IDH-1 47	ADA-1 47	AK-1 47	ALD-1 47	AAT-2 47	CK-1 47	DIA-1 47	EST-1 47	EST-2 47	FUM-1 47	GPI-1 47	GPI-2 47
A	0.554	0.149	0.777	0.989	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
B	0.435	0.840	0.223	0.011	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
C	0.011	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
D	0.000	0.011	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
H	0.504	0.271	0.347	0.021	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
H(UNB)	0.509	0.274	0.351	0.021	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
H(D.C.)	0.696	0.234	0.319	0.021	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000

	LOCUS AND SAMPLE SIZE														
ALLELE	G6PDH 47	GDH-1 47	G3P-1 47	GAPDH 47	LDH-1 47	LDH-2 47	MDH-1 47	MDH-2 47	ME-1 47	MPI-1 47	PGM-1 47	PGM-2 47	PGD-1 47	SDH-1 47	SOD-1 47
A	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
B	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
C	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
D	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
H	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
H(UNB)	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
H(D.C.)	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000

MEAN HETEROZYGOSITY PER LOCUS (BIASED ESTIMATE) = 0.038 (S.E. 0.022)

MEAN HETEROZYGOSITY PER LOCUS (UNBIASED ESTIMATE) = 0.039 (S.E. 0.022)

MEAN HETEROZYGOSITY PER LOCUS (DIRECT-COUNT ESTIMATE) = 0.042 (S.E. 0.026)

MEAN NUMBER OF ALLELES PER LOCUS = 1.20 (S.E. 0.10)

PERCENTAGE OF LOCI POLYMORPHIC (0.95 CRITERION) = 10.00

PERCENTAGE OF LOCI POLYMORPHIC (0.99 CRITERION) = 13.33

PERCENTAGE OF LOCI POLYMORPHIC (NO CRITERION) = 13.33

ALLELE FREQUENCIES AND GENETIC VARIABILITY MEASURES
****

POPULATION: U. LAGUNA (BS6)

	LOCUS AND SAMPLE SIZE														
ALLELE	ADH-1 8	AAT-1 8	G3P-2 8	IDH-1 8	ADA-1 8	AK-1 8	ALD-1 8	AAT-2 8	CK-1 8	DIA-1 8	EST-1 8	EST-2 8	FUM-1 8	GPI-1 8	GPI-2 8
A	0.750	0.125	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
B	0.250	0.875	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
H	0.375	0.219	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
H(UNB)	0.400	0.233	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
H(D.C.)	0.500	0.250	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000

	LOCUS AND SAMPLE SIZE														
ALLELE	G6PDH 8	GDH-1 8	G3P-1 8	GAPDH 8	LDH-1 8	LDH-2 8	MDH-1 8	MDH-2 8	ME-1 8	MPI-1 8	PGM-1 8	PGM-2 8	PGD-1 8	SDH-1 8	SOD-1 8
A	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
B	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
H	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
H(UNB)	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
H(D.C.)	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000

MEAN HETEROZYGOSITY PER LOCUS (BIASED ESTIMATE) = 0.020 (S.E. 0.014)

MEAN HETEROZYGOSITY PER LOCUS (UNBIASED ESTIMATE) = 0.021 (S.E. 0.015)

MEAN HETEROZYGOSITY PER LOCUS (DIRECT-COUNT ESTIMATE) = 0.025 (S.E. 0.018)

MEAN NUMBER OF ALLELES PER LOCUS = 1.07 (S.E. 0.05)

PERCENTAGE OF LOCI POLYMORPHIC (0.95 CRITERION) = 6.67

PERCENTAGE OF LOCI POLYMORPHIC (0.99 CRITERION) = 6.67

PERCENTAGE OF LOCI POLYMORPHIC (NO CRITERION) = 6.67

ALLELE FREQUENCIES AND GENETIC VARIABILITY MEASURES
****

POPULATION: L. LAGUNA (BS7)

	LOCUS AND SAMPLE SIZE														
ALLELE	ADH-1 42	AAT-1 42	G3P-2 42	IDH-1 42	ADA-1 42	AK-1 42	ALD-1 42	AAT-2 42	CK-1 42	DIA-1 42	EST-1 42	EST-2 42	FUM-1 42	GPI-1 42	GPI-2 42
A	0.571	0.143	0.750	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
B	0.369	0.857	0.250	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
C	0.060	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
H	0.534	0.245	0.375	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
H(UNB)	0.540	0.248	0.380	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
H(D.C.)	0.571	0.238	0.357	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000

	LOCUS AND SAMPLE SIZE														
ALLELE	G6PDH 42	GDH-1 42	G3P-1 42	GAPDH 42	LDH-1 42	LDH-2 42	MDH-1 42	MDH-2 42	ME-1 42	MPI-1 42	PGM-1 42	PGM-2 42	PGD-1 42	SDH-1 42	SOD-1 42
A	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
B	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
C	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
H	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
H(UNB)	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
H(D.C.)	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000

MEAN HETEROZYGOSITY PER LOCUS (BIASED ESTIMATE) = 0.038 (S.E. 0.023)

MEAN HETEROZYGOSITY PER LOCUS (UNBIASED ESTIMATE) = 0.039 (S.E. 0.023)

MEAN HETEROZYGOSITY PER LOCUS (DIRECT-COUNT ESTIMATE) = 0.039 (S.E. 0.023)

MEAN NUMBER OF ALLELES PER LOCUS = 1.13 (S.E. 0.08)

PERCENTAGE OF LOCI POLYMORPHIC (0.95 CRITERION) = 10.00

PERCENTAGE OF LOCI POLYMORPHIC (0.99 CRITERION) = 10.00

PERCENTAGE OF LOCI POLYMORPHIC (NO CRITERION) = 10.00

TABLE # 7
(9 pages)

Summary of chi-square analyses for deviation from Hardy-Weinberg equilibrium, and coefficients of heterozygosity defficiency or excess. In cases where a significant deviation was indicated, tables detailing results from a 'pooled chi-square test are presented. (Aransas and Corpus Christi bays)

CHI-SQUARE TEST FOR DEVIATION FROM HARDY-WEINBERG EQUILIBRIUM

POPULATION: GALVESTON (BS1)

LOCUS	CLASS	OBSERVED FREQUENCY	EXPECTED FREQUENCY	CHI-SQUARE	DF	P
ADH-1						
	A-A	13	11.667			
	A-B	23	25.238			
	A-C	1	1.429			
	B-B	14	13.124			
	B-C	2	1.514			
	C-C	0	0.029	0.722	3	0.868
AAT-1						
	A-A	1	1.154			
	A-B	13	12.692			
	B-B	32	32.154	0.029	1	0.865
G3P-2						
	A-A	33	31.982			
	A-B	18	20.037			
	B-B	4	2.982	0.587	1	0.443

COEFFICIENTS FOR HETEROZYGOTE DEFICIENCY OR EXCESS

POPULATION: GALVESTON (BS1)

LOCUS	OBSERVED HETEROZYGOTES	EXPECTED HETEROZYGOTES	FIXATION INDEX (F)	D
ADH-1	26	28.181	0.069	-0.077
AAT-1	13	12.692	-0.035	0.024
G3P-2	18	20.037	0.093	-0.102

CHI-SQUARE TEST FOR DEVIATION FROM HARDY-WEINBERG EQUILIBRIUM

POPULATION: EAST MATAGORDA (BS2)

LOCUS	CLASS	OBSERVED FREQUENCY	EXPECTED FREQUENCY	CHI-SQUARE	DF	P
ADH-1						
	A-A	1	1.895			
	A-B	7	5.211			
	B-B	2	2.895	1.314	1	0.252
G3P-2						
	A-A	9	9.000			
	A-B	1	1.000			
	B-B	0	0.000	0.000	1	1.000

COEFFICIENTS FOR HETEROZYGOTE DEFICIENCY OR EXCESS

POPULATION: EAST MATAGORDA (BS2)

LOCUS	OBSERVED HETEROZYGOTES	EXPECTED HETEROZYGOTES	FIXATION INDEX (F)	D
ADH-1	7	5.211	-0.414	0.343
G3P-2	1	1.000	-0.053	0.000

CHI-SQUARE TEST FOR DEVIATION FROM HARDY-WEINBERG EQUILIBRIUM

POPULATION: SAN ANTONIO (BS3)

LOCUS	CLASS	OBSERVED FREQUENCY	EXPECTED FREQUENCY	CHI-SQUARE	DF	P
ADH-1						
	A-A	9	8.881			
	A-B	16	15.672			
	A-C	1	1.567			
	B-B	6	6.493			
	B-C	2	1.343			
	C-C	0	0.045	0.617	3	0.893
AAT-1						
	A-A	0	0.046			
	A-B	3	2.908			
	B-B	30	30.046	0.049	1	0.825
G3P-2						
	A-A	18	17.552			
	A-B	13	13.896			
	B-B	3	2.552	0.148	1	0.701

COEFFICIENTS FOR HETEROZYGOTE DEFICIENCY OR EXCESS

POPULATION: SAN ANTONIO (BS3)

LOCUS	OBSERVED HETEROZYGOTES	EXPECTED HETEROZYGOTES	FIXATION INDEX (F)	D
ADH-1	19	18.582	-0.038	0.022
AAT-1	3	2.908	-0.048	0.032
G3P-2	13	13.896	0.050	-0.064

CHI-SQUARE TEST FOR DEVIATION FROM HARDY-WEINBERG EQUILIBRIUM

POPULATION: ARANSAS (BS4)

LOCUS	CLASS	OBSERVED FREQUENCY	EXPECTED FREQUENCY	CHI-SQUARE	DF	P
ADH-1						
	A-A	21	17.128			
	A-B	28	33.759			
	A-C	0	1.986			
	B-B	18	16.156			
	B-C	4	1.929			
	C-C	0	0.043	6.320	3	0.097
AAT-1						
	A-A	0	0.319			
	A-B	9	9.291			
	A-C	1	0.071			
	B-B	61	60.390			
	B-C	0	0.929			
	C-C	0	0.000	13.434	3	0.004
G3P-2						
	A-A	43	43.298			
	A-B	25	24.404			
	B-B	3	3.298	0.043	1	0.835
IDH-1						
	A-A	69	69.007			
	A-B	2	1.986			
	B-B	0	0.007	0.007	1	0.932

COEFFICIENTS FOR HETEROZYGOTE DEFICIENCY OR EXCESS

POPULATION: ARANSAS (BS4)

LOCUS	OBSERVED HETEROZYGOTES	EXPECTED HETEROZYGOTES	FIXATION INDEX (F)	D
ADH-1	32	37.674	0.145	-0.151
AAT-1	10	10.291	0.021	-0.028
G3P-2	25	24.404	-0.032	0.024
IDH-1	2	1.986	-0.014	0.007

CHI-SQUARE TEST WITH POOLING
****

POPULATION: ARANSAS (BS4)

LOCUS	CLASS	OBSERVED FREQUENCY	EXPECTED FREQUENCY	CHI-SQUARE	DF	P
ADH-1	HOMOZYGOTES FOR MOST COMMON ALLELE	21	17.128			
	COMMON/RARE HETEROZYGOTES	28	35.745			
	RARE HOMOZYGOTES AND OTHER HETEROZYGOTES	22	18.128	3.381	1	0.066
AAT-1	HOMOZYGOTES FOR MOST COMMON ALLELE	61	60.390			
	COMMON/RARE HETEROZYGOTES	9	10.220			
	RARE HOMOZYGOTES AND OTHER HETEROZYGOTES	1	0.390	1.105	1	0.293

CHI-SQUARE TEST FOR DEVIATION FROM HARDY-WEINBERG EQUILIBRIUM

POPULATION: CORPUS CHRISTI (BS5)

LOCUS	CLASS	OBSERVED FREQUENCY	EXPECTED FREQUENCY	CHI-SQUARE	DF	P
ADH-1						
	A-A	10	14.011			
	A-B	31	22.418			
	A-C	0	0.560			
	B-B	4	8.571			
	B-C	1	0.440			
	C-C	0	0.000	8.147	3	0.043
AAT-1						
	A-A	2	0.978			
	A-B	10	11.892			
	A-D	0	0.151			
	B-B	34	33.129			
	B-D	1	0.849			
	D-D	0	0.000	1.568	3	0.667
G3P-2						
	A-A	29	28.258			
	A-B	15	16.484			
	B-B	3	2.258	0.397	1	0.529
IDH-1						
	A-A	46	46.000			
	A-B	1	1.000			
	B-B	0	0.000	0.000	1	1.000

COEFFICIENTS FOR HETEROZYGOTE DEFICIENCY OR EXCESS

POPULATION: CORPUS CHRISTI (BS5)

LOCUS	OBSERVED HETEROZYGOTES	EXPECTED HETEROZYGOTES	FIXATION INDEX (F)	D
ADH-1	32	23.418	-0.382	0.366
AAT-1	11	12.892	0.138	-0.147
G3P-2	15	16.484	0.080	-0.090
IDH-1	1	1.000	-0.011	0.000

CHI-SQUARE TEST WITH POOLING
****

POPULATION: CORPUS CHRISTI (BS5)

LOCUS	CLASS	OBSERVED FREQUENCY	EXPECTED FREQUENCY	CHI-SQUARE	DF	P
ADH-1	HOMOZYGOTES FOR MOST COMMON ALLELE	10	14.011			
	COMMON/RARE HETEROZYGOTES	31	22.978			
	RARE HOMOZYGOTES AND OTHER HETEROZYGOTES	5	9.011	5.734	1	0.017
AAT-1	HOMOZYGOTES FOR MOST COMMON ALLELE	34	33.129			
	COMMON/RARE HETEROZYGOTES	11	12.742			
	RARE HOMOZYGOTES AND OTHER HETEROZYGOTES	2	1.129	0.933	1	0.334

CHI-SQUARE TEST FOR DEVIATION FROM HARDY-WEINBERG EQUILIBRIUM

POPULATION: U. LAGUNA (BS6)

LOCUS	CLASS	OBSERVED FREQUENCY	EXPECTED FREQUENCY	CHI-SQUARE	DF	P
ADH-1						
	A-A	4	4.400			
	A-B	4	3.200			
	B-B	0	0.400	0.636	1	0.425
AAT-1						
	A-A	0	0.067			
	A-B	2	1.867			
	B-B	6	6.067	0.077	1	0.782

COEFFICIENTS FOR HETEROZYGOTE DEFICIENCY OR EXCESS

POPULATION: U. LAGUNA (BS6)

LOCUS	OBSERVED HETEROZYGOTES	EXPECTED HETEROZYGOTES	FIXATION INDEX (F)	D
ADH-1	4	3.200	-0.333	0.250
AAT-1	2	1.867	-0.143	0.071

CHI-SQUARE TEST FOR DEVIATION FROM HARDY-WEINBERG EQUILIBRIUM
****

POPULATION: L. LAGUNA (BS7)

LOCUS	CLASS	OBSERVED FREQUENCY	EXPECTED FREQUENCY	CHI-SQUARE	DF	P
ADH-1						
	A-A	13	13.590			
	A-B	19	17.928			
	A-C	3	2.892			
	B-B	5	5.602			
	B-C	2	1.867			
	C-C	0	0.120	0.289	3	0.962
AAT-1						
	A-A	1	0.795			
	A-B	10	10.410			
	B-B	31	30.795	0.070	1	0.791
G3P-2						
	A-A	24	23.530			
	A-B	15	15.940			
	B-B	3	2.530	0.152	1	0.697

COEFFICIENTS FOR HETEROZYGOTE DEFICIENCY OR EXCESS
****

POPULATION: L. LAGUNA (BS7)

LOCUS	OBSERVED HETEROZYGOTES	EXPECTED HETEROZYGOTES	FIXATION INDEX (F)	D
ADH-1	24	22.687	-0.071	0.058
AAT-1	10	10.410	0.028	-0.039
G3P-2	15	15.940	0.048	-0.059

TABLE # 8

Summary of 2 x 2 contingency table analysis (similar to Fisher's exact probabilities test). Included to prevent erroneous data interpretation resulting from deviations due to use of Levene' correction factor in the chi-square test (BIOSYS-I User's Manual).

SIGNIFICANCE TEST USING EXACT PROBABILITIES
****

POPULATION: GALVESTON (BS1)

LOCUS	R1	R2	R3	P
ADH-1	14	25	14	0.783
AAT-1	32	13	1	1.000
G3P-2	33	18	4	0.466

POPULATION: EAST MATAGORDA (BS2)

LOCUS	R1	R2	R3	P
ADH-1	2	7	1	0.520
G3P-2	9	1	0	1.000

POPULATION: SAN ANTONIO (BS3)

LOCUS	R1	R2	R3	P
ADH-1	9	17	8	1.000
AAT-1	30	3	0	1.000
G3P-2	18	13	3	0.690

POPULATION: ARANSAS (BS4)

LOCUS	R1	R2	R3	P
ADH-1	21	28	22	0.095
AAT-1	61	9	1	0.342
G3P-2	43	25	3	1.000
IDH-1	69	2	0	1.000

POPULATION: CORPUS CHRISTI (BS5)

LOCUS	R1	R2	R3	P
ADH-1	10	31	5	0.020
AAT-1	34	11	2	0.313
G3P-2	29	15	3	0.672
IDH-1	46	1	0	1.000

POPULATION: U. LAGUNA (BS6)

LOCUS	R1	R2	R3	P
ADH-1	4	4	0	1.000
AAT-1	6	2	0	1.000

POPULATION: L. LAGUNA (BS7)

LOCUS	R1	R2	R3	P
ADH-1	13	22	7	0.760
AAT-1	31	10	1	1.000
G3P-2	24	15	3	0.694

TABLE # 9

Tables of genetic similarity/distance generated with various analyses devised by M. Nei (1972; 1978).

MATRIX OF GENETIC SIMILARITY AND/OR DISTANCE COEFFICIENTS
****

BELOW DIAGONAL: NEI (1972) GENETIC IDENTITY

ABOVE DIAGONAL: NEI (1972) GENETIC DISTANCE

POPULATION	1	2	3	4	5	6	7
1 GALVESTON	*****	0.002	0.001	0.000	0.000	0.004	0.001
2 EAST MATAGORDA	0.998	*****	0.002	0.001	0.002	0.004	0.003
3 SAN ANTONIO	0.999	0.998	*****	0.000	0.001	0.004	0.001
4 ARANSAS	1.000	0.999	1.000	*****	0.000	0.004	0.001
5 CORPUS CHRISTI	1.000	0.998	0.999	1.000	*****	0.003	0.000
6 U. LAGUNA	0.996	0.996	0.996	0.996	0.997	*****	0.003
7 L. LAGUNA	0.999	0.997	0.999	0.999	1.000	0.997	*****

BELOW DIAGONAL: NEI (1978) UNBIASED GENETIC IDENTITY

ABOVE DIAGONAL: NEI (1978) UNBIASED GENETIC DISTANCE

POPULATION	1	2	3	4	5	6	7
1 GALVESTON	*****	0.001	0.000	0.000	0.000	0.003	0.000
2 EAST MATAGORDA	0.999	*****	0.001	0.001	0.001	0.002	0.002
3 SAN ANTONIO	1.000	0.999	*****	0.000	0.000	0.004	0.000
4 ARANSAS	1.000	0.999	1.000	*****	0.000	0.003	0.000
5 CORPUS CHRISTI	1.000	0.999	1.000	1.000	*****	0.002	0.000
6 U. LAGUNA	0.997	0.998	0.996	0.997	0.998	*****	0.002
7 L. LAGUNA	1.000	0.998	1.000	1.000	1.000	0.998	*****

BELOW DIAGONAL: NEI (1978) UNBIASED MINIMUM DISTANCE

ABOVE DIAGONAL: NEI (1972) MINIMUM DISTANCE

POPULATION	1	2	3	4	5	6	7
1 GALVESTON	*****	0.002	0.001	0.000	0.000	0.004	0.000
2 EAST MATAGORDA	0.001	*****	0.002	0.001	0.002	0.004	0.003
3 SAN ANTONIO	0.000	0.001	*****	0.000	0.001	0.004	0.000
4 ARANSAS	0.000	0.001	0.000	*****	0.000	0.004	0.001
5 CORPUS CHRISTI	0.000	0.001	0.000	0.000	*****	0.003	0.000
6 U. LAGUNA	0.003	0.002	0.003	0.003	0.002	*****	0.003
7 L. LAGUNA	0.000	0.002	0.000	0.000	0.000	0.002	*****

TABLE # 10

Summary of genetic similarity/distance indices resulting from analyses devised by Rogers (1972), Wright (1978) and Cavalli-Sforza and Edwards (1967).

MATRIX OF GENETIC SIMILARITY AND/OR DISTANCE COEFFICIENTS
****

BELOW DIAGONAL: MODIFIED ROGERS DISTANCE (WRIGHT,1978)

ABOVE DIAGONAL: PREVOSTI DISTANCE (WRIGHT,1978)

POPULATION	1	2	3	4	5	6	7
1 GALVESTON	*****	0.013	0.007	0.005	0.004	0.018	0.005
2 EAST MATAGORDA	0.046	*****	0.013	0.011	0.015	0.016	0.017
3 SAN ANTONIO	0.025	0.046	*****	0.005	0.007	0.020	0.007
4 ARANSAS	0.017	0.036	0.014	*****	0.005	0.018	0.008
5 CORPUS CHRISTI	0.014	0.047	0.023	0.018	*****	0.015	0.004
6 U. LAGUNA	0.065	0.060	0.066	0.061	0.054	*****	0.015
7 L. LAGUNA	0.022	0.054	0.022	0.023	0.012	0.054	*****

BELOW DIAGONAL: ROGERS (1972) GENETIC SIMILARITY

ABOVE DIAGONAL: ROGERS (1972) GENETIC DISTANCE

POPULATION	1	2	3	4	5	6	7
1 GALVESTON	*****	0.013	0.007	0.005	0.004	0.018	0.005
2 EAST MATAGORDA	0.987	*****	0.012	0.011	0.015	0.016	0.017
3 SAN ANTONIO	0.993	0.988	*****	0.005	0.007	0.019	0.006
4 ARANSAS	0.995	0.989	0.995	*****	0.005	0.018	0.007
5 CORPUS CHRISTI	0.996	0.985	0.993	0.995	*****	0.015	0.004
6 U. LAGUNA	0.982	0.984	0.981	0.982	0.985	*****	0.014
7 L. LAGUNA	0.995	0.983	0.994	0.993	0.996	0.986	*****

BELOW DIAGONAL: CAVALLI-SFORZA & EDWARDS (1967) CHORD DISTANCE

ABOVE DIAGONAL: CAVALLI-SFORZA & EDWARDS (1967) ARC DISTANCE

POPULATION	1	2	3	4	5	6	7
1 GALVESTON	*****	0.062	0.025	0.024	0.021	0.070	0.017
2 EAST MATAGORDA	0.061	*****	0.053	0.051	0.061	0.061	0.066
3 SAN ANTONIO	0.025	0.053	*****	0.021	0.031	0.076	0.022
4 ARANSAS	0.024	0.051	0.021	*****	0.024	0.070	0.027
5 CORPUS CHRISTI	0.021	0.061	0.031	0.024	*****	0.065	0.025
6 U. LAGUNA	0.070	0.061	0.075	0.069	0.065	*****	0.070
7 L. LAGUNA	0.017	0.066	0.022	0.027	0.025	0.069	*****

TABLE # 11
(2 pages)

Indices of genetic similarity/distance for each of the variable loci.

MATRIX OF SINGLE-LOCUS GENETIC SIMILARITY OR DISTANCE COEFFICIENTS
****

COEFFICIENT: NEI (1978) UNBIASED GENETIC IDENTITY

LOCUS: ADH-1

POPULATION	1	2	3	4	5	6	7
1 GALVESTON	*****						
2 EAST MATAGORDA	1.000	*****					
3 SAN ANTONIO	1.000	1.000	*****				
4 ARANSAS	1.000	1.000	1.000	*****			
5 CORPUS CHRISTI	0.999	1.000	1.000	1.000	*****		
6 U. LAGUNA	0.903	0.886	0.952	0.922	0.966	*****	
7 L. LAGUNA	0.982	0.980	1.000	0.990	1.000	0.992	*****

COEFFICIENT: NEI (1978) UNBIASED GENETIC IDENTITY

LOCUS: AAT-1

POPULATION	1	2	3	4	5	6	7
1 GALVESTON	*****						
2 EAST MATAGORDA	0.984	*****					
3 SAN ANTONIO	0.992	1.000	*****				
4 ARANSAS	0.996	0.998	1.000	*****			
5 CORPUS CHRISTI	1.000	0.987	0.994	0.998	*****		
6 U. LAGUNA	1.000	0.999	1.000	1.000	1.000	*****	
7 L. LAGUNA	1.000	0.988	0.996	0.999	1.000	1.000	*****

MATRIX OF SINGLE-LOCUS GENETIC SIMILARITY OR DISTANCE COEFFICIENTS
****

COEFFICIENT: NEI (1978) UNBIASED GENETIC IDENTITY

LOCUS: G3P-2

POPULATION	1	2	3	4	5	6	7
1 GALVESTON	*****						
2 EAST MATAGORDA	0.975	*****					
3 SAN ANTONIO	1.000	0.958	*****				
4 ARANSAS	1.000	0.980	1.000	*****			
5 CORPUS CHRISTI	1.000	0.980	1.000	1.000	*****		
6 U. LAGUNA	0.958	1.000	0.937	0.965	0.964	*****	
7 L. LAGUNA	1.000	0.970	1.000	1.000	1.000	0.952	*****

COEFFICIENT: NEI (1978) UNBIASED GENETIC IDENTITY

LOCUS: IDH-1

POPULATION	1	2	3	4	5	6	7
1 GALVESTON	*****						
2 EAST MATAGORDA	1.000	*****					
3 SAN ANTONIO	1.000	1.000	*****				
4 ARANSAS	1.000	1.000	1.000	*****			
5 CORPUS CHRISTI	1.000	1.000	1.000	1.000	*****		
6 U. LAGUNA	1.000	1.000	1.000	1.000	1.000	*****	
7 L. LAGUNA	1.000	1.000	1.000	1.000	1.000	1.000	*****

TABLE # 12

Summary of populational subdivision indices. Contingency chi-square test and F-Statistics. (Wright 1978)

CONTINGENCY CHI-SQUARE ANALYSIS AT ALL LOCI
****

LOCUS	NO. OF ALLELES	CHI-SQUARE	D.F.	P
ADH-1	3	12.203	12	0.42948
AAT-1	4	19.826	18	0.34272
G3P-2	2	9.786	6	0.13395
IDH-1	2	3.957	6	0.68254
(TOTALS)		45.772	42	0.31842

SUMMARY OF F-STATISTICS AT ALL LOCI
****

LOCUS	F(IS)	F(IT)	F(ST)
ADH-1	-0.134	-0.096	0.034
AAT-1	0.002	0.038	0.036
G3P-2	0.044	0.109	0.068
IDH-1	-0.013	-0.004	0.009
MEAN	-0.057	-0.010	0.044

TABLE # 13

Summary of non-hierarchical F-Statistics, indicated for use in cases where sample size deviations are suspected. (Wright 1978)

NON-HIERARCHICAL F-STATISTICS
****

LOCUS	ALLELE	MEAN FREQUENCY	SAMPLING VARIANCE	ACTUAL VARIANCE	LIMITING VARIANCE	F DT
ADH-1						
	A	0.54359	0.00535	0.00333	0.24810	0.013
	B	0.43198	0.00533	0.00295	0.24537	0.012
	C	0.02443	0.00027	0.00017	0.02383	0.007
	TOTAL	---	0.01094	0.00645	0.51730	0.012
AAT-1						
	A	0.09939	0.00175	0.00149	0.08951	0.017
	B	0.89809	0.00177	0.00158	0.09153	0.017
	C	0.00101	0.00001	0.00000	0.00101	0.000
	D	0.00152	0.00002	0.00000	0.00152	0.000
	TOTAL	---	0.00354	0.00307	0.18356	0.017
G3P-2						
	A	0.82036	0.00175	0.00834	0.14737	0.057
	B	0.17964	0.00175	0.00834	0.14737	0.057
	TOTAL	---	0.00350	0.01667	0.29474	0.057
IDH-1						
	A	0.99647	0.00003	0.00000	0.00352	0.001
	B	0.00353	0.00003	0.00000	0.00352	0.001
	TOTAL	---	0.00006	0.00000	0.00704	0.001
AVERAGE		----	0.00451	0.00655	0.25066	0.026

TABLE # 14

Summary of population divergence data with East Matagorda and Upper Laguna Madre samples removed; to indicate source of slight divergence indicated by previous data (Table # 13).

SUMMARY OF F-STATISTICS AT ALL LOCI
****

LOCUS	F(IS)	F(IT)	F(ST)
ADH-1	-0.052	-0.045	0.007
AAT-1	0.033	0.054	0.022
G3P-2	0.049	0.051	0.003
IDH-1	-0.013	-0.005	0.008
MEAN	-0.003	0.005	0.008

NON-HIERARCHICAL F-STATISTICS
****

LOCUS	ALLELE	MEAN FREQUENCY	SAMPLING VARIANCE	ACTUAL VARIANCE	LIMITING VARIANCE	F DT
ADH-1						
	A	0.52103	0.00268	0.00000	0.24956	0.000
	B	0.44478	0.00264	0.00000	0.24695	0.000
	C	0.03420	0.00037	0.00000	0.03303	0.000
	TOTAL	---	0.00569	0.00000	0.52953	0.000
AAT-1						
	A	0.11414	0.00108	0.00113	0.10111	0.011
	B	0.88232	0.00111	0.00115	0.10383	0.011
	C	0.00141	0.00001	0.00000	0.00141	0.000
	D	0.00213	0.00002	0.00000	0.00212	0.000
	TOTAL	---	0.00222	0.00228	0.20847	0.011
G3P-2						
	A	0.75850	0.00198	0.00000	0.18318	0.000
	B	0.24150	0.00198	0.00000	0.18318	0.000
	TOTAL	---	0.00395	0.00000	0.36635	0.000
IDH-1						
	A	0.99506	0.00004	0.00000	0.00492	0.000
	B	0.00494	0.00004	0.00000	0.00492	0.000
	TOTAL	---	0.00008	0.00000	0.00984	0.000
AVERAGE		----	0.00299	0.00057	0.27855	0.002

TABLE # 15
(4 pages)

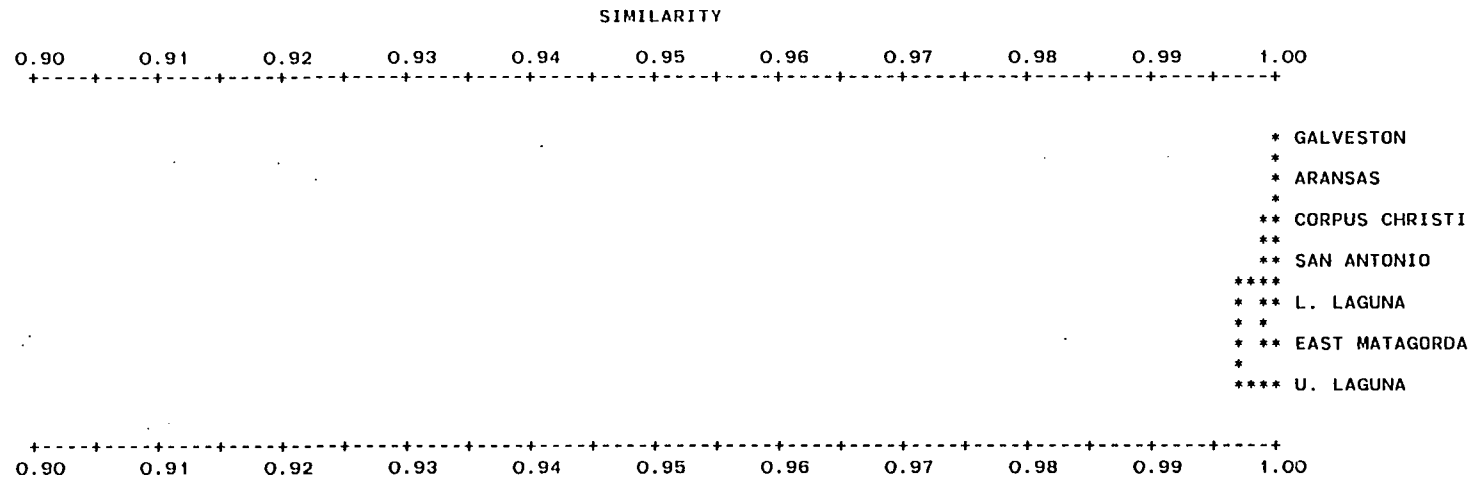
Phenograms of samples analysed using four different similarity/distance indices. Goodness of fit levels as well as index upon which phenogram is based are included for each cluster analysis.

FARRIS (1972) "F" = 0.005

PRAGER AND WILSON (1976) "F" = 0.026

PERCENT STANDARD DEVIATION (FITCH AND MARGOLIASH, 1967) = 0.041

COPHENETIC CORRELATION = 1.581



CLUSTER ANALYSIS USING UNWEIGHTED PAIR GROUP METHOD

COEFFICIENT USED: NEI (1978) UNBIASED GENETIC IDENTITY

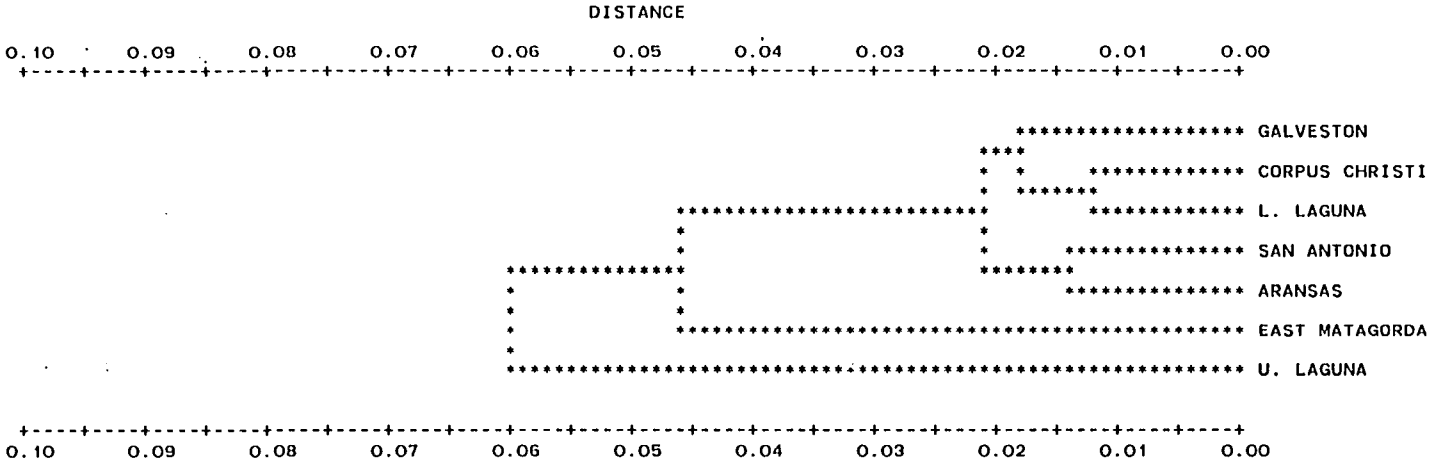
POPULATION OR CLUSTER NUMBERS JOINED		CLUSTERING LEVEL	CYCLE
1	4	1.00000	1
1	5	1.00000	2
3	7	1.00000	2
1	3	0.99992	3
1	2	0.99860	4
1	6	0.99724	5

FARRIS (1972) "F" = 0.067

PRAGER AND WILSON (1976) "F" = 8.633

PERCENT STANDARD DEVIATION (FITCH AND MARGOLIASH, 1967) = 13.704

COPHENETIC CORRELATION =0.973



CLUSTER ANALYSIS USING UNWEIGHTED PAIR GROUP METHOD

COEFFICIENT USED: MODIFIED ROGERS DISTANCE (WRIGHT, 1978)

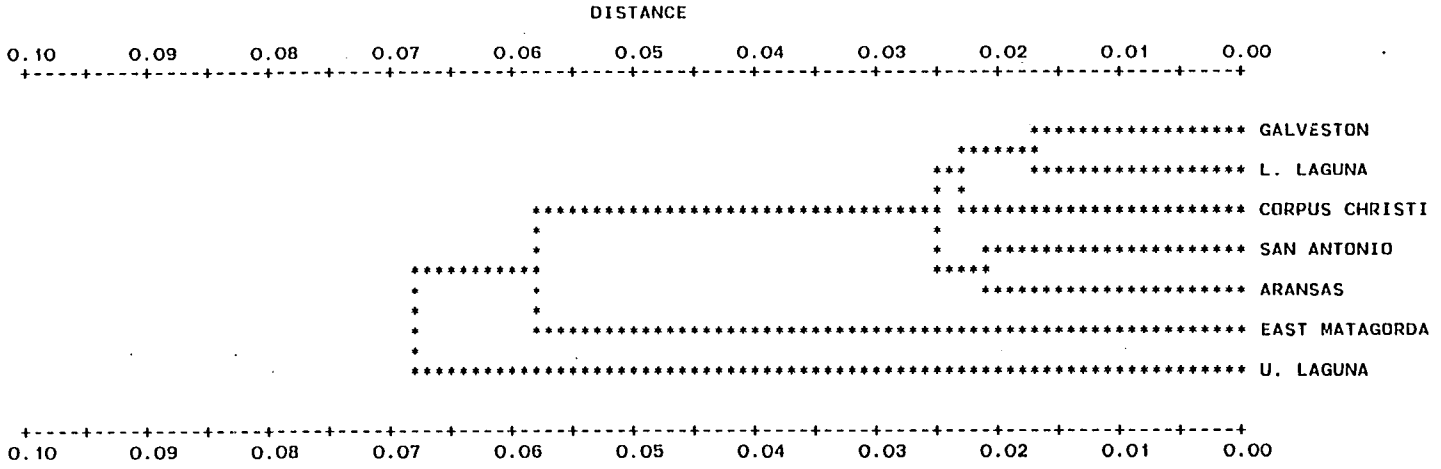
POPULATION OR CLUSTER NUMBERS JOINED		CLUSTERING LEVEL	CYCLE
3	4	0.01397	1
5	7	0.01228	1
1	5	0.01819	2
1	3	0.02139	3
1	2	0.04560	4
1	6	0.06000	5

FARRIS (1972) "F" = 0.063

PRAGER AND WILSON (1976) "F" = 6.752

PERCENT STANDARD DEVIATION (FITCH AND MARGOLIASH, 1967) = 8.706

COPHENETIC CORRELATION =0.982



CLUSTER ANALYSIS USING UNWEIGHTED PAIR GROUP METHOD

COEFFICIENT USED: CAVALLI-SFORZA & EDWARDS (1967) CHORD DISTANCE

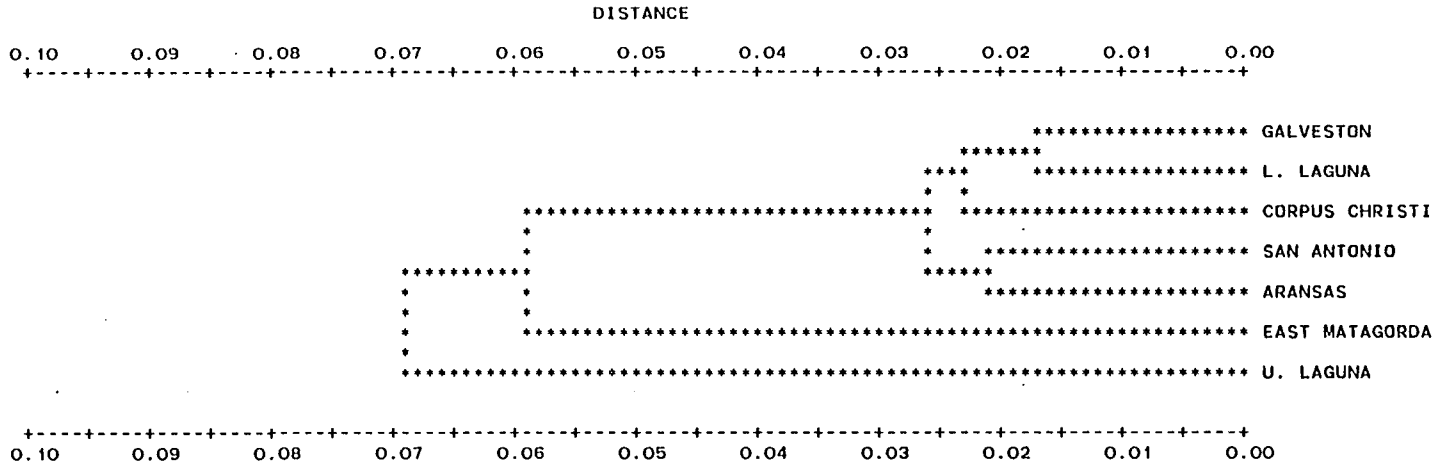
POPULATION OR CLUSTER NUMBERS JOINED		CLUSTERING LEVEL	CYCLE
1	7	0.01703	1
3	4	0.02078	1
1	5	0.02262	2
1	3	0.02548	3
1	2	0.05833	4
1	6	0.06810	5

FARRIS (1972) "F" = 0.064

PRAGER AND WILSON (1976) "F" = 6.831

PERCENT STANDARD DEVIATION (FITCH AND MARGOLIASH, 1967) = 8.779

COPHENETIC CORRELATION =0.982



CLUSTER ANALYSIS USING UNWEIGHTED PAIR GROUP METHOD

COEFFICIENT USED: CAVALLI-SFORZA & EDWARDS (1967) ARC DISTANCE

POPULATION OR CLUSTER NUMBERS JOINED		CLUSTERING LEVEL	CYCLE
1	7	0.01705	1
3	4	0.02079	1
1	5	0.02264	2
1	3	0.02551	3
1	2	0.05859	4
1	6	0.06866	5

TABLE # 16

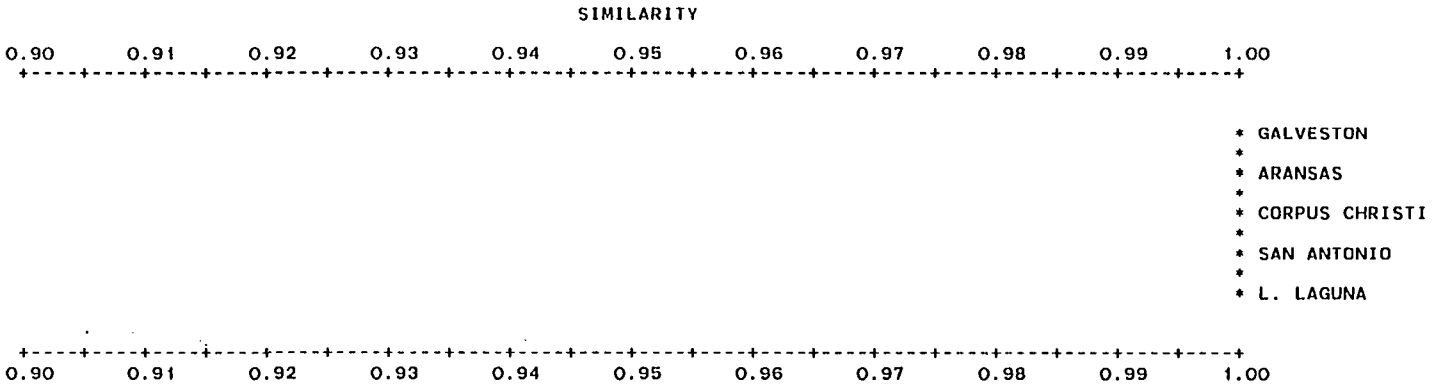
Phenogram of data set excluding East Matagorda and Upper Laguna samples, demonstrating lack of any divergence.

FARRIS (1972) "F" = 0.000

PRAGER AND WILSON (1976) "F" = 0.004

PERCENT STANDARD DEVIATION (FITCH AND MARGOLIASH, 1967) = 0.006

COPHENETIC CORRELATION =0.873



CLUSTER ANALYSIS USING UNWEIGHTED PAIR GROUP METHOD

COEFFICIENT USED: NEI (1978) UNBIASED GENETIC IDENTITY

POPULATION OR CLUSTER NUMBERS JOINED		CLUSTERING LEVEL	CYCLE
1	3	1.00000	1
1	4	1.00000	2
2	5	1.00000	2
1	2	0.99992	3