COOPERATIVE INSTITUTE FOR
FISHERIES MOLECULAR BIOLOGY:

FISHTEC

Study Report
A Synopsis of Research Efforts
Covering the Years: 1991-2006

Submitted by

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to

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Author's Preface

Although FISHTEC began in 1991, reporting commenced in 1992. Therefore, the results are compiled herein starting with the 1992-1995 reports submitted by the principal investigators. At that time, progress reports were required every three years. In latter stages of the project, reports were due annually and semi-annually. For the sake of convenience, results from these reports are presented on an annual basis.

There are many ways to organize 16 years of data, and it was decided that organizing research results by year(s) would be easiest for the reader to follow.

One of the goals of the FISHTEC project – one of great utility – was to develop primers (gene sequences) that would be made available to other researchers in the fields studied by the principal investigators of FISHTEC. These tools may be found in the section titled*: "Genomic Techniques Employed in FISHTEC"*.

The FISHTEC program is a cooperative enterprise consisting of university, state, and federal agencies whose individual efforts are markedly enhanced by providing a cooperative mechanism to plan and execute joint fisheries research. Participating organizations in FISHTEC include:

- The University of South Carolina
- National Ocean Service (NOS) - Center for Coastal Environmental Health and Biomolecular Research at Charleston
- Hollings Marine Laboratory, Charleston
- S.C. Department of Natural Resources - Marine Resources Division (MRD)
- S.C. Sea Grant Consortium
- Medical University of South Carolina

Furthermore, other institutions, some of which are global, contributed to FISHTEC by providing species or tissue samples for investigation. These additional organizations include: University of Maryland; Spanish Institute of Oceanography; California State University at Northridge; CSIRO, Tasmania; Girona University, Spain; National Research Institute of Far Seas Fisheries, Japan; North Carolina Department of Marine Resources; University of New Orleans; Advisory Committee to the US Commissioners for ICCAT; Richland Northeast High School; Louisiana State University; National Marine Fisheries Service (Ft. Johnson and Gulf of Mississippi); Grice Laboratory (University of Charleston); the Mississippi Department of Natural Resources; and the Florida Department of Natural Resources.

Executive Summary of FISHTEC Research

Pressures on the commercial and recreational fishing industries continue to exacerbate an already over fished and overcapitalized state for many fishery resources.Recent reports in the press make note of scientific studies which suggest the commercial fishes of the oceans may be completely fished out by 2050. Pollution and habitat degradation and loss have further stressed fisheries stocks, and the multi-national interests in stock management issues have sometimes confounded national policy development. Consequently, the Cooperative Institute for Fisheries Molecular Biology (FISHTEC) was established in 1991 to promote and conduct research needed to address fisheries management, habitat, and marketing issues that may be resolved through the use of biotechnology – specifically marine genomics. The FISHTEC program ended in 2006.

This report is a compilation of sixteen years of research by principal investigators, primarily from the University of South Carolina (Columbia, SC) and the South Carolina Department of Natural Resources – Marine Resources Division (Charleston, SC). It focuses on their research and the important outcomes of those efforts. Biographical information about the investigators is found in the "Profiles of Principal Investigators" section at the end of the report.

During the reporting period over eighty (80) marine species were examined for both nucleotide sequence library construction and to answer questions about population structure and phylogenetic histories. Marine species were studied from places as far away as Abidjan (Ivory Coast), the Iberian Peninsula, the Ionian Sea, and Brazil and Uruguay in the Southern Hemisphere. Species run the gamut from the American alligator to the Zebrafish. Principal investigator Joe Quattro discovered a completely new species of shark, the cryptic hammerhead, *Sphyrna nsp*.

The investigators published more than seventy (70) papers since the inception of FISHTEC, and more are *in press* or review. Over the 16-year period of the FISHTEC project, numerous presentations were given.

Another important aspect to the FISHTEC enterprise is training students as scientists in the technologies of the future, in this case primarily genomics. Throughout the life of FISHTEC, more than twenty-five (25) graduate students were supported by the various research projects. Numerous undergraduate students were supported as well.

The heart of this report focuses on the research undertaken by the investigators and the important outcomes of those efforts. The genomics work performed by the investigators is thoroughly summarized in the section titled "Genomic Techniques Employed in FISHTEC." This section is also important because it offers a "tool box" of gene sequencing information for eighty-plus marine species that will be valuable to other researchers in the field. These findings, as well as the report itself, will be posted on the Internet at the South Carolina Sea Grant Consortium's Web site: www.scseagrant.org.

As fisheries management issues now focus on Ecologically Significant Units, Marine Protected Areas, and Essential Fish Habitat, the genomics work of FISHTEC will provide important and useful tools and research approaches as a stair step up toward exploring and solving present and future fishery management issues.

Introduction to FISHTEC and its History

Recent developments in molecular biology provided tools that can be utilized to address a number of fisheries issues, including stock identification and the enhancement of fish reproduction and growth. FISHTEC was created to apply these new techniques, along with conventional approaches, to resolve key questions concerning fisheries.

In addition, the scientific programs of FISHTEC were integrated with policy issues so research projects have a direct relationship to users through the activities and responsibilities of the National Ocean Service, the Marine Resources Division of the S.C. Department of Natural Resources, and the S.C. Sea Grant Extension Program. The establishment of a FISHTEC Scientific Advisory Council provided an additional avenue for the identification of priority issues and applicable research. During the course of the FISHTEC project there has been a Board of Directors as well as a management component to guide research efforts.

During the past 16 years, FISHTEC addressed the following primary objectives:

- Incorporate biotechnology into ongoing state and federal monitoring and assessment programs that evaluate the effectiveness of fishery management policies and actions;
- Develop and test biotechnology for rapid and accurate identification of genetic stocks;
- Develop and test biotechnology for stock enhancement programs that minimize adverse impacts on the genetic diversity of wild stocks;
- Develop and test biotechnology for manipulation of reproductive performance and growth of cultured stocks;
- Develop biotechnology for assessing the health of individual organisms composing both wild and cultured populations;
- Develop biotechnology for producing broodstocks for aquaculture and stock enhancement programs; and
- Develop biotechnology for assessing the extent, severity and consequences of high risk pathogens on cultured and wild stocks.

In another effort to synchronize FISHTEC objectives with the evolving needs of fisheries management, the forensics needs of fisheries managers became a priority in 2004. This effort was supportive of the mission and strategic goals of the NOAA/NOS CCEHBR (Center for Coastal Environmental Health and Biomolecular Research) laboratory in Charleston, SC.

Further efforts in this area provided support to state and federal management personnel charged with the enforcement of those authorities established by the Magnuson-Stevens Fishery Conservation and Management Act (1966) and the Endangered Species Act (1973). DNA sequence analysis was used to resolve issues of species identification. An important outcome of these activities was the development of a curated DNA database, which may be found in the section titled: *"Genomic Techniques Employed in FISHTEC"*.

Initially, FISHTEC research appropriately focused on the development of genetic tools to assess the population structure of commercially and recreationally important species. This effort has expanded our understanding of the biology of these species and made significant contributions to management plans and deliberations. However, these studies targeted individual species of concern as commercial or recreational fisheries and did not deal with broader issues associated with management of multi-species assemblages or habitats. Aquatic Protected Areas is one such issue.

Aquatic Protected Areas (APA's) have been widely touted as an additional management tool for the conservation of species. APA's are thought to (1) protect spawning stocks, (2) elevate recruitment rates, (3) maintain stable population structure, (4) preserve ecological balances and (5) conserve the genetic resources underpinning life history characteristics. FISHTEC researchers applied their research tools to APAs in the study of spotted sea trout, red drum and weakfish. Results showed that the spotted sea trout has a very restricted migration pattern as an adult and the genetic data indicate substantial differentiation on a regional scale. This suggested that recruitment is restricted geographically and that the impact of an APA would be similarly restricted. In contrast, the genetic data on red drum and weakfish showed little evidence of geographic structure indicating no restriction on gene exchange and, hence, the benefits of an APA would be of great benefit. In other words, as important as these studies were to the management plans of the individual species, collectively they have ramifications far beyond the target species.

The benefits of FISHTEC-funded research to individual species management and marine APAs notwithstanding, FISHTEC researchers also were actively involved in the conservation of rare, threatened, and endangered freshwater species. The definition of freshwater APAs has become a priority issue in North American fisheries and conservation management since 37 percent of all North American fish species are extinct or seriously imperiled. Recent estimates suggest a current extinction rate for freshwater biodiversity approximately five times that for terrestrial fauna, and a projected future extinction rate of four percent per decade (a rate that rivals that estimated for tropical rain forests). This problem is particularly acute for the southeastern United States, a recognized center of endemism for freshwater fish biodiversity (where 500 of approximately 800 North American fish species occur in the Southeast).

This brief history of FISHTEC demonstrates how new technologies can be applied beyond individual species identification to far more complex and promising avenues of research.

General Summary of FISHTEC Research

Since its creation, the FISHTEC program has provided financial opportunity for a multitude of research projects focusing on the molecular study of over eighty species of both freshwater and saltwater fish species from around the globe. Projects ranged from population studies to ecotoxicology work and incorporated the cooperation of numerous local, national, and international organizations.

Reporting was organized by three-year report summaries unless otherwise noted below. Studies are distinguished by research host institution and major project foci. Glossary terms are **bolded** and can be referenced at the end of the report.

1992-1995

University of South Carolina; PIs: Bert Ely, Joseph M. Quattro

Research focused on the development of PCR-based assays from three anonymous nuclear loci (Leclerc et al., 1996) which allowed for the detection of genetic variation in striped bass (known to be historically difficult). Four hybridization and eight PCR-based assays were developed to discriminate between striped bass and white bass alleles. Five of the markers segregated randomly when known backcross progeny were tested. The markers were also used to demonstrate that hybrid striped bass (Chesapeake Bay) spawn with the native striped bass indicating backcross progeny. Analysis of age, length, and sex data indicated female hybrid striped bass are significantly longer than males at age 5 and 7 even though growth rate is the same suggesting female hybrids must have a faster period of growth at some earlier age. The analysis demonstrated backcrosses between hybrid striped bass and native striped bass in the Chesapeake Bay (Harrell et al., 1993), further verified purity of striped bass broodstock (Woods et al., 1995) and confirmed gynogen production (Leclerc et al., 1995).

DNA was isolated from 24 larval winter flounder and 150 swordfish of varying geographic origin.

Chemoluminescent detection techniques were found to be as sensitive as radioactive detection techniques for analysis of single copy genes. Incorporation of modified nucleotides into the probe for chemoluminescent detection was not reliable although teliable probes could be generated using gene amplification techniques (PCR).

Researchers experimented with random amplification of polymorphic DNA polymerase chain reaction (**RAPD-PCR**) for providing additional genetic markers. RAPD markers are not co-dominant which renders them inappropriate for use in genetic analyses of population sub-structure. However, they could prove useful in species identification although reproducibility is difficult to achieve.

Comparison of hybridization techniques for the detection of single copy nuclear DNA genes were conducted and showed these PCR techniques are more efficient for large scale population analyses.

Techniques for the isolation of DNA from larval fish were optimized via developed procedures to obtain DNA from a single larval fish that would be sufficient for more than 100 PCR reactions (Leclerc et. al., 1995).

Primers were designed based on conserved and anonymous nuclear DNA sequences which could amplify six different nuclear loci based on conserved DNA sequences. These primers were used to amplify bluefin tuna whose sequences were used to develop other primers. Cloned and sequenced DNA fragments amplified from five perciform fish taxa at second locus (*tpiB*) to were used to develop specific oligonucleotides. Researchers cloned and sequenced DNA amplified from the creatine kinase loci from a range of actinopterygian fishes which allowed for the designation of two anonymous bluefin tuna loci. Primers were designed to amplify either 5s ribosomal DNA or *ldhA* locus which amplified corresponding striped bass and white bass genes. Assays were developed to discriminate both amplified 5s rDNA and *ldhA* locus from the two species.

The extent of genetic variation among striped bass samples was determined utilizing three single copy nuclear loci (8-2, SSR14, SSR83) allele frequencies in seven different striped bass population samples from rivers throughout the natural range of the species. Significant differences in allele frequencies were observed with the largest difference occurring in populations at the extremes of the range (i.e. Appalachicola River on the Gulf Coast and the Shubenacadie and the Tabusintac Rivers in the Canadian maritime provinces). A hatchery-induced genetic bottleneck was determined to have caused a change in allele frequencies in the Santee-Cooper system. There appeared to be population subdivision within the Santee-Cooper system in the absence of obvious physical barriers to prevent mixing. Preliminary results indicated each river contained a genetically distinct population of striped bass.

Mitochondrial DNA (**mtDNA**) analysis of swordfish was used to develop a **PCR-RSP** assay to distinguish samples among three phylogenetic groups observed. The Pacific, Mediterranean, South Atlantic and North Atlantic Oceans were shown to contain genetically distinct populations (Alvarado Bremer et. al., 1995;1996). Another result of the study was the development of an inexpensive method for purifying DNA for DNA sequence analysis.
PCR-RSP assays were also developed for swordfish nuclear genes utilizing primers originally developed to amplify the corresponding portion of the tuna gene. A polymorphic site was revealed using various restriction enzymes. The site was absent in Pacific (n=30) and Mediterranean (n=50) samples and showed a 7% frequency in Atlantic samples (n=150). These results supports data that Atlantic population is genetically distinct.

Yellowfin and bigeye tuna samples were collected from the South Pacific, Indian Ocean, and South Atlantic Ocean. Atlantic bluefin were sampled from the Mediterranean as well.

An analysis of hybridization among various species of tuna was conducted. Each species of tuna was shown to have characteristic nucleotide sequences within the mtDNA d-loop region. Mitochondrial DNA sequences of two sub-species of Northern Bluefin tuna were as different from each other as they were from those of other tuna species. Pacific northern bluefin tuna mtDNA was most closely related to that of the albacore. The species status of Atlantic and Pacific northern bluefin tunas should be reconsidered from the findings of the study. Both tropical and temperate tuna subgenera were found to have monophyletic origins. Bigeye tuna were intermediate between the two groups, but appeared to be more closely related to tropical tunas. Researchers observed two clades of bigeye tuna which were as different from each other as were closely related species such as yellowfin and blackfin tuna.

A study focudes on the stock structure of bigeye tuna. For this, three RFLP assays of the d-loop region of bigeye tuna mtDNA were developed based on DNA sequences of d-loop regions from four individuals. Population analyses using assays resulted in an array of ten genotypes. One of the three RFLP assays was able to correctly distinguish between the two bigeye mtDNA clades. The other two assays were subject to misinterpretation since different mutations led to the loss of the same RFLP sites. This emphasize the importance of DNA sequence analysis for the interpretation of RFLP data. The study indicated bigeye tuna from the Atlantic were genetically distinct form those found in the Pacific or Indian Oceans. No significant differences were detected between samples from the Gulf of Guinea and the North Atlantic or between samples from the Pacific and Indian Oceans

Intraspecific genetic diversity in summer flounder was examined through the implementation of a comprehensive genetic survey of summer flounder population throughout its known range (n=20-25 individuals per putative population) utilizing the amplification of the mtDNA control region (d-loop).

A separate focus of USC's FISHTEC research was the study of population genetics of rare and threatened fish populations. During the reporting period, a population genetics study of rare freshwater fish species in North and South Carolina was initiated. Preliminary data suggested previously described populations of endemic species are in fact morpholigcally cryptic taxa. Specimens of broadtail madtoms from North and South Carolina were collected for the a comprehensive survey of the phylogenetic relationships among the many described species utilizing mtDNA d-loop, 12S rRNA, and 16s rRNA loci. Phylogenetic analyses of all described forms of madtom catfishes were performed allowing for unequivocal assignment of ancestry to undescribed forms. Population level surveys of genetic diversity within undescribed forms and their ancestors using *cyt*B and ND2 mtDNA genes were also conducted.

Beyond fish species, select invertebrates were also studied for intraspecific genetic diversity. A comprehensive genetic survey of market squid (*Loligo pealei*) populations throughout the known range (Canada to Argentina) was initiated with the aim to determine genetic stocks along the Atlantic Coast. ND4 and *cyt*B successfully amplified in samples from South Carolina and New Jersey estuaries and 6PGD nuclear encoded locus primers were designed. Work was conducted in an effort to develop a simple and economical molecular assay that identifies post-larvae of commercially important penaeid shrimp species (*Penaeus setiferus, P. aztecus, P. duorarum*). An examination of both intra-and inter-specific genetic diversity utilizing mtDNA *cyt*B nucleotide sequence comparisons delineated all three commercially important species as readily distinguishable at the nucleotide sequence level. This provided diagnostic nucleotide polymorphisms to aid in species identification.

PCR-based assay of mtDNA d-loop was constructed to discriminate between northern and southern forms of striped killifish (*Fundulus majalis*). The assay was used to study the distribution of mtDNA haplotypes across the hybrid zone were the two forms of the species come in contact. The transition zone was found to be in actuality less than 3 miles long although allozyme markers indicated a transition zone of at least 18 miles. Following suit, researchers developed a PCR-based assay to discriminate between mtDNA of Florida and Northern subspecies of largemouth bass as well as in American alligators. Results indicated alligators have a unique gene arrangement that is different from that found in either birds or other vertebrates.

1992-1995

Marine Resources Research Institute, SCDNR; PIs: Robert W. Chapman and George R. Sedberry

MRRI researchers determined the genetic structure of red drum (*Sciaenops ocellatus*) along the Atlantic Coast and in the Gulf of Mexico using available allozyme markers and mtDNA markers which could distinguish between Gulf and Atlantic populations. This research showed a need to develop other mtDNA and microsatellite markers to distinguish among Atlantic populations. Surveys of the **ACE Basin** (Ashepoo, Combahee, and Edisto Rivers), Charleston area (Ashley and Wando Rivers), and Cape Romain showed the areas receive the same recruits each year differing significantly from year to year. Successful spawners constitute only a small fraction of the adult population. Data suggests management of the species should include provisions to limit harvests of the adults in order to protect the relatively few individuals that contribute to a year class. These regulations should not be enforced simply to restore a depleted resource, but should be maintained in the long term to prevent future declines.

Genetic analysis of spotted sea trout was aimed at testing the hypothesis of limited migration by this species results in genetically differentiated populations in major estuaries. Available molecular markers lacked sufficient power to resolve the problem and microsatellite markers for sea trout were under development. Analysis of fish samples from Chesapeake Bay, ACE basin, SC, Charleston area, SC, Cape Romain, SC,

Brunswick, GA, Indian River, FL, and Choctawhatchee Bay, FL suggested gene flow may be sufficient among adjacent estuaries as to preclude differentiation and identified containment at zoogeographic boundaries (Cape Hatteras, Cape Canaveral, Appalachicola Bay, FL). Data recommends management policies should be coordinated among states within the same zoogeographic province (e.g. Georgia, South Carolina, southern North Carolina) and stocks on opposite sides of the zoogeographic barriers should be managed as separate entities.

Research also focused on examining the population structure of weakfish using genetic analysis and testing the hypothesis of the existence of a single stock along the Atlantic coast.

Five hundred and twenty-five samples were collected from 15 different sciaenid species beginning in June 1995 in both inshore and offshore collection efforts. Isolated genomic DNA was amplified in the 16s **rRNA** regions of **mtDNA**. The amplifications were screened with 23 different restriction endonucleases with 4-base recognition sequences (*Alu* I, *Aci* I, *aTaq* I, *Bfa* I, *Bsa* I, *Bsa*A I, *BsoF* I, *BstU* I, *BstY* I, *Dde* I, *Dpn* I, *Hae*1i1, *Hinc* II, *Hinf* I, *HinP* I, *Hpa* II, *Mse* I, *Nla* 1i1, *Rsa* I, *Sau*96 I, *Scr*F I), The results indicated *Mse* I distinguishes all species that spawn inshore at the same time of year. Those not distinguishable by *Mse* I can be differentiated using *Dde* I. Eggs were collected in the field, amplified, digested with *Mse* I and visualized to identify species.

Another key focus of this research period was the development of techniques that would permit rapid unambiguous identification of shark species based on various tissues (both fresh and degraded). Primers were developed which target a 1400 bp region of the mtDNA genome spanning 12s and 16s rRNA regions. Amplification products were digested with *Alu* I, *Bfa*I, *Dde* I, *Dpni* I, *Hae* IiI, *Hinc*i I *Hin*P II, *Mse* I, *Msp* I, *Nla* IiI, *Rsa* I, *Scr*F I. *Msp* I and *Nla* IiI showed little variation within profiles while *Mse*I and *Alu* I produced complex patterns (due to many restriction sites). *Bfa*I, *Dde* I, *Dpni* I, *Hae* IiI, *Hin*P II, *Rsa* I were the most informative enzymes providing unambiguous identification based on a single digest. The region amplified showed significant interspecific variation, but no intraspecific variation. RFLP patterns were found to sometimes be difficult to interpret due to co-migration of fragments of approximately the same length but derived from different recognition sites which might appear as a "doublet" that can vary in its resolution from gel to gel. Length polymorphisms of homologous bands produced by insertions and deletions of nucleotides also appeared as bands migrating different distances in a gel but do not represent actual restriction site differences. Small restriction length fragments which would either migrate off the gel or be too week (diffuse) to be detected by conventional staining methods. Due to these inconsistencies, it was decided to sequence fragments to avoid RFLP analysis issues. Two hundred bases for each of the three primers were collected among *Carcharhinus altimus,* Blacknose shark (*Carcharhinus acronotus*), Spinner shark (*Carcharhinus brevipinna*), Finetooth shark (*Carcharhinus isodon*), Blacktip shark (*Carcharhinus limbatus*), Sandbar shark (*Carcharhinus plumbeus*), White shark (*Carcharadon carcharias*), Basking shark (*Cetorhinum maximus),* Smooth dogfish (*Mustelus canis*), Florida smooth hound (*Mustelus* norris), Sand tiger (*Odontaspis taurus*), Bigeye thresher

(*Alopias superciliosus*), Blue shark (*Prionace glauca*), Atlantic sharpnose (*Rhizoprionodon terraenovae*), Scalloped hammerhead (*Sphyrna lewini*), Bonnethead (*Sphyrna tiburo*), Tiger shark (*Galeocerdo cuvieri*).

1992-1995

Marine Biomedical and Environmental Sciences; PI: Karen G. Burnett

Researchers evaluated the possibility that the status of immunological defenses in natural fish populations would be compromised in habitats with high anthropogenic stress. Some red drum populations were exposed to sufficiently high levels of bacteria against which they developed immune responses. Anti-bacterial immune responses of these feral animals could be detected by testing their sera for antibodies against indigenous bacterial isolates using an enzyme-linked immunosorbent assay (**ELISA**). There were clear seasonal and age-related changes in serum antibacterial responses which varied strikingly among natural fish populations. The percentage of animals which developed antibacterial responses was highest in population which resided in microhabitats with the lowest apparent anthropogenic stress. It was concluded immunological assays might be used to evaluate the health of natural fish stocks.

The possibility that stressors found in these microhabitats cause the kind of immunosuppression observed in the red drum study was validated by analyzing biphasic *in vitro* sensitivity of red drum peripheral blood lymphocytes (**PBL**) to inorganic and organic mercuric contaminants ($HgCl_2$ and CH_3HgCl_2). The effects of $HgCl_2$ on several critical components of the signal transduction pathway in fish PBL was profiled in order to explain the biphasic response to mercury. Components included calcium flux, intracellular calcium levels, and tryosine phosphorylation. Researchers identified multiple functional isoforms of protein kinase C (**PKC**) – critical component in signal transduction pathway which may be a target for the action of $HgCl_{2,\ i}$dentifying importance in mercury toxicity. It was demonstrated that low concentrations of $CdCl_2$ did not enhance red drum PBL growth. Higher doses were found to be toxic. Cadmium did not cause tyrosine phosphorylation or extensive changes in intracellular calcium. PBL were found to be 10-100 times more sensitive to the toxic effects of methylmercuric chloride than mercuric chloride

The effects of $HgCl_2$ and $CdCl_2$ were tested in low and high concentration mixtures. Results of the study indicated the two metals had additive toxicities at high concentrations and had synergistic effects at low concentrations. $HgCl_2$ caused increased extracellular calcium into red drum PBL independent of voltage-dependent calcium channels. This may represent a general breakdown of the cell membrane. It was predicted and tested the possibility that low *in vivo* doses of $HgCl_2$ could produce symptoms of autoimmunity in teleost fish. Preliminary data supported contention that low dose enhancement of fish immune system had pathological consequences which causes an increase in total serum antibody levels.

The expansion of techniques for monitoring immune function in eggs, fry, juvenile and sub-adult red drum encompassed the detection of immunoglobulin (**Ig**) in red drum eggs using monoclonal antibody, RDG048 which reacts with the heavy chain of red drum Ig. A technique was instituted to lyse individual eggs onto nitrocellulose and minimize chorion interference. Evaluated large batches of eggs by Western blot for the presence of a protein which behaved like an authentic Ig molecule showed red drum eggs did not contain sufficient Ig to assure reproducible detection.

Feral animals aged 12 to 48 months were also tested for serum anti-bacterial responses. Few young animals (12 months) displayed strong responses and animals aged 15 months or older responded well to natural bacterial exposure.

Developed procedures for assessing respiratory burst activity of anterior kidney macrophages of 50-75 mm length in the mummichog (*Fundulus heteroclitus*) were also applied to red drum specimens.

Alternative strategies in molecular immunology for assessing long-term health of natural fish populations was evaluated utilizing samples of *Sciaenops ocellatus* from low impact (Grice Cove) and high impact (Ashley River) locations with a plan to expand to North Inlet (pristine). Serum antibodies against indigenous bacteria and serum complement activity were used for the analysis

1992-1995

Medical University of South Carolina; PI: Lee Chao

A key facet of the MUSC based FISHTEC research was the establishment of DNA banks for marine vertebrates. Specimens were under collection at the National Marine Fisheries Service (**NMFS**) at Fort Johnson as well as with the South Carolina Wildlife and Marine Resources Department. Species collected included sword fish (*Xiphias gladius*), South Atlantic coastal rays, Atlantic stingray (*Sasyatis sabina*), rainbow trout (*Salmo gairdneri*), Pigmy whale (*Kogia breviceps*), bowfin (*Amia calva*), Longnose gar (*Lepisosteus osseus*), and the Atlantic sharpnose shark (*Rhizotrionodon terraenovae).* Beyond tissue samples, a repository of DNA libraries, probes and primers was also established. DNA and RNA extractions to construct cDNA and genomic libraries from black sea bass were chosen due to interest by NMFS laboratory and **SCWMFS** laboratory for stock identification studies. Several primers were included in the library for stock identification. Genomic libraries for little skate (*Raja erin*), sea lamprey (*Petromyzon marinus*), snapping turtle (*Chelydra serpentina*), Hagfish (*Eptatretus stoutii*), horned shark (*Heterodontus francisci*), caiman (*Caimen crocodylus*), catfish (*Ictalurus punctatus*), and carp (*Cyprinus carpio*) were also developed.

Two oligonucleotides for Dr. Bert Ely were designed based on homeobox consensus sequences for stock identification. Seven oligonucleotides were made for Dr. Robert Chapman as sequencing primer for stock identification.

CA repeat for stock identification
5'-CAC ACA CAC ACA CAC ACA CA-3'
FISH 22 sequencing primer for Dr. Cheryl Woodley
5'-GTT TTC CCA GTC ACG AC-3'
FISH 23 sequencing primer for Dr. Cheryl Woodley
5'-AGC GGA TAA CAA TTT CAC ACA GGA-3'
FISH 24 stock identification primer for Dr. Bert Ely
5'-ACT GCA GGA TCC TAC CAG AC(C/G) (C/T)TG CA(A/G) CTG GAG AA(A/G) GA(A/G) TT(C/T)-3'
FISH 25 stock identification primer for Dr. Bert Ely
5'-AGA ATT CAA GCT T(C/T)T TCC A(C/T)_ TCA T(C/G)C (C/T)NC G(A/G)T TCT G(A/G)A ACC AGA T-3'
Stock identification primer L6462
5'-TAT TTG GTG CCT GAG CCG-3'
Stock identification primer L4920
5'-AAG CTT TCG GGC CCA TAC-3'
Stock identification primer L3330
5'-AAT CCA GGT CAG TTT CTA TC-3'
Stock identification primer L10700
5'-TCC CTA TTC TGC TCA TTC TA-3'
Stock identification primer H6440
5'-GGC TCA GGC ACC AAA TAC AA-3'
TC repeats for microsatellite screening (TC-1)
5'-TCT CTC TCT CTC TCT CTC TCT CTC-3'
L15998 Pro
5'-TAC CCC AAA CTC CCA AAG CTA-3'
CRCSBD-H
5'-TGA AAT TAG GAA CCA GAT GCC AG-3'
LCR456
5'-TCC ATT ACC CA(A/C) CAT GCC-3'
GT repeat
5'-GTG TGT GTG TGT GTG TGT GTG TGT GTG TGT-3'

Collected sword fish tissue samples for mtDNA analysis with aid from Regional Marine Fisheries Science Center (Miami, FL) were analyzed employing random DNA primers (RAPD) of 10 bases to generate fragments from genomic DNA based on PCR. This technique was proven to be effective in genome mapping and stock identification and preliminary results indicated careful selecting and testing of random primers are potentially useful in stock identification. A total of 20 primers were tested for their specificity and usefulness. Some were not specific or reproducible (due to ability to bind to many site under marginal conditions) and some primers appeared to be highly specific especially in the identification of gar, black seabass, swordfish and Atlantic sturgeon. The use of microsatellite genetic markers was found beneficial to complement RAPD analysis. Sequences were unstable due to their ability to contract and expand rapidly from generation to generation. The sequences were determined to be useful markers in

genome mapping and stock identification and most commonly employs (CA) or (TC) repeats which were ubiquitous among samples.

Beyond stock analysis, genes involved in inflammation and disease were also studied. Efforts to identify and eventually clone fish genes encoding Kallikrein, kininogen, alpha-1-antitrypsin provided primers developed from consensus sequences from amplified confirmed Kallikrein regions (by Southern hybridization). A variety of tissues from several lower vertebrates (including fish) were screened for immunoreactivity toward HUK antibody. The muscles of black sea bass (*Centropristis striata*) and a close relative (rock sea bass) were most immunoreceptive.

1995-1998

University of South Carolina; PIs: Bert Ely, Joseph M. Quattro, Jaime R. Alvarado Bremer

It was demonstrated populations of Mediterranean swordfish are genetically distinct from Atlantic swordfish populations. Therefore, fishing practices in the Mediterranean do not have a direct effect on the Atlantic fishery. Analysis used a 330 base pair mtDNA control region I used for analysis.

Primers (Alavarado Bremer, 1994)
L-Strand : L15998-PRO
(5'-TAC CCC AAA CTC CCA AAG CTA -3')
H-Strand: CSBDH
(5'-TGA ATT AGG AAC CAG ATG CCA G-3')

Two hundred and nineteen swordfish individuals were included in the study from commercial fishing operations from two regions adjacent to the Strait of Gibraltar. One hundred and thirty individuals were collected from the Mediterranean side [Alboran Sea (n=74); Gulf of Valencia (n=56)] and 89 individuals from the Atlantic side [Iberia10-9192 (n=16); Iberia10-9596 (n=20); Iberia15-9192 (n=35); Iberia20-9596 (n=18)]. Researchers identified 117 haplotypes within the 219 individuals sampled. The distribution of haplotypes was not homogeneous with some occurring only once in most instances with no more than twice within any Atlantic samples. Fifty percent mtDNA types could be identified to one of three haplotypes within the Mediterranean samples. (Clade I haplotypes 21, 26 and Clade II haplotype 20 - clades identified in Alvarado Bremer, 1994) Haplotype 26 was most common among all samples showing 27-35% frequency within the Mediterranean samples and 3.4% frequency within Atlantic which were found in samples taken adjacent to Strait of Gibraltar. Clade II was divided into two lineages (bootstrap >60%) and no significant differences were found in haplotype frequency among samples within each region as determined by Monte Carlo randomization (Roff and Bentzen, 1989). All binary comparisons between Atlantic samples and Mediterranean Sea samples were found to be significant after Bonferroni correction ($P<0.05$) which was never seen in large pelagic fish population studies before and is exceptionally surprising considering the geographic proximity of the populations.

Fish belonging to either Clade I or II were found to be more closely related within region than among region as determined by hierarchical analysis of nucleotide diversity. Tests also showed genetic diversity to be greater in Atlantic samples. Clade II was separated into two monophyletic groups, theta-med and theta-atl. Theta-med comprised 30% of Mediterranean fish and 6 fish with this haplotype were found within the Iberian samples indicating some movement. Theta-atl comprised 17% of Atlantic samples and 0% of Mediterranean samples. F_{st} values indicated by number of migrants per generation had genetic exchange rates of 9.9 for Mediterranean and IBE10 and 5.5 for Mediterranean and IBE15&20 regions. This finding assumes shared haplotypes result from genetic exchange and the data represents very low total genetic exchange.

It was also demonstrated that swordfish in the Northwest Atlantic are genetically distinct form those in the South Atlantic. Thus, it is appropriate that these two stocks continue to be managed separately. A 330 base pairs (bp) region of the mtDNA control region was amplified in 397 individuals (Northwest Atlantic: Georges Banks (n=45), Caribbean (n=16); South Atlantic: Gulf of Guinea (n=83), Brazil-Uruguay (n=95); Indian Ocean : West of Madagascar (n=48); Mediterranean: Ionian Sea (n=81); North Pacific: Hawaii (n=29) A hierarchical analysis of nucleotide diversity was used to analyze data (g_{st}) which indicated a high haplotypic diversity. Haplotypes differed from each other by one mutational event in most instances and showed no geographic association to this co-ancestry. Three hundred and twenty distinct haplotypes were sequenced for 397 individuals with most occurring at very low frequencies with the exception of the Ionian Sea. Clade I nucleotide diversity for all populations was 0.0179-0.0320 and the lowest value was found in the Ionian Sea. All other sample areas had nucleotide diversity greater than 0.0246 which was in agreement with Bremer *et. al.* (SCRS/98/128) which found Mediterranean samples had lower diversity values than all other locations.

The South Atlantic clade II could not be differentiated from the other Atlantic clade II lineages (P=0.225). Georges Bank and Caribbean clade II members were not significantly different (P=0.600) from each other, but were individually different than the Ionian samples (P<0.0001). The Indian and Pacific clade I samples were significantly different than Atlantic (P<0.0001) but there was no differentiation detected between Indian and Pacific samples. The spawning ground of Brazil-Uruguay and feeding ground of Gulf of Guinea (both South Atlantic) were more closely related to each other than to any other samples from entire global study. The same was found within North Atlantic feeding (Georges Bank) and spawning (Caribbean) grounds. Ionian and Northwest Atlantic population samples were very distinctly unassociated (P<0.0001).

The data further supports the hypothesis that each of the five regions examined breed independently with limited exchange between Atlantic and Indian Ocean/Mediterranean populations. As mtDNA data might be biased due to possible swordfish segregation latitudinally by sex in many areas and other female biases, it becomes more difficult to conduct reliable mixed stock analyses (MSA). Another assay was thus used incorporating a region containing a (GT) microsatellite. Several alleles were present and varied in repeat length.

Another key species in genetic analyses was the big eye tuna. It was hypothesized bigeye tuna from the Atlantic are genetically distinct from those found in the Pacific or Indian Oceans. However, no significant differences were detected between samples from the Gulf of Guinea and the North Atlantic or between samples from the Pacific and Indian Oceans using 348 base pairs (**bp**) of mtDNA control region to examine 248 specimens. A minimum of two genetically distinct population of bigeye tuna (4.9+/- 0.1% divergence) were shown to exist which demonstrated the importance of DNA sequence analysis to support the interpretation of RFLP data. 73% of Atlantic individuals were clade II classified and 90% of Indo-Pacific individuals were clade I classified. Both 1995 and 1996 samplings yielded similar results. The null hypothesis of a single panmictic unit for all oceans was rejected as chi-square analysis with Bonferroni corrections for multiple tests indicated all samples from the Atlantic were significantly different from those in the Indo-Pacific. Heterogeneity within oceans was to be addressed at a later time as sampling was limited in locales within basins.

A separate study demonstrated that little tunny from Bermuda and the Ivory Coast were genetically quite different indicating a lack of any trans-Atlantic gene flow. A 380 base pair segment (primers L15998 and CSBDH) of the mtDNA control regions I and II were used to characterize 27 individuals, 14 from Bermuda and 13 from Abidjan, Ivory Coast. Fifty-one sites were found to be polymorphic. Little tunny sequences were determined to be monophyletic (500 replicate bootstrap values of 100%) after comparison with bullet mackerel (*Auxis rochei*), frigate mackerel (*Auxis thazard*), skipjack tuna (*Katsuwonus pelamis*), Atlantic bonitos (*Sarda sarda*), and Pacific bonitos (*Sarda chilensis*). The Bermuda and Ivory Coast populations were found to be reciprocally monophyletic with 21 fixed differences and an average of 19.8 nucleotide substitutions. The net number of nucleotide substitutions per site between populations was 0.086+/-0.011 (SD). Both nucleotide differences (k) and nucleotide diversity (π) were higher in Bermuda ($k=8.37$, $\pi = 0.024$) than in the Ivory Coast ($k = 3.45$, $\pi = 0.010$). The data strongly suggested there is no trans-Atlantic mtDNA gene flow.

Studies also provided preliminary evidence for the existence of more than one population of Atlantic northern bluefin tuna.

Researchers developed several nuclear markers that are useful for the genetic analysis of swordfish, tunas and other bony fishes utilizing intron variation in aldolase C (aldC) and lactate dehydrogenase A (ldhA) for nuclear markers to confirm mtDNA findings concerning geographic partitioning. Nuclear markers eliminate possibility for female demographic biases in genetic analyses. Intron 6 of ldhA from universal primers were developed by Quattro and Jones. Mitochondrial DNA analyses yielded high degree of nucleotide diversity and haplotype heterogeneity within the global swordfish population through examination of northwest Atlantic Ocean, Mediterranean Sea and north Pacific Ocean regions. Comparisons across time (years for Mediterranean Sea and Pacific Ocean, months for Atlantic Ocean) showed regional marker stability which allowed for temporal samples to be pooled for regional sampling. Through all three sampling locales, both loci showed high degrees of heterogeneity ($P<0.01$) as demonstrated through pairwise comparisons to test for genetic homogeneity. The *ald*C genetic variation was

detected through five size variants in a (CA) repeat and *ldh*A intron 6 showed four polymorphic sites giving six total alleles. All pairwise comparisons between sampling locations were highly significant ($P<0.01$) and the results were concordant with mtDNA data. Some primers were designed for intron sequences within triosephosphate isomerase-A gene in use with *Fundulus* and *Paralichthys* and others for the amplification of swordfish catalase and mannose phosphate isomerase genes.

The notion that striped bass lack genetic variation was disproved though primers which previously showed Santee-Cooper striped bass to have two to ten alleles at GT repeat loci. Five alleles were identified at the SB83 locus using restriction fragment length polymorphisms (RFLP). Four of the alleles were examined and found to be associated with an array of microsatellite alleles in Santee-Cooper populations. The fifth RFLP allele (A5) was associated with a single microsatellite allele. The neighboring region contained a GT microsatellite which gave the opportunity to analyze the evolution of microsatellite alleles through different DNA lineages. Each RFLP allele was an independent DNA lineage with the original microsatellite allele having had mutated to form a family of alleles differing only in the number of (GT) repeats. It was determined that the Congaree, Wateree, and Santee Rivers contained genetically distinct populations.

An assay was developed to distinguish the mitochondrial DNA of the two subspecies (Florida and Northern sub-species) of largemouth bass utilizing PCR-RFLP techniques.

Utilizing mtDNA d-loop region of 200 summer flounder individuals, an analysis of variance indicated a majority of the total genetic variation was within populations with only 2-3% of the variation existing among populations. This indicated large amounts of interpopualation gene flow with any significant variation among populations due to random fluctuations in haplotype frequencies.

A substantial amount of time was dedicated to the study of rare, threatened, and endangered Carolina endemic fishes. A considerable degree of genetic diversity was identified among endangered shortnose sturgeons captured in South Carolina. Eleven halpotypes were observed in the analyzed segments of the mtDNA control region within 50 individuals. Single-copy nuclear gene assays were developed for all shark species targeting triosephosphate isomerase (**TPI**) and muscle-type lactate dehydrogenase (**LDH**). Samples were collected and made available at the NMFS lab at Fort Johnson.

MRRI scientists sequenced the cytochrome-b (cytb) mtDNA locus of three squid genera: *Loligo* (n=30, 2 haplotypes, 3% and 97% frequency), *Illex* (n=10, 2 haplotypes, 6% and 94% frequency), and *Loliguncula* (n=12, 3 haplotypes, 30%, 30% and 40% frequencies). Particular focus was given to examining the limited genetic diversity in the long-finned squid (*Loligo pealei*) which is heavily fished from Cape Hatteras to Cape Cod. It was planed to expand the study to between year comparisons and include the short-finned squid (*Illex illecebrosus*). Two nuclear gene loci, rhodopsin and arginine kinase, were targeted in primer development.

An exciting product of this summary period was the creation of simple pedigree development using molecular markers. Individuals were genotyped for molecular marker loci and scored in quantitative traits utilizing maximum likelihood and linear estimator statistical procedures to determine the degree of relatedness between pairs of individuals (i.e. 50% related for full-sibs, 25% related for half-sibs). Combined estimates of relatedness with quantitative trait data in a mixture model was used to infer heritabilities and genetic correlations. The same approach was used to analyze summer flounder (*Paralichthys dentatus*) populations. Morphometric differences between two distinct "stocks" were noted near Cape Hatteras, NC which could be environmentally determined instead of genetically based as there was no genetic evidence for separation of populations. This finding is appropriate for wild fish populations as it does not require any controlled breeding.

1995-1998

SCDNR-MRRI; PI: Robert Chapman and George Sedberry

The characterization of the population structures of several species including red drum, spotted sea trout, weakfish, dolphin, wreckfish, gag, Nassau grouper, black sea bass, white grunt and white shrimp were studied.

Fifteen hundred samples of red drum were analyzed utilizing the *Soc*029 microsatellite locus and 2526 samples were analyzed using *Cne612* microsatellite locus. Five alleles were identified among those samples and nine other individuals were amplified at the *Soc*013 and *Soc017* loci. The CNE-611 locus was also amplified and shown to yield five alleles. Alleles 122, 127 and 129 were fairly common while 132 and 136 were rarer.

Forty-eight individuals collected in South Carolina rivers by MRRI Mariculture Section were successfully DNA extracted and 62 samples were collected from the Broad River and other locations in South Carolina using glass bead protocol. DNA was isolated from 244 samples collected from the Colleton River and various South Carolina estuaries and 23 of the Broad River samples. Several loci were used in analysis including *Soc*029 (380 samples, 218 samples scored, 25 alleles identified), *Soc*014 (571 samples, 305 samples scored, 6 alleles identified), *Cne*612 (319 samples, 264 samples scored, 27 alleles identified), *Soc*012 (40 samples, 40 samples scored, 1 allele identified) and *Soc*017 (40 samples, 40 samples scored, 1 allele identified). 16srRNA was the only region of mtDNA that consistently amplified in red drum individuals and yielded polymorphisms. The Roff-Bentzen Chi-Square analysis (Chi-square = 15.79) did not find evidence of spatial heterogeneity among collecting locations (Ace Basin, Banard Cove, Cape Romain, Charleston Harbor, Grice Cove, Lower Wando, Upper Wando, Wando River). The data indicated significant spatial heterogeneity among sampling locations in contrast with the mtDNA data. This may be an indicator of the resolving power of the two approaches as mtDNA haplotypes were dominated by the A profile (~80%). The frequency distribution may not have sufficient statistical power to resolve the differences that exist which may indicate sexual asymmetry in migratory or reproductive patterns thus reflecting a real biological phenomenon. Temporal variation was also shown to be

quite high indicated by a substantial change in the spawning stock from year to year as recruitment may be dependent upon a relatively small portion.

Four alleles were identified in twenty-four examined weakfish. No allele was present at frequency greater than 50%. Data indicated a mixing of northern and southern populations of weakfish in the South Atlantic Bight. South populations made little or no contribution to populations north of the Chesapeake Bay. Researchers performed an analysis of genetic variation in weakfish at the Cne612 locus on 126 samples of which twenty-two alleles were identified. Inconsistent amplifications occurred at the Soc29 locus and Soc12 locus studies suggested the locus to be monomorphic in weakfish from southeastern US. Soc14 and Soc17 primers were under optimization experiments.

Commercially important shrimp species were scored among 315 samples from Florida, South Carolina, and North Carolina (collected by SEAMAP) for Pse028 and Pse036 loci. Extensive variation was found at both loci (Pse028 = 65 alleles, Pse036=30 alleles of which one allele was present at 31% frequency). Heterozygote deficiency was observed possibly due to simultaneous collection of distinct subpopulations (Wahlund effect). Pse028 differentiated among some of the collection sites and geographic clines were apparent. Genetic sampling may lead to short-term variation among sites so the Charleston samples were amplified over a time series of samplings to look for temporal differences. No differentiations among allele frequency distributions were observed at either locus. A white shrimp genomic DNA library was created consisting of 400-1000 bp fragments and 270 samples were acquired from the Gulf of Mississippi courtesy of NMFS laboratory in Pascagoula, MS. Samples were also received from two river systems in Georgia (St. Andrews and Cumberland). Using microsatellite locus from *P. vannamei* for amplification, the amplification of the samples targeted several loci using PCR primers complementary to ligated adaptors followed by hybridization to oligonucleotides bound to small nylon filters. Several shrimp species (*Penaeus aztecus, P. duorarum, P. vannamei, P. stylirostris)* were analyzed with same suite of microsatellites (6 loci, 5 polymorphic in *P. setiferus)* used in the *P. setiferus* study.

*Pse*002
- *P. setiferus* (n=20, 1 allele)
- *P. aztecus* (n=11, 3 alleles)
- *P. duorarum* (n=10, 1 allele)
- *P. vannamei* (n=11, 5 alleles)
- *P. stylirostris* (n=21, 5 alleles)

*Pse*004
- *P. setiferus* (n=22, 21 allele)
- *P. aztecus* (n=10, 7 alleles)
- *P. duorarum* (n=8, 3 alleles)
- *P. stylirostris* (n=46, 7 alleles)

*Pse*017
- *P. setiferus* (n=6, 5 allele)

*Pse*028
- *P. setiferus* (n=90,49 alleles)

P. stylirostris (n=6, 5 alleles)

*Pse*035

P. setiferus (n=6, 6 alleles)

*Pse*036

P. setiferus (n=90, 23 alleles)
P. aztecus (n=8, 13 alleles)
P. duorarum (n=10, 4 alleles)
P. vannamei (n=10, 5 alleles)
P. stylirostris (n=48, 4 alleles)

The study further focused on *P. styliostris* to analyze differences between Hawaiian origin (n=19, from Maka Farms), Venezuelan origin (n=25, "Supershrimp"), and wild origin (n=24). Samples were contributed by Bill Cox and Chad Evangelista of Island Fresh Seafood. *Pse*004 was used to identify Hawaiian (2 distinct alleles not found in Venezuelan populations) and Venezuelan stock (shared 4 alleles with captured). *Pse*036 was also used and showed the shrimp to be monomorphic in Hawaiian stock and polymorphic in Venezuelan stock (3 alleles, one of which was found in Hawaiian samples). Four alleles were delineated in the captured shrimp (1 unique to group) which suggests Venezuelan origin but cannot be confirmed with only one locus. 100% of the captured shrimp were determined to be from Venezuela according to MSA program GIRLSEM.

Spotted sea trout were analyzed using CNE 611 and CNE 42 microsatellite loci. One thousand seven hundred and sixty-five red drum specimens analyzed with CNE612 locus and SOC029. MRRI was working to optimize SOC012 and SOC014 amplifications. The 151 individuals examined for CNE 611 indicated genetic differences (Total Chi-square = 6.51, $p<0.05$) among collecting sites (Charleston Harbor non-spawning, Charleston Harbor spawning, Cape Romain, Ace Basin, Indian River Lagoon) which supports earlier findings that spotted sea trout are residential and do not undertake significant migrations between estuaries.

A microsatellite library was constructed for the American shad.

Completed studies of species identification in sciaenids found *Mse*I and *DdeI* to provide positive identifications for all the species included in the study. MRRI abandoned the whole cell hybridization approach to egg identification due to project breadth. Eggs were unsuccessful in amplification and it was believed the genetic material in the eggs had degraded (age, preservation method, prior analysis) to a point that an 800bp mtDNA region could not be amplified.

Studies of serranid species identification continued as the 12s rRNA region of mtDNA genome was found useful in species identification amongst the common Caribbean grouper species. Primers 12sai/12sbi were used to yield a 450 bp sequence in eleven of the twelve species tested (did not amplify in *Mycteroperca interstitialis*, yellowmouth grouper). The product was digested with with *Rsa*I restriction endonuclease which showed a high degree of RFLPs. The banding patterns were monomorphic within all

species tested. Three species pairs were distinguishable using *Hae* III digestions and a nuclear genome screening revealed a 300-800 bp (GT) and (GAGT) microsatellite primers that could have been useful for population studies.

Species identification studies of sharks continued including the cloning and sequencing of ~2000 bases of mtDNA genome spanning the 12s and 16s regions in six species. Preliminary studies indicated the Carcharhinidae family is polyphyletic. The shark forensics program covered approximately 21 species of sharks. The work would allow for the identification of shark meat and cored (headed, gutted, finned) shark carcasses.

The identification and characterization of the vitellogenin receptor and another low-density lipoprotein receptor in the white perch was conducted by Dr. Craig Sullivan during his sabbatical at MRRI.

It was discovered that the white spot syndrome-like virus was present in a variety of native crustacean species and had been widely distributed in the southeastern US since 1988. This finding impacted SCDNR policy regarding aquaculture and the importation of non-native species.

*The following research summaries are organized annually as per the summary report organizations.

1998-1999

University of South Carolina; PIs: Bert Ely and Joseph Quattro

A collaboration with two Spanish groups yielded over 700 Mediterranean and Atlantic swordfish mtDNA sequences which identified a strong mixing zone where both Atlantic and Mediterranean swordfish coexisted. Mediterranean, North Atlantic and South Atlantic populations were found to be genetically distinct. Nuclear DNA markers were used to confirm mtDNA analyses. The ldhA and aldC loci showed significant allele differences between population samples and allele frequencies were stable over time in the northwest Atlantic.

The utilization of the mtDNA control region in 140 individuals was used to examine the possibility of Atlantic northern bluefin tuna existing as a panmictic unit. Individuals from the Mediterranean Sea and western Atlantic Ocean showed no differences between locations within Mediterranean or between sampling times. Both hierarchical analysis of nucleotide diversity and Chi-square analysis using Monte Carlo randomizations showed a departure from homogeneity resulting in rejection of the null hypotheses of panmixis. This was due to the distribution of haplotypes as monophyletic groups of mtDNA lineages existed in the Atlantic (0.11 frequency) and Mediterranean (0.31 frequency) samplings. Nucleotide sequence analysis of the mtDNA control regions of samples from four different year classes of the Mediterranean and two different size classes of the Atlantic determined if differences observed are stable over time.

A third primer set was designed that amplifies polymorphic locus (Aldolase B intron 'G') within actinopterygian fishes which could be ised in *Menidia* and *Fundulus* studies.

The use of microsatellite DNA analyses to examine bottleneck implications in closed colonies of *Peromyscus* had the potential to detect over-fishing. K. Scribner of Michigan State University provided eight primer pairs of which seven had been amplified in three subspecies. The USC research group was developing cDNA libraries to identify microsatellites for application in multiple paternity and population genetics studies throughout *Carcharinus* specifically targeting the sandbar (*Carcharhinus plumbeus*), bonnethead (*Sphyrna tiburo*)and blacktip (*Carcharhinus limbatus*) shark species. Eight species of shark had been sampled from both the Gulf of Mexico and Southeastern Atlantic examining the impacts of the Florida peninsula on life-histories. The primer sets proved effective in fifteen species of sharks and several species of skates.

Lake Waccamaw endemic catfish morphotypes were examined among rivers and lakes. Morphotypes were determined from sympatric species, *Noturus insignis.* Lake Waccamaw endemics were most closely related to Waccamaw River morphotypes. Cape Fear drainage river morphotypes were found to be independently derived from *N. insignis* and not closely related to either Waccamaw morphotype. The study was expanded to broadtail madtoms from the Edisto River.

The determination of Evolutionary Significant Units (**ESU**) focused on the rare, Carolina endemic *Elassoma.* The mtDNA and nDNA analyses supported reciprocally monophyletic assemblages of pygmy sunfishes inhabiting four river systems in the Carolinas. In some instances only allele frequencies varied between river systems. The threatened Carolina darters have a range from the Saluda River, SC to Roanoke River, VA. Within river populations are very small and vulnerable to extirpation as each river drainage contains reciprocally monophyletic populations as determined through both mtDNA and nDNA. These results recommend a drainage-by-drainage management approach. A specific look at the sandhills region of the Carolinas showed several endemic and putative endemic species in the region which represented deep evolutionary divergences compared to those that typify the fauna of Lake Waccamaw. These divergences were evident in chubs (*Semotilus* and *Hybopsis*) and tree frogs (*Hyla*).

An interesting find was of the threatened *Fundulus waccamensis*, thought to be endemic to Lake Waccamaw, in Phelps Lake. It was determined the Phelps Lake population was not the product of a recent introduction of the Lake Waccamaw population. The stretched phenotype indicative of *F. waccamensis* and other Waccamaw endemics evolved independently in the Phelps Lake system and was presumed to be an adaptation to the bay-lake ecosystems. Testing of multiple loci to examine complex evolutionary history included Pamlico Sound, Virginia and New Jersey sample locations. Researchers assayed *Menidia* species to determine time of colonization by threatened Lake Waccamaw endemic species of silversides, *Menidia extensa. Menidia* was foound to be significantly genetically diverged from its putative closest relative, *M. beryllina* and may have had an ancient marine phase.

Fundulus bermudae and *F. relictus* are both native freshwater ichthyofauna of Bermuda. The two species were indistinguishable over 350 bp of cytochrome b (cytb) and identical to a haplotype sampled from *F. heteroclitus* (from SE United States). One population of *F. bermudae* was highly divergent from other populations and is most likely representative of a second, more ancient colonization event. Mitochondrial DNA analysis using the quickly evolving control region indicated the five remnant populations on Bermuda were distinct genetic and evolutionary units. The relationships among the two formally described species are still paraphyletic

The examination mtDNA variation among populations of shortnose sturgeon (*Acipenser brevirostrum*) aimed to estimate gene flow among river systems. It provided valuable management information for this federally endangered species. Sequencing of the mtDNA control region in over 200 individuals representing four rivers in South Carolina was coupled with samples from Altahama River, Ogeechee River, Georgia, and the St. John River in Canada. Two mtDNA and three nDNA genes were used to estimate phylogenetic relationships among species of pygmy sunfishes (*Elassoma*). The gene-gene concordance approach was used to observe a significant degree of concordance among independent gene loci having very different sequence properties (e.g. transition/transversion ratios, A+T content, % variable sites, etc.). This described a sister-group relationship between two threatened Carolina endemic species, *E. boehlkei* and *E. okatie*

A repeated sequence present in multiple copies as a tandem repeat was identified in striped bass and represented about 5% of the genome. Six cloned repeat monomers from each of the four North American *Morone* species (*M. saxatilis, M. chrysops, M. americana, M. mississippiensis*) showed conservation of the mesmeric unit. Intraspecific sequence variation ranged from 3.2-5.4% suggesting most of the intraspecies variation may be due to variation among copies of the repeat. Interspecific sequence variation ranged from less than 4.6% (between *M. americana* and *M. mississippiensis*) to about 16% (between other *Morone* species pairs) indicating *M. americana* and *M. mississippiensis* were more closely related to each other than to any other pairs of *Morone* species. The *Morone* phylogeny was reconstructed using twenty-two previously described morphological characters. Congruent relationships were obtained between both sets of data and *Morone* was determine to be composed of two sets of sister taxa (*M. saxatilis/M. chrysops* and *M. americana/M. mississippiensis*). Several polymorphic loci were delineated. One loci consisted of a microsatellite locus and a variable flanking region which was uUsed to identify stock structure within the Santee-Cooper system.

Bigeye tuna from the Atlantic Ocean were found to be genetically distinct from those of the Indian or Pacific oceans. Although pronounced levels of genetic differentiation between two trans-Atlantic samples of little tunny were found, researchers were unable to detect genetic differences between samples of yellowfin tuna from the Northwest Atlantic and the Eastern Pacific when nucleotide sequences of d-loop regions were compared.

1998-1999

SCDNR-MRRI; PIs: Robert W. Chapman and George R. Sedberry
Six hundred and fifty-two croaker individuals were collected from North and South Carolina. Genetic analysis was conducted using the mtDNA region encompassing part of the 16srRNA and ND-1 region amplified from primers developed through other fish species. Primers were under redevelopment to produce more consistent amplifications.

Two hundred horseshoe crabs were collected from South Carolina (n=191) and Massachusetts (n=99).

Two hundred and forty-five Southern flounders samples were collected from South Carolina from Sept. through Dec. by Inshore Fisheries and Southeast Area Monitoring and Assessment Program (SEAMAP) programs of the South Carolina Department of Natural Resources (SCDNR). Primers were developed to target a 700 base pair segment of 16s rRNA and ND-1 regions. The amplification products were analyzed for genetic variation using restriction fragment length polymorphisms (RFLP's).

Microsatellite loci *Soc*29 (6 alleles described) and *Cne*612 (12 alleles described) were utilized in red drum population studies. Other loci analyzed included: *Soc*201 (19 alleles, observed heterozygosity = 0.496, expected heterozygosity = 0.757, *Soc*014 (22 alleles, observed heterozygosity = 0.648, expected heterozygosity = 0.802), *Soc*017 (24 alleles, observed heterozygosity = 0.789, expected heterozygosity = 0.746), *Cne*612 (8 alleles, observed heterozygosity = 0.603, expected heterozygosity = 0.601), *Soc*029 (6 alleles, observed heterozygosity = 0.583, expected heterozygosity = 0.611). Two thousand five hundred samples were collected and analyzed which proved highly informative in population structure analyses. A continued analysis of four additional loci utilizing fluorescent labeled PCR products was planned.

A microsatellite analysis of weakfish populations indicated mixing of northern and southern populations of weakfish in the South Atlantic Bight (**SAB**). Southern populations were shown to make little or no contribution to populations north of the Chesapeake Bay.

The study of white shrimp (*Penaeus setiferus*) genetics was expanded to sampling locations through the Atlantic coast and Gulf of Mexico encompassing a range including Charleston Harbor, SC and Camepeche, Mexico. Nine sets of microsatellite primers targeting tri- and tetranucleotide repeat markers were developed and labeled with fluorescent dyes for easier analysis. DNA was sequenced from 176 clones and 31 unique microsatellites were defined. Six were polymorphic and tree amplified consistently and were used in the study. These loci were scored on samples from Charleston Harbor, SC; GA; Florida/west; Mississippi River and West Delta, LA; Corpus Christi, TX; Mexico which showed little geographic or temporal differentiation among the allele frequency. Heterozygosity ranged from 0.18-0.68 which is much lower than the (GT) dinucleotide repeat loci. After the process was repeated, eluted fragments containing microsatellite repeats were cloned (70 clones). Thirty-four clones contained greater than ~100 bp inserts and most microsatellites contained $(GGA)_n$ repeats. Three clones were present 2-4 times in the library and were selected for primer designation

1999-2000

University of South Carolina; PIs: B. Ely and J.M. Quattro

Swordfish stock structure analyses continued utilizing nDNA marker analyses to reflect mtDNA analyses. Both *ldhA* and *aslC* loci indicated significant differences in allele frequencies among population samples from different ocean basins. Replicate sample analysis demonstrated allele frequencies were stable over time. Northwest Atlantic and South Atlantic swordfish samples showed distinct genetic variability.

The mtDNA control regions of samples of bluefin tuna from four different year classes from the Mediterranean and two size classes of Atlantic bluefin tuna were examined to confirm the existence of defined sub-populations. The survey indicated differences observed were actually artifacts of sample collection. AMOVA analysis suggested nearly all of the genetic variation occurred within individual samples and there was no significant differences observed between regions or among samples. Similar results were collected with the nuclear ldhA locus analysis. One Mediterranean year class appeared to be genetically distinct but this could have been a reflection of year class separations or sampling artifacts. This conclusion demonstrated the importance of analyzing samples from different year classes to avoid being mislead by a single sample. Researchers were unable to distinguish Mediterranean and North Atlantic blue fin tuna through this genetic study.

Researchers developed nuclear and mitochondrial markers to be used across all shark species for phylogenetic and population genetic analyses. These markers were planned to be used to analyze gene flow, genetic drift, sex-biased migration and selection within natural populations. The use of multiple loci (both mtDNA and nDNA) in a study increases accuracy of analyses and allows for inferences about sex-specific patterns of gene flow. This analysis can then be relied upon to delineates population structure and the genetic history of the species. Genetic markers consisted of mtDNA d-loop (haploid, non-coding region, maternally inherited, relatively high mutation rate, small effective population size) and 6th nuclear ldhA intron (diploid, non-coding region, both maternally and paternally inherited, lower mutation rates, larger effective population size). Both were successfully amplified in five orders of sharks and one order of rays. All species tested had unique sequences that could be used for forensic identification and/or establish evolutionary relationships. Within 12 species of Atlantic sharks, numerous intraspecific polymorphisms and high intraspecific genetic diversity were observed. Analysis continued in population genetic studies of barndoor skates (*Dipturus laevis*), blacknose sharks (*Carcharhinus acronotus*), and shortfin makos (*Isurus oxyrinchus*).

A focus on rare and endangered species suggested populations of Carolina darters are completely isolated from adjacent populations indicating the species should be managed on a drainage-by-drainage basis. Other data recommended shortnose sturgeon of South Carolina waters should be managed on a river-by-river basis as each of the four rivers examined contained a genetically unique population.

Researchers were unable to detect genetic differences between samples of yellowfin tuna from the Northwest Atlantic and the Eastern Pacific using d-loop mtDNA nucleotide sequences The sample size was expanded and samples from the Gulf of Guinea and Indian Ocean were added to the sample pool. Although high levels of genetic variation existed within the samples, no genetic differentiation was evident through the mtDNA d-loop region.

1999-2000

SCDNR-MRRI; PI: Robert W. Chapman and George R. Sedberry

Microsatellite primers were obtained for Southern flounder from *P. olivaceus* and *P. dentatus.*

Horseshoe crabs continued to be sampled and numbers reached 214 from South Carolina, 100 from Maryland, and 99 from Massachusetts.

652 total samples of croaker were collected from four locations: Florida/east coast (n=90), Georgia (n=89), South Carolina (n=202), North Carolina (n=271). Twenty-four samples from NC and FL were amplified with ND1 targeting mtDNA primers. Using restriction enzymes (*Dpn*II, *Msp*I, *Nla*I, *ScrF*I), only one North Carolina sample was fond to have a different haplotype. The same samples were amplified with 12S rDNA mtDNA primers and only one variant was observed once again. Twenty-four samples from South Carolina were amplified and digested and a single haplotype was observed after digestion by *Rsa*I and one other after *Srf*I restriction. This study indicated there did not appear to be enough variation to warrant population studies utilizing mitochondrial DNA markers. The development of microsatellite tools was hoped to provide some variation.

2000-2001

University of South Carolina; PIs: Bert Ely and Joseph M. Quattro

Nuclear DNA marker analyses was found to coincide with mtDNA findings. Both *ldhA* and *aldC* loci indicated significant differences in allele frequencies among population samples from different ocean basins. The analysis of replicate samples from the northwest Atlantic demonstrated allele frequencies were stable over time in this region and the Atlantic and South Atlantic were genetically distinct.

The mtDNA control region (d-loop) of 140 swordfish individuals from the Mediterranean Sea and western Atlantic Ocean were analyzed. The results suggested the rejection of the null hypothesis of panmixis. However, it was determined the results were due to sampling artifacts so there was in fact no significant difference between regions. Researchers observed a highly divergent mtDNA lineage at a low frequency (~4%) in the Mediterranean, but not in the Atlantic samples. The DNA of an additional 193 individuals was examined to confirm this finding. However, the divergent lineage was

present at a frequency of only 1.6% so the finding was rejected. There was no statistically significant differences between the Atlantic and Mediterranean year class samples utilizing *ldhA*. One Mediterranean year class appeared to be genetically distinct from all other samples and further testing was conducted.

An aldolase B intron ‘G’ primer marker was found useful in genetic studies involving darters, *Fundulus,* and *Menidia.*

Among sharks, successfully amplified target nDNA and mtDNA genes were isolated in five orders of sharks and one order of rays (skates). Decreasing genetic similarity in increasingly more inclusive taxa was found which supports the assumption that conserved primers were amplifying the target genes. All shark and skate species had unique sequences which is useful for forensic identification as well as evolutionary relationships. Intraspecific genetic surveys within 12 species of Atlantic sharks revealed numerous intraspecific polymorphisms and high intraspecific genetic diversity. The role of life-history strategies in determining relative levels of within species genetic diversity was under study.

The study of Evolutionary Significant Units (ESU) in rare, Carolina endemic *Elassoma* was completed. Mitochondrial DNA gene phylogenies supported reciprocally monophyletic assemblages of pygmy sunfishes inhabiting four river systems in the Carolinas. Nuclear DNA analysis was concordant with only allele frequencies variant among river systems in some instances. A sister-group relationship was described between two threatened Carolina endemic species, *E. boehlkei* and *E. okatie*. *U*tilizing mtDNA and nDNA markers to describe ESU in threatened Carolina darters, it was found that in most cases throughout its range from Saluda River, SC to Roanoke River, VA (with extremely small in-river populations which are vulnerable to extirpation), each river drainage harbored populations that were reciprocally monophyletic for both nuclear and mtDNA markers. Populations of Carolina darters were found to be completely isolated from adjacent populations and each species should be managed on a drainage by drainage basis as opposed to the state by state scheme.

The discovery of *Fundulus waccamensis* (threatened, thought to only inhabit Lake Waccamaw) in Phelps Lake was further examined and concluded to not be a recent introduction. The stretched phenotype evolved independently as a possible adaptation to bay-lake ecosystems.

Lake Waccammaw’s endemic threatened silverside species, *Menidia extensa,* was studied for a possible ancient life-history including a marine phase. The silverside displayed the same stretched morphology of *F. waccamensis.*

The completed sequence analysis of *Fundulus bermudae* and *F. relictus*, two native freshwater ichthyofauna species of Bermuda indicated the two species were indistinguishable over 350 base pairs of cytochrome B (cytB) and shared an identical haplotype to one found in *F. heteroclitus* (southeastern US). One population of *F. bermudae* was highly divergent from other populations (possible remnant of second,

more ancient colonization event) and each remaining *Fundulus* population was genetically distinct and should be managed in isolation.

Described mtDNA variation among shortnose sturgeon populations in South Carolina from the sequenced 200 bp control region (d-loop) segment from individuals sampled from four rivers of South Carolina revealed a population structure most conducive to management on a river-by-river basis.

Developed suite of highly variable microsatellite markers for genetic analyses of striped bass and other *Morone* species were utilized to determine the effective population size of the Congaree River spawning population. A previous study unearthed the effective population size to be 0.1% of the number of sexually mature adults.

A nucleotide sequence analysis of mtDNA control region (d-loop) skipjack tuna from South Pacific, northwestern Atlantic and South Atlantic indicated ~8% nucleotide diversity with no evidence of population subdivision. The mtDNA control region was found to be too variable to detect genetic variation. The study was continuing with *ldhA* and *cytB* analyses.

2000-2001

SCDNR-MRRI; PIs: Robert W. Chapman

Within the studies of red drum genetics, the completed genetic analysis of 5 microsatellite loci in 750 specimens (6 to 24 alleles each; observed heterozygotes = 0.4-0.8) highlighted all populations deviated from Hardy-Weinberg Equilibrium (**HWE**) at RD201 locus (likely due to null alleles). Allelic variations at the loci were characterized by imperfect repeats (amplified products ranged from 224-241 bp). All loci showed most of the variation was concentrated within sampling locations except *Cne*612 which indicated most of the variation was found between populations. It was determined the mtDNA control region (d-loop) proved to be an adequate fingerprinting tool for individuals and was used to analyze broodstock components and examine stock enhancement.

The studies of white shrimp genetics continued with the completion of collection efforts of *P. setiferus*. Samples were scored at 2 $(GT)_n$, 3 $(CCT)_n$, and 1 $(CCCT)_n$ microsatellite loci. Other loci examined included: *Pse*028 (sample number/n=1160, 76 alleles, *Pse*036 (n=1172, 32 alleles), *Pse*101 (n=1132, 19 alleles), *Pse*343 (n=1151, 10 alleles), *Pse*C48 (n=1156, 53 alleles), and *Pse*G42 (n=1138, 11 alleles). One locus was dropped due to extreme heterozygote deficiency (due to presence of null alleles). Little geographic or temporal differentiation among allele frequency distributions existed when the sites were considered separately. A slight difference was noted between Gulf and Atlantic samples when sites were grouped together.

Two hundred and forty-five summer flounder samples were collected from South Carolina. Utilizing microsatellite markers developed from *P. olivaceus* and *P. dentatus,* genetic analysis began.

Isolated DNA was extracted from all 652 samples of croaker from four locations: Florida/east coast (n=90), Georgia (n=89), South Carolina (n=202), and North Carolina (n=271).

Horseshoe crab collection continued with 413 total samples from three locations: South Carolina (n=214), Massachusetts (n=99), and Maryland (n=100).

2001-2002

MRRI- SCDNR; PI: Robert W. Chapman

Studies of southern flounder continued on the 263 samples collected from the east cost. A lack of HWE at separate locations may have reflected a population substructure, assortative breeding or null alleles at some of the loci. After Bonferroni correction, no significant differences in allele frequency distributions among any of the populations were noted. Loci examined revealed relatively high levels of polymorphism which is reflective of high genetic diversity at these non-coding and presumable non-selected loci. Coupled with the lack of genetic diversity from North Carolina to Georgia, the species may be immune to many of the genetic issues stocking can cause.

Allozyme and mtDNA variation along the Atlantic coast appeared to be limited among croaker samples. There were only slight differences between Louisiana and Atlantic coast sampled observed in mtDNA markers.

The abundant population of scup south of Cape Hatteras may indicate a nursery area. Haplotype diversity was relatively low (avg. 0.13 across five enzymes). Three to five profiles were observed at each enzyme with major the haplotypes occurring at a frequency >0.80.

2002-2003

University of South Carolina; PIs: Bert Ely and Joseph Quattro

Development of PCR-based methodologies for shark population genetics continued with the successful amplified mtDNA control region (d-loop) in 12 species of Atlantic sharks. These amplifications revealed levels of genetic diversity useful for population-level studies and determination of deep phylogenetic lineages within certain species (namely scalloped hammerheads and shortfin makos). Shortfin mako variation appeared to be due to isolation in the past followed by more recent coalescence while the scalloped hammerhead division in the Atlantic could indicate cryptic species. The search continued for single-copy nuclear polymorphic loci for use in examining non-sex-biased population genetic studies.

Researchers completed the population-level assays of blacknose, scalloped hammerhead and shortfin mako sharks. The scalloped hammerhead and shortfin mako sharks exhibited deep phylogenetic lineages which were under examination for concordance with mtDNA and nDNA analyses. Hammerhead data indicated the presence of sympatric, non-interbreeding taxa which appeared to be geographically restricted to the Southern Atlantic Coast. Sampling began at other nursery areas in NC, GA, and FL in order to locate the "new" species in FL and NC on Atlantic coast. Also, research began on developing microsatellite loci markers. Upon analyzing smoothhound and tiger shark genetics, two species of smoothhound sharks were found to be indistinguishable genetically. Adding nDNA to tiger shark mtDNA analysis indicated significant differentiation between Atlantic and Pacific populations. Microsatellite markers were under development to further resolve scalloped hammerhead populations along the east coast of the US. Designed markers for mtDNA control region (d-loop), cytochrome B (*cyt*B) and 12s rRNA were under investigation in order to resolve Atlantic stingray riverine and coastal populations.

Thirty-seven percent of all North American fish species are extinct or seriously imperiled. Five hundred of the 800 North American native fish species occur in the Southeast United States. Researchers determined several species of fishes (federally endangered *Notropis mekistocholas*, undescribed form of *Hbopsis,* darter *Etheostoma,* chub Semotilus) are found solely within the Sand Hills region of the Carolinas.

Analysis was completed of skipjack and yellowfin mtDNA d-loop/ control region sequencing and nearly every haplotype was found to be unique. There was no significant difference among samples from the world's major oceans which could be explained by either both species being relatively young and thus their effective population sizes were so large that population differentiation had not had time to occur or was prevented by low levels of mixing or the d-loop region was mutating at such a high rate that recurring mutations obscured the existing genetic differentiation. The samples were re-analyzed using mtDNA *cyt*B and nDNA *ldh*A and no difference in allele frequencies between Atlantic and Pacific were disclosed using the *ldh*A marker. The study did identify polymorphism in the *cyt*B gene of skipkjack which led to the conclusion large effective population size in skipjack tuna prevented population differentiation. Significant allele frequency differences between Atlantic and Pacific yellowfin tuna samples was consistent with reduced population differentiation due to large population sizes rather than confounding effects of homoplasy.

Through testing the effects of sample size, number of alleles, and allele frequencies on effective population size estimates of striped bass, new alleles were found in the SB83 locus in Choptank River (spawning population for Chesapeake Bay) samples. Researchers found most of the new alleles in the Roanoke, Delaware an Hudson river samples. These alleles were useable in distinguishing South Carolina striped bass populations from more northern populations although they could not distinguish populations within the northern region. It was determined that smaller populations had a greater probability of having divergent allele frequencies than larger populations. Small

populations in close proximity to each other tended to have similar allele frequencies as well.

The **BASSMAP** project was established to develop a domesticated broodstock with superior growth characteristics. FISHTEC researchers were developing microsatellite markers through gynogen progeny to be used in the analysis of genotype progeny of test crosses designed to provide rapid development of a medium density genetic map. The use of this map would be to facilitate selection of a high performing broodstock.

Research also began in the field of evolutionary ecotoxicology with the development of genetic variation assays at three loci: gamma-aminobutyric acid receptor (**GABA**; focus of pesticide resistance), aryl hydrocarbon receptor (**AHR**; implicated in differential sensitivity to xenobiotics), and androgen receptors (**AR**; steroid receptor that interacts with organochlorine pesticides, alkylphenols, and fungicides). Sequences were characterized in 50 individuals of *Menidia beryllina* for five mtDNA loci, two 'neutral' nDNA loci, and two loci putatively under selection for contaminant exposure. Construction of cDNA libraries from brain and muscle RNA began in an effort to expand toxicological endpoints to include changes in gene expression in meiobenthic copepod (*Amphiascuc tenuiremis*) and grass shrimp (*Palaemonetes pugio*). Researchers also constructed serial analysis of gene expression (**SAGE**) libraries to characterize "transcriptome."

2002-2003

MRRI-SCDNR; PI: Robert W. Chapman

Studies of southern flounder continued with the collection of 293 samples from the east coast (mostly South Carolina) during the time from 1998-2002. The data collecteed from the amplification of the NADH dehydrogenase subunit I region from mtDNA of NC samples using primers L330 (5'-AAT CCA GGT CAG TTT CTA TC-3') and H4715 (5'-TAC ATG TTT GGG GTA TGG GC-3') was not useful in population analysis but did aid in recognizing misidentification of samples in the field.

Nine hundred and forty-seven total samples of croaker were collected form the Atlantic coast of the US. They were shown to lack polymorphisms in mtDNA analyses using *Alu*I, *Hae*III and *Scr*FI restriction enzymes.

Studies of 565 scup samples collected from the Atlantic coast of US and Gulf of Mexico were analyzed using mtDNA loci which indicated a great amount of polymorphism. Identifying microsatellite loci became the next goal of the research program.

2003-2004

University of South Carolina; PIs: B. Ely and J.M. Quattro

Genetic surveys began to determine if Gulf of Mexico and Atlantic/Bay of Campeche populations of yellowedge grouper could be managed as separate populations. The oceanic origins of seven samples of swordfish were also successfully identified in a blind study.

The intergenic region between ATPase6 and COIII genes of yellowfin tuna DNA was evaluated and preliminary results indicated an allele frequency differentiation existed between Atlantic and Pacific samples. This finding was consistent with reduced population differentiations due to large population sizes rather than confounding effects of homoplasy. Utilizing **AFLP** techniques, it was determined a 3-decade long association of hybrid striped bass with the Savannah River striped bass population had not resulted in detectable introgression of white bass genes into the striped bass gene pool.

2002-2003

MRRI-SCDNR; PI: Robert W. Chapman

Collection of southern flounder ended with the addition of 99 flounder from Texas. Within croakers, 1046 total samples were collected and analyzed at mtDNA ND1 locus as well as microsatellites utilized primers MunND-R (5'-AAG GAG TTG GGG GGA GTT-3') and Mun-ND-F (5'-ACG AAA GGA CCG AAA AGA AAG-3') which yielded a ~1200 bp sequence. There was no evidence for differentiation and only two variants were observed with *Hae*III enzyme and none with either *Scr*FI or *Alu*I digestions.

Studies of scup consisted of 565 samples collected from the Atlantic US coast and Gulf of Mexico. Eight polymorphic microsatellite loci were identified of which 216 fish had been scored. *Sch*009 locus was at HWE for most of the population while *Sch*030 and *Sch*031 were determined to be out of HWE. Preliminary analysis suggested possible differences between Gulf of Mexico and the Atlantic samples as well as between populations from the Mid Atlantic Bight and South Atlantic Bight.

2004-2005

University of South Carolina; PIs: B. Ely and J.M. Quattro

The collection of 40-55 species of shark and commercially important finfish from western Atlantic and Gulf of Mexico allowed for the determination that a portion of the mtDNA control region could prove useful in discriminating closely related species of shark and snapper/grouper. The samples were also incorporated into the building of a photo ID database to accompany catch record.

It was resolved the newly discovered cryptic species of hammerhead shark used South Carolina estuaries exclusively as pupping grounds which underscores the importance of critical habitat management. Collection of 100 samples of *Sphyrna nsp.* and several *S. lewini* specimens were employed for comparison which included the noticed vertebral

count differences. The plan became to continue genetic analyses in order to define the distribution of this unique hammerhead shark which were confirm dependent upon SC waters.

Based on reports from (Bosma et al., 2001) who studied **GAD** mRNA expression in goldfish, GAD65 levels are consistently two-fold higher in males than females. Also, the GAD65/GAD67 ratio was found to be approximately 1:1 in males and 1:2 in females. This suggested GAD would serve as an excellent candidate for a sexing tool. Researchers developed oligonucleotides that amplifues GAD mRNA form *Fundulus heteroclitus* and *Menidia beryllinai* and characterized GAD67 in both species. They developed four **RT-PCR** primers:

GAD18F 5'-ACN YTN AAR AAR ATG MGN GA-3'
GAD59F 5'-GAR GTN AAR RMN AAR GGN ATG-3'
GAD137R 5'-TC RAA NGC NCC RTA NAC NGT-3'
GAD 149R 5'-C CCA NGC NCC RTC NAC RTG-3'

Utilizing microsatellite DNA markers to uncover "hidden" variation among *Palaemonetes pugio* (a sentinel species in South Carolina ecotoxicology studies), numerous clones were located with useable flanking regions for primer design. Mitochondrial DNA analysis of cytochrome B (cytB) from 200 individuals showed a deep phylogenetic "break" between Atlantic populations and those collected from the Gulf of Mexico in both *P. pugio* and *P. vulgaris.* The Gulf of Mexico samples were more diverse than Atlantic sampled possibly due to a relatively recent colonization of northern estuaries from a small southern refugium. This inferred a larger degree of ecological stability in the Gulf due to greater degree of genetic divergence in Gulf estuaries. Male *P. pugio* (collected from Leadenwah Creek, SC) were exposed in static renewal bioassays according to ASTM guidelines(2002) to several estuarine contaminants (including fipronil and endosulfan – pesticides – and metal cadmium). RNA was isolated from flash frozen samples at the conclusion of the exposure tests. From these samples, d expressed sequence tag (**EST**) and **cDNA** libraries were constructed. Preliminary screenings yielded *PmA V* (involved in virus resistance; Luo et al, 2003), *Peroxiredoxin* and *cytochrome P450* (involved in oxidative stress response), *HMG-like protein* (involved in gene expression), and *Ubiquitin* (involved in many cellular regulatory mechanisms such as protein degradation). Work continued in the direction of developing a large database of grass shrimp sequences and a molecular based assay of environmental stress to make available to resource managers.

USC researchers tested microsatellite loci and optimized conditions for mutiplex analyses in pursuit of inferring relative survival of wild and stocked red drum as well as the effect of natural genetic variation on survival. To perform this research, it was necessary to develop assays for 5 microsatellite loci (4 to 21 alleles per locus) for black sea bass. This was also used to determine if local nursery areas were important for maintaining adult populations on nearby reefs which provided information about the number of parents contributing to each year class on a particular reef and if the fish on the same reef tended to be genetically related (from local nursery area). Two mtDNA loci were analyzed for

yellowfin and skipjack tuna and it was found the degree of genetic differentiation between Atlantic and Pacific samples correlated with population size. Swordfish and bluefin tuna mtDNA analyses correlated with geologic events to develop hypotheses about the regional phylogenies of these two highly migratory fishes. Swordfish mtDNA from Atlantic samples was correlated with spawning and feeding areas demonstrating spawning site fidelity as well. The data illustrated North and South Atlantic locales contained separate breeding stocks.

Short tandem repeats (**STR**) loci were foud useful in identifying family relationships in wild fish (such as juvenile striped bass in Congaree River, SC). It was demonstrated ~30% of a relatively small 1992 year class was produced by two pairs of parents. The 1993 year class was larger and no pair of parents contributed more than 5% of the total progeny. It was found necessary to increase sample sizes to eliminate potential half-sibs and false positives in the study. The research provided evidence that pair matings of striped bass occured in the wild and males outnumbered females which suggested multiple males fertilized the eggs of each female. Data indicated the majority of the 1992 year class siblings in each of the two large families were sired by a single male.

No evidence suggested stocked red drum reproduce in the wild. STR loci and mtDNA were used to identify families and follow them through time. It was found though the 8000 collected samples red drum exhibited high mtDNA variation.

2004-2005

MRRI-SCDNR; PIs: Robert W. Chapman and Amy O. Ball

Eight microsatellite loci were optimizes for use in parentage analysis. Scientists genotyped recaptured one-year old fish (sampled from cohort fingerlings from 1999, 2000, 2001 stocking events and SCDNR experimental stock enhancement research project available broodstock) and classified fish matching parental genotypes at 6 or more loci (*Soc*014-GenBank accession number AF183146 = 14 alleles, 5.2 effective number of alleles; *Soc*017 – GenBank accession number AF183147 = 14 alleles, 6.5 effective number of alleles; *Soc*029 – GenBank accession number AF183148 = 4 alleles, 2.9 effective number of alleles; *Soc*060 – GenBank accession number AF073267 = 5 alleles, 2.4 effective number of alleles; *Soc*083 – GenBank accession number AF073269 = 14 alleles, 7.3 effective number of alleles; *Soc*129 – GenBank accession number AF073275 = 16 alleles, 10.1 effective number of alleles; *Cne*612 – unpublished= 7 alleles, 2.5 effective number of alleles) as hatchery produced or not. Wild stock were determined as failing to match at two or more loci. The loci were chosen based on levels of polymorphisms and the ease/consistency of amplification and scoring. They were multiplexed in three groups for efficiency of cost and time. The 1999 collection set consisted of fish from the Ashley River stock enhancement project (broodstock, 50 chemically marked (oxytetracycline); fingerlings sampled from each of seven groups of fish retained in the laboratory for mark validation, and recaptured one-year-old fish from three sample strata – Ashley and Wando Rivers, and Charleston Harbor. The 2000 data set was comprised of broodstock, 138 fingerlings sampled from six releases, and over 20

recaptured while the 2001-year class was broodstock, 70 fingerlings sampled from 5 release batches, and wild caught fish (30 caught outside three sample strata). Wild/cultured designation of the samples was determined by the presence or absence of an oxytetracycline mark on the otolith determined by epifluorescent microscope. It was confirmed genetically through either the exclusion method or maximum likelihood method. The likelihood of mistakenly identifying a fish as cultured with these broodstock and eight loci was on the magnitude of $\sim 1 \times 10^{-9}$ which showed genetic tags to be a non-lethal and effective method in identifying cultured fish that had been released to the wild.

In the continued studies of southern flounder, mtDNA RFLP assays indicated low levels of polymorphisms and no evidence of population structure which were valuable in identifying flounder misidentified in the field. Microsatellite loci showed high levels of polymorphism and evidence for division between Gulf of Mexico and US east coast populations. Four hundred and thirty-three samples were collected from the east coast between 1998 and 2002 (mostly from South Carolina) and 138 samples were taken from the Gulf of Mexico. Little variation was observed in mtDNA patterns among east coast US samples with only four of the 185 individuals showing variant haplotypes (h=0.147 Nei calculation). This result indicated mtDNA analysis was not informative for population studies. However, researchers did identify several clearly distinct RFLP patterns within samples which were useful in correctly identifying *P. albiguttta* which were originally misidentified as *P. lethostigma.* Lrge sequence differences were shown among three members of *Paralichthys* (computed by Tamura-Nei's D with 851 bp of ND1 mtDNA region). *P. lethostigma* from SC, NC, Georgia and Gulf of Mexico were tested with six microsatellite loci: Plet*022* (n=321, 8 alleles), Plet*038* (n=297, 6alleles), Plet*022* (n=321, 8 alleles), Plet*042* (n=320, 11 alleles), Plet*205* (n=317, 24 alleles), and Plet*022* (n=320, 18 alleles). The data collected did not indicate significant structure along the east coast but did suggest high levels of genetic diversity allowing for individual and/or family identification in any stocking or culture program.

Studies of croaker revealed little or no variation in DNA RFLP studies within samples obtained from Texas both within region and with comparison to east coast samples. Little variation was also found within the mtDNA ND1 locus (h = 0.116) thus proving phylogenetically uninformative. Six microsatellite loci showed typical high polymorphism generally found in teleosts: Mun*GT65* (number of samples/n=685, 43 alleles; consistently outside of HWE), Soc*012* (GenBank accession number AF183145, n=671, 46 alleles), Soc*014* (GenBank accession number AF183146, n=696, 22 alleles), Soc*029* (GenBank accession number AF183148, n=697, 8 alleles), Soc*050* (GenBank accession number AF073266, n=692, 20 alleles; consistently outside of HWE), and Soc*140* (GenBank accession number AF073277, n=690, 17 alleles). Little population structure was indicated within or between the Gulf of Mexico and US east coast populations. Five locations were found to be outside of Hardy-Weinberg Equilibrium (HWE) and there was little to no population structure evident throughout the sampled range of *M. undulatus.*

Samples of scup were collected from the Gulf of Maine and underwent mtDNA RFLP assays whichindicated relatively low levels of population structure. There was no indication of differentiation along the US east coast. However, a higher haplotype diversity was found in scup than that observed in croaker or flounders (h=0.366 according to Nei). Microsatellite data indicated high levels of polymorphism among the three major sampling bodies (northeastern US, southeastern US, Gulf of Mexico) utilizing three loci: *Sch*009 (sample number/n=540, 22 alleles), *Sch*030 (n=543, 49 alleles), and *Sch*031 (n=519, 42 alleles). Data suggested significant population differentiation across the range of scup which infers some separation along the east coast. It was found all significant pairwise differences either involved Rhode Island or the Gulf of Mexico.

2005-2006

University of South Carolina; PI: Bert Ely and Joseph M. Quattro

During this time period, researchers collected 100 samples of *Sphyrna nsp.*, the cryptic hammerhead species, and developed new genetic surveys for in-field identification and population surveys.

The microsatellite DNA containing library from *Palaemonetes pugio* was completed and was considered to be of high-quality as most inserts contained microsatellite repeats. Numerous clones had 'useable' flanking regions for primer design of with researchers developed four putative loci. Also developed was a 16s rRNA mtDNA locus marker which consistently amplified and was found to be as variable as cytB. Samples were further collected to cover both spatial and temporal scales.

Scientists completed the testing of microsatellite loci in red drum and optimized the mutiplex analyses conditions. Assays for 5 microsatellite loci were further developed. The number of alleles present for each locus (4-21) was determined in 50 black sea bass during the continuation of sample collections.

Genomic Techniques Employed in FISHTEC

A large variety of molecular techniques were employed during FISHTEC's existence. Longstanding and well-respected techniques such as DNA amplification by polymerase chain reaction (PCR), restriction fragment length polymorphisms (RFLP), Southern hybridization and enzyme-lined immunosorbent assays (ELISA) were used in conjuction with more novel techniques such as random amplification of polymorphic DNA PCR (RAPD-PCR), PCR-RSP, and DNA extraction from eggs. FISHTEC researchers critiqued and improved upon traditional techniques and created new uses for those forms of analysis such as with RAPD-PCR which was found to be better suited for species identification in contrast to population genetic studies.

A multitude of primers were developed to target mitochondrial and nuclear DNA at a number of loci including microsatellites and expressed sequence tags (EST). A brief list of these primers follows. Please refer to the publications list to reference all primers developed. These tools will be included in a digital reference toolbox.

PRIMERS:

The primer name is followed by nucleotide sequence in the 5'-3' direction as indicated.

Microsatellites

Stock identification (Chao)

Stock identification primer L6462

5'-TAT TTG GTG CCT GAG CCG-3'

Stock identification primer L4920

5'-AAG CTT TCG GGC CCA TAC-3'

Stock identification primer L3330

5'-AAT CCA GGT CAG TTT CTA TC-3'

Stock identification primer L10700

5'-TCC CTA TTC TGC TCA TTC TA-3'

Stock identification primer H6440

5'-GGC TCA GGC ACC AAA TAC AA-3'

Stock identification primer H4715

5'-TAC ATG TTT GGG GTA TGG GC-3'

TC repeats for microsatellite screening (TC-1)

5'-TCT CTC TCT CTC TCT CTC TCT CTC-3'

L15998 Pro

5'-TAC CCC AAA CTC CCA AAG CTA-3'

CRCSBD-H

5'-TGA AAT TAG GAA CCA GAT GCC AG-3'

LCR456

5'-TCC ATT ACC CA(A/C) CAT GCC-3'

GT repeat

5'-GTG TGT GTG TGT GTG TGT GTG TGT GTG TGT-3'

CA repeat for stock identification

5'-CAC ACA CAC ACA CAC ACA CA-3'

FISH 22 sequencing primer for Dr. Cheryl Woodley

5'-GTT TTC CCA GTC ACG AC-3'

FISH 23 sequencing primer for Dr. Cheryl Woodley

5'-AGC GGA TAA CAA TTT CAC ACA GGA-3'

FISH 24 stock identification primer for Dr. Bert Ely

5'-ACT GCA GGA TCC TAC CAG AC(C/G) (C/T)TG CA(A/G) CTG GAG AA(A/G) GA(A/G) TT(C/T)-3'

FISH 25 stock identification primer for Dr. Bert Ely

5'-AGA ATT CAA GCT T(C/T)T TCC A(C/T)_ TCA T(C/G)C (C/T)NC G(A/G)T TCT G(A/G)A ACC AGA T-3'

Sciaenid fishes (Chapman)

SOC 012f 5'-GAT CTA ACT CAC CGA CTC CAC-3'

SOC 012R 5'-CGC ATA GAG CCG TGT CAG TG-3'

SOC 014F(AF183146)5'-GTA TGT ATT AAG GGC ACA AGG TG-3'

SOC 014R 5'-GAT TGC TGC TGG ACA GAC TG-3'

SOC 017 F(AF183147)5'-CGC CCG TCT ACG TGA CAG TAT G-3'

SOC 017R 5'-ATA GCT GCG CAT CAT TCG GTT G-3'

S0C 029F(AF183148)5'-GGC AAA TAG TAC AGA AAA TTA CAT GG-3'

SOC 029R 5'-GAT TTC TCT CAG TGA CTG GCA GTT-3'

CNE4-2F 5'-GCT AAT AAA TAA ACC ATC CA-3'

CNE4-2R 5'-AGG GAG GAA AAA AGG AGA-3'

CNE612F 5'-CAA GTG CAC GGT ATG TGA TG-3'

CNE612R 5'AGG AAC CTG ACC AAT CCA AA-3'

White shrimp (Chapman)

PSE002F	5'-CTG AAA TAC AAC CAC TTT GC-3'
PSE002R	5'-CGG GAT TCG TGC TTG AGG G-3'
PSE004F	5'-GAT CAC GTG ACT CTG CAA AG-3'
PSE004R	5'-CGT TCA GAT TGT CAA CTT CGC G-3'
PSE012F	5'-GCG CGT CTA TTC CGA AAA GC-3'
PSE012R	5'-AAT CAA AGG TGT GAA GAG AAA TAC-3'
PSE017F	5'-GAT CTC GCT CAT CGC TTC AAG C-3'
PSE017R	5'-TTG TGA AAA TCG TAT TGC GCT GTC-3'
PSE028F	5'-GAT CCT TCT AGC TAA ATG GG-3'
PSE028R	5'-GAT CGA AGG TAA ACT TTA TTA TC-3'
PSE035F	5'-CAC GTG AGG GAC AAG AGC ATT G-3'
PSE035R	5'-CTT TCA TAC TCA CGC TAA CAT TTG-3'
PSE046F	5'-GAC TTT GTA TTT TCA TAA ACG CTG-3'
PSE036R	5'-CGC TAT ATT TCG CAG TAA GGC TAC-3'

Scup (*Stenotomus chrysops*)

*Sch*009F	5'-TAT TCC CCA CAA GGC TCA GA-3'
*Sch*009R	5'-GCA TAC GCA ACT TTT CAG GA-3'
*Sch*030F	5'-TCT GTG TGG TTT GGC AGA AG-3'
*Sch*030R	5'-CAG CGG TGA CAC GTT ATG AA-3'
*Sch*031F	5'-TGC AAC TCG ACT TCA TGT CA-3'
*Sch*031R	5'-ATT CAG GAG GGA GGT GGT GT-3'

mtDNA Loci

ND1	
MunND-R	5'-AAG GAG TTG GGG GGA GTT-3'
Mun-ND-F	5'-ACG AAA GGA CCG AAA AGA AAG-3'

RAPD *(Chao)*

RAPD1	5'-CTC CCG GAA G-3'
RAPD2	5'-GAC CCA TGA G-3'
RAPD3	5'-AGC CGC AAG C-3'
RAPD4	5'-GCG AGT TGC C-3'
RAPD5	5'-TCC GAG CGA C-3'
RAPD6(ap3)	5'-TCG TAG CCA A-3'
RAPD7	5'-CGC GAT CCA A-3'
RAPD8	5'-AGT CGG CGT C-3'
RAPD9	5'-TCG CGA GTT G-3'
RAPD10	5'-TGC TTG GAT C-3'
RAPD11	5'-GAT GCC CAC G-3'
RAPD12	5'-CCA GTG GTC C-3'
RAPD13	5'-CCG TGC ACT A-3'
RAPD14	5'-GGA CTC GCA C-3'
RAPD15	5'-TCC TCG AGA G-3'
RAPD16	5'-CCG CTG AGC A-3'
RAPD17	5'-CTA CGT GGC G-3'
RAPD18	5'-GGC AGT CGT C-3'
RAPD19	5'-CCA GTA CGC C-3'
RAPD20	5'-CGC GTC GTC G-3'
RAPD21	5'-GAC TCG CGT G-3'
RAPD22	5'-GAT CGG CTC G-3'
RAPD23	5'-GCA CCG AGT G-3'
RAPD24	5'-GCA TGG CCC G-3'
RAPD25	5'-TGC GAG CCA G-3'
RAPD26	5'-ACG CGA TTG T-3'

RAPD27	5'-GGA TGC CAG T-3'
RAPD28	5'-CCT ATC GGC A-3'
RAPD29	5'-GGC TAG TCG A-3'
RAPD30	5'-CGC CAG TGT C-3'
RAPD31	5'-ATC ACG TGC C-3'
RAPD32	5'-AAT CTG GCC G-3'
RAPD33	5'-AGG CCA CTT G-3'
RAPD34	5'-ACG TCC TAC G-3'
RAPD35	5'-CGC AGT TCC A-3'
RAPD36	5'-GGC TCA GCA T-3'

RT-PCR

Quattro, *Fundulus heteroclitus* and *Menidia beryllina* for GAD65, GAD67, GAD3

GAD18F	5'-ACN YTN AAR AAR ATG MGN GA-3'
GAD59F	5'-GAR GTN AAR RMN AAR GGN ATG-3'
GAD137R	5'-TC RAA NGC NCC RTA NAC NGT-3'
GAD 149R	5'-C CCA NGC NCC RTC NAC RTG-3'

Application of Genomics to Fisheries Management Issues

A root purpose of the FISHTEC program was to employ genetic techniques in the fields of fisheries management and conservation sciences. From the very start of the program, a focus was placed on commercially important species as well as threatened populations. As the years passed, forensics work in species identification and ecotoxicology also took a forefront but the main goals remained the same. Scientists at participating institutions specifically targeted the development of new and better molecular techniques and tools in species identification and population delineation.

Immense libraries of PCR primers and DNA samples for a multitude of genera and species were constructed for use by both research and management organizations. The information collected has unending potential in species identification from both fresh and preserved specimens which can aid in catch monitoring programs, illegal trade surveillance (esp. in shark species), and future research. The primers and techniques perfected under FISHTEC ensure more reliable and affordable identification of fish stocks which promotes the use of molecular techniques in governmental management bodies.

FISHTEC population studies had huge implications in management strategies. Studies on swordfish indicated two distinct populations, one of the Atlantic Ocean and one of the Mediterranean Sea. This result overturned previous policy and suggested each population should be managed separately as they do not intermingle at a high enough rate to consider them as one manageable unit. Other genetic studies found population distinctions among panoceanic populations of species such as bigeye tuna, as well as others including endemic darters, chubs, grass shrimp, Bermuda killifish, and commercially important shrimp species. Other species which were once thought to be separate populations were found not to be genetically distinct as in the case of the bluefin tuna.

Population studies aided in the recognition of Evolutionary Significant Units (ESU), such as among the Carolina darters which are imperative in conservation work. Upon identification as an ESU, species and habitats encompassing those populations become a focus of management and scientific work in the pursuit of species protection. This work was augmented by specific research foci on endangered, threatened and rare species such as the shortnose sturgeon and several shark species. One FISHTEC study even discovered a previously undescribed cryptic hammerhead shark species which has tremendous implications in South Carolina coastal water management.

With each FISHTEC finding, scientists recommended how management details should be organized whether by river system, state, region, or oceanic body. Other studies in the field of ecotoxicology further expanded this cooperation into the chemical field and drew in pollution monitoring organizations as well. This coupling of research and fisheries management in a multitude of fields created a mutually beneficial bond between scientific institutions and governing bodies.

Species-Specific Research Projects and Findings

Throughout its existence, FISHTEC research has targeted over 80 species spanning a multitude of genera and several taxonomic families. Research projects in this section are organized by species common name and dated by project summary reporting periods. Each research benchmark is marked by the institution performing the study as well.

A list of the organisms by common name, followed by scientific name in parentheses follows:

American alligator (*Alligator mississippiensis*)

(1992-1995, USC)

Alligators have unique gene arrangement that is different from that found in either birds or other vertebrates as determined by mtDNA analysis

Atlantic sharpnose shark (*Rhizotrionodon terraenovae*)

(1993-1994, MUSC)

DNA bank

Atlantic stingray (*Sasyatis sabina*)

(1993-1994, MUSC)

DNA bank

(2002-2003, USC)

Designed mtDNA control region (d-loop), cytochrome B (cytB) and 12s rRNA markers to resolve riverine and coastal populations

Atlantic sturgeon (*Acipenser oxyrhynchus*)

(1992-1993, MUSC)

Stock identification primers

Barndoor skates (*Dipturus laevis*)

(1999-2000, USC)

Population genetic study

Basking shark (*Cetorhinum maximus*)

(1992-1995, MRRI)

Identified three primers for identification

Bermuda killifish (*Fundulus bermudae*)

(1998-1999, USC)

Analyzed with *F. relictus* with 350 bp region of cytB as ichthyofauna study of Bermuda

One population is highly divergent than other populations most likely due to ancient colonization event

mtDNA analysis indicated five remnant populations on Bermuda are distinct genetic and evolutionary units

(2000-2001, USC)

Completed sequence analysis

Indistinguishable from *F. relictus* over 250 bp of cytB

Share identical haplotype from *F. heteroclitus*

Bigeye thresher (*Alopias superciliosus*)

(1992-1995, MRRI)

Identified three primers for identification

Bigeye tuna (*Thunnus obesus*)

(1992-1995, USC)

Obtained samples from South Pacific, Indian Ocean, and South Atlantic Ocean

Found to be intermediate between tropical and temperate tuna subgenera and monophyletic origins

Observed two clades as different from each other as were closely related species (i.e. yellowfin and blackfin tuna)

Performed RFLP assays of mtDNA d-loop region

Resulted in array of 10 genotypes

One of the three RFLP assays was able to correctly distinguish between two bigeye mtDNA clades

Other two were subject to misinterpretation since different mutation led to the loss of the same RFLP sites

Emphasizes importance of DNA sequence analysis for the interpretation of RFLP data

Bigeye tuna from the Atlantic are genetically distinct from Pacific/Indian Oceans

No significant differences were detected between samples from the Gulf of

Guinea and the North Atlantic or between samples from the Pacific and Indian Oceans

(1995-1998, USC)

Demonstrated that Atlantic big eye tuna are genetically distinct from those found in the Pacific and Indian Oceans. Therefore, the harvest of Atlantic bigeye tuna should be managed as a discrete unit

348 base pairs (bp) of mtDNA control region used to examine 248 specimens

See (Bremer et al., 1998) for primers

Incorporated polymerase chain reaction (PCR) and then restriction endonuclease assay (RFLP)

Revealed two monophyletic clades with 4.9+/- 0.1% divergence

Similar results to those found within yellowfin, blackfin and longtail tuna mtDNA control region analyses

Clades were distinguishable through presence/absence of *AccI* restriction endonuclease cleavage site

73% of Atlantic individuals were clade II classified

90% of Indo-Pacific individuals were clade I classified

Both 1995 and 1996 samplings yielded similar results; time-stable

Null hypothesis of a single panmictic unit for all oceans was rejected as chi-square analysis with Bonferroni corrections for multiple tests indicated all samples from the Atlantic were significantly different from those in the Indo-Pacific

Heterogeneity within oceans is to be determined as sampling was limited in locales within basins

(1997-1998, USC)

Stock structure studies of bigeye tuna

Bigeye tuna from Atlantic are genetically distinct from those found in the Pacific or Indian Oceans

No significant differences were detected between samples from the Gulf of Guinea and the North Atlantic or between samples from the Pacific and Indian Oceans

Minimum of two genetically distinct population of bigeye tuna

Demonstrated the importance of DNA sequence analysis to support the interpretation of RFLP data

(1998-1999, USC)

Bigeye tuna from the Atlantic Ocean are genetically distinct from those of the Indian or Pacific oceans

Bignose shark (*Carcharhinus altimus*)

(1992-1993, MUSC)

Identified three primers for identification

Black sea bass (*Centropristis striata*)

(1993-1995, MUSC)

DNA and RNA extractions to construct cDNA and genomic libraries from black sea bass chosen due to interest by NMFS laboratory and SCWMFS laboratory for stock identification studies

Screened variety of tissues from several lower vertebrates (including fish) for immunoreactivity toward HUK antibody

Muscles of black sea bass (*Centropristis striata*) and a close relative rock sea bass were most immunoreceptive

(2004-2005, USC)

Developed assays for 5 microsatellite loci (4 to 21 alleles per locus) for black sea bass to determine if local nursery areas are important for maintaining adult populations on nearby reefs

Provide information about number of parents contributing to each year class on a particular reef and if fish on the same reef tent to be genetically related (from local nursery area)

(2005-2006, USC)

Developed assays for 5 microsatellite loci and evaluated number of alleles present for each locus (4-21) in 50 black sea bass

Beginning to collect samples

Blackfin tuna (*Thunnus atlanticus*)

(1992-1995, USC)

Showed similar differentiation to closely related species such as yellowfin as found within two clades of bigeye tuna

Blacknose shark (*Carcharhinus acronotus*)

(1992-1995, MRRI)

Developed three primers for species identification

(1999-2000, USC)

Examined population genetic structure

Blacktip shark (*Carcharhinus limbatus*)

(1992-1995, MRRI)

Developed three primers for species identification

Blue shark (*Prionace glauca*)

(1992-1995, MRRI)

Developed three primers for species identification

(1995-1998, USC)

Samples collected from NMFS at Fort Johnson, Charleston, SC

Bluebarred pygmy sunfish (*Elassoma okatie*)

Bluefin tuna (*Thunnus thynnus*)

(1995-1998, USC)

Provided preliminary evidence for the existence of more than one population of Atlantic northern bluefin tuna

(1998-1999, USC)

Utilized mtDNA control region in 140 individuals to examine if Atlantic northern bluefin tuna exist as a panmictic unit

Individuals from Mediterranean Sea and western Atlantic Ocean

No differences between locations within Mediterranean or between sampling times

Both hierarchical analysis of nucleotide diversity and Chi-square analysis using Monte Carlo randomizations showed a departure from homogeneity resulting in rejection of the null hypotheses of panmixis

Due to distribution of haplotypes

Monophyletic groups of mtDNA lineages in Atlantic (0.11 frequency) and Mediterranean (0.31 frequency)

Nucleotide sequence analysis of the mtDNA control regions of samples from four different year classes of the Mediterranean and two different size classes of the Atlantic

Will determine if differences observed are stable over time

(1999-2000, USC)

Analyzed mtDNA control regions of samples from four different year classes of Mediterranean bluefin tuna and two size classes of Atlantic bluefin tuna to confirm the existence of defined sub-populations

Indicated differences observed were actually artifacts of sample collection

AMOVA analysis indicated near all of the genetic variation occurred within individual samples

No significant differences observed between regions or among samples

Similar results with nuclear ldhA locus analysis

One Mediterranean year class appeared to be genetically distinct

Could reflect year class separations or a sampling artifacts

Demonstrated importance of analyzing samples from different year classes to avoid being mislead by a single sample

Cannot distinguish Mediterranean and North Atlantic blue fin tuna

(2000-2001, USC)

Analyzed mtDNA control region (d-loop) of 140 individuals from Mediterranean Sea and western Atlantic Ocean

Rejected null hypothesis of panmixis

Determined results due to sampling artifacts so there was no significant difference between regions

Observed highly divergent mtDNA lineage at a low frequency (~4%) in the Mediterranean (not in Atlantic)

Examined DNA of additional 193 individuals to confirm (only present in 1.6% so rejected findings)

No statistically significant differences were observed between the Atlantic and Mediterranean year class samples utilizing *ldhA*

One Mediterranean year class appeared to be genetically distinct from all other samples

(2004-2005, USC)

Bluefin tuna mtDNA analyses correlated with geologic events to develop hypotheses about the regional phylogenies of these two highly migratory fishes

Bonnethead (*Sphyrna tiburo*)

(1992-1995, USC)

Developed three primers for species identification

(1998-1999, USC)

Developing cDNA libraries to identify microsatellites for application in multiple paternity and population genetics studies

Bowfin (*Amia calva*)

(1993-1994, MUSC)

Established DNA bank

Broadtail madtoms (*Noturus spp*.)

(1992-1995, USC)

Collected samples from North and South Carolina

Phylogenetic analyses of all described forms of madtom catfishes were performed

Allows for unequivocal assignment of ancestry to undescribed forms

(1998-1999, USC)

Examining Edisto River population structure

Brown shrimp (*Penaeus aztecus*)

(1992-1995, USC)

Developing simple and economical molecular assay that identifies post-larvae

(1995-1998, MRRI)

Amplified using PCR primers complementary to ligated adaptors followed by hybridization to oligonucleotides bound to small nylon filters

Analyzed *Penaeus aztecus, P. duorarum, P. vannamei, P. stylirostris* DNA with same suite of microsatellites

Caiman (*Caimen crocodylus*)

(1993-1994, MUSC)

Developed genomic libraries

Cape Fear shiner (*Notropis mekistocholas*)

(2002-2003, USC)

Determined found solely in Sand Hills region

Carolina darter (*Etheostoma collis*)

(1995-1998, USC)

Developing oligonucleotides to target single copy intron in aldolase-B locus

Paired with LDH-A to estimate relationships among populations of threatened darter species (*Etheostoma collis*)

(1998-1999, USC)

Range: Saluda River, SC to Roanoke River, VA

Within river populations are very small and vulnerable to extirpation

Each river drainage contains reciprocally monophyletic populations as determined through both mtDNA and nDNA

Recommend drainage-by-drainage management approach

(1999-2000, USC)

Suggest populations of Carolina darters are completely isolated from adjacent populations

Species should be managed on a drainage-by-drainage basis

(2000-2001, USC)

Utilized mtDNA and nDNA markers to describe ESU in threatened Carolina darters

Range from Saluda River, SC to Roanoke River, VA with extremely small in-river populations (vulnerable to extirpation)

In most cases each river drainage harbors populations that are reciprocally monophyletic for both nuclear and mtDNA markers

Populations of Carolina darters are completely isolated from adjacent populations

Each species should be managed on a drainage by drainage basis as opposed to the state by state scheme

Carolina pygmy sunfish (*Elassoma boehlkei*)

Carp (*Cyprinus carpio*)

(1993-1994, MUSC)

Developed genomic library

Catfish (*Ictalurus punctatus*)

(1993-1994, MUSC)

Developed genomic library

(1998-1999, USC)

Examined Lake Waccamaw endemic catfish morphotypes among rivers and lakes

Determined all morphotypes from sympatric species, *Noturus insignis*

Lake Waccamaw endemic is most closely related to Waccamaw River morphotypes

Cape Fear drainage river morphotypes are independently derived from *N. insignis*

Not closely related to either Waccamaw morphotype

Chubs (*Semotilus* and *Hybopsis*)

(1998-1999, USC)

Looking at the sandhills region of the Carolinas

Several endemic and putative endemic species in the region represent deep evolutionary divergences compared to those that typify the fauna of Lake Waccamaw

Diverges evident in chubs (*Semotilus* and *Hybopsis*) and tree frogs (*Hyla*)

Incorporating nDNA studies to complement the mtDNA analyses

(2002-2003, USC)

Determined several species of fishes (federally endangered *Notropis mekistocholas*, undescribed form of *Hbopsis,* darter *Etheostoma,* chub Semotilus) are found solely within the Sand Hills region of the Carolinas

Copepod (*Amphiascuc tenuiremis*)

(2002-2003, USC)

Expanded toxicological endpoints to include changes in gene expression in meiobenthic copepod (*Amphiascuc tenuiremis*) and grass shrimp (*Palaemonetes pugio*)

Constructing serial analysis of gene expression (SAGE) libraries to characterize "transcriptome"

Croaker (*Micropogonias undulatus*)

(1998-1999, MRRI)

Collected samples for croaker (652 individuals from North and South Carolina)

Used mtDNA region encompassing part of the 16srRNA and ND-1 region amplified from primers developed through other fish species

Redesigning primers to be more consistent in amplification

(1999-2000, MRRI)

652 total samples were collected from four locations

Florida/east coast (n=90), Georgia (n=89), South Carolina (n=202), North Carolina (n=271)

Twenty-four samples each from NC and FL were amplified with ND1 targeting mtDNA primers

Using restriction enzymes (*Dpn*II, *Msp*I, *Nla*I, *ScrF*I), found only one North Carolina sample had a different haplotype

Amplified same samples with 12S rDNA mtDNA primers and only one variant was observed

Twenty-four samples from South Carolina were amplified and digested

Single haplotype was observed after digestion by *Rsa*I and another after *Srf*I restriction

Does not appear to be enough variation to warrant population studies

Developing microsatellite tools

(2000-2001, MRRI)

Isolated DNA from all 652 samples from four locations

Florida/east coast (n=90), Georgia (n=89), South Carolina (n=202), North Carolina (n=271)

(2001-2002, MRRI)

Allozyme and mtDNA variation along the Atlantic coast appeared to be limited

Slight differences between Louisiana and Atlantic coast observed in mtDNA markers

(2002-2003, MRRI)

947 total samples were collected form the Atlantic coast of the US

Lack of polymorphisms in mtDNA analyses using *Alu*I, *Hae*III and *Scr*FI restriction enzymes

(2003-2004, MRRI)

1046 total samples collected and analyzed at mtDNA ND1 locus as well as microsatellites

Utilized primers MunND-R (5'-AAG GAG TTG GGG GGA GTT-3') and Mun-ND-F (5'-ACG AAA GGA CCG AAA AGA AAG-3') yielding ~1200 bp sequence

No evidence for differentiation

Only two variants observed with *Hae*III enzyme and none with either *Scr*FI or *Alu*I

(2004-2005, MRRI)

Little or no variation in DNA RFLP studies of croaker obtained from Texas within region or with comparison to east coast samples

Little variation found within mtDNA ND1 locus (h = 0.116) thus proving phylogenetically uninformative

Six microsatellite loci showed typical high polymorphism generally found in teleosts

Mun*GT65* (number of samples/n=685, 43 alleles; consistently outside of HWE), Soc*012* (GenBank accession number AF183145, n=671, 46 alleles), Soc*014* (GenBank accession number AF183146, n=696, 22 alleles), Soc*029* (GenBank accession number AF183148, n=697, 8 alleles), Soc*050* (GenBank accession number AF073266, n=692, 20 alleles; consistently outside of HWE), Soc*140* (GenBank accession number AF073277, n=690, 17 alleles)

Little population structure indicated within or between Gulf of Mexico/US east coast populations

Five locations found to be outside of Hardy-Weinberg Equilibrium (HWE)

Little to no population structure throughout sampled range of *M. undulatus*

Deer mice (*Peromyscus spp*.)

(1998-1999, USC)

Use microsatellite DNA analyses to examine bottleneck implications in closed colonies of *Peromyscus*

Dolphin (*Coryphaena hippurus*)

(1995-1998, MRRI)

Characterized population structure

Finetooth shark (*Carcharhinus isodon*)

(1992-1995, MRRI)

Developed three primers for species identification

Fundulus relictus (common name to be determined)

(1998-1999, USC)

Examined *Fundulus bermudae* and *F. relictus,* both native freshwater ichthyofauna of Bermuda

Two species were indistinguishable over 350 bp of cytochrome b (cytb) and identical to a haplotype sampled from *F. heteroclitus* (from SE United States)

One population of *F. bermudae* is highly divergent from other populations

Most likely represents a second, more ancient colonization event

mtDNA analysis using the quickly evolving control region indicates the five remnant populations on Bermuda are distinct genetic and evolutionary units

The relationships among the two formally described species are still paraphyletic

(2000-2001, USC)

Completed sequence analysis of *Fundulus bermudae* and *F. relictus*, two native freshwater ichthyofauna species of Bermuda

Two species indistinguishable over 350 base pairs of cytochrome B (cytB) and share identical haplotype from *F. heteroclitus* (southeastern US)

One population of *F. bermudae* is highly divergent from other populations (remnant of second, more ancient colonization event)

Each remaining *Fundulus* population is genetically distinct and should be managed in isolation

Fundulus waccamensis (common name to be determined)

(1998-1999, USC)

Threatened *Fundulus waccamensis*, thought to be endemic to Lake Waccamaw found in Phelps Lake

Determined Phelps Lake population not to be recent introduction of Lake Waccamaw population

Stretched phenotype indicative of *F. waccamensis* and other Waccamaw endemics evolved independently in the Phelps Lake system

Presumable to be adaptation to bay-lake ecosystems

Testing multiple loci to examine complex evolutionary history and including Pamlico Sound, Virginia and New Jersey sample locations

(2000-2001, USC)

Examined discovery of *Fundulus waccamensis* (threatened, thought to only inhabit Lake Waccamaw) in Phelps Lake

Not a recent introduction

Stretched phenotype evolved independently (possible adaptation to bay-lake ecosystems)

Gag (*Mycteroperca microlepis*)

(1995-1998, MRRI)

Characterized population structure

Grass shrimp (*Palaemonetes pugio*)

(2002-2003, USC)

Expanded toxicological endpoints to include changes in gene expression in meiobenthic copepod (*Amphiascuc tenuiremis*) and grass shrimp (*Palaemonetes pugio*)

Constructing serial analysis of gene expression (SAGE) libraries to characterize "transcriptome"

(2004-2005, USC)

Utilizing microsatellite DNA markers to uncover "hidden" variation among *Palaemonetes pugio* which is a sentinel species in South Carolina ecotoxicology Studies

Constructed microsatellite DNA containing library from *P. pugio*

Identified numerous clones with useable flanking regions for primer design

Developed microsatellite assays with four putative loci

Further screening the library and developed appropriate assays

mtDNA analysis of cytochrome B (cytB) from 200 individuals shows deep phylogenetic "break" between Atlantic populations and those collected from Gulf of Mexico in both *P. pugio* and *P. vulgaris*

Gulf of Mexico samples more diverse than Atlantic possibly due to relatively recent colonization of northern estuaries from a small southern refugium

Inferred larger degree of ecological stability in Gulf due to greater degree of genetic divergence in Gulf estuaries

Exposed male *P. pugio* (collected from Leadenwah Creek, SC) in static renewal bioassays according to ASTM guidelines(2002) to several estuarine contaminants (including fipronil and endosulfan – pesticides – and metal cadmium)

RNA was isolated from flash frozen samples at conclusion of exposure

Constructed expressed sequence tag (EST) and cDNA libraries

Preliminary screen yielded *PmA V* (involved in virus resistance; Luo et al, 2003), *Peroxiredoxin* and *cytochrome P450* (involved in oxidative stress response), *HMG-like protein* (involved in gene expression), *Ubiquitin* (involved in many cellular regulatory mechanisms such as protein degradation)

Working towards developing a large database of grass shrimp sequences and a molecular based assay of environmental stress to make available to resource managers

(2005-2006, USC)

Constructed microsatellite DNA containing library from *Palaemonetes pugio*

High-quality as most inserts contain microsatellite repeats

Numerous clones have 'useable' flanking regions for primer design

Developed four putative loci

Developed 16s rRNA mtDNA locus marker which consistently amplifies and is as variable as cytB

Gained samples that cover both spatial and temporal scales

Hagfish (*Eptatretus stoutii*)

(1993-1994, MUSC)

Developed genomic library

Horned shark (*Heterodontus francisci*)

(1993-1994, MUSC)

Developed genomic library

Inland silverside (*Menidia beryllina*)

(1998-1999, USC)

Developed a third primer set that amplifies polymorphic locus (Aldolase B intron 'G') within actinopterygian fishes

Assaying *Menidia* species to determine time of colonization by threatened Lake Waccamaw endemic species of silversides, *Menidia extensa* to determine

Follow same stretched morphology as other Lake Waccamaw endemics

Menidia is significantly genetically diverged from putative closest relative, *M. beryllina*

May have had ancient marine phase

(2000-2001, USC)

Aldolase B intron 'G' useful in genetic studies involving darters, *Fundulus,* and *Menidia*

(2002-2003, USC)

Characterized sequence variation in 50 individuals of *Menidia beryllina* for five mtDNA loci, two 'neutral' nDNA loci, and two loci putatively under selection for contaminant exposure

Constructing cDNA libraries from brain and muscle RNA

(2004-2005, USC)

Developed oligonucleotides that amplify GAD mRNA form *Fundulus heteroclitus* and *Menidia beryllina*

Characterized GAD67 in both species

Developed four RT-PCR primers

GAD18F 5'-ACN YTN AAR AAR ATG MGN GA-3'

GAD59F 5'-GAR GTN AAR RMN AAR GGN ATG-3'

GAD137R 5'-TC RAA NGC NCC RTA NAC NGT-3'

GAD 149R 5'-C CCA NGC NCC RTC NAC RTG-3'

Largemouth bass (*Micropterus salmoides*)

(1992-1995, USC)

Developed PCR-based assay to discriminate between mtDNA of Florida and Northern subspecies of largemouth bass

(1995-1998, USC)

PCR-RFLP assay was used to discriminate between mtDNA of Florida and Northern sub-species

Little skate (*Raja erin*)

(1992-1995, MUSC)

Developed genomic library

Little tunny (*Euthynnus alletteratus*)

(1995-1998, USC)

Demonstrated that little tunny from Bermuda and the Ivory Coast are genetically quite different. Thus, there does not appear to be any trans-Atlantic gene flow.

380 base pairs corresponding to mtDNA control regions I and II were used to characterize 27 individuals, 14 from Bermuda and 13 from Abidjan, Ivory Coast

Primers L15998 and CSBDH

51 sites were polymorphic

Little tunny sequences are monophyletic (500 replicate bootstrap values of 100%) after comparison with bullet mackerel (*Auxis rochei*), frigate mackerel (*Auxis thazard*), skipjack tuna (*Katsuwonus pelamis*), Atlantic bonitos (*Sarda sarda*), Pacific bonitos (*Sarda chilensis*)

The Bermuda and Ivory Coast populations were found to be reciprocally monophyletic with 21 fixed differences and an average of 19.8 nucleotide substitutions

Net number of nucleotide substitutions per site between populations was 0.086+/-0.011 (SD)

Both nucleotide differences (k) and nucleotide diversity (π) were higher in Bermuda ($k=8.37$, $\pi = 0.024$) than in the Ivory Coast ($k = 3.45$, $\pi = 0.010$)

Data strongly suggests there is no trans-Atlantic mtDNA gene flow

(1998-1999, USC)

Pronounced levels of genetic differentiation between two trans-Atlantic samples of little tunny

Loliguncula

(1997-1998, USC)

Sequenced the cytochrome-b (cytb) mtDNA locus of three squid genera

Loliguncula (n=12, 3 haplotypes, 30%, 30% and 40% frequencies)

Longfinned squid (*Loligo pealei*)

(1992-1995, USC)

Initiated comprehensive genetic survey of market squid (*Loligo pealei*) populations throughout the known range (Canada to Argentina)

Aim to determine genetic stocks along the Atlantic Coast

ND4 and *cyt*B successfully amplified in samples from South Carolina and New Jersey estuaries

Developing 6PGD nuclear encoded locus primers

(1997-1998, USC)

Sequenced the cytochrome-b (cytb) mtDNA locus of three squid genera

Loligo (n=30, 2 haplotypes, 3% and 97% frequency)

Particular focus will be given to examining the limited genetic diversity in the long-finned squid (*Loligo pealei*)

Heavily fished from Cape Hatteras to Cape Cod

Longnose gar (*Lepisosteus osseus*)

(1993-1994, MUSC)

Established DNA Bank

Developed primers for species identification

Margined madtom (*Noturus insignis*)

(1998-1999, USC)

Determined all morphotypes from sympatric species, *Noturus insignis*

Mummichog (*Fundulus heteroclitus*)

(1992-1995, MBES)

Developed procedures for assessing respiratory burst activity of anterior kidney macrophages of 50-75 mm length in the mummichog (*Fundulus heteroclitus*)

(1995-1998, USC)

Developing primers for intron sequences within triosephosphate isomerase-A gene

(1998-1999, USC)

Developed a third primer set that amplifies polymorphic locus (Aldolase B intron ‘G’) within actinopterygian fishes

(2004-2005, USC)

Developed oligonucleotides that amplify GAD mRNA form *Fundulus heteroclitus* and *Menidia beryllina*

Characterized GAD67 in both species

Developed four RT-PCR primers

GAD18F 5'-ACN YTN AAR AAR ATG MGN GA-3'

GAD59F 5'-GAR GTN AAR RMN AAR GGN ATG-3'

GAD137R 5'-TC RAA NGC NCC RTA NAC NGT-3'

GAD 149R 5'-C CCA NGC NCC RTC NAC RTG-3'

Nassau grouper (*Epinephelus striatus*)

(1995-1998, MRRI)

Characterization of population structure in red drum, spotted sea trout, weakfish, dolphin, wreckfish, gag, Nassau grouper, black sea bass, white grunt and white shrimp.

1500 samples of red drum were analyzed utilizing *Soc*029 microsatellite locus

2526 samples were analyzed using *Cne612* microsatellite locus

Five alleles were identified

Nine individuals were amplified at *Soc*013 and *Soc017* loci

Forty-eight individuals collected in South Carolina rivers by MRRI Mariculture Section were successfully DNA extracted

Penaeis shrimp (*Penaeus stylirostris*)

(1995-1998, MRRI)

Amplified using PCR primers complementary to ligated adaptors followed by hybridization to oligonucleotides bound to small nylon filters

Analyzed *Penaeus aztecus, P. duorarum, P. vannamei, P. stylirostris* DNA with same suite of microsatellites (6 loci, 5 polymorphic in *P. setiferus)* used in *P. setiferus* study

Pigmy whale (*Kogia breviceps*)

(1993-1994, MUSC)

Established DNA bank

Pink shrimp (*Penaeus duorarum*)

(1992-1995, USC)

Developing simple and economical molecular assay that identifies post-larvae of commercially important penaeid shrimp species (*Penaeus setiferus, P. aztecus, P. duorarum*)

Examine both intra-and inter-specific genetic diversity utilizing mtDNA nucleotide sequence comparisons using mtDNA *cyt*B

Indicate all three commercially important species are readily distinguishable at the nucleotide sequence level

Identifying diagnostic nucleotide polymorphisms to aid in species identification

(1995-1998, MRRI)

Amplified using PCR primers complementary to ligated adaptors followed by hybridization to oligonucleotides bound to small nylon filters

Analyzed *Penaeus aztecus, P. duorarum, P. vannamei, P. stylirostris* DNA with same suite of microsatellites (6 loci, 5 polymorphic in *P. setiferus)* used in *P. setiferus* study

Rainbow trout (*Salmo gairdneri*)

(1993-1994, MUSC)

Established DNA bank

Red drum (*Sciaenops ocellatus*)

(1992-1995, MRRI)

Determine the genetic structure of red drum (*Sciaenops ocellatus*) along the Atlantic Coast and in the Gulf of Mexico

Available allozyme markers and mtDNA markers could distinguish between Gulf and Atlantic populations

Needed to develop other mtDNA and microsatellite markers to distinguish among Atlantic populations

Surveyed ACE Basin (Ashepoo, Combahee, and Edisto Rivers), Charleston area (Ashley and Wando Rivers), and Cape Romain

Areas receive same recruits each year differing significantly from year to year

Successful spawners constitute only a small fraction of the adult population

Data suggests management of the species should include provisions to limit harvests of the adults in order to protect the relatively few individuals that contribute to a year class

Should not be enforced simply to restore a depleted resource, but should be maintained in the long term to prevent future declines

(1992-1995, **MBES**)

Evaluate the possibility that the status of immunological defenses in natural fish populations would be compromised in habitats with high anthropogenic stress

Some red drum populations were exposed to sufficiently high levels of bacteria that they developed immune responses against their indigenous

Anti-bacterial immune responses of these feral animals could be detected by testing their sera for antibodies against indigenous bacterial isolates using an enzyme-linked immunosorbent assay (ELISA)

There were clear seasonal and age-related changes in serum antibacterial responses

Anti-bacterial responses varied strikingly among natural fish populations

Percentage of animals which develop antibacterial responses was highest in population which reside in microhabitats with lowest apparent anthropogenic stress

Concluded immunological assays might be used to evaluate the health of natural fish stocks

Tested the possibility that stressors found in these microhabitats cause the kind of immunosuppression observed in the red drum study

Validated biphasic *in vitro* sensitivity of red drum peripheral blood lymphocytes (PBL) to inorganic and organic mercuric contaminants ($HgCl_2$ and CH_3HgCl_2)

Profiled the effects of $HgCl_2$ on several critical components of the signal transduction pathway in fish PBL in order to explain the biphasic response to mercury

Components include calcium flux, intracellular calcium levels, tryosine phosphorylation

Identified multiple functional isoforms of protein kinase C (PKC) – critical component in signal transduction pathway which may be a target for the action of $HgCl_2$

Identifying importance in mercury toxicity

Demonstrated that low concentrations of $CdCl_2$ did not enhance red drum PBL growth

Higher doses were toxic

Cadmium did not cause tyrosine phosphorylation or extensive changes in intracellular calcium

PBL 10-100 times more sensitive to the toxic effects of methylmercuric chloride than mercuric chloride (1994)

Tested the effects of $HgCl_2$ and $CdCl_2$ in low and high concentration mixtures

Two metals had additive toxicities at high concentrations

Effects synergistic at low concentrations

$HgCl_2$ causes increased extracellular calcium into red drum PBL

Independent of voltage-dependent calcium channels

May represent a general breakdown of the cell membrane

Predicted and tested the possibility that low *in vivo* doses of $HgCl_2$ could produce symptoms of autoimmunity in teleost fish

Preliminary data supports contention that low dose enhancement of fish immune system has pathological consequences

Causes increase in total serum antibody levels

Develop techniques for monitoring immune function in eggs, fry, juvenile and sub-adult red drum

Tested immunoglobulin (Ig) could be detected in red drum eggs using monoclonal antibody, RDG048

Reacts with heavy chain of red drum Ig

Developed techniques to lyse individual eggs onto nitrocellulose and minimize chorion interference

Evaluated large batches of eggs by Western blot for the presence of a protein which behaved like an authentic If molecule

Red drum eggs were found to not contain sufficient If to assure reproducible detection

Tested feral animals ages 12 to 48 months for serum anti-bacterial responses

Few young animals (12 months) displayed strong responses

Animals aged 15 months or older responded well to natural bacterial exposure

Developed procedures for assessing respiratory burst activity of anterior kidney macrophages of 50-75 mm length in the mummichog (*Fundulus heteroclitus*)

Can apply techniques to red drum

Develop a simple field-based assay for immunological health

Evaluate alternative strategies in molecular immunology for assessing long-term health of natural fish populations

Samples *Sciaenops ocellatus* from low impact (Grice Cove) and high impact (Ashley River) – plan to expand to North Inlet (pristine)

Look at serum antibodies against indigenous bacteria and serum complement activity

(1995-1998, MRRI)

Characterization of population structure in red drum, spotted sea trout, weakfish, dolphin, wreckfish, gag, Nassau grouper, black sea bass, white grunt and white shrimp.

1500 samples of red drum were analyzed utilizing *Soc*029 microsatellite locus

- 2526 samples were analyzed using *Cne612* microsatellite locus
    - Five alleles were identified
- Nine individuals were amplified at *Soc*013 and *Soc017* loci
- Forty-eight individuals collected in South Carolina rivers by MRRI Mariculture Section were successfully DNA extracted

Utilized large portion of mtDNA molecule and microsatellite locus in the nuclear genome (CNE-611) to analyze small scale population structuring

- 16srRNA was the only region of mtDNA that consistently amplified in red drum individuals and yielded polymorphisms
    - Roff-Bentzen Chi-Square analysis (Chi-square = 15.79) did not find evidence of spatial heterogeneity among collecting locations (Ace Basin, Banard Cove, Cape Romain, Charleston Harbor, Grice Cove, Lower Wando, Upper Wando, Wando River)
- CNE-611 amplified nuclear locus with at least five alleles
    - 122, 127 and 129 were fairly common while 132 and 136 were rarer
    - Data indicated significant spatial heterogeneity among sampling locations in contrast with the mtDNA data
        - May be difference in resolving power of approaches as mtDNA haplotypes were dominated by the A profile (~80%) and the frequency distribution may not have sufficient statistical power to resolve the differences that exist.
        - May indicate sexual asymmetry in migratory or reproductive patterns – reflecting a real biological phenomenon
    - Showed temporal variation to be quite high
        - Indicated a substantial change in the spawning stock from year to year or recruitment is dependent upon a relatively small portion of the available spawning stock

Hope to find differences between local groups and other populations along the Atlantic Coast

(1996-1997, MRRI)

Collected 62 samples from Broad River and other locations in South Carolina using glass bead protocol

Isolated DNA from 244 samples collected from the Coleton River and various South Carolina estuaries and 23 of the Broad River samples

Amplified 25 of Colleton River samples at CNE612 locus

Amplified 142 Colleton River samples at Soc29 locus

1765 red drum specimens analyzed with CNE612 locus and SOC029

(1998-1999, MRRI)

Determine population structure of red drum (*Sciaenops ocellatus*) utilizing microsatellite loci *Soc*29 (6 alleles described) and *Cne*612 (12 alleles described)

*Soc*201 (19 alleles, observed heterozygosity = 0.496, expected heterozygosity = 0.757, *Soc*014 (22 alleles, observed heterozygosity = 0.648, expected heterozygosity = 0.802), *Soc*017 (24 alleles, observed heterozygosity = 0.789, expected heterozygosity = 0.746), *Cne*612 (8 alleles, observed heterozygosity = 0.603, expected heterozygosity = 0.601), *Soc*029 (6 alleles, observed heterozygosity = 0.583, expected heterozygosity = 0.611)

2500 samples collected

Informative in population structure analyses

Continuing analyses of four additional loci utilizing fluorescent labeled PCR products

(2000-2001, MRRI)

Completed genetic analysis of 5 microsatellite loci in 750 specimens (6 to 24 alleles each; observed heterozygotes = 0.4-0.8)

All populations deviated from HWE at RD201 locus (likely due to null alleles)

Allelic variations at locus were characterized by imperfect repeats (amplified products ranged from 224-241 bp)

All locus showed most of the variation concentrated within sampling locations except Cne612 which indicated most of the variation was found between populations

Determined mtDNA control region (d-loop) adequate fingerprinting tool for individuals

Used to analyze broodstock and examine stock enhancement

(2004-2005, USC)

Tested microsatellite loci and optimized conditions for mutiplex analyses in pursuit of inferring relative survival of wild and stocked red drum and affect of natural genetic variation on survival

Examined impact of stocking on native red drum populations

No evidence stocked red drum reproduce

STR loci and mtDNA used to identify families and follow them through time

Have high mtDNA variation

8000 samples available (collected by SCDNR since 1995)

(2004-2005, MRRI)

Optimized eight microsatellite loci for use in parentage analysis

Genotyped recaptured one-year old fish (sampled from cohort fingerlings from 1999, 2000, 2001 stocking events and SCDNR experimental stock enhancement research project available broodstock

Classified fish matching parental genotypes at 6 or more loci (Soc014-GenBank accession number AF183146 = 14 alleles, 5.2 effective number of alleles; Soc017 – GenBank accession number AF183147 = 14 alleles, 6.5 effective number of alleles; Soc029 – GenBank accession number AF183148 = 4 alleles, 2.9 effective number of alleles; Soc060 – GenBank accession number AF073267 = 5 alleles, 2.4 effective number of alleles; Soc083 – GenBank accession number AF073269 = 14 alleles, 7.3 effective number of alleles; Soc129 – GenBank accession number AF073275 = 16 alleles, 10.1 effective number of alleles; Cne612 – unpublished= 7 alleles, 2.5 effective number of alleles) as hatchery

Wild stock determined as failing to match at two or more loci

Loci chosen based on levels of polymorphisms and ease/consistency of amplification and scoring

Multiplexed in three groups for efficiency of cost and time

1999 Ashley River stock enhancement project (broodstock, 50 chemically marked (oxytetracycline) fingerlings sampled from each of seven groups of fish retained in the laboratory for mark validation, recaptured one-year-old fish from three sample strata – Ashley and Wando Rivers, and Charleston Harbor

2000 data set is comprised of broodstock, 138 fingerlings sampled from six releases, over 20 recaptured

2001-year class is broodstock, 70 fingerlings sampled from 5 release batches, wild caught fish (30 caught outside three sample strata)

Wild/cultured designation determined by presence or absence of oxytetracycline mark on otolith determined by epifluorescent microscope

Determined genetically through exclusion method or maximum likelihood method

Likelihood of mistakenly identifying a fish as cultured with these broodstock and eight loci was $\sim 1 \times 10^{-9}$

Shows genetic tags to be non-lethal and effective in identifying cultured fish that have been released to the wild

(2005-2006, USC)

Completed testing of microsatellite loci in red drum

Optimized mutiplex analyses conditions

Sand tiger (*Odontaspis taurus*)

(1992-1995, MRRI)

Developed three primers for species identification

Sandbar shark (*Carcharhinus plumbeus*)

(1992-1995, MRRI)

Developed three primers for species identification

(1998-1999, USC)

Developing cDNA libraries to identify microsatellites for application in multiple paternity and population genetics studies throughout *Carcharinus*

Scalloped hammerhead (*Sphyrna lewini*)

(1992-1995, MRRI)

- Developed three primers for species identification

(2004-2005, USC)

- Collected 100 samples of *Sphyrna nsp.* and several *S. lewini* specimens for comparison
  - Noticed vertebral count differences in new species
- Successfully amplified mtDNA control region (d-loop) in 12 species of Atlantic sharks
  - Revealed levels of genetic diversity useful for population-level studies and deep phylogenetic lineages within certain species (namely scalloped hammerheads and shortfin makos)
  - Scalloped hammerhead division in Atlantic could indicate cryptic species

Scup (*Stentomus chrysops*)

(2001-2002, MRRI)

- Abundant populations south of Cape Hatteras may indicate nursery area
- Haplotype diversity was relatively low (avg. 0.13 across five enzymes)
  - 3-5 profiles observed at each enzyme with major haplotypes occurring at a frequency >0.80

(2002-2003, MRRI)

- Collected 565 samples from Atlantic coast of US and Gulf of Mexico
  - New Jersey (n=100), Rhode Island (n=100), Virginia (n=58), Georgia (n=92), South Carolina (n=100), Florida (n=15), Gulf of Mexico (n=100)
- mtDNA analysis indicated great amount of polymorphism
- Identifying microsatellite loci
- Successfully amplified mtDNA control region (d-loop) in 12 species of Atlantic sharks
  - Revealed levels of genetic diversity useful for population-level studies and deep phylogenetic lineages within certain species (namely scalloped hammerheads and shortfin makos)

Shortfin mako variation due to isolation in the past followed by more recent coalescence

Scalloped hammerhead division in Atlantic could indicate cryptic species

(2003-2004, MRRI)

565 samples collected from Atlantic US coast and Gulf of Mexico

Identified eight polymorphic microsatellite loci of which 216 fish have been scored and one or more

*Sch*009 is at HWE for most of population while *Sch*030 and *Sch*031 are out of HWE

Preliminary analysis suggests possible differences between Gulf of Mexico and the Atlantic, and between populations from Mid Atlantic Bight and South Atlantic Bight

(2004-2005, MRRI)

Acquiring samples from Gulf of Maine

mtDNA RFLP assays indicated relatively low levels of population structure

No indication of differentiation along the US east coast

Higher haplotype diversity than observed in croaker or flounders (*h*=0.366 according to Nei)

Microsatellite data indicate high levels of polymorphism and three major groups (northeastern US, southeastern US, Gulf of Mexico)

*Sch*009 (sample number/n=540, 22 alleles), *Sch*030 (n=543, 49 alleles), *Sch*031 (n=519, 42 alleles)

Data suggests significant population differentiation across range of scup

Suggest some separation along east coast as all significant pairwise differences either involve Rhode Island or Gulf of Mexico

Sea lamprey (*Petromyzon marinus*)

(1993-1994, MUSC)

Established genomic library

Short finned squid (*Illex illecebrosus*)

(1997-1998, USC)

Sequenced the cytochrome-b (cytb) mtDNA locus of three squid genera

- *Illex* (n=10, 2 haplotypes, 6% and 94% frequency)

Plan to expand study to between year comparisons and include the short-finned squid (*Illex illecebrosus*)

- Developed primers for two nuclear gene loci, rhodopsin and arginine kinase

Shortfin makos (*Isurus oxyrinchus*)

(1995-1998, USC)

Developing single-copy nuclear gene assays for all shark species

- Targeting triosephosphate isomerase (TPI) and muscle-type lactate dehydrogenase (LDH)

Samples from northern Atlantic available at NMFS lab at Fort Johnson

- Also using outside researchers for blue shark and shortfin mako

(1999-2000, USC)

Underwent population genetics society

(2002-2003, USC)

Successfully amplified mtDNA control region (d-loop) in 12 species of Atlantic sharks

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Shortnose sturgeon (*Acipenser brevirostrum*)

(1995-1998, USC)

- Identified considerable genetic diversity among endangered shortnose sturgeons captured in South Carolina.
  - mtDNA control region used to analyze 50 individuals
  - Eleven haplotypes were observed

(1998-1999, USC)

- Examined mtDNA variation among populations of shortnose sturgeon (*Acipenser brevirostrum*)
  - Aiming to estimate gene flow among river systems
    - Provide valuable management information for this federally endangered species
  - Sequenced mtDNA control region in over 200 individuals representing four rivers in South Carolina
    - Also have samples from Altahama River, Ogeechee River, Georgia, St. John River in Canada

(1999-2000, USC)

- Suggest populations of Carolina darters are completely isolated from adjacent populations
  - Species should be managed on a drainage-by-drainage basis
- Data recommends shortnose sturgeon of South Carolina waters be managed on a river-by-river basis as each of the four rivers examined contained a genetically unique population

(2000-2001, USC)

- Described mtDNA variation among shortnose sturgeon populations in South Carolina
  - Sequenced 200 control region (d-loop) from individuals sampled from four rivers of South Carolina
  - Management should be on river-by-river basis

Silversides (*Menidia extensa*)

(1998-1999, USC)

Assaying *Menidia* species to determine time of colonization by threatened Lake Waccamaw endemic species of silversides, *Menidia extensa* to determine

Follow same stretched morphology as other Lake Waccamaw endemics

Menidia is significantly genetically diverged from putative closest relative, *M. beryllina*

May have had ancient marine phase

(2000-2001, USC)

Examined endemic threatened silverside species of Lake Waccamaw, *Menidia extensa* for possible ancient life-history including marine phase

Same stretched morphology of *F. waccamensis*

Skipjack tuna (*Katsuwonus pelamis*)

(1995-1998, USC)

Compared for phylogenetic analysis to little tunny

(2000-2001, USC)

Performed nucleotide sequence analysis of mtDNA control region (d-loop) skipjack tuna from South Pacific, northwestern Atlantic and South Atlantic

~8% nucleotide diversity with no evidence of population subdivision

mtDNA control region too variable to detect genetic variation

continuing study with *ldhA* and *cytB*

(2002-2003, USC)

Completed analysis of skipjack and yellowfin mtDNA d-loop/ control region

Nearly every haplotype was unique and there was no significant difference among samples from the world's major oceans

Explained by either:

a. both species are relatively young and effective population sizes are so large that population differentiation has not had time to occur or is prevented by low levels of mixing

b. d-loop region is mutating at such a high rate that recurring mutations obscure the existing genetic differentiation

Re-analyzed samples using mtDNA *cyt*B and nDNA *ldh*A

No difference in allele frequencies between Atlantic and Pacific using *ldh*A marker

Identified polymorphism in *cyt*B gene of skipkjack

Concluded large effective population size in skipjack tuna prevented population differentiation

Significant allele frequency differences between our Atlantic and Pacific yellowfin tuna samples

Data consistent with reduced population differentiation due to large population sizes rather than confounding effects of homoplasy

(2004-2005, USC)

Two mtDNA loci analyzed for yellowfin and skipjack tuna

Degree of genetic differentiation between Atlantic and Pacific samples correlates with population size

Smooth dogfish (*Mustelus canis)*

(1992-1995, MUSC)

Developed three primers for species identification

Snapping turtle (*Chelydra serpentina*)

(1993-1994, MUSC)

Developed genomic library

Spinner shark (*Carcharhinus brevipinna*)

(1992-1995, MUSC)

Developed three primers for species identification

Spotted sea trout (*Cynoscion nebulosus*)

(1992-1995, MRRI)

Testing hypothesis limited migration by this species results in genetically differentiated populations in major estuaries

Available molecular markers lacked sufficient power to resolve the problem

Developed microsatellite markers for sea trout

Analyzed fish samples from Chesapeake Bay, ACE basin, SC, Charleston area, SC, Cape Romain, SC, Brunswick, GA, Indian River, FL, and Choctawhatchee Bay, FL

Data suggests gene flow may be sufficient among adjacent estuaries as to preclude differentiation

Constrained at zoogeographic boundaries (Cape Hatteras, Cape Canaveral, Appalachicola Bay, FL)

Recommend management policies should be coordinated among states within the same zoogeographic province (e.g. Georgia, South Carolina, southern North Carolina)

Stocks on opposite sides of the zoogeographic barriers should be managed as separate entities

(1995-1998, MRRI)

Characterization of population structure in red drum, spotted sea trout, weakfish, dolphin, wreckfish, gag, Nassau grouper, black sea bass, white grunt and white shrimp.

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Striped bass (*Morone saxatilis*)

(1992-1995, USC)

Development of PCR-based assays from three anonymous nuclear loci (Leclerc et al., 1996)

Detection of genetic variation in striped bass was historically difficult

Four hybridization and eight PCR-based assays were developed to discriminate between striped bass and white bass alleles

Demonstrated backcrosses between hybrid striped bass and native striped bass in the Chesapeake Bay (Harrell et al., 1993), verify purity of striped bass broodstock (Woods et al., 1995) and verify gynogen production (Leclerc et al., 1995)

Primers were designed to amplify either 5s ribosomal DNA or *ldhA* locus

Amplify corresponding striped bass and white bass genes

Developed assays to discriminate both amplified 5s rDNA and *ldhA* locus from the two species

Determined the extent of genetic variation among striped bass samples form SC rivers

Utilized three single copy nuclear loci (8-2, SSR14, SSR83) allele frequencies in seven different striped bass population samples from rivers throughout the natural range of the species

Significant differences in allele frequencies were observed

Largest difference occurring in populations at the extremes of the range (i.e. Appalachicola River on the Gulf Coast and the Shubenacadie and the Tabusintac Rivers in the Canadian maritime provinces)

Determined hatchery-induced genetic bottleneck caused change in allele frequencies in the Santee-Cooper system

Appeared to be population subdivision within the Santee-Cooper system in the absence of obvious physical barriers to prevent mixing

Preliminary results indicate each river contains a genetically distinct population of striped bass

(1993-1994, USC)

Developed genomic markers to discriminate between striped bass and white bass

Five of the markers segregated randomly when known backcross progeny were tested

Used markers to demonstrate that hybrid striped bass (Chesapeake Bay) spawn with the native striped bass – backcrossed progeny

Analysis of age, length, and sex data indicate female hybrid striped bass are significantly longer than males at age 5 and 7 even though growth rate is the same

Female hybrids must have a faster period of growth at some earlier age

(1995-1998, USC)

Disproved the notion that striped bass lack genetic variation by demonstrating that striped bass do contain regions of genetic variability.

Primers developed previously show Santee-Cooper striped bass have two to ten alleles at GT repeat loci

Determining nucleotide sequence

Developed assays to analyze the genetic variability of striped bass populations and demonstrated that each river system studied contains a genetically unique population of striped bass.

Identified five alleles at the SB83 locus using restriction fragment length polymorphisms (RFLP)

Four of the alleles were examined and found to be associated with an array of microsatellite alleles in Santee-Cooper populations

The fifth RFLP allele (A5) is associated with a single microsatellite allele

G to T transition interrupts the GT repeat

Neighboring region contains GT microsatellite

Gives opportunity to analyze the evolution of microsatellite alleles through different DNA lineages

Each RFLP allele is an independent DNA lineage

Original microsatellite alleles have mutated to form a family of alleles differing only in the number of GT repeats

Determined Congaree, Wateree, and Santee rivers contain genetically distinct populations

(1998-1999, USC)

Identified repeated sequence present in multiple copies as a tandem repeat

Represents about 5% of the genome

Six cloned repeat monomers from each of the four North American *Morone* species (*M. saxatilis, M. chrysops, M. americana, M. mississippiensis*) showed conservation of the mesmeric unit

Intraspecific sequence variation ranged from 3.2-5.4%

Same variation found within the same variation suggesting most of the intraspecies variation may be due to variation among copies of the repeat

Interspecific sequence variation ranged from less than 4.6% (between *M. americana* and *M. mississippiensis*) to about 16% (between other *Morone* species pairs)

Analysis indicated *M. americana* and *M. mississippiensis* were more closely related to each other than to any other pairs of *Morone* species

Reconstructed *Morone* phylogeny using twenty-two previously described morphological characters

Congruent relationships were obtained between both sets of data

Morone composed of two sets of sister taxa (*M. saxatilis/M. chrysops* and *M. americana/M. mississippiensis*)

Identified several polymorphic loci

One loci consisted of a microsatellite locus and a variable flanking region

Used to identify stock structure within the Santee-Cooper system

(2000-2001, USC)

Developed suite of highly variable microsatellite markers for genetic analyses of striped bass and other *Morone* species

Utilizing markers to determine effective population size of Congaree River spawning population

Previous study indicated effective population size to be 0.1% of number of sexually mature adults

(2002-2003, USC)

Estimates of effective population size of striped bass

Testing effects of sample size, number of alleles, and allele frequencies on effective population size estimates

Found new alleles of SB83 locus in Choptank River (spawning population for Chesapeake Bay) samples

Found most of new alleles in Roanoke, Delaware an Hudson river samples

Alleles are useable in distinguishing South Carolina striped bass populations from more northern populations although cannot distinguish populations within northern region

Determined smaller populations have greater probability of having divergent allele frequencies than larger populations

Small populations in close proximity to each other tend to have similar allele frequencies

(2003-2004, USC)

Utilized AFLP technique to determine 3-decade long association of hybrid striped bass with the Savannah River striped bass population has not resulted in detectable introgression of white bass genes into the striped bass gene pool

(2004-2005, USC)

Short tandem repeats (STR) loci useful in identifying family relationships in wild fish (such as juvenile striped bass in Congaree River, SC)

Demonstrated ~30% of relatively small 1992 year class produced by two pairs of parents

1993 was larger and no pair of parents contributed more than 5% of total progeny

Need to increase sample size to eliminate potential half-sibs and false positives

Provided evidence that pair matings of striped bass occur in the wild

Males outnumbered females so assumed multiple males fertilized the eggs of each female

Data indicated majority of 1992 year class siblings in each of the two large families were sired by a single male

Striped kilifish (*Fundulus majalis*)

(1992-1995, USC)

Developed PCR-based assay of mtDNA d-loop to discriminate between northern and southern forms of striped killifish (*Fundulus majalis*)

Used assay to study distribution of mtDNA across the hybrid zone were the two forms come in contact

The transition zone is in actuality less than 3 miles long although allozyme marker indicated a transition zone of at least 18 miles

Summer flounder (*Paralichthys dentatus*)

(1992-1995, USC)

Implemented comprehensive genetic survey of summer flounder population throughout its known range (n=20-25 individuals per putative population)

Amplifying mtDNA control region (d-loop)

(1995-1998, USC)

Identified significant variation in summer flounder mitochondrial DNA, but found no evidence for genetically distinct subpopulations.

Utilized mtDNA d-loop region of 200 individuals in study

Analysis of variance indicated a majority of the total genetic variation is within populations

Only 2-3% of variation is among populations

Large amounts of interpopualation gene flow

Any significant variation among populations is due to random fluctuations in haplotype frequencies

(1997-1998, USC)

Intraspecific genetic diversity in summer flounder

200 individuals were sequenced for the mitochondrial d-loop

Majority of total genetic variance lies within each population as determined by analysis of variance

2-3% of the total variation lies among populations

Populations characterized by large amounts of interpopulation gene flow

Any significant differences among populations are due to random fluctuations in haplotype frequencies

Developed novel approach to determining heritability

Create simple pedigree using molecular markers

Genotype individuals for molecular marker loci and score quantitative traits

Utilize maximum likelihood and linear estimator statistical procedures to determine the degree of relatedness between pairs of individuals (i.e. 50% related for full-sibs, 25% related for half-sibs)

Combines estimates of relatedness with quantitative trait data in a mixture model to infer heritabilities and genetic correlations

Used this approach to analyze summer flounder (*Paralichthys dentatus*) populations

Morphometric differences between two distinct "stocks" noted near Cape Hatteras, NC could be environmentally determined instead of genetically based

No genetic evidence for separation of populations

Sword fish (*Xiphias gladius*)

(1992-1995, USC)

mtDNA analysis of swordfish

Developed PCR-RSP assay to distinguish among three phylogenetic groups observed

Showed Pacific, Mediterranean, South Atlantic and North Atlantic Oceans contain genetically distinct populations (Alvarado Bremer et. al., 1995;1996)

Developed inexpensive method for purifying DNA for DNA sequence analysis

Develop PCR-RSP assays for swordfish nuclear genes

Utilized primers capable of amplifying portion of the tuna gene to amplify corresponding region in swordfish DNA

Polymorphic site was revealed using various restriction enzymes

Site absent in Pacific (n=30) and Mediterranean (n=50) samples

7% frequency in Atlantic samples (n=150)

Supports data that Atlantic population is genetically distinct

(1993-1994, USC)

Isolated DNA from 150 swordfish of varying geographic origin

mtDNA d-loop was PCR amplified

Screening swordfish genomic library

(1993-1994, MUSC)

Developed set of 20 primers for species identification

(1995-1998, USC)

Demonstrated populations of Mediterranean swordfish are genetically distinct from Atlantic swordfish populations. Therefore, fishing practices in the Mediterranean do not have a direct effect on the Atlantic fishery

330 base pair mtDNA control region I used for analysis

Primers (Alavarado Bremer, 1994)

L-Strand : L15998-PRO

(5'-TAC CCC AAA CTC CCA AAG CTA -3')

H-Strand: CSBDH

(5'-TGA ATT AGG AAC CAG ATG CCA G-3')

219 swordfish individuals included in study

From commercial fishing operations from two regions adjacent to the Strait of Gibraltar

130 individuals from Mediterranean side

Alboran Sea (n=74)

Gulf of Valencia (n=56)

89 individuals from Atlantic side

Iberia10-9192 (n=16)

Iberia10-9596 (n=20)

Iberia15-9192 (n=35)

Iberia20-9596 (n=18)

Identified 117 haplotypes within the 219 individuals sampled

Distribution of haplotypes was not homogeneous

- Haplotypes occurred only once in most instances with no more than twice within any Atlantic samples
- 50% of mtDNA types could be identified to one of three haplotypes within Mediterranean samples
    - Clade I haplotypes 21, 26 and Clade II haplotype 20 (Clades identified in Alvarado Bremer, 1994)

Haplotype 26 was most common among all samples

- 27-35% frequency within Mediterranean
- 3.4% frequency within Atlantic
    - Found in samples taken adjacent to Strait of Gibraltar

Clade II divided into two lineages (bootstrap >60%)

No significant differences in haplotype frequency among samples within each region as determined by Monte Carlo randomization (Roff and Bentzen, 1989)

All binary comparisons between Atlantic samples and Mediterranean Sea samples were found to be significant after Bonferroni correction ($P<0.05$)

- Never seen in large pelagic fish population studies before
- Surprising considering geographic proximity of populations

Fish belonging to either Clade I or II were found to be more closely related within region than among region as determined by hierarchical analysis of nucleotide diversity

- Test also showed genetic diversity grater in Atlantic samples

Clade II separated into two monophyletic groups, theta-med and theta-atl

- Theta-med comprises 30% of Mediterranean fish
    - 6 fish with this haplotype were found within the Iberian samples indicating some movement
- Theta-atl comprises 17% of Atlantic samples and 0% of Mediterranean

F_{st} values indicated by number of migrants per generation genetic exchange rates of 9.9 for Mediterranean and IBE10 and 5.5 for Mediterranean and IBE15&20 regions

Very low genetic exchange

Calculations assume shared haplotypes result from genetic exchange

Demonstrated that swordfish in the Northwest Atlantic are genetically distinct form those in the South Atlantic. Thus, it is appropriate that these two stocks continue to be managed separately.

330 base pairs (bp) of mtDNA control region from 397 individuals

Northwest Atlantic

Georges Banks (n=45)

Caribbean (n=16)

South Atlantic

Gulf of Guinea (n=83)

Brazil-Uruguay (n=95)

Indian Ocean

West of Madagascar (n=48)

Mediterranean

Ionian Sea (n=81)

North Pacific

Hawaii (n=29)

Utilized hierarchical analysis of nucleotide diversity to analyze data (g_{st})

High haplotypic diversity

Haplotypes differ from each other by one mutational event in most instances

No geographic association to this co-ancestry

Agreed with Bremer *et. al.* (SCRS/98/128)

320 distinct haplotypes for 397 individuals

Most occurred at very low frequencies with the exception of the Ionian Sea

Clade I nucleotide diversity for all populations was 0.0179-0.0320

Lowest value in Ionian Sea

All other sample areas had nucleotide diversity greater than 0.0246

In agreement with Bremer *et. al.* (SCRS/98/128) which found Mediterranean samples had lower diversity values than all other locations

South Atlantic clade II cannot be differentiated from other Atlantic clade II lineages (P=0.225)

Georges Bank and Caribbean clade II members were not significantly different (P=0.600)

Above were different than Ionian samples (P<0.0001)

The Indian and Pacific clade I samples were significantly different than Atlantic (P<0.0001)

No differentiation detected between Indian and Pacific

Spawning ground of Brazil-Uruguay and feeding ground of Gulf of Guinea (both South Atlantic) were more closely related to each other than to any other samples from entire global study

Same found within North Atlantic feeding (Georges Bank) and spawning (Caribbean) grounds

Ionian and Northwest Atlantic were very distinctly unassociated (P<0.0001)

Data further supports each of the five regions examined breed independently

Feeding and spawning grounds within each region were closely related

Limited exchange between Atlantic and Indian Ocean/Mediterranean populations

Determined mtDNA data is significant enough to distinguish NW Atlantic and South Atlantic feeding/spawning grounds

mtDNA data might be biased

Swordfish segregate latitudinally by sex in many areas

Other female biases

More difficult to conduct mixed stock analyses (MSA)

Developed several nuclear markers that are useful for the genetic analysis of swordfish, tunas and other bony fishes.

Utilized intron variation in aldolase C (aldC) and lactate dehydrogenase A (ldhA) for nuclear markers to confirm mtDNA findings concerning geographic partitioning

Nuclear markers eliminate possibility for female demographic biases in genetic analyses

Intron 6 of ldhA from universal primers developed by Quattro and Jones (

aldC primers from (Lessa and Applebaum, 1993)

mtDNA analyses yielded high degree of nucleotide diversity and haplotype heterogeneity within the global swordfish population

Examined northwest Atlantic Ocean, Mediterranean Sea and north Pacific Ocean regions

Comparisons across time (years for Mediterranean Sea and Pacific Ocean, months for Atlantic Ocean) showed regional marker stability

Temporal samples pooled for regional sampling

Through all three sampling locales, both loci showed high degree of heterogeneity ($P<0.01$) demonstrated through pairwise comparisons to test for genetic homogeneity

*ald*C genetic variation was detected through five size variants in a CA repeat

*ldh*A intron 6 showed four polymorphic sites giving six total alleles

All pairwise comparisons between sampling locations were highly significant ($P<0.01$)

All results concordant with mtDNA data

Developing primers to amplify swordfish catalase and mannose phosphate isomerase genes

(1997-1998, USC)

Swordfish stock structure analyses

mtDNA determined Mediterranean and Atlantic theta lineages are clearly distinct

Six alleles were identified from the amplified region of aldolase C gene

Only three of these alleles were found in Atlantic fishes

Another assay used a region containing a (GT) microsatellite

Several alleles present, varying in repeat length

(1998-1999, USC)

Collaboration with two Spanish groups yielded over 700 Mediterranean and Atlantic swordfish mtDNA (SCRS/98/128 & SCRS/98/127)

Identified strong mixing zone where both Atlantic and Mediterranean swordfish inhabit

Mediterranean, North Atlantic and South Atlantic populations are genetically distinct

Nuclear DNA markers were used to confirm mtDNA analyses (SCRS/98/129)

ldhA and aldC loci showed significant allele differences between population samples

Allele frequencies are stable over time in the northwest Atlantic

(1999-2000, USC)

nDNA marker analyses reflected mtDNA analyses

Used *ldhA* and *aslC* loci

Indicated significant differences in allele frequencies among population samples from different ocean basins

Replicate sample analysis demonstrated allele frequencies stable over time

Northwest Atlantic and South Atlantic swordfish samples showed distinct genetic variability

(2000-2001, USC)

nDNA marker analyses coincide with mtDNA findings

Both *ldhA* and *aldC* loci indicated significant differences in allele frequencies among population samples from different ocean basins

Analysis of replicate samples from the northwest Atlantic demonstrated allele frequencies are stable over time in this region

Atlantic and South Atlantic are genetically distinct

(2003-2004, USC)

Marine forensics

Successfully identified ocean origins of seven samples of swordfish in blind study

(2004-2005, USC)

Swordfish and bluefin tuna mtDNA analyses correlated with geologic events to develop hypotheses about the regional phylogenies of these two highly migratory fishes

Swordfish mtDNA from Atlantic samples was correlated with spawning and feeding areas demonstrating spawning site fidelity

Data illustrates North and South Atlantic contain separate breeding stocks

Tiger shark (*Galeocerdo cuvieri*)

(1992-1995, MRRI)

Developed three primers for species identification

(2002-2003, USC)

Adding nDNA to tiger shark mtDNA analysis which indicates significant differentiation between Atlantic and Pacific populations

Tree frogs (*Hyla spp*.)

(1995-1998, USC)

Several endemic and putative endemic species in the region represent deep evolutionary divergences compared to those that typify the fauna of Lake Waccamaw

Diverges evident in chubs (*Semotilus* and *Hybopsis*) and tree frogs (*Hyla*)

Undescribed form of *Hbopsis*

(2002-2003, USC)

Determined several species of fishes (federally endangered *Notropis mekistocholas*, undescribed form of *Hbopsis,* darter *Etheostoma,* chub Semotilus) are found solely within the Sand Hills region of the Carolinas

Weakfish (*Cynoscion regalis*)

(1992-1995, MRRI)

To examine the population structure of weakfish using genetic analysis

Testing hypothesis single stock exists along the Atlantic coast

(1995-1998, MRRI)

Weakfish were analyzed using three microsatellite loci

Analyzed using *Soc*029 (380 samples, 218 samples scored, 25 alleles identified), *Soc*014 (571 samples, 305 samples scored, 6 alleles identified), *Cne*612 (319 samples, 264 samples scored, 27 alleles identified), *Soc*012 (40 samples, 40 samples scored, 1 allele identified), *Soc*017 (40 samples, 40 samples scored, 1 allele identified)

Data indicated mixing of northern and southern populations of weakfish in South Atlantic Bight

South populations make little or no contribution to populations north of Chesapeake Bay

Identified four alleles in twenty-four examined weakfish

No allele present at frequency greater than 50%

(1996-1997, MRRI)

Performed analysis of genetic variation in weakfish at the Cne612 locus on 126 samples

Twenty-two alleles were identified

Inconsistent amplifications of Soc29 locus

Soc12 locus studies suggest locus is monomorphic in weakfish from southeastern US

Optimizing Soc14 and Soc17

Scored 315 samples from Florida, South Carolina, and North Carolina (collected by SEAMAP) for Pse028 and Pse036

Found extensive variation at both loci (Pse028 = 65 alleles, Pse036=30 alleles of which one allele was present at 31% frequency)

Heterozygote deficiency was observed – due to simultaneous collection of distinct subpopulations (Wahlund effect)

Pse028 differentiate among some of the collection sites

Geographic clines were apparent

Genetic sampling may lead to short-term variation among sites

Amplified Charleston samples to look for temporal differences

(1998-1999, MRRI)

Indicate mixing of northern and southern populations of weakfish in the South Atlantic Bight (SAB)

Southern populations make little or no contribution to populations north of the Chesapeake Bay

White bass (*Morone chrysops*)

(1992-1995, USC)

Four hybridization and eight PCR-based assays were developed to discriminate between striped bass and white bass alleles

Demonstrated backcrosses between hybrid striped bass and native striped bass in the Chesapeake Bay (Harrell et al., 1993), verify purity of striped bass broodstock (Woods et al., 1995) and verify gynogen production (Leclerc et al., 1995)

Primers were designed to amplify either 5s ribosomal DNA or *ldhA* locus

Amplify corresponding striped bass and white bass genes

Developed assays to discriminate both amplified 5s rDNA and *ldhA* locus from the two species

(1993-1994, USC)

Developed genomic markers to discriminate between striped bass and white bass

Five of the markers segregated randomly when known backcross progeny were tested

Used markers to demonstrate that hybrid striped bass (Chesapeake Bay) spawn with the native striped bass – backcrossed progeny

Analysis of age, length, and sex data indicate female hybrid striped bass are significantly longer than males at age 5 and 7 even though growth rate is the same

Female hybrids must have a faster period of growth at some earlier age

(1998-1999, USC)

Six cloned repeat monomers from each of the four North American *Morone* species (*M. saxatilis, M. chrysops, M. americana, M. mississippiensis*) showed conservation of the mesmeric unit

Reconstructed *Morone* phylogeny using twenty-two previously described morphological characters

Congruent relationships were obtained between both sets of data

Morone composed of two sets of sister taxa (*M. saxatilis/M. chrysops* and *M. americana/M. mississippiensis*)

(2003-2004, USC)

Utilized AFLP technique to determine 3-decade long association of hybrid striped bass with the Savannah River striped bass population has not resulted in detectable introgression of white bass genes into the striped bass gene pool

White grunt (*Haemulon plumieri*)

(1995-1998, MRRI)

Defined population genetic structure

White perch (*Morone americana*)

(1995-1998, MRRI)

Identification and characterization of the vitellogenin receptor and another low-density lipoprotein receptor in the white perch. Research conducted by Dr. Craig Sullivan during his sabbatical at MRRI.

White shark (*Carcharadon carcharias*)

(1992-1995, MRRI)

Determined three primers for species identification

White shrimp (*Penaeus setiferus*)

(1992-1995, USC)

Developing simple and economical molecular assay that identifies post-larvae of commercially important penaeid shrimp species (*Penaeus setiferus, P. aztecus, P. duorarum*)

Examine both intra-and inter-specific genetic diversity utilizing mtDNA nucleotide sequence comparisons using mtDNA *cyt*B

Indicate all three commercially important species are readily distinguishable at the nucleotide sequence level

Identifying diagnostic nucleotide polymorphisms to aid in species identification

(1995-1998, MRRI)

White shrimp genomic DNA library was created consisting of 400-1000 bp fragments

Amplified using PCR primers complementary to ligated adaptors followed by hybridization to oligonucleotides bound to small nylon filters

(1998-1999, MRRI)

Studies of white shrimp (*Penaeus setiferus*) genetics

Expanded sampling locations through Atlantic and Gulf of Mexico to Charleston Harbor, SC and Camepeche, Mexico

Nine sets of microsatellite primers targeting tri- and tetranucleotide repeat markers were developed and labeled with fluorescent dyes for easier analysis

DNA was sequenced from 176 clones

31 unique microsatellites

Six were polymorphic

Three amplified consistently and were used in the study

Scored on samples from Charleston Harbor, SC; GA; Florida/west; Mississippi River and West Delta, LA; Corpus Christi, TX; Mexico

Little geographic or temporal differentiation among the allele frequency

Heterozygosity ranged from 0.18-0.68 which is much lower than the (GT) dinucleotide repeat loci

After process was repeated, eluted fragments containing microsatellite repeats were cloned (70 clones)

34 clones contained greater than ~100 bp inserts

Most microsatellites contained $(GGA)_n$ repeats

Three clones were present 2-4 times in the library and selected for primer designation

(2000-2001, MRRI)

Completed collection of *P. setiferus*

Scored samples at 2 $(GT)_n$, 3 $(CCT)_n$, and 1 $(CCCT)_n$ microsatellite loci

*Pse*028 (sample number/n=1160, 76 alleles, *Pse*036 (n=1172, 32 alleles), *Pse*101 (n=1132, 19 alleles), *Pse*343 (n=1151, 10 alleles), *Pse*C48 (n=1156, 53 alleles), *Pse*G42 (n=1138, 11 alleles)

One locus was dropped due to extreme heterozygote deficiency (due to presence of null alleles)

Little geographic or temporal differentiation among allele frequency distributions when sites considered separately

Slight difference noted between Gulf and Atlantic when sites were grouped

White shrimp (*Penaeus vannamei*)

(1995-1998, MRRI)

Amplified using PCR primers complementary to ligated adaptors followed by hybridization to oligonucleotides bound to small nylon filters

(1996-1997, MRRI)

Collected samples from Charleston over several and analyzed two loci to determine stability of allele frequencies over time

No differentiation among allele frequency distributions were observed at either locus

Acquired 270 samples from the Gulf of Mississippi from NMFS laboratory in Pascagoula, MS

Received samples from two river systems in Georgia (St. Andrews and Cumberland)

Using microsatellite locus from *P. vannamei* for amplification

Wreckfish (*Polyprion americanus*)

(1995-1998, MRRI)

Characterized population structure

Yellow bass (*Morone mississippiensis*)

(1998-1999, USC)

Six cloned repeat monomers from each of the four North American *Morone* species (*M. saxatilis, M. chrysops, M. americana, M. mississippiensis*) showed conservation of the mesmeric unit

Intraspecific sequence variation ranged from 3.2-5.4%

Same variation found within the same variation suggesting most of the intraspecies variation may be due to variation among copies of the repeat

Interspecific sequence variation ranged from less than 4.6% (between *M. americana* and *M. mississippiensis*) to about 16% (between other *Morone* species pairs)

Analysis indicated *M. americana* and *M. mississippiensis* were more closely related to each other than to any other pairs of *Morone* species

Reconstructed *Morone* phylogeny using twenty-two previously described morphological characters

Congruent relationships were obtained between both sets of data

Morone composed of two sets of sister taxa (*M. saxatilis/M. chrysops* and *M. americana/M. mississippiensis*)

Identified several polymorphic loci

One loci consisted of a microsatellite locus and a variable flanking region

Used to identify stock structure within the Santee-Cooper system

Yellowfin tuna (*Thunnus albacares*)

(1992-1995, USC)

Obtained samples of yellowfin and bigeye tuna from South Pacific, Indian Ocean, South Atlantic Ocean

Observed two clades of bigeye tuna

As different from each other as were closely related species such as yellowfin and blackfin tuna

(1995-1998, USC)

Revealed two monophyletic clades with 4.9+/- 0.1% divergence within bigeye

Similar results to those found within yellowfin, blackfin and longtail tuna mtDNA control region analyses

(1998-1999, USC)

Unable to detect genetic differences between samples of yellowfin tuna from the Northwest Atlantic and the Eastern Pacific when nucleotide sequences of d-loop regions were compared

(1999-2000, USC)

Unable to detect genetic differences between samples of yellowfin tuna from the Northwest Atlantic and the Eastern Pacific using d-loop mtDNA nucleotide sequences

Expanded sample size and included samples from the Gulf of Guinea and Indian Ocean

Although high levels of genetic variation exists with the samples, no genetic differentiation has occurred in the mtDNA d-loop region

(2002-2003, USC)

Completed analysis of skipjack and yellowfin mtDNA d-loop/ control region

Nearly every haplotype was unique and there was no significant difference among samples from the world's major oceans

Explained by either:

a. both species are relatively young and effective population sizes are so large that population differentiation has not had time to occur or is prevented by low levels of mixing

b. d-loop region is mutating at such a high rate that recurring mutations obscure the existing genetic differentiation

Re-analyzed samples using mtDNA *cyt*B and nDNA *ldh*A

No difference in allele frequencies between Atlantic and Pacific using *ldh*A marker

Identified polymorphism in *cyt*B gene of skipkjack

Concluded large effective population size in skipjack tuna prevented population differentiation

Significant allele frequency differences between our Atlantic and Pacific yellowfin tuna samples

Data consistent with reduced population differentiation due to large population sizes rather than confounding effects of homoplasy

(2003-2004, USC)

Analyzed intergenic region between ATPase6 and COIII genes of yellowfin tuna DNA

Preliminary results indicate an allele frequency differentiation exists between Atlantic and Pacific samples

Consistent with reduced population differentiations due to large population sizes rather than confounding effects of homoplasy

(2004-2005, USC)

Two mtDNA loci analyzed for yellowfin and skipjack tuna

Degree of genetic differentiation between Atlantic and Pacific samples correlates with population size

Zebrafish (*Brachydanio rerio*)

(1998-1999, USC)

Developed a third primer set that amplifies polymorphic locus (Aldolase B intron 'G') within actinopterygian fishes

Working on zebrafish

FISHTEC Species Listing by Scientific and Common Name

Note: Listed by scientific name followed by common name in parentheses. Reference the common name listed here for research summaries in previous section *"Species-Specific Research Projects and Findings"*.

Acipenser brevirostrum (Shortnose sturgeon)
Euthynnus alletteratus (Little tunny)
Acipenser oxyrhynchus (Atlantic sturgeon)
Alligator mississippiensis (American alligator)
Alopias superciliosus (Bigeye thresher)
Amia calva (Bowfin)
Amphiascuc tenuiremis (Copepod)
Brachydanio rerio (Zebrafish)
Caimen crocodylus (Caiman)
Carcharadon carcharias (White shark)
Carcharhinus acronotus (Blacknose shark)
Carcharhinus altimus (Bignose shark)
Carcharhinus brevipinna (Spinner shark)
Carcharhinus isodon (Finetooth shark)
Carcharhinus limbatus (Blacktip shark)
Carcharhinus plumbeus (Sandbar shark)
Carcharhinus plumbeus (Sandbar shark)
Centropristis striata (Black sea bass)
Cetorhinum maximus (Basking shark)
Chelydra serpentina (Snapping turtle)
Coryphaena hippurus (Dolphin)
Cynoscion nebulosus (Spotted sea trout)
Cynoscion regalis (Weakfish)
Cyprinus carpio (Carp)
Dipturus laevis (Barndoor skates)
Elassoma boehlkei (Caroline pygmy sunfish)
Elassoma okatie (Bluebarred pygmy sunfish)
Epinephelus striatus (Nassau grouper)
Eptatretus stoutii (Hagfish)
Etheostoma collis (Carolina darter)
Etheostoma collis (Darter)
Fundulus bermudae (Bermuda killifish)
Fundulus heteroclitus (Mummichog)
Fundulus majalis (Striped kilifish)
Fundulus relictus
Fundulus waccamensis
Galeocerdo cuvieri (Tiger shark)
Haemulon plumieri (White grunt)
Hbopsis (Undescribed form)
Heterodontus francisci (Horned shark)

Hyla spp.(Tree frogs)
Ictalurus punctatus (Catfish)
Illex illecebrosus (Short finned squid)
Isurus oxyrinchus (Shortfin makos)
Katsuwonus pelamis (Skipjack tuna)
Kogia breviceps (Pigmy whale)
Lepisosteus osseus (Longnose gar)
Loligo pealei (Longfinned squid)
Loliguncula
Menidia beryllina (Inland silverside)
Menidia extensa (Silversides)
Micropogonias undulatus (Croaker)
Micropterus salmoides (Largemouth bass)
Morone americana (White perch)
Morone chrysops (White bass)
Morone mississippiensis (Yellow bass)
Morone saxatilis (Striped bass)
Mustelus canis (Smooth dogfish)
Mycteroperca microlepis (Gag)
Notropis mekistocholas (Cape Fear shiner)
Noturus insignis (Margined madtom)
Noturus spp. (Broadtail madtoms)
Odontaspis taurus (Sand tiger)
Palaemonetes pugio (Grass shrimp)
Paralichthys dentatus (Summer flounder)
Penaeus aztecus (Brown shrimp)
Penaeus duorarum (Pink shrimp)
Penaeus setiferus (White shrimp)
Penaeus stylirostris (Penaeid shrimp)
Penaeus vannamei (White shrimp)
Peromyscus spp. (Deer mice)
Petromyzon marinus (Sea lamprey)
Polyprion americanus (Wreckfish)
Prionace glauca (Blue shark)
Raja erin (Little skate)
Rhizotrionodon terraenovae (Atlantic sharpnose shark)
Salmo gairdneri (Rainbow trout)
Sasyatis sabina (Atlantic stingray)
Sciaenops ocellatus (Red drum)
Semotilus and ***Hybopsis*** (Chubs)
Sphyrna lewini (Scalloped hammerhead)
Sphyrna tiburo (Bonnethead)
Stentomus chrysops (Scup)
Thunnus albacares (Yellowfin tuna)
Thunnus atlanticus (Blackfin tuna)
Thunnus obesus (Bigeye tuna)

Thunnus thynnus (Bluefin tuna)
Xiphias gladius (Sword fish)

FISHTEC Research Projects by Geographic Deployment

Below is an alphabetical listing of locations with species common names that were studied under FISHTEC research projects. Please refer to species list or general summary sections for specific project details and scientific names of species. Institutions that provided species samples for sequencing are identified in the *"Author's Preface"* section.

Abidjan, Ivory Coast
Little tunny

ACE Basin (Ashepoo, Combahee, Edisto)
Red drum, spotted sea trout

Alboran Sea
Swordfish

Altahama River, GA
Shortnose sturgeon

Appalachicola, FL
Spotted sea trout, striped bass

Ashley River, SC
Red drum

Barnard Cove
Red drum

Bermuda
Little tunny, endemic freshwater *Fundulus spp.*

Brazil-Uruguay
Swordfish

Broad River, SC
Red drum

Brunswick, GA
Spotted sea trout

Campeche, Mexico
Grouper

Canadian Maritime Provinces - Shubenacadie River and Tabusintac River
Striped bass

Cape Cod, MA
Long finned squid

Cape Fear Drainage River, NC
Catfish

Cape Hatteras, NC
Scup, spotted sea trout, summer flounder, long finned squid

Cape Romain, SC
Red drum, spotted sea trout

Caribbean Sea
Swordfish

Charleston, SC
Red drum, shrimp, spotted sea trout, weakfish

Chesapeake Bay
Spotted sea trout, striped bass, weakfish, white bass

Choctawhatchee Bay, FL
Spotted sea trout

Choptank River, MD
Striped bass

Coleton River, SC
Red drum

Congaree River, SC
Striped bass

Corpus Christi, TX
White shrimp

Cumberland River, GA
Shrimp

Florida
Scup, sharks, white shrimp, croaker

Georges Bank, MA
Swordfish

Grice Cove, SC
Red drum

Gulf Coast
Scup, grouper, grass shrimp, southern flounder, croaker

Gulf of Guinea
Bigeye tuna, swordfish

Gulf of Mississippi
Shrimp

Gulf of Velencia
Swordfish

Hawaii
Shrimp, swordfish

Hudson River, NY
Striped bass

Iberian Peninsula
Swordfish

Indian Ocean
Yellowfin tuna, bigeye tuna, swordfish

Indian River, FL
Spotted sea trout

Ionian Sea
Swordfish

Lake Waccamaw, NC
Endemic darter, catfish, silversides

Leetown, WV
Horseshoe crab

Lower Wando, SC
Red drum

Maryland

Horseshoe crabs

Massachusetts
Horseshoe crabs

Mediterranean Sea
Swordfish, bluefin tuna

Mississippi River
Shrimp

New Jersey
Long finned squid, silverside, scup

North Atlantic
Bluefin tuna, swordfish, bigeye tuna

North Carolina
Spotted sea trout, shrimp

North Inlet, SC
Red drum

Ogeechee River, GA
Shortnose sturgeon

Pacific Ocean
Swordfish, yellowfin tuna, bigeye tuna, bluefin tuna, little tunny

Pamlico Sound, NC
Silverside

Phelps Lake, NC
Fundulus waccamensis

Roanoke River, VA
Darters, striped bass

Saluda River, SC
Carolina darter,

Sandhills Region of SC
Chub, darter

Santee-Cooper System

Striped bass, yellow bass

Savannah River, GA
Striped bass, white bass

South Atlantic Bight
Weakfish, scup

St. Andrews River, GA
Shrimp

St. John River, Canada
Shortnose sturgeon

Strait of Gibraltar
Swordfish

Upper Wando, SC
Red drum

Wateree River, SC
Striped bass

FISHTEC Publications

Note: FISHTEC related publications are organized alphabetically and by year, starting with the most recent publications.

2006

Quattro, J.M., Stoner D.S., Driggers, W.B., Anderson, C.C., Priede, K.A., Hoppmann, E.C., Campbell, N.H., Duncan, K.M. and Grady, J.M. 2006. Genetic evidence of cryptic speciation within hammerhead sharks (Genus *Sphyrna*). Marine Biology 148:1143-1155. **FISHTEC 06-01.**

2005

Ball, A.O. , Beal, M.G., Chapman, R.W., Sedberry, G.R. (2005) "Stock Structure of Red Porgy, *Pagrus pagrus*, in the North Atlantic." Project Final Report, MARFIN Project Number NA17FF2008, June 28, 2005. South Carolina Department of Natural Resources, Charleston, SC. **FISHTEC 05-01**

Grieg, T.W., M.K. Moore, C.M. Woodley and J.M. Quattro. 2005. Mitochondrial gene sequences useful for species identification of western North Atlantic Ocean sharks. *Fishery Bulletin*. 103(3):516-523.

Robbins, S.N., A.O. Ball, M. R. Denson, W. E. Jenkins, T.I.J. Smith and R.W. Chapman

"The Use of Genetic Tags to Assess Stock Enhancement of red drum, *Sciaenops ocellatus,* in South Carolina" abstract submitted for the 2005 AFS annual meeting, Sept 11-15, Anchorage, Al. **FISHTEC 05-02**

2004

Hiramatsu, N., R.W. Chapman, J.K. Lindzey, M.R. Haynes, and C.V. Sullivan. 2004. Molecular characterization and expression of vitellogenin receptor from white perch (*Morone americana*). *Bio. Reprod.* Online first: 10.1095/biolreprod.103.023655

Zalcoff, M.S., A.O. Ball, and G.R. Sedberry. 2004. Population genetic analysis of red grouper (*Epinephelus morio*) and scamp (*Mycteroperca phenax*) from the southeastern US Atlantic and Gulf of Mexico. *Mar. Biol.* online first: DOI: 10.1007/s00227-003-1236-z.

2003

Ball, A.O., Sedberry, G.R. and Chapman, R.W. 2003. Large scale Genetic Differentiation of *Pagrus pagrus* (L.) in the Atlantic. Journal of Fish Biology. **FISHTEC 03-01**

Chapman, R.W., A.O. Ball, and L.R. Marsh. 2003. Spatial homogeneity and temporal heterogeneity of red drum, *Sciaenops ocellatus*, microsatellites: effective population sizes and management implications. *Mar. Biotech.* 4:589-603.

Loefer, J.K. and G.R. Sedberry. 2003. Life history of the Atlantic sharpnose shark (*Rhizoprionodon terraenovae*) (Richardson, 1836) off the southeastern United States. *Fishery Bulletin*. 101:75-88.

2002

Chapman, R.W., A.O. Ball, and L.R. Mash. 2002. Spatial homogeneity and temporal heterogeneity of red drum, *Sciaenops ocellatus,* microsatellites: effective population sizes and management implications. *Mar. Biotech.* 4:589-603.

Ely, B., D. S. Stoner, J. R. Alvarado Bremer, J. M. Dean, P. Addis, A. Cau, E. J. Thelen, W.J. Jones, D.E. Black, L. Smith, K. Scott, I. Naseri and J. M. Quattro. 2002. Analyses of Nuclear ldhA Gene and mtDNA Control Region Sequences of Atlantic Northern Bluefin Tuna Populations. Marine Biotechnology 4:583-588. **FISHTEC 02-01**

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Graduate Students Receiving Technical Training

- Jaime Alvarado Bremer. University of South Carolina. Postdoctoral fellow
- Christopher Anderson. Ph.D.
- Amy Ball. The Medical University of South Carolina. Ph.D. graduate. Dissertation title: Population Genetics of White Shrimp, *Penaeus setiferus.*
- Rosemary Bennetts. University of South Carolina. M.S. graduate. Thesis topic:
- Taxonomy and Evolution of Broadtail Madtoms (Genus *Noturus*).
- Dave Carbreara. University of Charleston. M.S. student.
- Marilyn Diaz. University of South Carolina. Ph.D. candidate. Dissertation topic: Genetic Variation in Natural Populations of Striped Bass.
- Gus DiNovo. Medical University of South Carolina. Ph.D. graduate.
- W. B. Driggers. Ph.D. postdoctoral fellow.
- S. Finkenbine. University of Charleston. M.S. student.
- Thomas Greig. University of South Carolina. Ph.D. student. Dissertation topic:
- Molecular Ecology of Swordfish.
- Kai Ping Han. University of South Carolina. Postdoctoral Fellow. Project title:
- Population Genetics of Striped Bass.
- William J. Jones. University of South Carolina. M.S. graduate. Thesis topic: Population Genetics of Summer Flounder (*Paralichthys dentatus*).
- Gilles Leclerc. University of South Carolina. Postdoctoral Fellow.
- P. Mericko. University of Charleston. M.S. student.
- Thomas Merritt. University of South Carolina M.S. graduate. Thesis title: Conservation Genetics of Carolina Darters (*Ethoestoma collis*).
- Michael Musyl. National Marine Fisheries Service. Postdoctoral Fellow.
- Kenneth Oswald. University of South Carolina. M.S. student. Thesis topic: Phylogenetic Relationships of the Carolina Darter.
- Kristine Priede. University of South Carolina. Ph.D. student. Dissertation topic:
- Bottleneck Effects on Genetic Diversity in Natural Populations of Fishes.
- Shane Sarver. University of South Carolina. Postdoctoral Fellow.
- Duane Stephenson. University of Charleston, SC. M.S. graduate. Thesis title: Stock Structure of Nassau Grouper, *Epinephelus striatus,* Using Microsatellite DNA analysis.
- Douglas S. Stoner. Ph.D., Postdoctoral fellowship.
- Brad Wiley. University of Charleston, SC. M.S. graduate. Thesis title: Stock Structure of Spotted Sea Trout, *Cynosion nebulosis*, in the Southeastern U.S.
- Robyn Wingrove. University of Charleston, SC. M.S. student. Thesis title: Population Genetics of Dolphin, *Coryphena hippurus.*
- Michelle Zatcoff. University of Charleston, SC. M.S. student. Thesis title: Population Genetics of Red and Black Grouper.
- Liang Zhang. University of South Carolina. M.S. Thesis title: Striped bass odorant receptor genes.

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Glossary of Terms and Acronyms

ACE Basin – Ashepoo, Combahee and Edisto River Basin

AFLP – Amplified Fragment Length Polymorphism

AHR - Aryl Hydrocarbon Receptor

AR – Androgen Receptor

BASSMAP – Tools for the genetic improvement of sea bass

Bp – Base Pairs

cDNA – Complementary DNA

DNA – Deoxyribonucleic Acid

ELISA - Enzyme-linked Immunosorbent Assay

ESU – Evolutionary Significant Unit

EST – Expressed Sequence Tags

HWE – Hardy-Weinberg Equilibrium

GABA – Gamma-aminobutyric Acid Receptor

GAD – Glutamic Acid Decarboxylase

Ig - Immunoglobin

LDH – Lactate Dehydrogenase

MBES – Marine Biomedical and Environmental Sciences Center (MUSC)

MRRI – Marine Resources Research Institute

mtDNA – Mitochondrial DNA

nDNA - Nuclear DNA

NMFS – National Marine Fisheries Service

PBL – Peripheral Blood Lymphocytes

PKC – Protein Kinase C

PCR – Polymerase Chain Reaction

RAPD-PCR – Random Amplification of Polymorphic DNA PCR

RFLP – Restriction Fragment Length Polymorphism

RNA – Ribonucleic Acid

rRNA –Ribosomal RNA

RT-PCR – Reverse Transcriptase PCR

SAB – South Atlantic Bight

SAGE – Serial Analysis of Gene Expression

SCDNR – South Carolina Department of Natural Resources

SCWMFS – South Carolina Wildlife and Marine Fisheries Service

STR – Short Tandem Repeats

TPI – Triosephosphate Isomerase