CONTEMPORARY POPULATION STRUCTURE AND HISTORICAL DEMOGRAPHY OF SAILFISH (*Istiophorus platypterus*) IN THE ATLANTIC OCEAN

A Thesis

by

JESSICA BANGMA

Submitted to the Office of Graduate Studies of
Texas A&M University
in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

August 2006

Major Subject: Wildlife and Fisheries Sciences

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Approved by:

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Committee Members, John Gold
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Head of Department, Delbert Gatlin

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ABSTRACT

Contemporary Population Structure and Historical Demography of Sailfish (*Istiophorus platypterus*) in the Atlantic Ocean. (August 2006)

Jessica Bangma, B.Sc., University of Victoria, British Columbia, Canada

Chair of Advisory Committee: Dr. Jaime Alvarado-Bremer

The Atlantic sailfish (*Istiophorus platypterus*) is considered over-fished in U.S. waters. Furthermore, preliminary analyses of abundance indicate that a decline in biomass has occurred. While seminal studies have provided useful baseline data about intra- and inter-oceanic variation within sailfish, such studies may have underestimated the amount of genetic variability as a result of small sample sizes and the poor resolution of restriction fragment length polymorphism data. We used 263 base pairs from the hypervariable mitochondrial control region and fragment polymorphisms from five microsatellite loci to assess the contemporary population structure between eastern (n = 30) and western (n = 192) Atlantic sailfish stocks. We failed to reject the hypothesis of panmixia in Atlantic sailfish; however, higher levels of genetic variation were observed within the eastern Atlantic sailfish, and the nature of this difference needs to be investigated further. Having found no evidence of transatlantic differentiation, we pooled the Atlantic samples (n = 222) and compared them to a sample from the eastern Pacific (n = 22) and rejected the null hypothesis, concluding that sailfish from separate ocean basins do not share a common gene pool. We also found evidence of a recent sudden expansion of Clade I (the ubiquitous clade found in both Atlantic and Pacific Oceans) sailfish into the Atlantic that appears to have occurred between 164000 and 351000 years ago, coinciding with interglacial periods during the Pleistocene. This study also presents the first evidence of a recent sudden expansion of sailfish into the eastern Pacific, roughly 85400 to 173000 years ago, following a period when cooling in the eastern Pacific would have restricted the sailfish range to the warmer waters of the western Indo-Pacific. We emphasize that sailfish from this region of the Pacific are phenotypically distinct from those in the rest of the species' range and encourage further studies in order to determine if the eastern Pacific sailfish population should be managed as a separate stock.

This thesis is dedicated to my soon-to-be husband, Kurt. Thank you for your constant encouragement and support and for providing me with the best distractions (New York City). On to the next thing!

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER I

INTRODUCTION

Sailfish (*Istiophorus platypterus*) is a highly migratory, epipelagic, circumtropically distributed istiophorid species found commonly in neritic waters (Ovchinnikov 1970, Beardsley et al. 1972, Hoolihan 2004), but distributed widely through the tropical Atlantic. As a result of bycatch in commercial fisheries targeting tunas and swordfish, the Atlantic sailfish population is considered over-fished in U.S. waters. In addition, preliminary analyses of abundance indices indicate that a decline in biomass has occurred in the Atlantic (Restrepo et al. 2003). Currently, this species in the Atlantic Ocean is managed by the International Commission for the Conservation of Atlantic Tunas (ICCAT), under a two-stock hypothesis (East and West). There is little evidence to support this hypothesis of population structure, and the possibility of a single panmictic unit exists. While sailfish distribution, derived from fisheries catch data, could be interpreted as evidence of a single population, it has to be noted the catch data may not accurately reflect seasonal changes in the distribution of temporally and or geographically discrete subpopulations of a species with high migratory potential. Despite these uncertainties, there is little information about levels of genetic variability in Atlantic sailfish, and in particular of its genetic population structure. This thesis attempts to fill this void by presenting an analysis of the contemporary population structure of sailfish. In addition, there is little understanding about the mechanisms -current and historical- that may explain the observed levels of genetic variation. Thus, genetic variability in Atlantic sailfish is analyzed in reference to biogeography, historical demography, and the potential of variance in reproductive success.

This thesis is divided into five chapters, and include: this Introduction, three main research chapters intended to be stand-alone manuscripts which after some modifications will be submitted for publication, and final chapter that summarizes the major findings. Chapter II addresses the need to differentiate among tissue of billfish species. Several techniques for the forensic identification of istiophorid billfish primarily with the purpose of law enforcement have been developed previously. However, these DNA-based forensic ID approaches are also presently being employed to determine the temporal occurrence of istiophorid billfish larvae as a means of identifying spawning and nursery habitats of these highly migratory fishes (Hyde et al. 2005; McDowell and Graves 2002). The present study is part of a larger project at Texas A&M University, Galveston (TAMUG), the Atlantic Billfish Research Program, that has a similar goal: to survey the waters of the northern Gulf of Mexico (GoM) to determine the importance of this basin as spawning and nursery habitat for istiophorids. Thus, Chapter II discusses the advantages and limitations of previous molecular forensic approaches, and based upon the larval

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This thesis follows the style and format of Conservation Biology.

billfish collected from the Gulf of Mexico and reference adult samples of different billfish, utilizes the mitochondrial DNA control region I sequence as an alternative to identify billfish larvae in the Atlantic. This chapter also presents summary data pertaining to the genetic diversity within sailfish and the other two billfish species collected, namely white marlin and blue marlin.

In Chapter III, the positively identified larvae were then pooled with adult data sets to conduct a analysis of contemporary population structure and historical demography of Atlantic sailfish. Previous genetic studies have failed to find evidence of transatlantic differentiation (e.g., Graves and McDowell 1995). Here we analyze the mtDNA control region because the high levels of variability observed in this region may provide sufficient information to resolve intra-oceanic differentiation in sailfish. However, the uniparental mode of inheritance of mtDNA would provide only evidence from female mediated gene flow. Thus, we also included nuclear microsatellite loci, which are characterized by high mutation rates affecting short tandem repeats, resulting in size polymorphisms that can be distinguished by determining the exact length of the DNA fragment (Estroup et al. 1995). Due to this pronounced hypervariability, microsatellite loci are often used in conjunction with mtDNA data in order to improve the resolution of analyses. Thus, Chapter III consists of an examination of the contemporary population structure of sailfish in the Atlantic Ocean comparing eastern and western Atlantic sailfish stocks using mtDNA sequences as well as five microsatellite loci. This chapter also includes an assessment of the historical demography of sailfish.

The genetic variation contained in both the control region and microsatellite loci can also be used to assess kinship among larvae that share the same mitotype. Thus Chapter IV, includes the examination of the larval sailfish collected from the Gulf of Mexico for evidence of an over-representation of related individuals within geographically and temporally discrete sampling collections (tows). Female sailfish are highly fecund, capable of producing 2.3 million to 4.7 million eggs per spawning event (Voss 1953). In common with other highly fecund aquatic species that display Type III survivorship, sailfish experience high mortality during the early stages. Owing to high fecundity and the sweepstakes chance of individuals to match their reproductive activity to oceanographic conditions favorable for maturation, fertilization, larval development, and recruitment, Hedgecock (Hedgecock et al. 1982; Hedgecock 1994) hypothesized that marine animals have extremely large variance in individual reproductive success. Here we employed two approaches to test for evidence of variance in reproductive success in Atlantic sailfish. The first test was a direct comparison of the levels of genetic variability between larval and adult samples, where evidence of reproductive variance is inferred when larval samples contain significantly lower levels of genetic variation than adult samples (Chapman et al. 1999). As previously mentioned, we attempted this by comparing the levels of genetic variability of the mtDNA CR-I and five microsatellite loci, in larval

and adult collections of sailfish from the Atlantic Ocean. Adult data were then used as reference to examine the larval collections for significant heterozygote deficiencies, the presence of which would be indicative of the Wahlund effect (Wahlund 1928). The larvae were also examined for evidence of linkage disequilibrium at multiple loci which would suggest that some of the larvae were either full or half siblings (Chapman et al. 1999). Lastly, individuals collected in the same or proximal tows sharing the same mtDNA CR-I haplotype were tested for siblingship using the fragment polymorphism data from five microsatellite loci to determine whether they could represent the contribution of the same spawning event.

CHAPTER II

FORENSIC IDENTIFICATION OF ISTIOPHORID BILLFISH LARVAE

INTRODUCTION

Istiophorid billfish are euteleost, perciform pelagic fishes widely distributed in tropical and subtropical waters of the world's oceans. Sailfish (*Istiophorus platypterus*), blue marlin (*Makaira nigricans*), black marlin (*M. indica*), and shortbill spearfish (*Tetrapturus angustirostris*) are found in the Atlantic, the Indian, and the Pacific oceans (albeit with varying abundances). The longbill spearfish (*T. pfluegeri*) and white marlin (*T. albidus*) appear restricted to the Atlantic. The striped marlin (*T. audax*) is primarily confined to the Indian and the Pacific oceans; however concentrations around the southern tip of South Africa are not uncommon (FishBase 2006). As a result of depleted stock levels being observed in the U.S., the Fishery Management Plan for Atlantic Billfishes has prohibited the sale of istiophorid billfish caught in the Atlantic Ocean, while the sale of individuals from the Pacific Ocean is still permitted (McDowell and Graves 2001).

Adult istiophorid species can be readily identified based on morphology. However, once diagnostic characters are removed during processing, it is not possible to discriminate between billfish carcasses (Shepard and Hartmann 1996). Furthermore, inter-oceanic morphs of sailfish and blue marlin are virtually indistinguishable, facilitating the illegal sale of Atlantic catches of these species. Fueled in part by the need to be able to differentiate among billfish species as well as between oceanic subpopulations for law enforcement purposes, researchers have developed several techniques for the forensic identification of istiophorid billfish. Chow (1994) attempted to identify billfish species using a polymerase chain reaction restriction fragment length polymorphism (PCR-RFLP) assay that targeted the mitochondrial DNA (mtDNA) cytochrome b (Cyt b) gene but generated identical restriction profiles for white and striped marlin as well as for Atlantic and Pacific blue marlin. Alvarado Bremer (1994) conducted a RFLP assay of the entire mtDNA molecule with a battery of 6 restriction endonucleases and produced composite haplotypes diagnostic to identify sailfish, white marlin, striped marlin and blue marlin. In a more recent study McDowell and Graves (2002) attempted to develop mitochondrial and nuclear markers for specific identification of istiophorids, and while they were successful in differentiating among five species of istiophorids, they failed to discriminate between white and striped marlin and between Atlantic and Pacific specimens of sailfish and blue marlin, respectively. Innes et al. (1998) were successful in developing a genetic test for the identification of the five species of istiophorid billfish found in Australian waters based on an RFLP analysis of the hypervariable mitochondrial d-loop or control region, but Atlantic species were not characterized. Likewise, Hsieh et al. (2005) were able to differentiate the five billfish species from the Pacific using RFLPs in a portion of the Cyt b gene but, as in previous examples, no Atlantic samples were included. Hyde et al. (2005) developed a shipboard single-locus

multiplex PCR assay that targeted a segment of the mtDNA Cyt b gene for the five species of Indo-Pacific istiophorids. Although this study included individuals from both Atlantic and Pacific locations (where applicable) it made no effort to differentiate between these populations in the development of the multiplex assay and white marlin was not included. Accordingly, without modifications, this assay can not characterize Atlantic billfish. Moreover, the authors reported that *a posteriori* sequencing resulted in the detection of false positive species identifications. In summary, while these approaches are appealing for being rapid and relatively inexpensive, they lack the precision necessary when conducting unambiguous species identifications as well as sufficient resolution to detect intra-specific variation.

Some of the aforementioned techniques are presently being employed to determine the temporal occurrence of istiophorid billfish larvae as a means of identifying spawning and nursery habitats of these highly migratory fishes (Hyde et al. 2005; McDowell and Graves 2002). This study is part of a larger project with a similar goal: to survey the waters of the northern Gulf of Mexico (GoM) to determine whether it constitutes an important spawning and nursery habitat for istiophorids. The identification within this region of the GoM of a larval 'hot-spot' would allow for subsequent directed sampling efforts which would provide additional insight into the spawning ecology and reproductive biology of this species (Hyde et al. 2005). Studies conducted along the Florida coast report that sailfish spawn between April and October with a peak from June to July (deSilva and Breeder 1997; Voss 1953). However, this long spawning season overlaps with the spawning seasons of white marlin and blue marlin, whose peak spawning time in the GoM apparently occurs from March to June and July to October, respectively (deSilva and Breeder, 1997). Young istiophrids are very similar in their developmental stages, thus it is difficult to identify pre- and post-flexion larvae to the species level based on morphometric and meristic characters alone. Direct sequencing of the hypervariable control region (CR-I) of mitochondrial DNA is a useful tool for discriminating between virtually indistinguishable larvae of closely related tuna species (Alvarado Bremer et al. 1998a, Talley-Farnham 2003). Although this approach has been criticized for being both time-consuming and expensive (Chow 1994; Innes et al. 1998; Hyde et al. 2005), a reduction in costs (approximately $6.00 per specimen) and the advent of automated genetic analyzers allows for direct sequencing to be affordable and relatively fast. More importantly, the increases in both the accuracy of species identification and the wealth of information generated for subsequent population analyses are unprecedented.

Sequences from positively identified adult billfish representing three genera and four species in the Family Istiophoridae were used as references to identify the larval billfish collected during two research cruises in the Gulf of Mexico. Representatives of both Atlantic and Pacific populations of sailfish and blue marlin were included, as were reference samples from white marlin and striped marlin. While this study should have ideally included representatives of shortbill spearfish and black marlin, these istiophorids have only rarely been observed in the Atlantic Ocean, with only a few 'stray' individuals

having ever been captured (FishBase 2006). In addition, control region sequences for these two species have already been characterized (J. Graves pers. comm.; Professor of Marine Science, Virginia Institute of Marine Science, Gloucester Point, VA) and they possess a very distinct signature from the other species included in this study. Accordingly, any sequence that would fail to cluster among samples included in this study would have been forwarded to Dr. Graves for verification. After the larvae were identified their sequences were pooled with the corresponding adult sample and basic diversity statistics were calculated.

METHODS

Istiophorid larvae were collected from the northern Gulf of Mexico during two research cruises in May and July 2005 aboard the Oceanic Conservation Organization's (OCO's) research vessel, the *Holo Kai*. Two neuston nets with mesh sizes of 500 µm and 1200 µm were towed for 15 minutes at a time, 10 times per day, for a total of 60 sampling stations per cruise. Temperature, salinity, pH, and dissolved oxygen were measured at the beginning of each tow. This information is included in Appendix D (Table D-1) along with a map depicting the station locations (Figure D-1). Each billfish larva was assigned an identification number, digitally photographed, and preserved in 70% ethanol. The ethanol was changed once in order to minimize the dilution of ethanol below 70% by the presence of tissue fluids.

Fin clippings from positively identified adult billfish (sailfish, blue marlin, and white marlin) collected off Abidjan, Africa (Gulf of Guinea) were provided by Bernard Stequert (Centre IRD de Brest, France). Dr. Jaime Alvarado Bremer (Texas A&M University) collected liver tissue from adult sailfish (Pacific and Atlantic specimens) and striped marlin and these samples were also used as sources of adult DNA. Dr. Andre Landry (Texas A&M University, Galveston) also provided tissue samples obtained from Pacific sailfish. Tissue from a single blue marlin from Kona, Hawaii, supplied by Dr. Barbara Block (Hopkins Marine Station, Stanford University), was assayed to determine the mtDNA signature characteristic of Pacific blue marlin (Clade I; Finnerty and Block 1992). The mtDNA CR-I sequences generated from these samples provided the baseline data for the forensic identification of the larval billfish. Table 2-1 describes the sampling details of the positively identified adult istiophorids that were used to generate the reference sequences for the identification of the larvae.

Table 2-1. Sampling details for adult sailfish.

Region	Collector	Capture Date	n	Locality
SAILFISH – ATLANTIC				
Gulf of Guinea	B. Stequert	December – October 1998	30	05°N, 04°W
Cancun, Mexico	J. Alvarado Bremer	June 1989	11	21°N, 86°W
SAILFISH – PACIFIC				
Los Sueños, Costa Rica	A. Landry	January 2006	16	9°N, 84°W
Acapulco, Mexico	J. Alvarado Bremer	July 1989	6	16°N, 99°W
BLUE MARLIN – ATLANTIC				
Gulf of Guinea	B. Stequert	March – December 1998	30	05°N, 04°W
BLUE MARLIN – PACIFIC				
Kona, Hawaii	B. Block	1997	1	19°N, 159°W
WHITE MARLIN				
Gulf of Guinea	B. Stequert	September – November 1998	8	05°N, 04°W
Sable Island, Canada	J. Alvarado Bremer	September 1990	5	67°N, 42°W
STRIPED MARLIN				
Cabo San Lucas, Mexico	J. Alvarado Bremer	July 1989	5	22°N, 106°W

Protocols for tissue digestion, mtDNA extraction and isolation, polymerase chain reaction (PCR), and sequencing are described in Appendices A and B. The resulting mtDNA sequences were aligned using ClustalW (Thompson et al. 1994) and edited using the sequence alignment editor MEGA 3.1 (Kumar et al. 2004). MEGA was also used to generate the neighbor-joining tree used in the forensic identification of the istiophorid larvae. DnaSP 4.10 (Rozas et al. 2003) was used to obtain basic diversity estimates including haplotype diversity (*h*), nucleotide diversity (π), the mean number of pairwise nucleotide differences (k), as well as the number of haplotypes (M) and the number of segregating sites (S) for all sailfish, blue marlin, white marlin, and striped marlin sequences generated. This software was also used to determine the corrected sequence divergence between all species pairs as well as between separate clades in both sailfish and blue marlin.

RESULTS

Identification of Istiophorid Larvae

The length of unambiguous nucleotide sequence of the mtDNA CR-I ranged from roughly 264 base pairs (bp) for sailfish to 350 bp for white marlin. Sequences were aligned and edited manually, and a consensus alignment 288 bp long was generated using sailfish as a reference. Although three distinct genera of the Family Istiophoridae were aligned, their sequences contained no tandem repeats and only a

few singleton insertion-deletion (indels) events were necessary to optimize the multiple sequence alignment. A CR-I alignment of a subsample of istiophorids (35 adults and 29 larvae) is shown in Figure 2-1. A comparison of 244 sailfish with 44 blue marlin showed a high corrected mean divergence value (D_A) of 35.6%. The respective comparisons of sailfish with white marlin (n=22) and with striped marlin (n=5) yielded higher nucleotide divergence values at 45.2% and 48.2%. Conversely, the nucleotide divergence between the blue marlin and white marlin was 47.0%, and between blue marlin and striped marlin was 50.0%. Finally, the lowest value of nucleotide sequence divergence was obtained between white marlin with striped marlin, with sequences diverging on average by only 5.8%. With exception of this last value, it should be noted that the extreme values of divergence most likely represent an underestimate given that it is probable that many nucleotide sites have suffered multiple hits. Accordingly, this segment contains little information to resolve the phylogenetic relationship among istiophorids. Instead, the large sequence divergence values underline the robustness of the CR-I to unambiguously identify each species of billfish assayed. Figure 2-2 represents the neighbor-joining tree generated from the sequences in Figure 2-1. A tree that includes all the larvae identified in this study can be found in Appendix D (Figure D-2). Each of the 204 larvae clustered among the positively identified adults of each of the four Atlantic istiophorid species used as reference in this study. Statistical support for nodes was estimated with 1000 non-parametric bootstrap replicates (Felsenstein 1985). The majority of the nodes grouping the branches of each species were extremely well-supported (>99%), illustrating the reliability of this approach for istiophorid species identification. Furthermore, the respective clades of sailfish (Graves and McDowell 1995) and blue marlin (Finnerty and Block 1992) are also extremely well supported (>92%). The corrected nucleotide divergence between Clades I and II of sailfish was 7.9%. The same comparison for blue marlin clades was three-and-a-half times higher (27.8%). The divergence between these clades and the phylogeographic interpretation addressing their origin will be discussed further in Chapter III.

```
IPLA283750003          CACACCCCGA CCCCGCA-A- -ATAAGCATA TAGCTTCTTG TATTTTAACC ATGGTGGGTC ACCATGACTT GA-TTGTAAC CATACAAGCA TTGACATTCT ATGTATTATC GTACATAAA- GTACA [125]
IPLA283750002          .......... .......-.- -......... .......... .......... .......... .......... ..-....... .......... C......... .......... .........- ..... [125]
IPLA283150004          .......... .......-.T -......... .....C.... ...C...... .........T .......... ..A..A.... .......... C.A....C.. .......... .........- ..... [125]
IPLA2851120002         .......... .......-.T -......... .....C.... ..CC...... .........T .......... ..-..A.... .......... C.A....C.. .......... .........- .C... [125]
IPLA284450001          .......... .......-.T -......... .....C.... ...C...... .........T .......... ..-..A.... .......... C.A....C.. .......... .........- ..... [125]
IPLA2841120003         .......... .......-.- -....-.... .......... ........T. ......A... .......... ..-....... .........G ........T. .......... .........- ..... [125]
IPLAnn01-28341200-004  .......... .......-.- -......... .......... C......... .......... .......... ..-....... .......... .......... .......... .........- ..... [125]
IPLAnn01-28311200-002  .......... .......-.- -......... .......... C......... .......... .......... ..-....... .......... .......... .......... .........- ..... [125]
IPLAnn01-28331200-005  .......... .......-.T -......... .....C.... ...C...... .......... .......... ..-..A.... .......... ..A....C.. .......... .......G.- ..... [125]
IPLAnn01-28321200-026  .......... .......-.T -......... .....C.... ...C...... .......... .......... ..-..A.... .......... C.A....C.. .......... .......G.- ..... [125]
IPLA077                .......... .......-.- -....-.... .......... ........T. ......A... .......... ..-....... .........G ........T. .......... .........- ..... [125]
IPLA126                .......... .......-.- -......... .......... .......... ......A... .......... ..-....... .......... .......... .......... .........- ..... [125]
IPLACAN5               .......... .......-.- -....-.... .......... ........T. ......A... .......... ..-....... .........G ........T. .......... .........- ..... [125]
IPLAPAC2               .......... .......-.T -......... .....C.... ...C...... .........T .......... ..-..A.... .......... C.A....C.. .......... .........- ..... [125]
IPLAPAC4               .......... .......-.T -......... .....C.... ...C...... .........T .......... ..-..A.... .......... C.A....C.. .......... .........- ..... [125]
IPLA257                .......... .......-.T -......... .....C.... ...C...... .........T .......... ..-..A.... .......... C.A....CT. .......... .........- ..... [125]
IPLA263                .......... .......-.T -......... .....C.... ...C...... .........T .......... ..-..A.... .......... C.A....C.. .......... .........- ..... [125]
IPLA272                .......... .......-.T -......... .....C.... ...C...... .........T .......... ..-..A.... .......... ..A....C.. .......... .........- ..... [125]
IPLA177                .......... .......-.- -......... .......... C......... .......... .......... ..-....... .......... .......... .......... .........- ..... [125]
IPLA163                .......... .......-.- -......... .......... .......... .......... ......G... ..-....... .......... .......... .......... .........- ..... [125]
MNIG283150011          ..T....... ....CA.A.A -TCG.A.GC. .GTT.C.C.. ..C.C..-TT .......A.T .....T.A.. ..-..A.... .......... .GATT.CAAC ...CT....T .G.......G AC.T. [125]
MNIG2729120003         ..T....... T...CACA.A -TC..ATGC. .GTT.C.... .....C.-.. .......... .....A.... ..-..A.... .......... .GATT..AAC ....C....T .G.......G AC.TG [125]
MNIG2761120001         ..T....... T...CA.A.A -TCG.A.GC. .GTT.C.C.. ..CCC..-TT .........T .....T.A.. ..-..A.... .......... .GATT.CAAC ...CT....T .G.......G AC.T. [125]
MNIG2831120004         ..T....... T...CA.A.A ATCG.A.GC. .GTTCC.C.. ..C.C..-TT .........T .....T.A.. ..-..A.... .......... .GACT.CAAC ....T..G.T .G.......G AC.T. [125]
MNIG272850001          ..T....... T...CACA.A -TC..ATGC. .GTT.C.C.. .....C.-.. ......A... .......... ..-..A.... .......... .GATT..AAC ....C....T .G.......G AC.TG [125]
MNIG272950002          ..T....... T...CACA.- -TC..ATGC. .GTT.C.C.. .....C.-.. .......... .....A.... ..-..A.... .......... .GATT..AAC ....C....T .G.......G AC.TG [125]
MNIG272950003          ..T....... T...CA.A.A -TCG.A.GC. .GTT.C.C.. ..C.C..-TT .......A.T .....T.A.. ..-..A.... .......... .GATT.CAAC ...CT....T .G.......G AC.T. [125]
MNIG272950004          ..T....... T...CA.A.A -TCG.A.GC. .GTTCC.C.. ..C....-TT .........T .....T.A.. ..-..A.... .......... .GACT.CAAC ...CT..G.T .G.......G AC.T. [125]
MNIG2728120001         ?????????? ?...CACA.A -TC..ATGC. .GTT.C.C.. .....C.-.. .......... .....A.... ..-..A.... .......... .GATT..AAC ....C....T .G.......G AC.TG [125]
MNIG2728120002         ..T....... T...CACA.A -TC..ATGC. .GTT.C.C.. .....C.-.. ......A... .......... ..-..A.... .......... .GATT..AAC ....C....T .G.......G AC.TG [125]
MNIG174                ..T....... T...CA.A.A -TCG.A.G-. .GTT.C.C.. ..CCC..-.T ........CT .....T.A.. ..-..A.... .......... .GATT.CAAC ...CT....T .G.......G AC.T. [125]
MNIG033                ..T....... T...CA.A.A -TCG.A.GC. .GTT.C.C.. ..CCC..-.T .......A.T .....T.A.. ..-..AC.... .......... .GATT.CAAC ...CT....T .G.......G AC.T. [125]
MNIG040                ..T....... T...CACA.A -TC..ATGC. .GTT.C.C.. .....C.-.. .......... .....A.... ..-..A.... .......... .GATT..AAC ....C....T .G.......G AC.TG [125]
MNIG089                ..T....... T...CACA.A -TC..ATGC. .GCT.C.C.. .....C.-.. .......... .....A.... ..-..A.... .......... .GATT..AAC ....C....T .G.......G AC.TG [125]
MNIG130                ..T....... T....ACA.A -TC..ATGC. .GTT.C.C.. .G...C.-.T ......A... ........C. ..-..A.... .......... .GATT..AAC ....C.C.CT .G.......G AC.TG [125]
MNIG164                ..T....... T...CACA.A -TC..ATGC. .GTT.C.C.. .....C.-.. .......... .....A.... ..-..A.... .......... .GATT..AGC ....C....T .G.......G .C.TG [125]
MNIG075                ..T....... T...CA.A.A -TCG.A.GC. .GTT.C.C.. ..CCC..-.T ........CT .....T.A.. ..-..A.... .......... .GATT.CAAC ...CT....T .G.......G AC.T. [125]
MNIG105                ..T....... T...CA.A.A -TCG.A.GC. .GTTCC.C.. ....C..-TT .........T .....T.A.. ..-..A.... .......... .GACT.CAAC ...CT..G.T .G.......G .C.T. [125]
MNIG123                ..T....... T...CA.A.A -TCG.A.GC. .GTT.C.C.. ..CCC..-.T .........T .....T.A.. ..-..A.... .......... .GATT.CAAC ...CT....T .G.......G AC.T. [125]
MNIG126                ..T....... T...CACA.A -TC..ATGC. .GTT.C.C.. .....C.-.. .......... .....A.... ..-..A.... .......... .GATT..AAC ....T....T .G.......G AC.TG [125]
TALBI2-1               ..T.T..TC. A..TA.GTGA -G...A.G.. .G--....CA ..CCC.G-.T .....TTA.T .....T.A.. T.-..A.... .......... .G.....G.- ....T..G.. .G.......C A.GGG [125]
TALBI2-10              ..T.T..TC. A..TA.GTGA -G...A.G.C CG--....CA ..CCC.G-.T .....TTA.T .....T.A.. T.-..A.... .......... .G.....GT- ....C..... .G.......C A.GGG [125]
TALBI2-12              ..T.T..TC. A..TA.GTGA -G...A.G.. .G--....CA ..CCC.G-.T .....TTA.T .....T.A.. T.-..A.... .......... .G.....G.- ....T..... .G.......C A.GGG [125]
TALBI2-13              ..T.T..TC. A..TA.GTGA -G...A.G.. .G--....CA ..CCC.G-.T .....TTA.T .....T.A.. T.-..A.... .......... .G.....G.- ....T..... .G.......C A.GGG [125]
TALBI020               ..T.T..TC. A..TA.GTGA -G...A.G.. .G--....CA ..CCC.G-.T .....TTA.T .....T.A.. T.-..A.... .......... .G.....G.- ....T..... .G.......C A.GGG [125]
TALBI022               ..T.T..TC. A..TA.GTGA -G...A.G.. .G--....CA ..CCC.G-TT .....TTA.T .....T.A.. T.-..A.... .......... .G.....G.- ....T..... .G.......C A.GGG [125]
TALBI027               ..T.T..TC. A..TA.GTGA -G...A.G.. .G--....CA ..CCC.G-.T .....TTA.T .....T.A.. T.-..A.... .......... .G.....G.- ....T....T .G.......C A.GGG [125]
TALBI028               ..T.T..TC. A..TA.GTGA -G...A.G.. .G--.....A ..CCC.G-.T .....TTA.T .....T.A.. T.-..A.... .......... .G.....GT- ....T..... .G.......C A.GGG [125]
TALBI030               ..T.T..TC. A..TA.GTGA -G...A.G.. .G--....CA ..CCC.G-.T .....TTA.T .....TGA.. T.-..A.... .......... .G.....G.- ....T..... .G.......C A.GGG [125]
TALBI032               ..T.T..TC. A..TA.GTGA -G...A.... .G--....CA ..CCC.G-TT .....TTA.T .....T.A.. T.-..A.... .......... .G.....G.- ....T..... .G.......C A.GGG [125]
TALBInn01-28351200-003 ..T.T..TC. A..TA.GTGA -G...A.... .G--....CA ..CCC.G-.T .....TTA.T .....T.A.. T.-..A.... .......... .G.....G.- ....T..... .G.......C A.GGG [125]
TALBInn01-27261200-002 ..T.T..TC. A..TA.GTGA -G...A.G.. .G--....CA ..CCC.G-.T .....TT..T .....TGA.. T.-..A.... .......... .G.....G.- ....T..... .G.......C A.GGG [125]
TALBInn01-28391200-005 ..T.T..TC. A..TA.GTGA -G...A.G.. .G--....CA ..CCC.G-.T .....TTA.T .....T.A.. T.-..A.... .......... .G.....G.- ....T..... .G.......C A.GGG [125]
TALBInn01-28341200-003 ..T.T..TC. A..TA.GTGA -G...A.G.. .G--....CA ..CCC.G-.T .....TTA.T .....T.A.. T.-..A.... .......... .G..T..G.- ....T..... .G.......C A.GGG [125]
TALBInn01-28341200-005 ..T.T..TC. A..TA.GTGA -G...A.G.. .G--....CA ..CCC.G-.T .....TTA.T .....T.A.. T.-..A.... .......... .G.....G.- ....T..... .G.......C A.GG. [125]
TALBInn01-28321200-021 ..T.T..TC. A..TA.GTGA -G...A.G.. .G--....CA ..CCC.G-.T .....TTA.T .....T.AC. T.-..A.... .......... .G.....G.- ....T..... .G.......C A.GGG [125]
TALBInn01-28321200-012 ..T.T..TC. A..TA.GTGA -G...A.G.. .G--....CA ..CCC.G-.T .....TTA.. .....T.A.. T.-..A.... .......... .G.....G.- ....T..... .G.......C A.GGG [125]
TALBInn01-28321200-014 ..T.T..TC. A..TA.GTGA -G...A.... .G--....CA ..CCC.G-.T .....TTA.T .....T.A.. T.-..A.... .......... .G.....G.- ....T..... .G.......C A.GGG [125]
TALBInn01-28321200-025 ..T.T..TC. A..TA.GTGA -G...A.G.. .G--....CA ..CCC.G-.. .....TTA.T .....T.A.. T.-..A.... .......... .G.....G.- ....T..... .G.......C A.GGG [125]
TAUD8                  ..T.T..AC. A...A.GTGA -G...A.G.. .G--....CA ..CC-.G-.T .....TTA.. .....T.A.C T.-...C... .......... .G.....G.- ....T....T .-......GC A.GG. [125]
TAUD3                  ..T.T..TC. A..T..GTGA -G...A.G.. .G--....CA ..CCC..-.T .....TTA.T .....T.AC. T.-..... .......... CG.....G.- ....T.C... .G......GC A.GA. [125]
TAUD4                  ?????????? ??.T..GTGA -G...A.G.. .G--....CA ..CCC..-.T .....TTA.. .....T.AC. T.-..... .......... .G.....G.- ....T.C... .G......GC A.GA. [125]
TAUD5                  ..T.T..TC. A..T..GTGA -G...A.G.. .G--....CA ..CCC..-.T .....TTA.T .....T.AC. T.-..... .......... .G.....G.- ....T.C... .G......GC A.GA. [125]
TAUD6                  ?????????? ???T..GTGA -G...A.G.C .G--....CA ...CC..-.T .....TTA.T .....T.A.. T.-..... .......... .G.....G.- ....T.C... .G......GC A.GA. [125]
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Figure 2-1. Consensus alignment of 288 bp long segment of the mtDNA CR-I of 64 istiophorids. IPLAn = Atlantic sailfish; IPLAPACn = Pacific sailfish; MNIGn = Atlantic blue marlin; TALBIn = white marlin; TAUDn = striped marlin; all other labels indicate istiophorid larvae.

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IPLA283750003          TGTACTGCTA AC---TGGAA TGTACTTGA- ATTCAAGTGA TAGCGTGCAT GGACTGAAGA ATCCAAT--- TACAGTCTTG CTTGCAACCG AACATACAGC TGCGG---TT AAGAGATTGT T---A [250]
IPLA283750002          .......... ..---..... .........- .......... .......... .......--- .......... .......... ...A.---.. .---. [250]
IPLA283150004          ....T..T.. GT---..... .........- .........G ...T.C.... .......... ......C--- ....A.T... .......T.. G......... ...A.---.C ...G...C.. .---. [250]
IPLA2851120002         .......T.G GT---..... .........- .......... ...T.C.... .......... ......C--- ....A.T... .......T.. G......... ...A.---.C ...G...C.. .---. [250]
IPLA284450001          ....T..T.. GT---..... .........- .......... ...T.C.... .......... ......C--- ....ACT... .......T.. G......... ...A.---.C ...G...C.. .---. [250]
IPLA2841120003         .......... ..---..... .........- .......... ...T..A... .......... ......C--- ....A..... .......... ........A. ...A.---.. .---. [250]
IPLAnn01-28341200-004  .......... ..---..... .........- .......... .......... .......... .......--- .......... .......... .........T ...A.---.. .---. [250]
IPLAnn01-28311200-002  .......... ..---..... .........- .......... .......... .......... .......--- .......... .......... ...A.---.. .---. [250]
IPLAnn01-28331200-005  ....T..T.. GT---..... .........- .......... ...T.C.... .......... ......C--- ....A.T... .......T.. G......... ...A.---.C ...G...C.C .---. [250]
IPLAnn01-28321200-026  ....T..T.. GT---..... .........- .......... ...T.C.... .......... ......C--- ....A.T... .......T.. G......... ...A.---.C ...G...C.C .---. [250]
IPLA077                .......... ..---..... .........- .......... ...T..A... .......... ......C--- ....A..... .......... ........A. ...A.---.. .---. [250]
IPLA126                .......... ..---..... .........- .......... .-........ .......... .......--- .......... .......... ...A.---.. .---. [250]
IPLACAN5               .......... ..---..... .........- .......... ...T..A... .......... ......C--- ....A..... .......... ........A. ...A.---.. .---. [250]
IPLAPAC2               ....T..T.. GT---..... .........- .......... ...T.C.... .......... ......C--- ....A.T... .......T.. G......... ...A.---.C ...G...C.. .---. [250]
IPLAPAC4               ....T..T.. GT---..... .........- .......... ...T.C.... .......... ......C--- ....A.T... .......T.. G......... ...A.---.C ...G...C.. .---. [250]
IPLA257                ....T..T.. GT---..... .........- .......... ...T.C.... .......... ......C--- ....A.T... .......T.. G......... ...A.---.C ...G...C.. .---. [250]
IPLA263                ....T..T.. GT---..... .........- .......... ...T.C.... .......... ......C--- ....A.T... .......T.. G......... ...A.---.C ...G...C.. .---. [250]
IPLA272                ....T..T.. GT---..... .........- .......... ...T.C.... .......... ......C--- ....A.T... .......T.. G......... ...A.---.C ...G...C.. .---. [250]
IPLA177                .......... ..---..... .........- .......... .......... .......... .......--- .......... .......... .......... ...A.---.. .---. [250]
IPLA163                .......... ..---..... .........- .......... .......... .......... .......--- .......... .......... .......... ...A.---.. .---. [250]
MNIG283150011          .ACTGACTA. C--A-.A..G .....C..C- .CCTG..CAG ..------CA AAGTAC..A- TC.TGG.TCA A...ACA..A A....G...A ..A-CTT... .ATCC-AGCC .G..AC.CAC ATAA. [250]
MNIG2729120003         .ACTGATTA. TTAAACA... ...G..C.C- G..TG.A.A. ..------CG A.GTAC..AT TCTGG.CCAA C.TGACA... GC.....T.A GG--CCTG.. CATCC-GG.C ....ATCCAC A-TA. [250]
MNIG2761120001         .ACTGACTA. C--A-.AA.G ........C- .CCTG.ACAG ..------CA AAGTAC..A- CC.TGG.TCA A...ACA..A A.C.TG...A GGA-CTT... .ATCC-AGCC ....AC.CAC ATAA. [250]
MNIG2831120004         .ACTGACTA. C--G-.AA.. .....C..C- ....G.ACA. .G------CA A.GTAC..A. CCTTGG.TTA AG..ACA..A A....G...A .GA-CCT... .ATCC-GG.C ..A.AC..AC ATAA- [250]
MNIG272850001          .ACTGATTA. TTAAACA... ...G..C.C- GC.TG.A.A. ..------CG A.GTAC..AT TCTGG.CCGA C.TGACA... GC.....T.A GG--CCTG.T CATCC-GG.C ....ATCCAC A-TA. [250]
MNIG272950002          .ACTGATTA. TTAAACA... ...G..C.C- G..TG.A.A. ..------CG A.GTAC..AT TCTGG.CCGA C.TGACA... G......T.A GG--CCTG.T CATCC-GG.C ....ATCCAC AATA. [250]
MNIG272950003          .ACTGACTA. C--A-.AA.G .....C..C- .CCTG..CAG ..------CA AAGTAC..A- TC.TGGCTCA A...ACA.CA A...TG...A ..A-CTT... .ATCC-AGCC .G..AC.CAC ATAA. [250]
MNIG272950004          .ACTGACTA. C--G-.AA.G .....C..C- ....G.ACA. .G------CA A.GTAC..A. CCTTGG.TTG AG..ACA..A A....G...A .GA-.CT... .ATCC-GGCC ..A.AC..AC ATAA- [250]
MNIG2728120001         .ACTGATTA. TTAAACA... ...G..C.C- G..TG.A.A. ..------CG A.GTAC..AT TCTGG.CCGA C.TGACA... GC.....T.A GG--CCTG.T CATCCCGG.C ....ATCC.C A-TA. [250]
MNIG2728120002         .ACTGATTA. TTAAACA... ...G..C.C- GC.TG.A.A. ..------CG A.GTAC..AT TCTGG.CCGA C.TGACA... GC.....T.A GG--CCTG.T CATCC-GG.C ....ATCCAC A-TA. [250]
MNIG174                .ACTGGCTA. C--A-.AA.G ........C- .CCTGGACAG ..------CA AAGTAC..A- CC.TGG.TCA A...ACA..A A.C.TG...A G.A-CTT... .ATCC-AGCC .....C.CAC ATAA. [250]
MNIG033                .ACTGACTA. C--AA.AA.G ........C- .CCTG.ACAG ..------CA AAGTAC..A- CC.TGG.TCA A...ACA..A A.C.TG...A G.A-CTT... .ATCC-AGCC ....AC.CAC ATAA. [250]
MNIG040                .ACTGATTA. TTAAACA... ...G..C.C- G..TG.A.A. ..------CG A.GTAC..AT TCTGGGCCAA C.TGACA... GC.....T.A GG--CCTG.T CATCC-GG.C ....ATCCAC A-TA. [250]
MNIG089                .ACTGATTA. TTAAACA... ...G..C.C- G..TG.A.A. ..------CG A.GTAC..AT TCTGG.CCGA C.TGACA... GC.....T.A GG--CCTG.T CATCC-GG.C ....ATCCAC A-TA. [250]
MNIG130                .ACTGGTTA. TTAAA.A.G. ...G..C.T- G..TG.A.A. ..------CG A.GTGC..AT TCTGGGCCAA C.TGACA... GC.....T.A GG--CCTG.. CATCC-GG.C ..A.ATC.AC A-TAG [250]
MNIG164                .ACTGATTA. TTAAACA... .C.G..C.C- G..TG.A.A. ..------CG A.GTAC..AT TCTGG.CCGA C.TGACA... GC.....T.A GG--CCTG.. CATCC-GG.C ....ATCCAC A-TA. [250]
MNIG075                .ACTGACTA. C--A-.AA.G ........C- .CCTG.ACAG ..------CA AAGTAC..A- CC.TG..TCA A...ACA..A A.C.TG...A G.A-CTT... .ATCC-AGCC ....AC.CAC ATAA. [250]
MNIG105                ..CTGGCTA. C--G-.AA.G .....C..C- ....G.ACA. .G------CA A.GTAC..A. CCTTGG.TTA AG..ACA..A A....G...A .GA-CCT... .ATCC-GG.C ..A.AC..AC ATAA- [250]
MNIG123                .ACTGACTA. C--A-.AA.G ........C- ..CTG.ACAG ..------CA AAGTAC..A- TC.TG..TCA A...ACA..A A.C..G...A G.A-CTT... .ATCC-AGCC ....AC.CAC ATAA. [250]
MNIG126                .ACTGATTA. TTAAACA... ...G..C.C- G..TG.A.A. ..------CG A.GTAC..AT TCTGG.CCGA C.TGACA... GC.....T.A GG--CCTG.T CATCC-GG.C .G..ATC??C A-TA. [250]
TALBI2-1               .A.TAA.GAG ..---CA.G. CA.G.A..CC G.A.....A. ..-T----T. A.CA.CT.A. T..T.GCTTG .G....A... T..T...A.C .G--.C.G.T CA.A----.. ...C..CCAC A---. [250]
TALBI2-10              .A.TAA.GAG ..---CA.GG CA.G.AC.CC G.A....CA. ..-T----T. A.CA.CT.A. T..T.GCTTG .G....A... ...C..GA.C .G--.C.GAT .A..----.. ..AC..CCAC A---. [250]
TALBI2-12              .A.TAA.GAG ..---CA.G. CA.G.A..CC G.A.....A. C.-T----T. A.CA.CT.A. T..T.GCTTG .G....A... ..CTT..A.C .G--.CTG.T CA.A----.. ...C..CCAC A---. [250]
TALBI2-13              .A.TAA.GAG ..---CA.G. CA.GTA..CC G.A.....A. ..-T----T. AACA.TT.A. T..T.GCTTG .G....A... ...T...A.C .G--.C.GAT CA.A----.. ...CA.CCAC A---. [250]
TALBI020               .A.TAA.GAG ..---CA.G. C..GTA..CC G.A.....A. C.-T----T. A.CA.CT.A. T..T.GCTTG .G....A... ...T...A.C .G--.C.G.T .A.A----.. ...CA.CCAC A---. [250]
TALBI022               .A.TAA.GAG ..---CA.G. CA.G.A..CC G.A.....A. C.-T----T. A.CA.CT.A. T..T.GCTTG .G....G... ...T...A.C .G--.CTG.. CA.A----.. ...C..CCAC A---. [250]
TALBI027               .A.TAA.GAG ..---CA.G. CA.GTA..CC G.A....CA. C.-T----T. AACA.CC.A. T..T.GCTTG .G....A... ...T...A.C .G--.C.G.T CA.A----.. ...CA.CCAC A---. [250]
TALBI028               .ACTAA.GAG ..---CA.G. CA.GTAC.CC G.A.....A. ..-T----T. A.CA.CT.A. T..T.GCTTG .G....A... ...TT..A.C .G--.TTG.T CA.A----.. ...C..CCAC A---. [250]
TALBI030               .A.TAA.GAG ..---CA.G. CA.G.A..CC G.A.....A. C.-T----T. A.CA.CT.A. T..T.GCTTG .G....A... ...TT..A.C .G--.CTG.T CA.A----.. ...C..CCAC A---. [250]
TALBI032               .A.TAA.GAG ..---CA.GG CA.G.AC.CC GCA..-.CA. ..-T----T. A.CA.CT.A. T..T.GCTTG .G....A... ...TT.GA.C .G--.C.GAT .A..----.. ...C..CCAC A---. [250]
TALBInn01-28351200-003 .A.TAA.GAG ..---CA.GG CA.G.AC.CC GCA..-.CA. ..-T----T. A.CA.CT.A. T..T.GCTTG .G....A... ...TT.GA.C .G--.C.GAT .A..----.. ...CA.CCAC A---. [250]
TALBInn01-27261200-002 .A.CAA.GAG ..---CA.G. CA.G.A..CC G.A.....A. ..-T----T. A.CA.CT.A. T..T.GCTTG .G....A... ...TT..A.C ..--.CTG.T CA.A----.. ...C..CCAC A---. [250]
TALBInn01-28391200-005 .???AA.GAG ..---CA... CA.G.A..CC G.A.....A. C.-T----T. A.CA.CT.A. T..T.GCTTG .G....A... ...TT..A.C .G--.CTG.. CA.A----.. ...C..CCAC A---. [250]
TALBInn01-28341200-003 .A.TAA.GAG ..---CA.GG CA.G.AC.CC G.A....CA. ..-T----T. A.CA.CT.A. T..T.GCTTG .G....A... ...C..GA.C .G--.C.GAT .A..----.. ...C..CCAC A---. [250]
TALBInn01-28341200-005 .A.TAA.GAG ..---CA.G. CA.G.A..CC G.A.....A. ..-.----C. A.CA.CTGA. T..T.GCTTG .G....A... ..CT...A.C .G--.CTG.T CA.A----.. ...CA.CCAC A---. [250]
TALBInn01-28321200-021 .A.TAA.GAG ..---CA.GG CA.G.AC.CC G.A....CA. ..-.----T. A.CA.CT.A. T..T.GCCTG .G....A... ...C..GA.C .G--.C.GAT .A..----.. ...C..CCAC A---. [250]
TALBInn01-28321200-012 .A.TAA.GAG ..---CA.G. CA.GTA..CC G.A.....A. ..-T----T. A.CA.CT.A. T..T.GCTTG .G....A... ...T...A.C .G--.CTG.. CA.A----.. ...C..CCAC A---. [250]
TALBInn01-28321200-014 .A.TAA.GAG ..---CA.GG CA.G.AC.CC GCA..-.CA. ..-T----C. A.CA.CT.A. T..T.GCTTG .G....A... ...TT.GA.C .G--.C.GAT .A..----.. ...C..CCAC A---. [250]
TALBInn01-28321200-025 .A.TAA.GAG ..---CA.G. CA.G.A..CC G.A.....A. C.-T----T. A.CA.CT.A. T..T.GCTTG .G....A... ...T...A.C .G--.C.G.T CA.A----.. ...C..CCAC A---. [250]
TAUD8                  .A.TAA.GAG ..---CA.G. CA.GTAC.CC G.A....C.. .G-.----T. A.TACAT.A. T....GCTTG ......A... ...T..GA.C .G--.C.GAT .A.A----.. ...CA.CCAC A---. [250]
TAUD3                  .A.TAA.AAG ..---CA.GG CA.GTAC.C- GCG....CA. ..AT----C. A.CA..T.A. T..T.GCTTG .G....A... ...T..GA.C .G--.CTGAT .A..----.. ...C...CAC A---. [250]
TAUD4                  .A.TAA.GAG ..---CA.GG CA.GTAC.C- G.G....CA. .GAT----CC A.CA..T.A. T..T.GCTTG .G....A... ...T..GA.C .G--.CTCAT .A.A----.. ...C...CAC A---. [250]
TAUD5                  .A.TAA.AAG ..---CA.GG CA.G.AC.C- GCA....CA. ..AT----CC A.CA..T.A. T..T.GCTTG .G....A... ...T..GA.C .G--.TTGAT .A.A----.. ...C....AC A---. [250]
TAUD6                  CA.TAA.GAG ..---CA.GG CA.G.AC.C- G.A....CA. ..ATT---CC A.CA..T.A. T..T.GCTTG .G....A... ...T..GA.C .G--.TTGAT .A.A----.. ...CAG.CAC A---. [250]
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Figure 2-1. Continued.

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IPLA283750003          AACTGTCAAT TAATAATGCC ATGCGCAGTA AGAGACCACC AAC [293]
IPLA283750002          .......... .......... .......... .......... ... [293]
IPLA283150004          ........G. CG........ .......... .......... ... [293]
IPLA2851120002         ........G. CG........ .......... .......... ... [293]
IPLA284450001          ........G. CG........ .......... .......... ... [293]
IPLA2841120003         .......... .......... .......... .......... ... [293]
IPLAnn01-28341200-004  .......... .......... .......... .......... ... [293]
IPLAnn01-28311200-002  .......... .......... .......... .......... ... [293]
IPLAnn01-28331200-005  ........G. CG........ .......... .......... ... [293]
IPLAnn01-28321200-026  ........G. CG........ .......... .......... ... [293]
IPLA077                .......... .......... .......... .......... ... [293]
IPLA126                .......... .......... .......... .......... ... [293]
IPLACAN5               .......... .......... .......... .......... ... [293]
IPLAPAC2               ........G. CG........ .......... .......... ... [293]
IPLAPAC4               ........G. CG........ .......... .......... ... [293]
IPLA257                ........G. CG........ .......... .......... ... [293]
IPLA263                ........G. CG........ .......... .......... ... [293]
IPLA272                ........G. CG........ .......... .......... ... [293]
IPLA177                .......... .......... .......... .......... ... [293]
IPLA163                .......... .......... .......... .......... ... [293]
MNIG283150011          CC..TGT.GC A.GC....TA ..AT...A.. .....T.... ..T [293]
MNIG2729120003         CC..TGT.GC AGGC....TA .......... ...A...... ..T [293]
MNIG2761120001         CC..TGT..C A.GCG...TA ..AT...A.. .....T.... ..T [293]
MNIG2831120004         CC..TGT.GC A.GCG...TA ...T...A.. ...A.T.... ..T [293]
MNIG272850001          CC..TGT.GC AGGC....TA .......... ...A...... ..T [293]
MNIG272950002          CC..TGT.GC AGG.....TA .......... ...A...... ..T [293]
MNIG272950003          CC..TGT.GC A.GC....TA ..AT...A.. .....T.... ..T [293]
MNIG272950004          CC..TGT.GC A.GCG...TA ...T...A.. ...A.T.... ..T [293]
MNIG2728120001         CC..TGT.GC AGGCC...TT .......... ...A...... ..T [293]
MNIG2728120002         CC..TGT.GC AGGC....TA .......... ...A...... ..T [293]
MNIG174                CC..TGT..C A.GCG...TA ..AT...A.. ...A.T.... ..T [293]
MNIG033                CC..TG...C A.GCG...TA ..AT...A.. .....T.... ..T [293]
MNIG040                CC..TGT.GC AGGC....TA .......... ...A...... ..T [293]
MNIG089                CC..TGT.GC AGGC....TA .......... ...A...... ..T [293]
MNIG130                CT..TGT.GC AGGC....TA ....A..... .....T.... ..T [293]
MNIG164                CT..TGT.GC AGGC....TA .......... ...A...... ..T [293]
MNIG075                CC..TGT..C A.GCG...TA ..AT...A.. .....T.... ..T [293]
MNIG105                CC..TGT.GC A.GCG...TA G..T...A.. ...A.T.... ..T [293]
MNIG123                CC..TGT..C A.GCG...TA ..AT...A.. .....T.... ..T [293]
MNIG126                CC..TGT.GC AGGC....TA .......... ...A...... ..T [293]
TALBI2-1               TC.CT.A.CA G.G....T.A .......... ...A...... ... [293]
TALBI2-10              TT.CT.A.CG G.G....T.A .......... ...A...... ... [293]
TALBI2-12              TC.CT.A.CA G.G....T.A .......... .......... ... [293]
TALBI2-13              TC.CT.A.CA G.G....T.A .......... ...A...... ... [293]
TALBI020               TC..T.A.CA G.G....T.A .......... ...A...... ... [293]
TALBI022               TC.CT.A.CA G.G....TTA .......... ...A...... ... [293]
TALBI027               TC.CT.A.CA G.G....T.A .......... ...A...... ... [293]
TALBI028               TC.CT.A.CA A.G....T.A .......... ...A...... ... [293]
TALBI030               TC.CT.A.CA G.G....T.A .......... ...A...... ... [293]
TALBI032               TC.CT.A.CA G.G....T.A .......... ...A...... ... [293]
TALBInn01-28351200-003 TC.CT.A.CA G.G....T.A .......... ...A...... ... [293]
TALBInn01-27261200-002 TC.CT.A.CA G.G....T.A .......... ...A...... ... [293]
TALBInn01-28391200-005 TC.CT.A.CA G.G....T.A .........G ...A...... ... [293]
TALBInn01-28341200-003 TT.CT.A.CG A.G....T.A .......... ...A...... ... [293]
TALBInn01-28341200-005 TC.CT.A.CA G.G....T.A .......... ...A...... ... [293]
TALBInn01-28321200-021 TT.CT.A.CG G.G....T.A .......... ...A...... ... [293]
TALBInn01-28321200-012 TC.CT.A.CA G.G....T.A .......... ...A...... ... [293]
TALBInn01-28321200-014 TC.CT.A.CA G.G...CT.A .......... ...A...... ... [293]
TALBInn01-28321200-025 TC.CT.A.CA G.G....T.A .......... ...A...... ... [293]
TAUD8                  TC.CT.A.CA A.G....T.A .......... .......... ... [293]
TAUD3                  TC.CT.A.CA AGGG...TTA .C........ ...A...... ... [293]
TAUD4                  TC.CT.A.CA AGG....TTA .......... ...A...... ... [293]
TAUD5                  TC.CT.A.CA AGG....TTA .......... ...A...... ... [293]
TAUD6                  TT.CT.A.CA AGG....TTA .......... ...A...... ... [293]
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Figure 2-1. Continued.

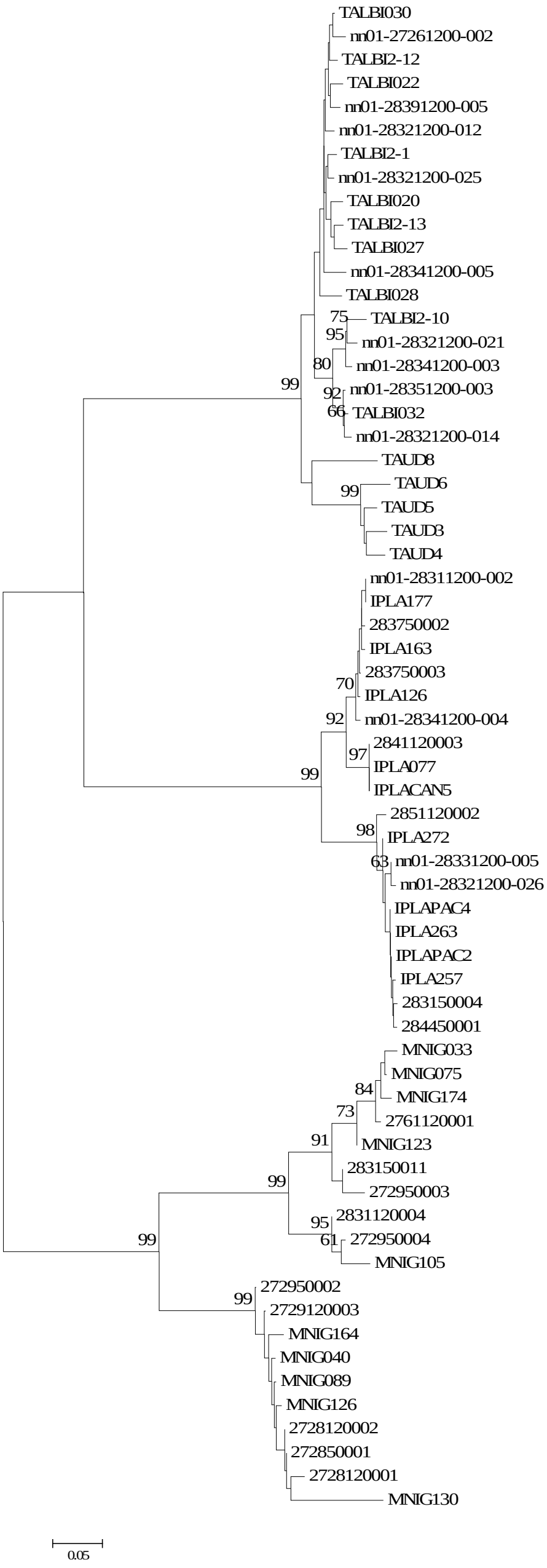


Figure 2-2. Example of a neighbor-joining tree, using Tamura-Nei distance, constructed for the purpose of the forensic identification of istiophorid larvae. A total of 1000 resampling replicates were conducted and bootstrap values above 60% are listed. IPLAn = Atlantic sailfish; IPLAPACn = Pacific sailfish; MNIGn = Atlantic blue marlin; TALBIn = white marlin; TAUDn = striped marlin; all other labels indicate istiophorid larvae. The tree is unrooted.

In total, 204 istiophorid larvae were collected during two research cruises conducted in 2005 and each larva was unambiguously identified to the species level (Table 2-2).

Table 2-2. Summary of catch numbers for istiophorid billfish larvae over 2 cruises.

	Sailfish	Blue Marlin	White Marlin	**TOTAL**
May 2005	46	-	9	55
July 2005	135	14	-	149
TOTAL	181	14	9	204

While sailfish larvae were collected during both cruises, white marlin were sampled only in the first trip (May 2005) and blue marlin only in the second trip (July 2005). A more detailed collection list illustrating the number of istiophorid larvae collected at each sampling location during each cruise is in Appendix D (Table D-2).

Once identified, the larval sequences were pooled with those of conspecific adults in order to obtain basic diversity estimates for each species (Table 2-3). Sailfish, white marlin, and striped marlin all exhibited similar levels of nucleotide diversity, ranging between 5.1 – 6.6%, while the level of nucleotide diversity observed for the blue marlin was nearly three times as high.

Table 2-3. Summary of diversity statistics for all istiophorid billfish in this study.

Species	n	S	M	*h* (SD)	π (SD)	k (SD)
Sailfish	244	81	121	0.954 (0.008)	0.05668 (0.0018)	11.222 (7.519)
Blue Marlin	44	103	39	0.994 (0.007)	0.17054 (0.00933)	32.232 (21.661)
White Marlin	22	53	22	1.000 (0.014)	0.05120 (0.00317)	11.519 (4.623)
Striped Marlin	5	45	5	1.000 (0.126)	0.06645 (0.01916)	20.200 (11.745)

DISCUSSION

Direct sequencing of the mtDNA CR-I of istiophorid billfish provided an unambiguous identification of istiophorid larvae. Specimens could be processed in only a couple days at the cost of no more than six dollars per sample. Moreover, the large nucleotide divergences between istiophorid species pairs provided assurance that the identifications of the larval samples were correct. Shepard and Hartmann (1996) developed a sandwich-style immunoassay (sEIA) for the rapid identification of sailfish, reporting that 18 samples could be processed in one hour in a non-laboratory setting. The multiplex PCR developed by Hyde et al. (2005) is also attractive because it supplies near real-time species identification in the field. However, very few studies would only be interested in species identification, and future amplification and

analysis would most likely be desired. By direct sequencing, not only were we able to obtain unambiguous species identifications, but in addition we acquired a wealth of information to investigate the contemporary population structure and historical demography and phylogeography of sailfish.

The values of haplotypic diversity obtained in this study give an insight into the amounts of genetic variation within four species of istiophorid billfishes. Graves and McDowell (1995) used RFLP data from whole mtDNA and found that haplotype diversity among sailfish was 0.85 in a pooled sample containing individuals from both the Atlantic and the Indo-Pacific. A possible explanation for the lower observed haplotypic diversities in this latter study is that the use of four-base pair restriction endonucleases would have resulted in the production of many short restriction fragments that most likely could not be scored in agarose gels. In a study of the Arabian Gulf sailfish for evidence intraspecific phylogeographic association, Hoolihan et al. (2004) used four, five, and six base pair restriction endonucleases to target the mtDNA CR-I and by separating restriction fragments on polyacrylamide gels were able to calculate haplotypic diversities ranging from 0.82 to 0.94. Thus, although the haplotypic diversity calculated here appears high (0.95) in comparison to the value obtained by Graves and McDowell (1995), it is probably a more accurate reflection of the actual nucleotide diversity present in the CR-I of sailfish. Using Cyt b sequence data Finnerty and Block (1992) estimated that haplotypic diversity was 0.74 for a sample of blue marlin from the Atlantic Ocean. However, we detected a considerably larger value in our Atlantic blue marlin sample (0.99). Similarly, nucleotide diversity values estimated here for white marlin (1.00) and in striped marlin (1.00) are considerably higher than those estimated by Graves and McDowell (1995) at 0.45, and 0.82, respectively.

The temporal distribution of the larvae of the three istiophorid billfishes identified in this study was consistent with previous studies (deSilva and Breeder 1997). Spawning of white marlin typically occurs from March to June, consistent with the May capture of white marlin larvae. Similarly, deSilva and Breeder (1997) reported that blue marlin spawning activity occurs primarily between July and October, again consistent with our larval catch. Finally, the extended spawning period of sailfish from April to October (Voss 1953) encompasses our collection of these larvae, and the observed increase in abundance during the July cruise is coincident with the peak spawning season of this species in June and July (deSilva and Breeder 1997). Thus, the northern Gulf of Mexico indeed appears to be a ‘hot spot’ for istiophorid larvae in general and sailfish larvae in particular. Richards et al. (1993) noted that the distribution of bluefin tuna larvae indicated that spawning was associated with the boundary of the Loop Current, the major hydrographic feature of the GoM. This current, which enters the GoM via the Yucatan Channel, proceeds northward, and then loops east to exit through the Florida Straits to eventually become the Gulf Stream, is fast-moving (50 to 200 cm/sec) and highly variable in position (Leipper 1970). At the time of our sampling, the Loop Current may have been in the midst of an intrusion, bringing its outer western edge to the outer eastern edge of our sampling grid (Figure D-1). Whether the istiophorid larvae identified in this study

resulted from a distant spawning event, being carried to their capture location via the swift-moving current, or if spawning and capture locations are proximal and there is larval retention occurring as a result of some association with the Loop Current remains to be assessed. Pending analyses of otolith chemistry and RNA/DNA ratios will hopefully provide some clues with respect to the spawning location of these istiophorid larvae.

CHAPTER III

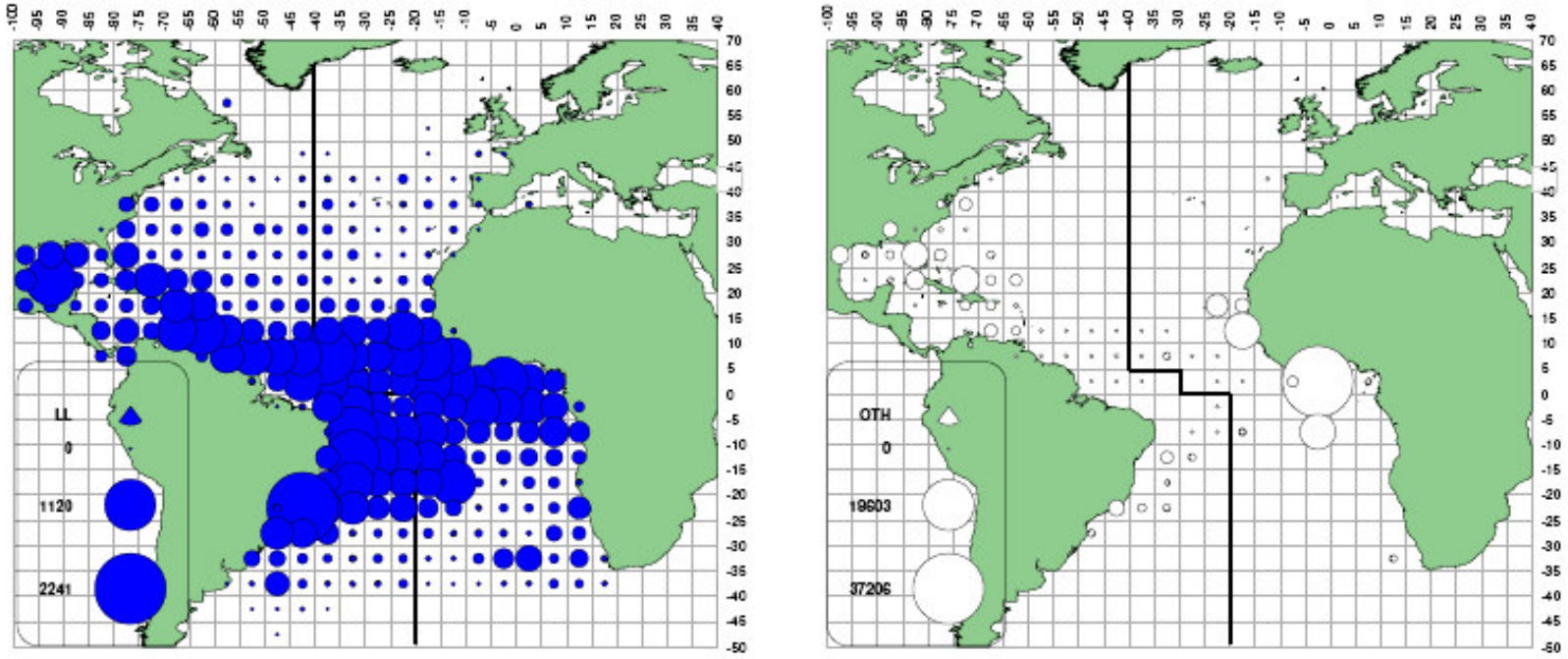
CONTEMPORARY POPULATION STRUCTURE AND HISTORICAL DEMOGRAPHY OF THE SAILFISH, *Istiophorus platypterus*

INTRODUCTION

Sailfish is a highly migratory, epipelagic, circumtropically distributed istiophorid species found commonly in neritic waters (Ovchinnikov 1970, Beardsley et al. 1972, Hoolihan 2004), but distributed widely through the tropical Atlantic (Figure 3-1). These fish predominantly swim above the thermocline in water ranging from 21 to 28° C (Nakamura 1985). Most reports of sailfish schooling behavior are vague; Voss (1953), however, observed that they appear diffusely distributed in warmer months but then congregate into schools upon the onset of cooler weather. Spawning occurs between April and October and the resulting larvae concentrate their feeding efforts on copepods (Ovchinnikov 1970). Adult sailfish, while primarily piscivorous, also feed on squid and other available invertebrates (Evans and Wares 1972). Sailfish are sexually dimorphic with females attaining larger sizes than males (Jolley 1977). The larger females are highly fecund, capable of producing 2.3 million to 4.7 million eggs (Voss 1953).

While the taxonomic status of sailfish was surrounded by controversy for decades, culminating with the recognition of two species, the Pacific sailfish (*Istiophorus platypterus*) and the Atlantic sailfish (*I. albidus*; Nakamura 1985), a thorough comparison of morphometric and meristic characters supports a single cosmopolitan species (Morrow and Harbo 1969). Genetic studies reveal that significant genetic differences exist between Atlantic and Pacific sailfish populations, but that such differences do not warrant their recognition as separate species (Graves and McDowell 2003). Instead, the observed genetic differences suggest that the Pacific and the Atlantic populations were separated by one or more vicariant events. Such events include closure of the Panama seaway during the Pleistocene (about 3 million years ago; Grant 1987), and cooling of water around the southern tip of South Africa during the Pliocene (approximately 1.8 million years ago; Graves and McDowell 1995). Here, sailfish (*Istiophorus platypterus*) is recognized as a monotypic species found in the Atlantic, Pacific, and Indian Oceans.

Because it is a component of bycatch in commercial fisheries targeting tunas and swordfish, the Atlantic sailfish population is considered over-fished in U. S. waters (Idrisi et al. 2003). Preliminary analyses of abundance indices indicate that a decline in biomass has occurred in the Atlantic (Restrepo et al. 2003; NMFS 2004). The U. S. Fisheries Management Plan for Atlantic billfish prohibits commercial harvest of sailfish, reserving these valuable stocks for recreational purposes only (Goodyear 1999). Two areas of concern in the conservation genetics of marine fishes include: (i) the genetic structure of populations, and (ii) the effects of over-exploitation on the levels of genetic variability. The first line of study is important because management decisions are based on the assumption of a particular population (= stock) structure. The second concern is relevant because of the potential for reduction in the effective size



Longline Data Other Gear

Figure 3-1. Distribution of Atlantic sailfish in metric tons based on commercial catch data from 1950-2003 (ICCAT 2005).

of the population, which may be critical when considering the long-term adaptability of a species (Hauser et al. 2002; Turner et al. 2002).

There is little information about levels of genetic variability in Atlantic sailfish. Graves and McDowell (1995) conducted a restriction fragment length polymorphism (RFLP) study on whole molecule mtDNA to examine the population structure of Atlantic sailfish, comparing 36 individuals from two locations in the Atlantic, and found no evidence of transatlantic differentiation. In the same study, Graves and McDowell (1995) compared the Atlantic sample to 33 individuals collected from two separate Pacific locations and found significant inter-oceanic differences in this species. These seminal studies, while they provided useful baseline data about intra- and inter-oceanic variation, may have underestimated the amount of genetic variability as a result of small sample sizes and because RLFP data from whole mtDNA molecule was characterized through agarose gels which lack the resolution necessary to resolve the small fragments generated by four base pair cutter restriction endonucleases. Finnerty and Block (1992) claimed that such a technique could not be expected to detect fine differences expected at an intra-ocean scale. In a PCR-RFLP study of the mtDNA CR-I, Hoolihan et al. (2004) characterized the fragment polymorphisms through acrylamide gels and were able to detect significant differences between sailfish from the Arabian Gulf compared to those outside the Gulf, despite the relatively close proximity of these populations. Thus, the mtDNA the control region appears to contain sufficient resolution to resolve intra-oceanic differentiation in sailfish. Furthermore, employing direct sequencing should enhance the resolution of this segment. In a study examining the intraspecific nucleotide variation in the control region of white sturgeon (*Acipenser transmontanus*) from the Fraser River in British Colombia, Canada and the Colombian River in Washington, USA, Brown et al. (1993) direct sequenced a 462 nucleotide portion of the control region and found 19 unique mtDNA CR-I haplotypes in 27 individuals compared with the detection of only 10 haplotypes in 178 individuals with RFLPs. By direct sequencing 330 base pairs of the CR-I of swordfish, Alvarado Bremer et al. (1996) demonstrated that the North Atlantic and Mediterranean populations of swordfish are genetically distinct.

Using mtDNA and microsatellite markers in conjunction with allozyme and single copy nuclear DNA, Buonaccorsi et al. (1999) detected a significant difference in genetic variation in another istiophorid, the blue marlin (*Makaira nigricans*), from Atlantic and Pacific Oceans. Microsatellites have high mutation rates often resulting in many alleles differing in the number of short tandem repeats, and can be distinguished by determining the exact length of the DNA fragment (Estroup et al. 1995). Due to this pronounced hypervariability, microsatellite loci are often used in conjunction with mtDNA data in order to improve the resolution of analyses. Here they were used to help detect intra- and inter-oceanic variation among sailfish populations as well as to assess kinship among larvae that share the same mitotype (Chapter IV).

I used 264 base pairs of sequence from the hypervariable mitochondrial control region (CR-I) and fragment polymorphisms from five microsatellite loci to make comparisons between 244 individuals from 3 Atlantic localities (n = 222) and two eastern Pacific localities (n = 22). This information was used to determine if there was any contemporary intra-oceanic genetic differentiation between eastern and western Atlantic populations of sailfish stocks (ICCAT 2004). While sailfish distribution may suggest the potential for considerable transatlantic movement (Figure 3-1), the catch data may not accurately reflect seasonal changes in the distribution of temporally and or geographically discrete subpopulations. In addition, inter-oceanic migration may be severely restricted. Land masses that extend into cold waters, such as the southern tip of South Africa, may present an additional barrier to dispersal, limiting the mixing of individuals from these separate ocean basins. Accordingly, I also used mtDNA and microsatellite data to resolve inter-oceanic differences in sailfish. In addition, of the historical demography of sailfish was reconstructed from the patterns of variation contained in the CR-I and this information was compared against the phylogeographic interpretations offered for other cosmopolitan species of highly migratory fishes as they relate to inter-oceanic connectivity and habitat availability determined by the paleoceanographic conditions of the Atlantic and Indo-Pacific during the Pliocene and Pleistocene.

METHODS

Protocols for tissue digestion, DNA extraction and isolation, polymerase chain reaction (PCR), sequencing, and fragment mobility analysis are described in Appendices A, B, and C. It should be noted that the DNA of the larvae from the second cruise was too degraded to obtain successful amplifications of the microsatellite loci, and thus only CR-I data was obtained for these specimens. GENEPOP 3.3 (Raymond and Rousset 1995) was used to calculate allele frequencies, the number of alleles per locus and conduct the exact test for Hardy-Weinberg equilibrium. Arlequin 3.0 (Excoffier et al. 2005) was used to perform the analyses of molecular variance for both mtDNA and microsatellite data (AMOVA; Excoffier et al. 1992), generate the mismatch distributions, calculate F_{ST} values, estimate the number of migrants between localities, and estimate past demographic parameters (θ_0, τ, θ_1), which were then used to approximate the historical female effective population size of sailfish in the Atlantic Ocean following the method of Rogers and Harpending (1992). Tajima's D (Tajima 1989) test of selective neutrality was computed with DnaSP 4.10 (Rozas et al. 2003) and used to test for the sudden expansion model. Any statements regarding statistical significance were based on $\alpha = 0.05$.

RESULTS

Genetic Diversity within Populations of Sailfish

mtDNA

264 base pairs (bp) of mtDNA CR-I sequence were generated from 244 sailfish, including larvae from the Gulf of Mexico (n = 181), adults from the eastern Pacific Ocean (n = 22), adults from the western Atlantic Ocean (Cancun; n = 11), and eastern Atlantic adults (Gulf of Guinea; n = 30). Collection

information for these fish was given in Chapter II (Table 2-1). Sailfish mtDNA CR-I contained a total of 81 segregating (polymorphic) sites, 59 of which were parsimony informative. These polymorphisms defined 121 haplotypes among 244 individuals, giving the sample an overall haplotypic diversity of 0.954 ± 0.008 and nucleotide diversity of 0.05668 ± 0.00118. Table 3-1 summarizes the basic diversity statistics for respective samples of sailfish according to the region of capture. Briefly, values of haplotypic diversity, nucleotide diversity, and mean number of pairwise differences were substantially lower in the Pacific sample than in the Atlantic samples, with the highest diversity values found the Eastern Atlantic sample.

Table 3-1. Summary of diversity indices for sailfish within the regions of capture.

Population	n	S	M	*h* (SD)	π (SD)	k (SD)
Cruise #1 Larvae	46	55	32	0.970 (0.014)	0.05665 (0.00513)	12.690 (9.061)
Cruise #2 Larvae	135	76	82	0.969 (0.009)	0.05539 (0.00209)	11.466 (7.731)
Eastern Atlantic	30	63	26	0.984 (0.016)	0.07006 (0.00429)	16.324 (10.213)
Western Atlantic	192	81	101	0.960 (0.009)	0.05352 (0.00185)	10.918 (7.486)
Atlantic pooled	222	80	114	0.952 (0.009)	0.05502 (0.00172)	10.895 (7.282)
Pacific	22	25	13	0.840 (0.078)	0.01130 (0.00370)	2.926 (3.431)

Microsatellite Assays

96 sailfish (43 larvae from the May 2005 cruise, 8 adults from the western Atlantic, 22 adults from the eastern Atlantic, and 21 Pacific adults) were successfully amplified targeting 5 tetranucleotide microsatellite loci. Four of the five microsatellite loci analyzed were found to be in Hardy-Weinberg Equilibrium (HWE) at all locations, with the exception of locus Mn60 (Table 3-2). This locus exhibited heterozygote deficiencies in all populations tested except for the eastern Atlantic, however, we are confident that the observed deviations from HWE are likely the result of the presence of null alleles and that the frequency of their occurrence is sufficiently low not to affect the interpretation of the data (for a more detailed discussion of null alleles see Chapter IV). Furthermore, analyses conducted with this locus omitted yielded the same conclusions as those obtained before its omission.

The microsatellite loci were also highly variable with a total of 115 alleles and averaging 23 alleles per locus. At locus Mn60, 39 alleles ranged in size from 232 to 448 bp. Locus Mn90 was represented by 36 alleles, ranging from 234 bp to 412 bp in length. Loci Mn01 and Mn08 had 12 and 11 alleles ranging from 232 bp to 288 bp and 226 bp to 277 bp, respectively. Lastly, 17 alleles ranging from 261 bp to 282 bp base pairs in length were observed at locus Mn10. A table containing the frequency of the alleles at the five microsatellite loci is located in Appendix D (Table D-3). As previously observed in the mtDNA genetic diversity assessment, the eastern Atlantic sailfish population had the highest (by a slight

margin) average gene diversity and the Pacific population exhibited the lowest value of diversity (Table 3-3).

Table 3-2. P-value (standard error) of Hardy-Weinberg Equilibrium Exact Test at each locus for the pooled sailfish sample, the eastern and western Atlantic populations, and the Atlantic and Pacific populations. The null hypothesis was that there was no evidence of heterozygote deficiency.

Population	Mn60	Mn90	Mn01	Mn08	Mn10
All Sailfish	0.0092	0.5164	0.7795	0.9962	0.0689
	(0.0092)	(0.0476)	(0.0195)	(0.0019)	(0.0162)
Eastern Atlantic	0.2897	1.0000	0.3579	0.9880	0.1996
	(0.0413)	(0.0000)	(0.0157)	(0.0043)	(0.0264)
Western Atlantic	0.0404	0.0879	0.7272	0.9440	0.4639
	(0.0191)	(0.0241)	(0.0184)	(0.0130)	(0.0381)
Atlantic pooled	0.0000	0.2681	0.6532	0.9993	0.2314
	(0.0000)	(0.0422)	(0.0237)	(0.0005)	(0.0293)
Pacific	0.0485	1.0000	1.0000	0.9625	0.5705
	(0.0179)	(0.0000)	(0.0000)	(0.0099)	(0.0158)

Table 3-3. Diversity indices calculated using 5 microsatellite loci.

Population	h	n	Gene Diversity (S.D.)	Mean Number of Pairwise Differences (S. D.)	Average Gene Diversity (S. D.)
All Sailfish	192	96	1.0000 (0.0005)	4.360329 (2.164702)	0.872066 (0.479093)
Eastern Atlantic	48	24	1.0000 (0.0043)	4.374113 (2.199198)	0.874823 (0.488197)
Western Atlantic	102	51	1.0000 (0.0014)	4.348088 (2.167639)	0.869618 (0.480158)
Atlantic pooled	150	75	1.0000 (0.0008)	4.353468 (2.164338)	0.870694 (0.479143)
Pacific	42	21	1.0000 (0.0052)	4.186992 (2.122429)	0.837398 (0.471437)

Genetic Differentiation between Populations of Sailfish

The corrected genetic distance (D_A) between larval samples collected during Cruises 1 and 2 yielded a very low value (0.01%). Also, the values for haplotypic diversity, nucleotide diversity, and mean pairwise nucleotide differences for Cruise 1 and 2 were nearly identical (Table 3-1). Accordingly, larval samples were pooled to increase the size of the GoM sample, and will be referred to hereafter as 'pooled larvae'. Furthermore, the nucleotide divergence between the pooled larvae and the adult sailfish from Cancun was similarly low (0.02%), thus these data were combined to constitute a western Atlantic sample.

Differentiation of eastern and western Atlantic Sailfish

Eastern (Gulf of Guinea) and western (Gulf of Mexico and Cancun) samples were compared to test the hypothesis of panmixia in Atlantic sailfish. The results of the AMOVA (Table 3-4) indicate that only a minute and not significant fraction (0.16%) of the total variation was explained by the among-groups component, thus failing to reject the null hypothesis of no differentiation. Similarly, the results of the microsatellite analysis showed no transatlantic differences with the among-group component yielding negative values of variance. In both mtDNA and microsatellite data the majority of the variation was explained by within population differences with no evidence of heterogeneity among the samples within respective groups. The global F_{ST} values were extremely low for mtDNA and not different from zero for

Table 3-4. AMOVA comparing transatlantic samples of sailfish. In mtDNA and microsatellite analyses groups were Western and Eastern Atlantic. In mtDNA analysis populations within groups were i) Western Atlantic: Cancun, Cruise 1 and Cruise 2 and ii) Eastern Atlantic: Gulf of Guinea. In microsatellite analysis populations within groups were i) Western Atlantic: Cruise 1 and Cancun samples and ii) Eastern Atlantic: Gulf of Guinea.

Source of Variation	mtDNA d.f.	Microsatellite d.f.	mtDNA Percentage of Variation (P)	Microsatellite Percentage of Variation (P)
Among groups	1	1	0.16 (0.25611)*	-1.00 (0.66178)**
Among populations within groups	2	1	-0.09 (0.88563)	0.98 (0.15836)
Within populations	218	147	99.64 (0.42033)	100.02 (0.17791)
TOTAL	221	149		

*F_{ST} = 0.00129
** F_{ST} = -0.01003

microsatellite data, and similar values were obtained for each of the pairwise F_{ST} values (Table 3-5). mtDNA CR-I data estimated that the effective number of migrants between eastern and western Atlantic sailfish populations was approximately 871 individuals per generation, while microsatellite data converged to an infinite effective number of migrants per generation. The comparison of the genetic distances for CR-I data confirms the absence of transatlantic partitioning (D_A = -0.00013 ± 0.00376) in sailfish.

Table 3-5. Fixation indices calculated from mtDNA CR-I sequences (below the diagonal) and microsatellite loci (above the diagonal) for pairwise comparisons between sailfish populations. 1 = Cruise 1 Larvae, 2 = Cruise 2 Larvae, 3 = Cancun Adults, 4 = Gulf of Guinea Adults. P-values are in parentheses.

	1	2	3	4
1	----	N/A	0.00987 (0.14414)	-0.00142 (0.54955)
2	-0.00145 (0.48649)	----	N/A	N/A
3	-0.02285 (0.55856)	0.00944 (0.22523)	----	0.00624 (0.11712)
4	0.00697 (0.26126)	-0.00264 (0.43243)	0.00287 (0.31532)	----

Differentiation of Atlantic and Pacific Sailfish Populations

The inter-ocean comparison of sailfish mtDNA data using AMOVA showed that although two-thirds of the observed variation corresponded to differences within populations, a large and significant proportion of the variation (36%) was explained by differences between the Atlantic and the Pacific populations (Table 3-6). Furthermore, when Clade II Atlantic sailfish were removed, a small (3.05%) yet

Table 3-6. Inter-oceanic comparison of sailfish. In mtDNA and microsatellite analyses populations were Atlantic and Pacific.

Source of Variation	mtDNA d.f.	Microsatellite d.f.	mtDNA Percentage of Variation (P)	Microsatellite Percentage of Variation (P)
Among populations	1	1	35.90 (0.0000)*	2.80 (0.0000)**
Within populations	242	190	64.10 (0.0000)	97.20 (0.0000)
TOTAL	243	191		

*F_{ST} = 0.35905
** F_{ST} = 0.02803

significant (p = 0.0000) percentage of the variation observed was attributed to inter-oceanic differences. While the microsatellite data also revealed significant inter-oceanic differences, the proportion of the differentiation (2.8%) was substantially smaller, with most of the variation explained by differences within-populations. This value did not change substantially when Clade II Atlantic sailfish were removed from the analysis (2.58%; p = 0.0000). Both mtDNA and microsatellite analyses indicated no differences among populations within groups, as emphasized by the negative and non-significant proportion of the variation explained by this component. The corrected mean divergence between the Atlantic and Pacific populations of sailfish for the mtDNA CR-I was 0.03786 ± 0.00376. Estimates of the effective number of migrants between Atlantic and Pacific Oceans was less than one individual per generation from mtDNA data, and in the order of 17 individuals per generation from microsatellite data.

Phylogenetic Analysis

Concordant with previous studies (Alvarado Bremer 1994, Graves and McDowell 1995) the NJ tree of sailfish mtDNA CR-I (Figure D-2; Appendix D) reveals two highly divergent clades with a corrected mean distance of 7.9% (± 0.18%). Clade I is ubiquitous, with lineages occurring in both Atlantic and Pacific Oceans, whereas Clade II is private to the Atlantic Ocean. The ubiquitous Clade I had higher haplotypic diversity than Clade II, with only seven haplotypes being repeated in Clade I compared 15 repeated haplotypes in Clade II. Furthermore, one of the seven repeated Clade I haplotypes occurred at high frequency in the Pacific sample (32%). Aside from the differences in haplotypic diversity the clades were comparable in the remaining aspects of their diversity (Table 3-7).

Table 3-7. Basic diversity statistics for the ubiquitous Clade I and the private Atlantic Clade II.

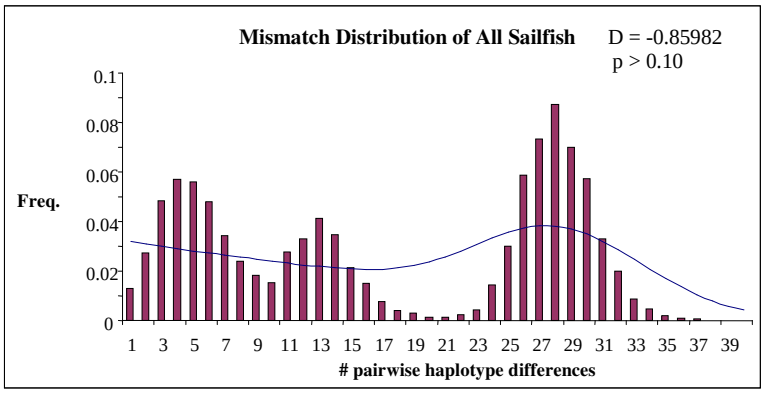
Population	n	S	M	*h* (SD)	π (SD)	k (SD)
Clade I*	68	57	52	0.990 (0.004)	0.02082 (0.00124)	4.622 (2.056)
Clade II	154	67	80	0.942 (0.013)	0.02645 (0.00116)	5.600 (3.725)

. *Clade I sailfish originating from the Pacific Ocean were excluded from the analysis.

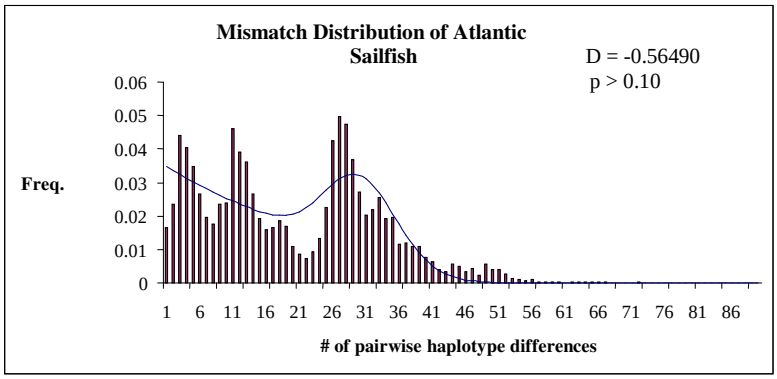
Historical Demography

In agreement with the observed multimodal mismatch distributions obtained from the analyses of the pooled data (Atlantic and Pacific sailfish; Figure 3-2a) and the total Atlantic population (Clades I and II; Figure 3-2b), non-significant neutrality tests were obtained. In contrast, the mismatch distributions generated for both the Pacific sailfish and Atlantic Clade I fish showed smooth unimodal curves indicative of sudden population expansion (Figure 3-2c and 3-2d, respectively). Furthermore, both of these populations had significantly negative values for Tajima's D and comparatively low τ values, also consistent with a sudden population expansion. Clade II sailfish had a bimodal mismatch distribution and a low, but not statistically significant Tajima's D value, suggesting that this clade has not undergone recent sudden expansion (Figure 3-2e).

(a)



(b)



(c)

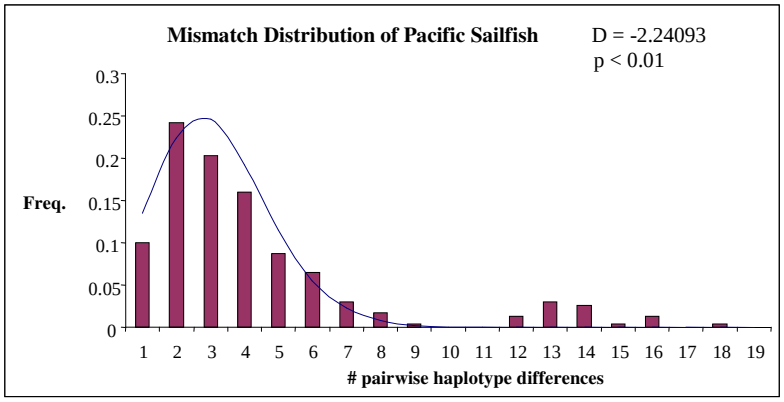
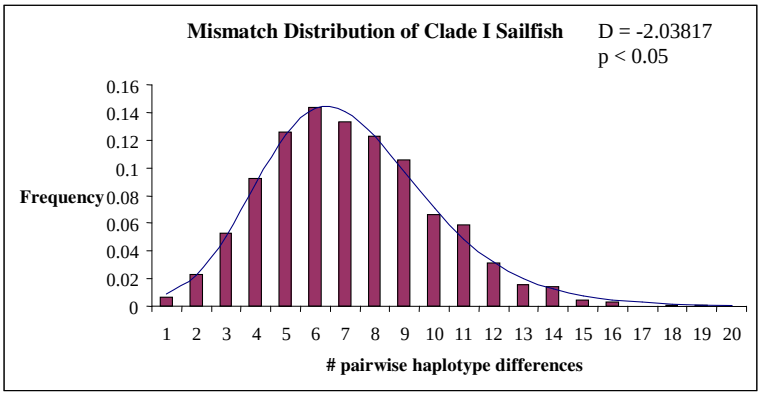


Figure 3-2. Mismatch distributions for sailfish. Included in figure is Tajima's D and associated p-value. (a) All sailfish, (b) Atlantic sailfish, (c) Pacific sailfish, (d) Clade I sailfish, and (e) Clade II sailfish.

(d)



(e)

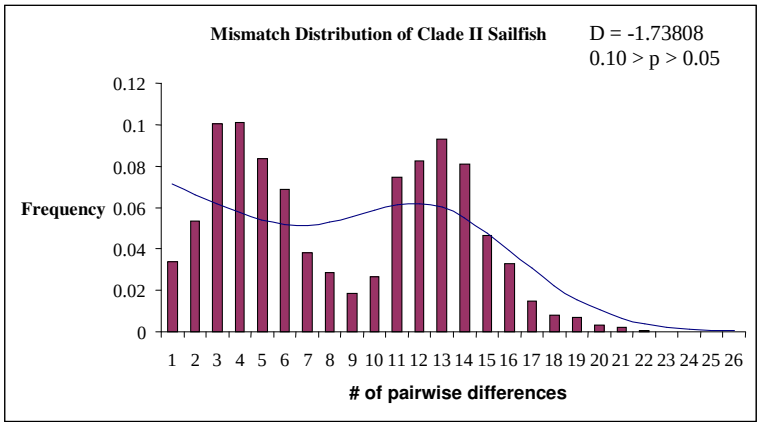


Figure 3-2. Continued.

The estimated historical demographic parameters are included in Table 3-8. As previously

Table 3-8. Estimates of historical demographic parameters for various groups of sailfish. τ = units of mutational time before the present with 95% confidence interval (CI) in parentheses, θ_0 = diversity before the expansion, and θ_1 = diversity after the expansion.

Population	θ_0	θ_1	τ (lower bound, upper bound)
All Sailfish	0.000	30.521	28.106 (17.739, 47.598)
Atlantic Sailfish (both clades)	0.071	27.780	28.321 (16.526, 46.290)
Clade I*	1.527	177.012	4.838 (3.054, 9.196)
Clade II	0.000	13.026	12.774 (6.309, 20.524)
Pacific Sailfish (Clade I)	0.000	14.109	2.442 (0.529, 4.852)

*Clade I sailfish originating from the Pacific Ocean were omitted from the analysis.

mentioned Clade II did not conform to sudden expansion, so only the estimates for Atlantic Clade I sailfish were used to calculate the time since expansion. Initial female effective population sizes prior to and after the expansion were also calculated for Atlantic sailfish. Based on mutation rates for teleosts discussed in Volckaert et al. (2002) and references therein, the lower bound of time since expansion was estimated using a rate of 5% per million years and the upper using a rate of 10% per million years. Considering that generation time in sailfish is approximately 3 years (Au 1994) we estimated that the female effective population size in the Atlantic after the expansion was between 324000 and 657000 fish and that Clade I sailfish began their expansion in the Atlantic approximately 164000 to 351000 years ago (95% CI = 104000 to 719000 years ago). As the eastern Pacific sailfish population also shows evidence of a recent evolutionary history, we estimated that this population began to expand approximately 78500 to 173000 years ago (95% CI = 18500 to 344000 years ago).

DISCUSSION

Eastern and Western Atlantic Sailfish Populations

A comparison of genetic diversity between populations of sailfish from the western Atlantic (Gulf of Mexico and Cancun) with those from the eastern Atlantic (Gulf of Guinea, Abidjan, South Africa) were extremely similar. AMOVAs based on mtDNA and microsatellite data determined that 0.16% ($p = 0.25611$) and -1.00% (0.66178), respectively, of the variation observed can be attributed to genetic differentiation between the eastern and western samples of Atlantic sailfish, thus, in both instances failing to reject the null hypothesis of a single panmictic unit. However, it should be noted that substantially higher values of nucleotide diversity were found in the eastern Atlantic sample, and thus larger samples from several localities of the eastern Atlantic should be surveyed to determine whether this difference is real.

The absence of differentiation between eastern and western Atlantic sailfish is in agreement with the results of the RFLP analysis of the entire mtDNA molecule conducted by McDowell and Graves (2001). Similarly no transatlantic differentiation was detected for blue marlin and white marlin (Graves and McDowell 2000). A possible explanation for the lack of population structure among these istiophorid species may be the relative size of the Atlantic Ocean (McDowell and Graves 2000). It should be noted that population structuring within the Atlantic has been reported for swordfish (Alvarado Bremer 1996, 2005a; Chow et al. 1997; Chow and Takeyama 2000). In addition, sailfish shows population differentiation in relatively close proximity (Hoolihan et al. 2004). However, Atlantic sailfish are known to perform long migrations on the order of several thousand kilometers (Beardsley et al. 1972), sufficient to maintain genetic homogeneity between distant regions in the Atlantic Ocean. Furthermore, migration on the order of only a handful of individuals per generation would be adequate to allow panmixia of the Atlantic sailfish population (Waples 1998).

Atlantic and Pacific Sailfish Populations: Unidirectional Gene Flow into the Atlantic

Similar to several other highly migratory species, including blue marlin, swordfish, bluefin

tuna, and bigeye tuna (Finnerty and Block 1992; Alvarado Bremer et al. 1996, 1998a, 1998b, 2005a and 2005b), and as previously reported by Graves and McDowell (1995), sailfish exhibit inter-oceanic differentiation between Atlantic and Pacific populations. An examination of the basic diversity statistics calculated from the mtDNA CR-I sequences showed that the Atlantic samples had higher haplotypic diversity than those from the Pacific and nucleotide diversity in the Atlantic was five times higher that in the Pacific. Diversity indices calculated from the microsatellite data were also higher for the Atlantic sample, although heterozygosity was only 3.33% higher than the estimate for the Pacific population. Evidence of higher haplotypic and nucleotide diversity in Atlantic populations has also been documented in blue marlin. Finnerty and Block (1992) found greater haplotypic diversity among Atlantic blue marlin and that nucleotide diversity was twice in the Atlantic what it was in the Pacific. Similarly, Buonnacorsi et al. (2001) found that nucleotide diversity among blue marlin was almost four times greater in Atlantic blue marlin when compared to values in the Pacific and suggested that a possible explanation for higher diversity being observed in the Atlantic may be that the Atlantic is acting as a sink for unidirectional migrants entering the Atlantic from the Indo-Pacific via the Cape of Good Hope around the southern tip of South Africa. Beardsley (1980) mentioned that when oceanographic conditions were favorable, sailfish could possibly move from the western Indian Ocean to the eastern Atlantic via this corridor. However, estimates of migration between these basins from mtDNA data appears to be very low (less than one individual per generation), although a substantially larger effective number of migrants (17 individuals per generation) between the Atlantic and Pacific was obtained with microsatellite data. Thus, the migration of individuals from the Indo-Pacific into the Atlantic is not sufficient to prevent significant differences from accumulating between members of these two ocean basins**.** Penrith and Cram (1972) reported that there had been no specimens of sailfish obtained in the longline fishery off of the Cape of Good Hope, suggesting that influx of Pacific individuals into the Atlantic is a rare, punctuated event.

The Eastern Pacific: Evidence of Recent Invasion?

The reduced levels of genetic diversity in the eastern Pacific sailfish sample corroborates the study of Graves and McDowell (1995), who reported that 19 out of 20 individuals (h = 0.1) from Cabo San Lucas, Mexico shared the same haplotype. By contrast, a western Pacific (Australia) sample exhibited much higher levels of haplotypic diversity (h = 0.897), comparable to the levels reported in the same study for the Atlantic samples. Although the reduction in variability in our Pacific sample appears not to be as pronounced - a difference that can be explained by the higher amount variation of CR-I compared to RFLP data of the entire genome - we also see a substantial reduction in nucleotide diversity and haplotypic diversity in the eastern Pacific, with 22 individuals being represented by 13 haplotypes. Also, eastern Pacific sailfish differ on average by three mutations compared to more than 10 in any of the Atlantic samples. In consequence, the historical demographic signal indicates that the eastern Pacific population

experienced a sudden expansion within the last 85400 to 173000 years, a period considerably more recent than that estimated for the Atlantic.

A reduction of genetic diversity within eastern Pacific populations has also been reported in two other species: the blacktip shark (*Carcharhinus limbatus*; Keeney and Heist, in review) and the olive ridley turtle (*Lepidochelys olivacea*; Bowen et al. 1998). Both species are thought to have re-invaded the eastern Pacific approximately 300000 years ago following a period when conditions in this region were too cold to sustain these tropical species (Keeney and Heist in review, Bowen et al. 1998). While similarities between sailfish, olive ridley turtles, and blacktip sharks are initially not apparent, they all share a tropical distribution and the ability to perform long-distance migrations. Such similarities have been invoked to explain concordance in the historical demographic signal of other species of pelagic fishes (Alvarado Bremer et al. 2005b). It has been suggested that the absence of genetic diversity among eastern Pacific populations of the olive ridley turtle and the blacktip shark is related to the fact that, during recent evolutionary time, the climate in the eastern tropical Pacific has not been stable. Any cold water extensions across the equator could have lead to the elimination of tropical faunas in this region, restricting these large pelagic fish to warmer waters of the southwest Pacific and Indian Ocean, a region that has been the site of the warmest water for the last 20 million years (Bowen et al. 1998). Currently, sailfish of the Pacific Ocean display a much more restricted latitudinal distribution on the eastern side of this basin (5° to 25°N) compared to their more extensive range on the western side (27°S to 40°N; Beardsley et al. 1972 and references therein). Kotilainen and Shackelton (1995) report that an increased discharge of icebergs into the North Pacific has occurred at high frequency (every 2000 to 3000 years) during the past 95000 years. This punctuated influx of cooler water in conjunction with the prevailing current pattern in the Pacific would result in a decrease in water temperature on predominantly the eastern side of this basin. The ongoing occurrence of such instability may explain the compressed distribution of sailfish in this area.

It is interesting to note that authors have long recognized the sailfish of the eastern Pacific as a population unique from the sailfish in the rest of the species' distribution. Individuals from this region are easily recognizable on the basis of morphological characteristics including dramatically larger body sizes and a more pronounced nuchal hump among individuals along the coasts of Mexico and Panama (Morrow and Harbo 1969). Also, Beardsley (1980) mentions that the formation of the spinous dorsal fin differs among individuals from the Atlantic and those from this area of the Pacific. These differences were previously thought to be the result of environmental conditions that promoted the attainment of larger size among eastern Pacific sailfish (Beardsley 1980) since heritability for traits such as body weight and length traditionally have been thought to be much lower within fish populations than within populations of other vertebrates. Thus, the larger phenotypic variation observed in fish species isn't necessarily associated with greater genetic variability (Allendorf et al. 1987). To the contrary, we have found significant genetic variation between the Atlantic sailfish population and the phenotypically unique sailfish of the eastern

Pacific. Further studies are required in order to determine what proportion of total phenotypic variation within the sailfish population is due to genetic differences and what proportion of variation is environmental in order to determine if the sailfish population of the eastern Pacific should be managed as a unique stock.

Phylogeography and Historical Demography

Unidirectional migration of individuals from the Pacific into the Atlantic Ocean is thought to result in the presence of two distinct clades of many highly migratory fish within the Atlantic. Clade I defines the ubiquitous clade, represented by individuals found in both Pacific and Atlantic Oceans while and Clade II is the private clade that includes haplotypes found in the Atlantic only. Species found to exhibit this pattern include blue marlin (Finnerty and Block 1992; Graves and McDowell 2000; Buonnacorsi et al. 2001), swordfish (*Xiphias gladius*; Alvarado Bremer 1994; Alvarado Bremer et al. 1995, 1996, 2005a, 2005b; Rosel and Block 1996) and bigeye tuna (*Thunnus obesus*; Alvarado Bremer et al. 1998a; Martinez et al. 2006). Based on the topology of the mtDNA CR-I tree, we too recognize two clades in sailfish as previously recognized by Graves and McDowell (1995).

All of these authors recognize that the origin of these two clades is associated with vicariant events. This hypothesis was first presented by Perrin et al. (1978) to account for the higher diversity of Atlantic delphinid fauna when compared to other tropical waters of the world. Their hypothesis, hereafter to be referred to as the Mitchell-Perrin model of inter-oceanic unidirectional gene flow is as follows:

> The tropical Atlantic has been separated from the eastern tropical Pacific by the Isthmus of Panama since the Pliocene. During the Pleistocene glacial periods, the Cape of Good Hope (southern tip of Africa) was a barrier isolating the tropical Atlantic from the Indo-Pacific. During interglacial periods, such as at the present, a south-running current, such as the Agulhas Current, sporadically carried warm water across the Cape, where it was moved northward by a north-running current, such as the Benguela Current. The Cape thus may have acted as a one-way filter, admitting tropical, Indo-Pacific pelagic forms to the tropical Atlantic.

Thus, the rise of the Isthmus of Panama approximately 3.5 million years ago (mya) would have terminated any faunal exchange that previously had been free to occur between the Atlantic and Pacific Oceans (Grant 1987), presenting a barrier to gene flow between sailfish residing in either basin. An intermittent barrier to gene flow may have been operating around the tip of South Africa, with lower water temperatures preventing any migration that may have previously occurred between these groups of temperature sensitive fish (Martinez et al. in press). The occurrence of Pacific mtDNA CR-I haplotypes in the Atlantic basin is explained by secondary contact of Atlantic and Indo-Pacific populations of sailfish, with westward migration of sailfish from the Indo-Pacific being facilitated by the strong westward flowing, warm waters of the Agulhas current around South Africa. Analysis of the Clade I sailfish indicated that these individuals had undergone a recent sudden expansion in the Atlantic during the last 164000 to 351000 years. Peeters et

al. (2004) collected sediment cores from beneath the Agulhas corridor and using planktonic foraminiferal assemblage data, reconstructed the history of the leakage of Indo-Pacific fauna into the Atlantic during the late Pleistocene. In the last 550000 years, Peeters et al. (2004) identified six major leakage events, three of which correspond to the time frame of the expansion of our Clade I sailfish. The earliest episode occurred between 340000 and 425000 years ago, followed by another event 300000 to 320000 years ago. The most recent influx of Indo-Pacific sailfish into the Atlantic appears to have taken place between 185000 and 240000 years ago. Thus, it appears that the presence of Clade I individuals in the Atlantic is the result of the punctuated influx of sailfish from the Indo-Pacific during these leakage events.

CHAPTER IV

EXAMINING ATLANTIC SAILFISH FOR EVIDENCE OF VARIANCE IN REPRODUCTIVE SUCCESS

INTRODUCTION

As previously noted in Chapter III, female sailfish are highly fecund, capable of producing 2.3 million to 4.7 million eggs per spawning event (Voss 1953). In common with other highly fecund aquatic species that display Type III survivorship, sailfish experience high mortality during the early stages. Owing to high fecundity and the sweepstakes chance of individuals to match their reproductive activity to oceanographic conditions favorable for maturation, fertilization, larval development, and recruitment, Hedgecock (Hedgecock et al. 1982; Hedgecock 1994) hypothesized that marine animals have extremely large variance in individual reproductive success. Evidence of variance in reproductive success has been documented by characterizing temporal shifts in allele frequency within populations of oysters (*Crassotrea gigas*) (Hedgecock et al. 1982) and other marine organisms (Hedgecock 1994). For free-living stages, a testable prediction would be to detect genetic heterogeneity among cohorts of larvae if a small proportion of adults produce them (Li and Hedgecock 1997; Ruzzante et al. 1996). This approach is particularly challenging when studying long-lived broadly distributed highly migratory species. However, because optimal oceanographic conditions tend to be patchily distributed in the oceanic realm (Turner et al. 2002), it is reasonable to expect that evidence of bias in reproductive output could be documented if spatially proximal surveys of cohorts of larvae contain an over-representation of related individuals. This alternative approach cannot document the magnitude of the variance in reproductive success in a population. Instead, it relies on the highly polymorphic mtDNA CR-I and microsatellites markers to establish the potential contribution of an individual female to a surveyed cohort. Recently, Talley-Farnham (2003) used this approach to demonstrate that sample collections of spatially proximal juvenile yellowfin tuna (*Thunnus albacares*) from the Gulf of Guinea contained full siblings. This special case of reproductive variance, wherein sampling results in the capture of larvae from a limited number of families (Saillant et al. 2006), is called the Allendorf-Phelps effect (Waples 1998).

Here we employed two approaches to test for evidence of variance in reproductive success in Atlantic sailfish. The first test was a direct comparison of the levels of genetic variability between larval and adult samples, where evidence of reproductive variance is inferred when larval samples contain significantly lower levels of genetic variation than adult samples (Chapman et al. 1999). We attempted this by comparing the levels of genetic variability of the mtDNA CR-I and five microsatellite loci, in larval and adult collections of sailfish from the Atlantic Ocean. Since, in the transatlantic analysis, there was no difference between eastern and western stocks, it was reasonable to assume that the genetic variability of the adults used for this comparison was representative of the variability that would have been observed in

adults from the Gulf of Mexico had they been available for this study. Adult data were then used as reference to examine the larval collections for significant heterozygote deficiencies, the presence of which would be indicative of the Wahlund effect (Wahlund 1928). The larvae were also examined for evidence of linkage disequilibrium at multiple loci which would suggest that some of the larvae were either full or half siblings (Chapman et al. 1999). Lastly, individuals collected in the same or proximal tows sharing the same mtDNA CR-I haplotype were tested for siblingship using the fragment polymorphism data from five microsatellite loci to determine whether they could represent the contribution of the same spawning event.

METHODS

The collection information is the same as that described in Chapter II. Protocol for tissue digestion, DNA extraction and isolation, polymerase chain reaction (PCR), sequencing, and fragment mobility analysis are described in Appendices A, B, and C. It should be noted that none of the larvae from the second cruise were included in the microsatellite assays as DNA degradation prevented successful amplifications of these loci.

The microsatellite assays were analyzed using the program GeneScan 3.7 (Applied Biosystems, Foster City, CA, USA). GENEPOP 3.3 (Raymond and Rousset 1995) was used to calculate allele frequencies, the number of alleles per locus, genic (allelic) differentiation between pairs of populations and conduct the exact test for Hardy-Weinberg equilibrium. An Excel macro written in Visual Basic and developed by W. Amos at the University of Cambridge was used to calculate the frequency of null alleles that would best fit the data (Allen et al. 1995). Arlequin 3.0 (Excoffier et al. 2005) was used to estimate F_{ST} values, conduct AMOVAs for both microsatellite and mtDNA data, and test for linkage disequilibrium among loci of adult and larval populations. Lastly, pairwise relatedness among members of the larval assemblage that were collected from the same or nearby tows and shared mtDNA CR-I haplotypes was tested with the program MARK (Lynch and Ritland 1999).

RESULTS

Heterozygote deficiency tests of Hardy-Weinberg Equilibrium (HWE) for all five microsatellite loci revealed no departures except for Locus Mn60 in the Pooled Atlantic (Larvae and Adults) sample (Table 4-1). Table 4-2 depicts the observed and expected number of homozygous and heterozygous individuals for each locus analyzed in the larval, Atlantic adults, and pooled Atlantic sailfish sampled. The pooled Atlantic sample has only a slight deficiency of heterozygotes (68) when compared to the expected number of heterozygous individuals (72.174) at Mn60. This deficiency could be explained by null alleles. An Excel macro developed by W. Amos at the University of Cambridge was used to calculate the frequency of null alleles that would best fit the data (Allen et al. 1995). This program predicted that the frequency of null alleles at locus Mn60 would have to be 2.6% among the pooled individuals in order to best explain this data. Neither of these values was significant at $\alpha = 0.05$, thus Mn60 was kept in the analysis. Furthermore, the omission of Mn60 from the analyses resulted in the same conclusions.

Table 4-1. P-value (standard error) of Hardy-Weinberg Equilibrium Test at each locus for the Cruise 1 larval collection, the Atlantic adult sample (sailfish from the Gulf of Guinea and Cancun) and the pooled Atlantic sample (containing all Atlantic adults and all Cruise 1 larvae),

Population	Mn60	Mn90	Mn01	Mn08	Mn10
Larvae	0.0204	0.5559	0.5453	0.9989	0.4759
	(0.0141)	(0.0473)	(0.0231)	(0.0006)	(0.0381)
Atlantic Adults	0.1182	1.0000	1.0000	0.9236	0.0796
	(0.0310)	(0.0000)	(0.0000)	(0.0112)	(0.0192)
Pooled Atlantic	0.0000	0.1470	0.6319	0.9986	0.2211
	(0.0000)*	(0.0334)	(0.0280)	(0.0008)	(0.0297)

*P<0.01 with Bonferroni adjustments for five simultaneous comparisons.

Table 4-2. Observed and expected (in parentheses) homozygosity and heterozygosity at each locus within the pooled Atlantic samples, the adult Atlantic sample and the larval sample.

Population	Mn60		Mn90		Mn01		Mn08		Mn10	
	Hom	Het	Hom	Het	Hom	Het	Hom	Het	Hom	Het
Larvae	3	40	2	41	6	37	7	36	5	38
	(1.729)	(41.270)	(1.706)	(41.294)	(6.835)	(36.165)	(12.588)	(30.412)	(5.329)	(37.671)
Atlantic	4	28	1	31	2	30	4	28	4	28
Adults	(1.349)	(30.651)	(1.540)	(30.460)	(4.524)	(27.476)	(6.984)	(25.016)	(5.937)	(26.063)
Pooled	7	68	3	72	8	67	11	64	9	66
Atlantic	(2.826)	(72.174)	(3.040)	(71.960)	(11.617)	(63.383)	(19.651)	(55.349)	(11.356)	(63.644)

None of the results from the linkage disequilibrium tests for each pair of loci in either the larval or adult Atlantic sample were significant. Thus, we found no evidence of nonrandom association of alleles among any of the 5 microsatellite loci. It should be noted however, that this test for linkage is not robust.

Genetic Diversity within Larval and Adult Populations of Atlantic Sailfish

mtDNA

The basic summary statistics for the larval collections, as well as adults from Cancun and the eastern Atlantic are presented in Table 4-3. The eastern Atlantic adult sample had slightly higher values of diversity but these differences were not statistically significant (Chapter III).

Table 4-3. Summary of basic diversity statistics for Cruise 1 and Cruise 2 larvae, and Atlantic adults from Cancun and the eastern Atlantic (Gulf of Guinea), and pooled Atlantic sailfish samples as calculated from mtDNA CR-I sequences.

Population	n	S	M	*h* (SD)	π (SD)	k (SD)
Cruise 1 (Larvae)	46	55	32	0.970(0.014)	0.05665(0.00513)	12.690(9.061)
Cruise 2 (Larvae)	135	76	82	0.969 (0.009)	0.05539 (0.00209)	11.466 (7.731)
Cancun (Adults)	11	46	11	1.000(0.039)	0.05924(0.01246)	14.927(10.172)
E. Atlantic (Adults)	30	63	26	0.984 (0.012)	0.07006 (0.00429)	16.324 (10.213)

Microsatellite Assays

No major differences in diversity values were obtained in the comparison of larvae (Cruise 1) and adult samples of Atlantic sailfish (Table 4-4). High levels of heterozygosity were observed in the larval collection and both adult samples, and these values were not significantly different from one another.

Table 4-4. Summary of diversity indices calculated for larvae, Atlantic adults, and pooled Atlantic sailfish samples using 5 microsatellite loci.

Population	h	n	Gene Diversity (S.D.)	Average Gene Diversity (S. D.)
Cruise 1 (Larvae)	86	43	1.0000 (0.0018)	0.868892 (0.480705)
Cancun (Adults)	16	8	1.0000 (0.0221)	0.858333 (0.502441)
E. Atlantic (Adults)	48	24	1.0000 (0.0043)	0.874823 (0.488197)

Genetic Differentiation between Larval and Adult Populations of Atlantic Sailfish

Table 4-5 shows the results of the AMOVAs performed to determine if differences in genetic variation among larval and adult samples of Atlantic sailfish exist. mtDNA data revealed that none of the variation observed was attributable to variation between larval and adult samples. Similarly, the results of the microsatellite analysis showed that none of the variation was due to differences in genetic variation between these two groups. Thus, in both mtDNA and microsatellite data the majority of variation was respectively contained within adult and larval samples. In consequence the global F_{ST} values for both mtDNA and microsatellite data were extremely low (Table 4-5). The genetic distance data between the larval sample and pooled Atlantic adult sample was not different from zero (D_A = -0.00010 ± 0.00271).

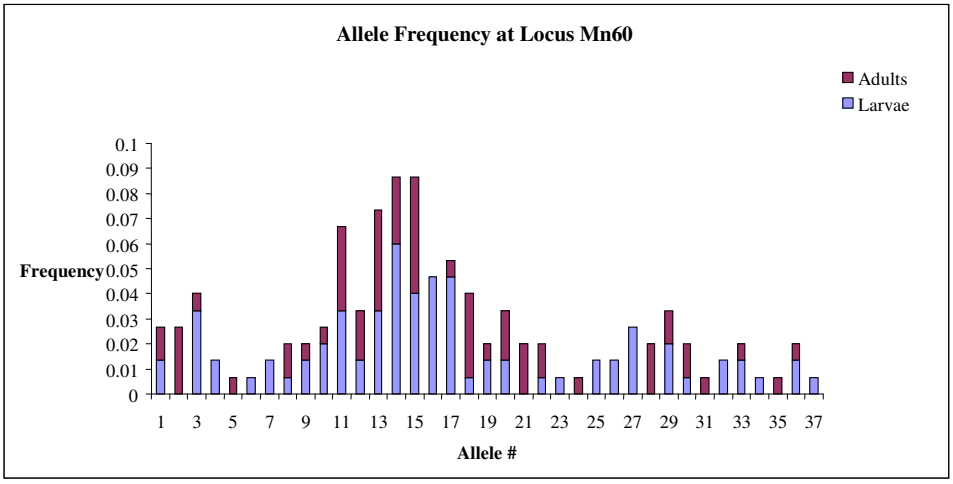
Table 4-5. AMOVA examining evidence of genetic heterogeneity in mtDNA CR-I sequences and five microsatellite loci between larval and adult samples of Atlantic sailfish. Samples were Atlantic adults and larvae for both AMOVAs. Larval mtDNA data includes specimens collected in both Cruise 1 and Cruise 2. Larval microsatellite data includes only specimens from Cruise I.

Source of Variation	mtDNA d.f.	Microsatellite d.f.	mtDNA Percentage of Variation (P)	Microsatellite Percentage of Variation (P)
Among groups	1	1	-0.02 (0.35582)*	0.02 (0.35582)**
Within groups	220	148	100.02	99.98
TOTAL	221	149		

*F_{ST} = -0.00022
** F_{ST} = 0.00021

Histograms generated from the allele frequencies at each locus indicated that in general, the majority of the alleles present were shared between adult and larval collections (Figure 4-1). Furthermore, larvae exhibited the same range of allele sizes as the adults at each locus, and the same relative frequency for both of the highly polymorphic loci (Mn60 and Mn90) as well as the more conserved loci (Mn01, Mn08, and Mn10).

(a)



(b)

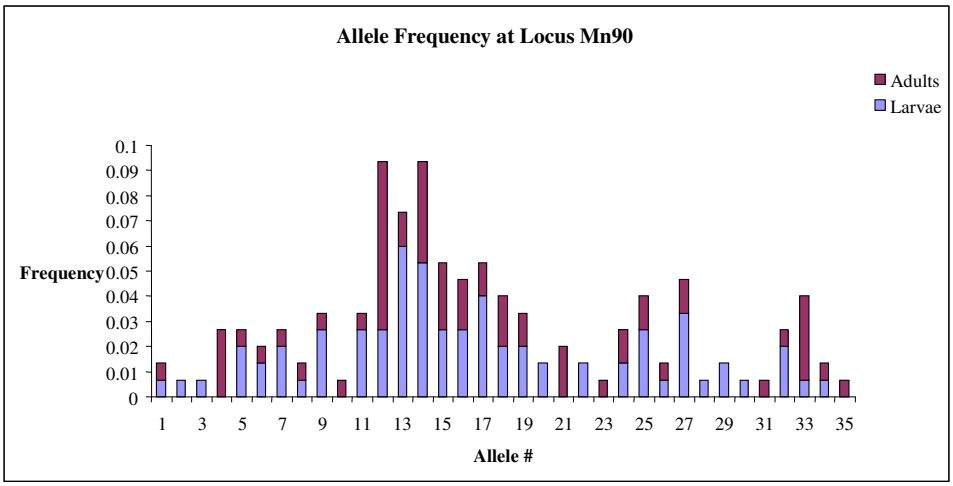
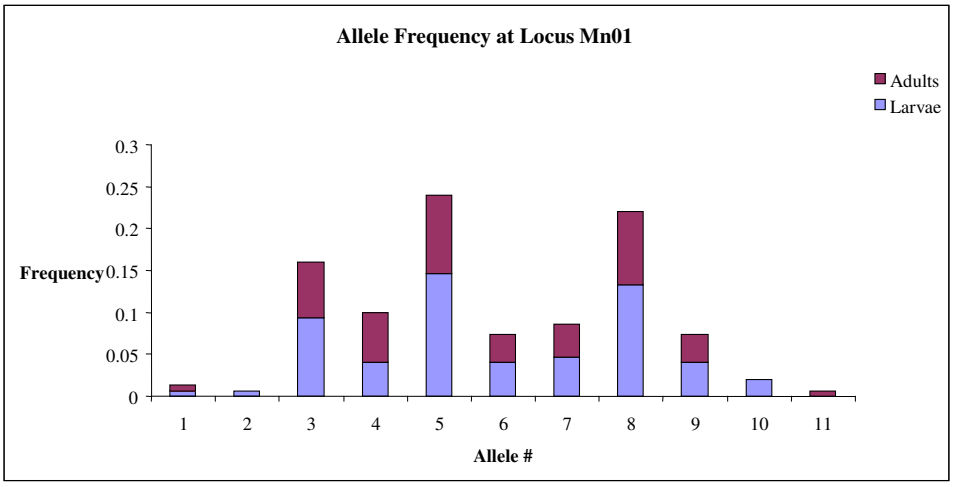


Figure 4-1. Allele frequencies at each of the five microsatellite loci for both larval and adult samples. (a) Allele frequency at Mn60, (b) Allele frequency at Mn90, (c) Allele frequency at Mn01, (d) Allele frequency at Mn08, and (e) Allele frequency at Mn10.

(c)



(d)

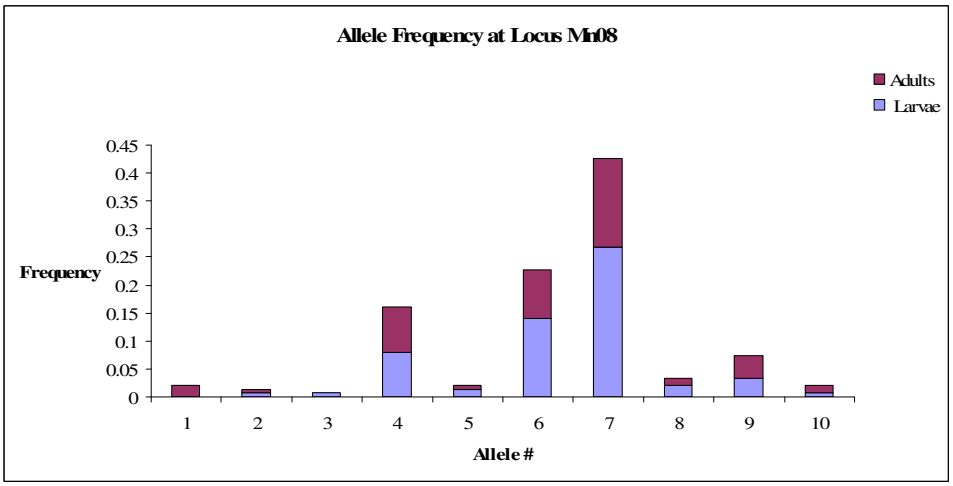


Figure 4-1. Continued.

(e)

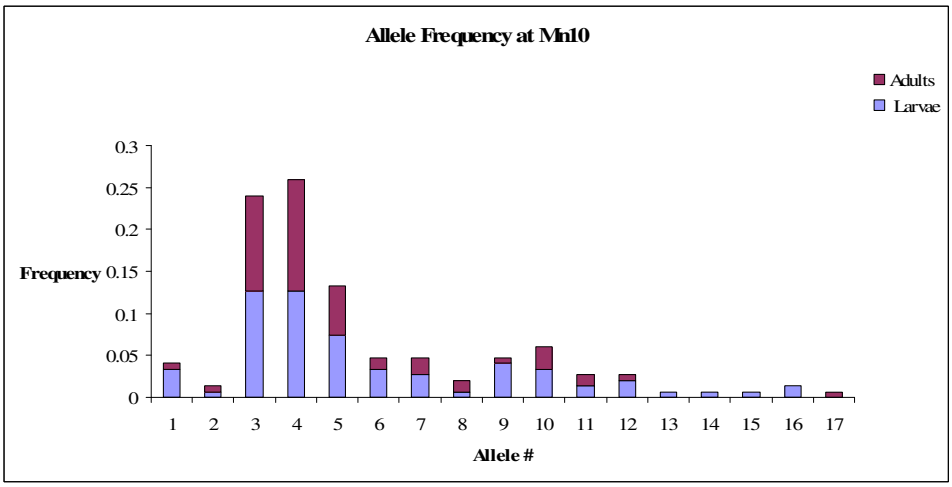


Figure 4-1. Continued.

Relatedness among Larvae

The neighbor-joining tree in Figure 4-2 revealed that 4 sets of larvae collected in the same or neighboring tows during Cruise I that shared the same mtDNA CR-I haplotype. The standard lengths of these larvae were also similar, indicating the possibility of being the product of the same spawning event. However, none of the spatially proximal larvae were related as indicated by the results from the pairwise relatedness analysis. Thus, we find no evidence of siblingship between any of the pairs of larvae.

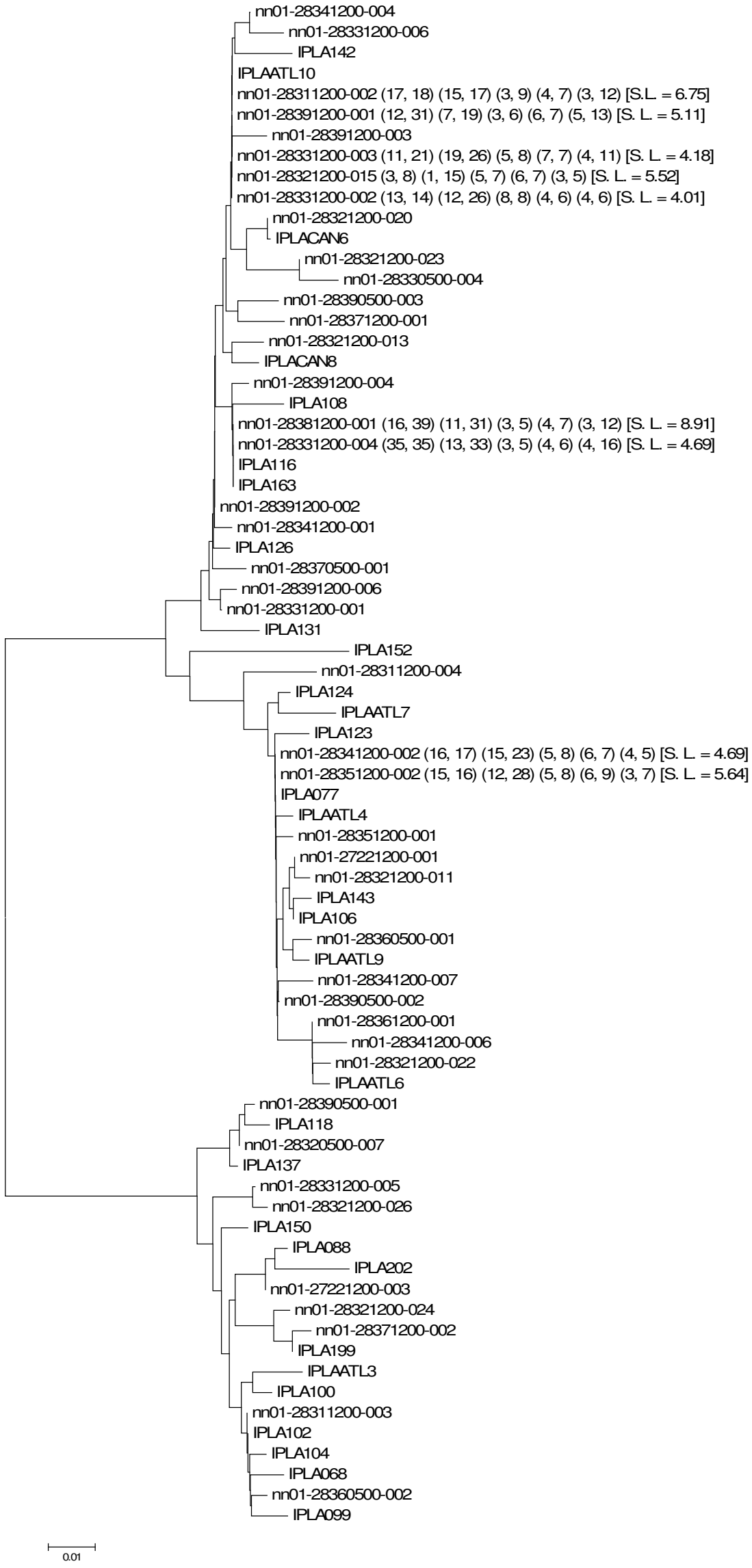


Figure 4-2. Neighbor-joining tree illustrating relationships among larvae from Cruise 1 (nn01-n) and Atlantic adults (IPLAn). Fragment size for alleles of each microsatellite locus are listed in Appendix D (Table D-3). Here, they are ordered, in parentheses, as follows: Mn60, Mn90, Mn01, Mn08, Mn10. Values in square brackets represent standard lengths (S. L.) of larvae (mm). Standard lengths of all larvae collected are listed in Appendix D (Table D-4).

DISCUSSION

Heterozygote Deficiencies and Null Alleles

Among the five microsatellite loci surveyed, only locus Mn60 deviated from HWE in the pooled Atlantic sailfish sample. Such deviations can be interpreted as either being due to excess homozygotes (Wahlund effect) or due to the presence of null alleles. Given than none of the comparisons between adult and larval samples were significant, suggesting a single panmictic unit for Atlantic sailfish, we feel that this heterozygote deficiency could be associated with the use of primers that were originally developed for blue marlin (Buonaccorsi et al. 2000) and not for sailfish. A point mutation in a flanking region of the microsatellite may result in the failure of the primer to anneal to the site and thus in unsuccessful amplification and the resultant detection of many homozygous phenotypes (Allen et al. 1995, Jarne and Lagoda 1996). However, the frequency of null alleles at locus Mn60 was very low (2.6%) and this low frequency of occurrence should not affect the interpretation of the data (Amos 2001).

Kinship within the Larval Assemblage

The larval sailfish population exhibited only marginally lower levels of genetic variation compared to the adult samples at both the mitochondrial DNA and microsatellite level, and the corrected mean distance between these adult and larval samples was not different from zero. AMOVAs failed to reveal any heterogeneity between adult and larval samples with both CR-I and microsatellite data.

While there were no obvious reductions in the levels of genetic diversity among larval and adult samples, the NJ tree (Figure 4-2) indicated that four spatially proximal groups of similarly-sized larvae shared the same mitotype. However, estimates of pairwise relatedness did not differ significantly from zero in all four instances. In addition, the pairwise relatedness among all the larvae captured, irrespective of the station they were captured, also failed to reveal any potential siblings. Thus, from the characterization of a total of 43 sailfish larvae captured in the Gulf of Mexico, we found no evidence within the larval samples indicative of a reproductive output from a single spawning event. External fertilization in highly fecund marine fauna enhances the potential for substantial variation in reproductive success among individuals, as fertilization success depends on the proximal and synchronous release of eggs and sperm. However, pairing of mates has been reported among sailfish (Voss 1953), and may reduce the reproductive variance associated with unequal fertilization success (Palumbi and Hedgecock 2005). However, the discovery of 6 full siblings in 3 discrete larval tows (2 siblings per tow) in yellowfin tuna (Talley-Farnham 2003) suggests that pairing on its own may not be determinant factor affecting individual reproductive output. Saillant et al. (2006) performed a similar comparison in order to determine if shrimp trawling was causing non-random mortality among age 0 red snapper (*Lutjanus campechanus*) as a result of individuals from the same spawning event remaining spatially proximal during part of their early life history. They concluded that red snapper from by-catch samples were not more closely related to each other than would be expected

had they been collected randomly. Thus, while the potential for variance in reproductive success may be present in free-living fishes, we find no evidence of its occurrence among Atlantic sailfish.

CHAPTER V

CONCLUSIONS

The major findings of this thesis can be summarized as follows: First, I was successful in forensically identifying billfish larvae belonging to three distinct genera and species of istiophorid billfish: sailfish (*Istiophorus platypterus*), blue marlin (*Makaira nigricans)*, and white marlin (*Tetrapterus albidus*), on the basis of their distinct nucleotide sequences of the mtDNA control region I. The collection in the northern Gulf of Mexico of high concentrations of billfish larvae in general and sailfish larvae in particular identifies this region as a larval 'hot-spot' for these species. Future analyses of otolith chemistry and RNA/DNA ratios may provide additional evidence to support the importance of this area as a critical spawning habitat for these istiophorid billfish. Second, using the patterns of variation of the mtDNA control region I examined the contemporary population structure and historical demography of the sailfish. The transatlantic comparison of eastern and western sailfish samples representative of the two putative stocks yielded no significant genetic difference between these regions, and these results were confirmed by microsatellite data. The ability for sailfish to perform long-distance migrations may result in gene flow at levels sufficiently large to maintain a single panmictic unit, although the possibility of seasonal mixing of separate eastern and western stocks has to be considered. Thus, the comparison of temporal samples representing different seasons within the same year could reveal the separation of eastern and western sailfish stocks. However, having found no significant genetic difference in the transatlantic comparison, I pooled the Atlantic sailfish samples and compared this population to a small sample from the eastern Pacific. This comparison revealed significant evidence of inter-oceanic differentiation with both the mtDNA and microsatellites. In addition, the phylogeographic association of mtDNA lineages revealed evidence of a recent invasion of Clade I sailfish (the ubiquitous clade) into the Atlantic, estimated to have taken place between 164000 to 351000 years ago. This invasion follows the Mitchell-Perrin model of inter-oceanic unidirectional gene flow, whereby Indo-Pacific individuals intermittently enter the Atlantic via the Agulhas current when conditions are favorable. Furthermore, evidence of a recent sudden expansion was detected among the eastern Pacific sailfish population, having taken place between 85400 and 173000 years ago. This recent sudden expansion in conjunction with the lower levels of genetic diversity observed in this eastern Pacific sample is consistent with the pattern observed in other tropically distributed, highly migratory species, specifically blacktip shark and the olive ridley sea turtle. Accordingly, sailfish reinvaded the eastern Pacific from the warmer western Indo-Pacific following a period when the conditions in the east were too cold for this tropical fish. The phenotypic differences associated with sailfish of the eastern Pacific combined with the observed genetic differences lead us to suspect that sailfish in this region may represent a unique stock and thus should be managed as such. Further studies with larger sample sizes are required in order to determine if this recommendation is appropriate. Lastly, I examined the larval sailfish

from the Gulf of Mexico for evidence of reproductive success. While the potential for such variance does exist in free-living species, I found no evidence of its occurrence among sailfish.

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APPENDIX A

Extraction of Mitochondrial and Microsatellite DNA

Larvae

Extraction of DNA followed the protocol described by Simpson et al. (1999) modified by Talley-Farnham (2003). Briefly, a portion of each larva was placed in 30 microliters (µl) of extraction buffer (50 mM KCl, 10 mM TrisHCl, pH 8.3, 2.5 mM $MgCl_2$, 0.01% gelatin, 0.9% Tween® 20) and 10µl of 30 mg/mL proteinase K in a 0.2 ml tube. Each sample was incubated for 60 min at 65°C and denatured for 15 min at 44°C. The supernatant was then used as DNA template.

Adults

A small piece of tissue, approximately 4 µg, was clipped from each sample using sterilized scissors and tweezers. Each sample was then be placed in a 1.5 ml microfuge tube to which 200 µl TENS solution (50 mM Tris-HCl (pH 8.0), 100 mM NaCl, 100 mM EDTA, and 1% SDS) and 20 µl proteinase K (10mg/ml) was added. The samples were then placed in a 55°C water bath for one hour, inverted, and left in the water bath overnight to ensure digestion.

DNA was precipitated by adding 20 µl 5M NaCl and two volumes of cold 95% ethanol to each tube. The samples were centrifuged (Fisher Scientific accuSpin™) for 10 minutes at 13000 rpm and the resulting supernatant was decanted. The remaining DNA pellets were washed with 300 µl cold 70% ethanol and centrifugation repeated. The supernatant was decanted again and the tubes will be left open, covered with Kimwipes®, and the pellets were allowed to dry overnight.

DNA was re-suspended in 100 µl of TE buffer (10 mM Tris-HCl (pH 8.0) and 1 mM EDTA) and incubated at 55°C in a water bath for 15 minutes.

APPENDIX B

MtDNA Amplification and Sequencing

Polymerase chain reactions (PCR) were prepared in 12.5 µl volumes containing: 8.4 µl ddH2O, 1.25 µl 10x buffer, 0.5 µl 2 mM $MgCl_2$, 0.25 µl dNTPs, 0.5 µl of each primer (L15998 and CSBD-H), 0.1 µl Platinum® *Taq* polymerase (Invitrogen), and 1 µl of isolated DNA template.

DNA amplification was carried out in an Eppendorf Mastercycler® Gradient thermal cycler. An initial denaturing step of 2 min at 94.0°C was followed by 36 cycles of strand denaturation at 94.0°C for 30 sec, primer annealing at 50.0°C for 45 sec, and extension at 72.0°C for 1 min. The final extension was at 72.0°C for 3 min. 5 µl of each PCR product was then be loaded into a 1% to 1.5% agarose gel pre-stained with 0.1 µg/ml ethidium bromide, allowed to run at 100 mV for 20 min, and viewed through an ultraviolet transilluminator to determine the quality of the amplifications.

Excess primers and dNTPs were removed from PCR products by the adding 2 µl of ExoSAP-IT™ (USB Corporation, Cleveland, Ohio) following the manufacturer's recommendations. The BigDye™ Terminator Cycle Sequencing Ready Reaction Kit (Perkin-Elmer Corporation, Foster City, California) was used in the cycle sequencing reaction. This reaction involved combining 2 µl each of BigDye, 5x dilution buffer, and clean PCR product, with 4 µl of L15998 primer diluted 3:1 in new 0.2 ml tubes. After a pulse in the centrifuge, the samples were reloaded into the thermal cycler for cycle sequencing, which consisted of 26 cycles of the following profile: 96.0°C for 10 sec, 50.0°C for 5 sec, and 60.0°C for 4 min.

DNA was precipitated by adding 1 µl 7.5 M ammonium acetate and 25 µl cold 95% ethanol to each 0.2 ml tube. The tubes were then inverted and pulsed in the centrifuge. After precipitating for 10 min at room temperature, the samples were placed into the Fisher Scientific accuSpin™ for 25 min at 2000 rpm, and then decanted by inversion. 150 µl of 70% ethanol was added to each tube and mixed by inverting. The samples were then returned to the centrifuge for 10 min at 13000 rpm. The resulting supernatant was removed and discarded and the samples covered with a Kimwipe® and allowed to dry for 20 min. In preparation for sequencing in the ABI 310 genetic analyzer (Perkin-Elmer Corporation, Foster City, California), 25 µl of formamide was added to each reaction, then vortexed for 10 seconds, and finally pulsed in the centrifuge. After being preheated in the thermal cycler for two minutes at 95°C the samples were placed on ice and subsequently loaded into the ABI 310 genetic analyzer.

APPENDIX C

Microsatellite DNA Amplification and Sequencing

Microsatellite loci were targeted in order to characterize polymorphisms in fragment mobility. The primers that were used were developed by Bournaccorsi and Graves (2000) and target 5 tetranucleotide microsatellite loci in billfish (Mn01, Mn08, Mn10, Mn60, and Mn90). The PCR reaction mix was the same as that described for mtDNA amplification but using each microsatellite primer pair instead. PCR began with an initial denaturing step at 94°C for 2 min followed by 30 cycles of the following profile: denaturation at 94°C for 30 sec, annealing at 55°C for 45 sec, and extension at 72°C for 1 min. The final extension ran for 5 min at 72°C. In preparation for size characterization, 0.5 µl of TAMRA™ Size Standard (ABI Prism, Applied Biosystems, Warrington, UK) and 22 µl of formamide was added to 1 µl of each amplified sample. Fragment mobility was then detected in an ABI 310 genetic analyzer (Perkin-Elmer Corporation, Foster City, California).

APPENDIX D

Table D-1. Physio-chemical parameters measured at surface of each station.

	May 2005				**July 2005**			
Station #	Temp. (°C)	Salinity (ppt)	[DO] (mg/L)	pH	Temp. (°C)	Salinity (ppt)	[DO] (mg/L)	pH
1	24.5	35.50	6.80	8.09	29.31	35.55	6.69	8.10
2	25.28	35.78	6.88	8.18	29.42	35.63	6.59	8.10
3	25.17	34.80	6.85	8.22	29.53	35.61	6.63	8.12
4	25.56	34.66	6.80	8.15	29.64	35.78	6.58	8.10
5	25.81	34.97	6.83	8.13	29.81	35.81	6.51	7.96
6	25.87	35.21	6.90	8.21	29.86	35.89	6.55	7.43
7	25.57	35.53	7.01	7.79				
8	25.85	35.23	7.18	8.15	30.14	36.01	8.93	7.24
9	26.14	34.79	7.29	8.17	30.04	36.03	10.30	8.00
10	25.40	35.68	7.15	8.02	29.86	35.99	10.21	8.10
11	25.20	35.85	10.82	8.24	29.63	35.90	10.42	8.09
12	25.09	35.46	6.47	8.24	29.52	36.01	10.00	8.17
13	25.12	35.64	6.61	8.22	29.57	36.01	10.30	8.15
14	25.46	35.68	6.38	8.17	29.72	35.98	6.40	8.14
15	25.57	35.61	6.60	7.85	29.85	36.06	6.42	8.12
16	26.13	35.60	6.59	7.57	29.95	36.07	6.19	7.99
17	25.96	35.75	6.77	7.51	30.53	36.16	8.03	7.56
18	26.40	35.43	6.71	7.91	30.73	36.17	10.50	7.76
19	26.29	35.71	6.93	8.02	31.56	36.19	6.51	7.74
20	26.15	34.24	7.00	8.08	31.10	36.30	6.53	7.95
21	26.45	36.05	6.78	8.25	29.70	36.07	6.64	8.10
22	26.72	34.89	6.84	8.25	29.81	36.15	6.22	8.15
23	27.03	35.98	6.77	8.25	29.76	36.10	6.23	8.11
24	26.86	35.99	6.70	8.23	29.74	35.94	6.28	8.03
25	27.15	35.56	6.79	8.23	30.17	36.02	6.20	8.13
26	27.71	35.85	6.30	8.08	30.34	36.09	6.18	8.02
27	27.82	36.03	6.87	7.62	30.59	36.12	6.18	8.02
28	27.70	35.96	7.02	7.68	30.36	36.12	6.26	8.01
29	27.74	36.10	6.81	7.91	30.48	36.13	6.17	7.95
30	27.03	35.55	7.03	7.92	30.35	36.07	6.17	7.84
31	27.17	36.22	6.33	8.21	29.92	36.02	6.28	8.07
32	27.88	36.07	6.69	7.98	30.01	36.05	6.36	8.04
33	28.02	36.10	7.73	8.06	30.21	36.09	6.38	8.01
34	28.13	36.08	7.06	8.07	30.22	36.07	6.44	7.92
35	28.04	35.97	7.01	8.01	30.43	36.16	6.41	7.88
36	27.81	35.94	9.20	8.18	31.90	36.21	6.29	7.96
37	28.04	35.56	6.71	8.14	32.51	36.08	6.18	8.04
38	27.85	36.33	6.76	8.22	30.40	36.21	6.35	8.02
39	27.84	35.79	7.46	8.22	31.76	36.16	6.23	7.98
40	27.59	36.23	6.67	8.23	31.36	36.18	6.20	8.07
41	27.16	36.45	6.65	8.21	30.51	33.75	6.24	8.11
42	25.99	36.23	8.92	8.18	30.57	33.76	6.27	8.09
43	26.14	35.36	7.43	7.86	30.68	33.78	6.24	7.85
44	26.27	36.45	7.05	5.63	31.01	33.09	6.16	7.93
45	25.93	36.03	6.96	7.25	31.09	33.80	6.50	7.71

Table D-1. Continued.

May 2005 Station #	Temp. (°C)	Salinity (ppt)	[DO] (mg/L)	pH	**July 2005** Temp (°C)	Salinity (ppt)	[DO] (mg/L)	pH
46	26.03	36.37	6.69	7.82	31.69	33.73	6.46	7.79
47	26.01	35.19	6.93	7.99	30.87	33.88	6.47	7.96
48	26.35	34.34	7.02	8.11	30.70	34.86	6.46	7.84
49	26.36	34.03	7.10	8.11	31.05	35.32	6.34	8.05
50	26.24	35.15	7.18	8.16	30.71	33.78	6.47	8.01
51	25.79	35.30	7.53	8.16	29.74	34.88	6.26	8.00
52	25.28	34.54	8.82	8.17	30.10	35.13	6.39	7.91
53	25.35	34.84	7.52	7.97	30.15	35.52	6.38	7.80
54	25.75	34.78	6.58	7.87	30.66	34.91	6.39	7.95
55	25.80	34.70	6.50	6.80	30.70	33.58	6.35	7.95
56	26.11	34.07	7.38	6.74	30.92	32.15	6.43	7.89
57	26.28	34.16	7.35	7.78	30.99	32.84	6.44	7.96
58	26.65	34.39	6.72	7.99	31.22	32.35	6.19	7.77
59	26.60	34.86	6.64	8.06	31.18	32.99	6.31	7.99
60	26.54	34.48	6.98	8.14	31.05	32.38	6.23	8.07
61					30.10	36.15	6.15	8.07
62					30.88	32.10	6.31	8.10

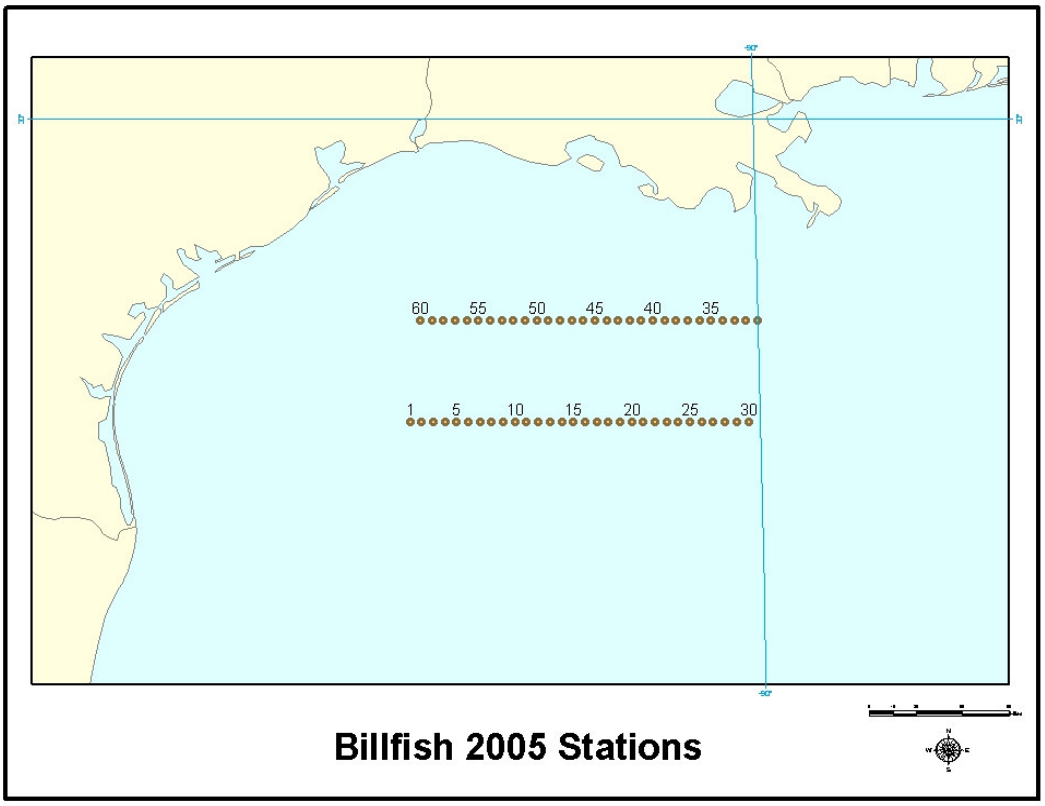

Figure D-1. Station locations for 2005 collections (Courtesy of C. Pratt; Research Engineer/Scientific Assistant, University of Texas at Austin Marine Science Institute, Port Aransas, TX).

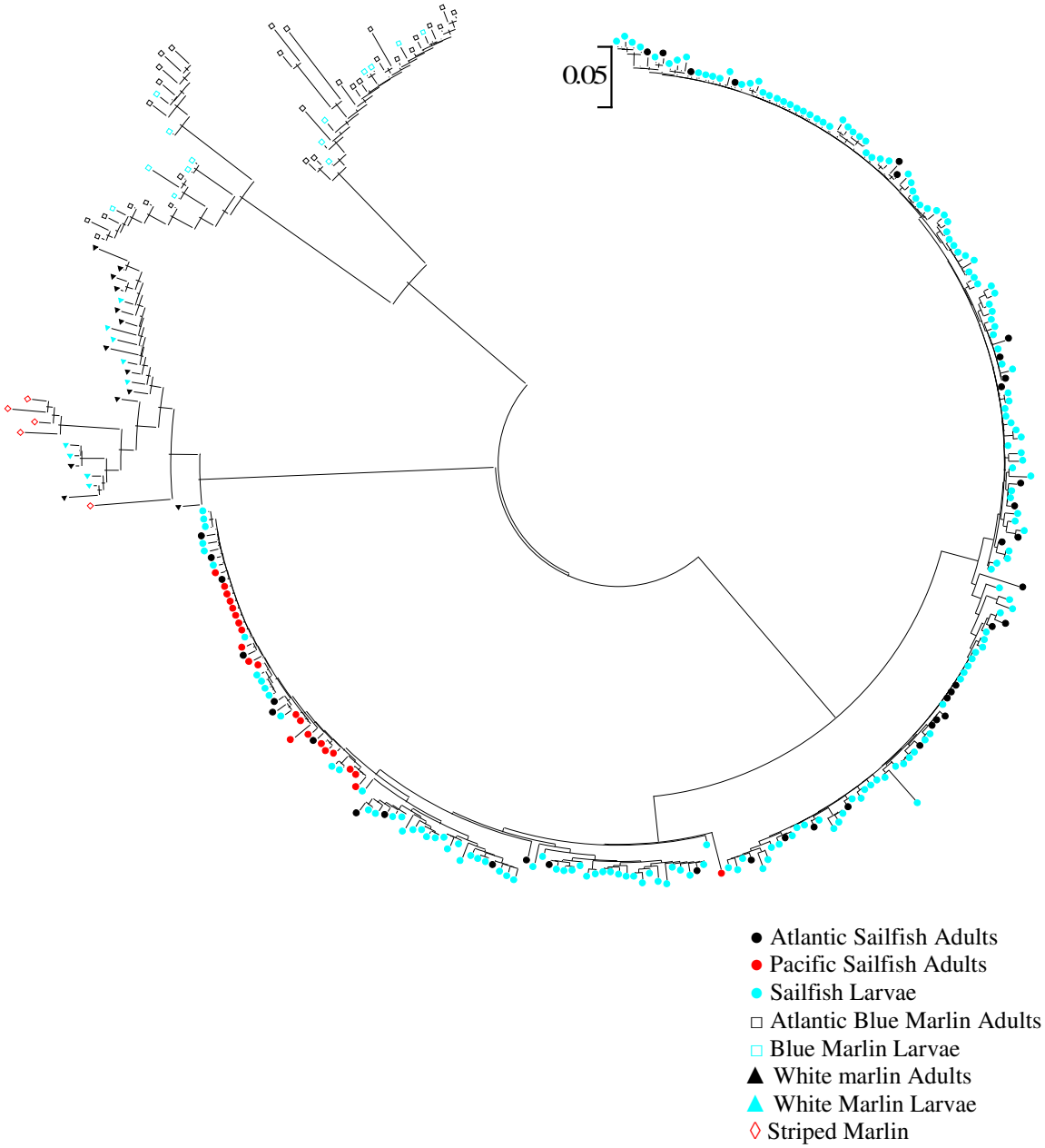

Figure D-2. Neighbor-joining tree used for identification of all istiophorid larvae collected in May and July of 2005.

Table D-2. Number of istiophorid larvae per station collected during Cruise 1 (May 2005) and Cruise 2 (July 2005). In the station numbering '27' indicates that the sample was collected along the 27th line of latitude and '28' that they were collected along the 28th line of latitude. The second two digits represent the station number itself as reported in Figure A-1.

May 2005			July 2005		
Station	Sailfish Larvae	White Marlin Larvae	Station	Sailfish Larvae	Blue Marlin Larvae
2722	3		2721	29	
2726	1	1	2723	1	
2831	4		2724	1	
2832	9	4	2728	1	4
2833	7		2729		6
2834	5	2	2761		1
2835	2	1	2831	12	3
2836	3		2835	32	
2837	3		2836	3	
2838	1		2837	6	
2839	8	1	2840	1	
			2841	3	
			2842	4	
			2844	1	
			2846	1	
			2848	1	
			2849	6	
			2850	12	
			2851	12	
			2853	4	
			2857	1	
			2858	1	
			2860	2	
			2862	1	
TOTAL	46	9	**TOTAL**	135	14

Table D-3. Frequency of alleles at 5 tetranucleotide microsatellite loci.

Allele #	Mn60		Mn90		Mn01		Mn08		Mn10	
	Length	Freq.	Length	Freq.	Length	Freq.	Length	Freq.	Length	Freq.
1	232	0.022	234	0.016	232	0.015	226	0.014	261	0.031
2	236	0.016	250	0.005	246	0.015	233	0.010	264	0.010
3	241	0.027	254	0.010	250	0.171	242	0.005	268	0.266
4	260	0.011	265	0.064	254	0.079	249	0.128	271	0.259
5	266	0.012	269	0.026	258	0.196	254	0.019	275	0.125
6	273	0.005	273	0.053	261	0.052	257	0.261	279	0.069
7	276	0.016	277	0.053	265	0.068	261	0.446	283	0.042
8	280	0.038	281	0.021	269	0.215	265	0.029	286	0.015
9	288	0.016	289	0.026	273	0.084	269	0.069	290	0.069
10	292	0.016	291	0.005	277	0.073	272	0.014	294	0.047
11	296	0.022	295	0.026	280	0.026	277	0.005	298	0.015
12	300	0.050	299	0.075	288	0.005			302	0.021
13	304	0.033	303	0.070					307	0.005
14	308	0.073	305	0.070					311	0.005
15	312	0.078	307	0.042					315	0.005
16	316	0.090	312	0.037					319	0.010
17	321	0.044	316	0.042					382	0.005
18	326	0.044	320	0.059						
19	329	0.033	325	0.037						
20	333	0.005	329	0.005						
21	337	0.016	336	0.010						
22	341	0.027	340	0.016						
23	345	0.022	345	0.010						
24	349	0.016	349	0.005						
25	360	0.011	352	0.026						
26	364	0.016	356	0.032						
27	368	0.011	359	0.010						
28	372	0.011	364	0.042						
29	376	0.033	367	0.010						
30	379	0.033	371	0.016						
31	384	0.033	377	0.010						
32	387	0.038	390	0.005						
33	393	0.016	398	0.021						
34	399	0.016	403	0.026						
35	404	0.016	408	0.010						
36	407	0.005	412	0.005						
37	419	0.005								
38	423	0.016								
39	448	0.005								

Table D-4. Sailfish larvae standard length (mm). 01-n = May 2005 Cruise; 02-n = July 2005 Cruise.

ID	SL	ID	SL	ID	SL	ID	SL
01-2721120001	11.34	02-2721050012	5.72	02-2835120015	7.62	02-2851050003	5.86
01-2722120002	5.71	02-2721050013	6.70	02-2835120016	5.25	02-2851050004	8.92
01-2722120003	5.65	02-2721050015	6.90	02-2835120017	5.37	02-2851050005	6.26
01-2726120001	5.92	02-2721050018	6.49	02-2835120018	4.86	02-2851050006	7.60
01-2831120001	7.55	02-2721050020	6.53	02-2835120019	3.89	02-2851050007	-
01-2831120002	6.75	02-2721050022	6.91	02-2835120020	4.68	02-2851050008	5.19
01-2831120003	4.51	02-2721050023	-	02-2835120021	8.84	02-2851120001	12.81
01-2831120004	5.11	02-2721050024	6.23	02-2835120022	6.92	02-2851120002	12.43
01-2832050007	5.58	02-2721050025	7.88	02-2835120025	6.70	02-2851120003	7.90
01-2832120011	4.55	02-2721050026	7.81	02-2835120026	6.81	02-2851120004	6.67
01-2832120013	5.02	02-2721050028	6.94	02-2835120027	6.08	02-2853050001	9.75
01-2832120015	5.52	02-2721050030	6.58	02-2835120028	6.96	02-2853120001	10.02
01-2832120020	5.58	02-2721050032	-	02-2835120029	5.30	02-2853120002	14.87
01-2832120022	5.86	02-2721050034	7.50	02-2835120030	6.91	02-2853120003	11.65
01-2832120023	5.94	02-2721120001	10.31	02-2836050001	15.83	02-2857050002	13.14
01-2832120024	5.98	02-2721120002	10.32	02-2836120001	7.83	02-2858050001	7.59
01-2832120026	7.20	02-2721120004	7.01	02-2836120002	-	02-2860050001	5.44
01-2833050004	6.90	02-2721120005	6.84	02-2837050001	7.18	02-2860120001	11.93
01-2833120001	3.86	02-2721120006	7.47	02-2837050002	5.88	02-2862120001	12.29
01-2833120002	4.01	02-2721120007	8.95	02-2837050003	6.88		
01-2833120003	4.18	02-2721120009	7.38	02-2837050005	5.95		
01-2833120004	4.69	02-2723050001	11.33	02-2837120001	6.60		
01-283312000	4.74	02-2724050001	6.80	02-2837120004	7.90		
01-2833120006	5.88	02-2728050002	5.87	02-2840120001	7.01		
01-2834120001	4.56	02-2831050002	5.81	02-2841120001	13.41		
01-2834120002	4.69	02-2831050003	6.17	02-2841120002	8.35		
01-2834120004	4.84	02-2831050004	6.68	02-2841120003	5.03		
01-2834120006	7.67	02-2831050005	5.82	02-2842050002	9.42		
01-2834120007	7.82	02-2831050006	5.78	02-2842120001	17.88		
01-2835120001	5.34	02-2831050007	5.98	02-2842120002	11.86		
01-2835120002	5.64	02-2831050008	6.20	02-2842120003	8.48		
01-2836050001	7.25	02-2831050009	6.35	02-2844050001	16.34		
01-2836050002	9.72	02-2831050010	5.92	02-2846120001	7.63		
01-2836120001	7.57	02-2831120001	6.48	02-2848120001	20.29		
01-2837050001	7.68	02-2831120002	6.54	02-2849050001	9.51		
01-2837120001	7.70	02-2831120003	6.95	02-2849050002	10.19		
01-2837120002	8.37	02-2835050001	10.69	02-2849050003	-		
01-2838120001	8.91	02-2835050002	6.76	02-2849120001	12.38		
01-2839050001	6.13	02-2835050003	8.05	02-2849120002	5.94		
01-2839050002	8.00	02-2835050004	6.77	02-2849120004	4.92		
01-2839050003	9.53	02-2835120001	8.31	02-2850050001	6.98		
01-2839120001	5.11	02-2835120002	6.19	02-2850050002	9.46		
01-2839120002	6.49	02-2835120003	4.17	02-2850050003	8.18		
01-2839120003	6.58	02-2835120004	13.00	02-2850050004	6.67		
01-2839120004	7.63	02-2835120005	12.90	02-2850050005	6.59		
01-2839120006	8.28	02-2835120006	10.38	02-2850120001	13.61		
02-2721050001	14.49	02-2835120007	8.37	02-2850120002	6.94		
02-2721050002	9.17	02-2835120008	9.31	02-2850120003	7.26		
02-2721050003	9.17	02-2835120009	9.94	02-2850120004	5.99		
02-2721050004	7.26	02-2835120010	7.93	02-2850120005	5.78		

Table D-4. Continued.

02-2721050005	6.75	02-2835120011	7.10	02-2850120006	-
02-2721050006	6.29	02-2835120012	3.95	02-2850120007	7.27
02-2721050009	7.03	02-2835120013	6.02	02-2851050001	7.93
02-2721050011	5.31	02-2835120014	6.49	02-2851050002	9.75

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- Fully competent with maintenance and use of ABI310 Genetic Analyzer and associated programs for DNA sequencing and fragment polymorphism (microsatellite) analyses
- Extensive knowledge of GENEPOP 3.3, Arlequin 3.0, MARK, DnaSP 4.10, and MEGA 3.1 computer programs for genetic analyses; fully capable of learning new software
- Familiar with programming Eppendorf Mastercycler® Gradient thermal cycler and autoclave for sterilization of equipment

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Publications:

Bangma, J. L. and R. L. Haedrich. A comparison of mesopelagic fish fauna in the Gulf of Mexico with that of the Caribbean and Sargasso Seas. Deep-Sea Research (in review).

Presentations:

Bangma, J. L. and J. R. Alvarado Bremer. 2005. Assessment of reproductive variance among sailfish larvae (*Istiophorus platypterus*) in the Gulf of Mexico. American Fisheries Society Annual Meeting, September 12-15, 2005 Anchorage, Alaska.

References:
Available upon request