HISTORICAL DEMOGRAPHY AND GENETIC POPULATION STRUCTURE OF THE BLACKFIN TUNA (*Thunnus atlanticus*) FROM THE NORTHWEST ATLANTIC OCEAN AND THE GULF OF MEXICO

A Thesis

by

BRANDON LAROY SAXTON

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

May 2009

Major Subject: Wildlife and Fisheries Sciences

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Approved by:

Chair of Committee,	Jaime Alvarado-Bremer
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ABSTRACT

Historical Demography and Genetic Population Structure of the Blackfin Tuna (*Thunnus atlanticus*) from the Northwest Atlantic Ocean and the Gulf of Mexico. (May 2009)

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Little is known about the population structure and genetic variability of blackfin tuna despite catch increases over the past 25 years. In this thesis, levels of genetic variation contained in 323bp of the mitochondrial DNA (mtDNA) control region-I (CR-I) and in six microsatellite loci were characterized for two regions: the Gulf of Mexico (GoM) and the Northwest Atlantic. Large amounts of mtDNA diversity (h>0.99; =0.047) were observed in both regions. Mismatch distribution analysis of the CR-I sequence data, using a mutation rate of 1.6% Ma-1for scombroid fishes, indicate blackfin tuna underwent population expansion about 1.4 Ma, a timeline concordant with the expansion of other tunas and billfishes. Estimates of female effective population size were very large at 7.8 million and 12.8 million individuals for the NW Atlantic and the GoM, respectively.

Both mtDNA and six microsatellite loci were used to determine blackfin tuna population structure. Microsatellite and mtDNA AMOVAs revealed significant differentiation (msat st=0.01, p=0.006 and mtDNA st=0.01, p=0.049) between the GoM and the NW Atlantic samples. Migration estimates using mtDNA data indicate

that twice as many females enter the NW Atlantic from the GoM (346 individuals/generation) than the opposite direction (150 individuals/generation). Migration estimates using microsatellite data were substantially smaller, with the Gulf receiving 7 individuals/generation and the NW Atlantic 4 individuals/generation.

Finally, low levels of genetic differentiation using microsatellite data have been reported in other highly abundant marine fishes, which have been attributed to homoplasy in allele size. To test this hypothesis, the allele frequency distributions of blackfin and yellowfin tuna at six microsatellite loci were compared. The distances between species were surprisingly small (D_a=4.0%, $(\delta\mu)^2$=1.08), with a large degree of similarity in frequency distributions at four loci. The comparison of bigeye tuna at two microsatellite loci revealed additional inter-specific similarities. A mutation rate for these loci was estimated by modifying an equation used to estimate time since divergence. Microsatellites in tunas appear to evolve at a rate (4.3×10^{-7} Ma^{-1}) that is two orders of magnitude slower than other fishes (1×10^{-5} Ma^{-1}). Accordingly, microsatellite allele size similarities are plesiomorphic and not due to homoplasy.

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CHAPTER I

INTRODUCTION

Blackfin tuna, *Thunnus atlanticus*, is a highly migratory, tuna that is present in pelagic waters of the western Atlantic (Adams 2004). Their range includes the entire Gulf of Mexico and the Caribbean Sea, and in the Atlantic from Martha's Vineyard off the coast of the U.S. (40°N) to Rio de Janeiro, Brazil (22°S), although it has been recorded as far as 31°S (Freire et al. 2005) (Fig. 1-1). Blackfin tuna reach a maximum size of 108cm in total length (TL), with males typically being larger than females when they attain sexual maturity around age 2 (Adams 2004; Freire et al. 2005; Gothreaux 2007). Around Florida and in the Gulf of Mexico, spawning occurs year round far offshore over epipelagic waters (Collette 1983). By contrast, spawning off northeastern Brazil occurs year round with a possible peak in December (Freire et al. 2005; Vieira et al. 2005).

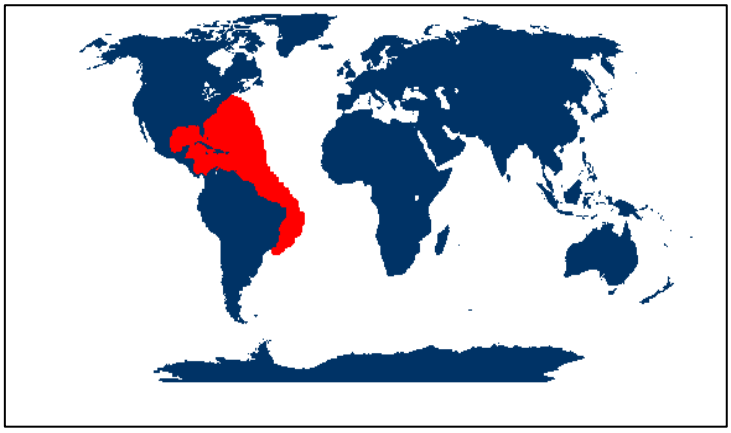


Figure 1-1. Geographic distribution of *T. atlanticus*, based on a map from the FAO species catalogue (Collette 1983).

This thesis follows the style of Journal of Heredity.

Blackfin tuna are members of the tropical subgenus *Neothunnus*; along with yellowfin tuna, *Thunnus albacares*, and longtail tuna, *T. tonggol* (Collette 1979). Internal synapomorphies of *Neothunnus* include the presence of central heat exchangers, multiple modifications in the vertebral column, a post cardinal vein that joins with the right cutaneous vein, and the ventral surface of the liver contains no striations with no vascular cones present on the dorsal surface (Gibbs and Collette 1967; Collette 1979). Phylogenetic analyses using mitochondrial DNA (mtDNA) control region (CR) sequence data confirms the very close relationship of the three *Neothunnus* species (Alvarado Bremer et al. 1997).

Blackfin tuna is subject to commercial and recreational exploitation and no management regulations are currently in place by the International Commission for the Conservation of Atlantic Tunas (ICCAT) (ICCAT 2008). Annual landings of blackfin tuna between 1980-2004 throughout all regions averaged 2,929 metric tons (MT). Although landings are characterized by substantial inter-annual variability, they have increased gradually over past 25-year period (Fig. 1-2) (ICCAT 2006). Blackfin tuna are caught with various types of gear and support various fisheries. For instance, in the US, blackfin tuna support important recreational fisheries in the Gulf of Mexico and northwestern Atlantic. In Cuba and the Lesser Antilles, blackfin tuna is captured primarily using hook and line as by-catch of skipjack tuna fishery (Collette 1983; Luckhurst et al. 2001). In Brazil, blackfin tuna are by-catch of the longline fisheries targeting other large pelagic species. However, in the Rio Grande do Norte state there is an artisanal handline fishery, which is economically important to this region (Freire et al.

2005). With such diverse fisheries data, correct estimates of abundance are difficult. However, with the imminent decline of other more valuable tuna stocks because of overfishing (Myers and Worm 2003), heavier fishing pressure could be placed on blackfin tuna in the near future.

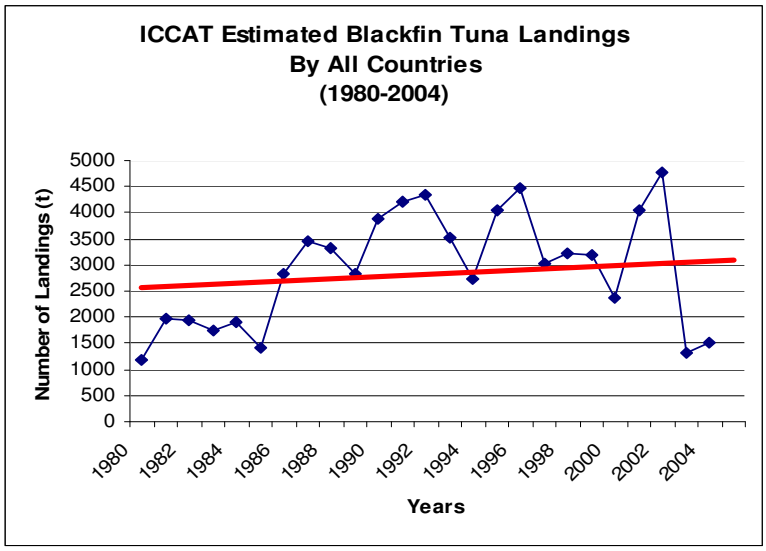


Figure 1-2. ICCAT reported numbers of *T. atlanticus* over a 25 year period based from data in SMT-Table-1 (ICCAT 2006). Regression line (red) represents the average increase in the number of landings over the time period.

Fisheries management involves an assessment of the fishery's health derived from estimates of abundance, recruitment, mortality, age structure, growth rates, and the reproductive structure, or the sex ratio and reproductive biomass, of the stock (Gulland 1983; Cooper 2006). Here, a stock corresponds to an intraspecific group of randomly mating individuals with temporal and spatial integrity (Ward 2000). Stock resolution is necessary for management because each stock is affected by it own set of environmental

conditions, and the goal for management should be to sustain long-term fisheries and reduce the risk of depletion (Laikre et al. 2005). Resolving stock structure can be accomplished through various means, including tagging studies, otolith chemistry, and genetic analyses (Alvarado Bremer 1998; Carlsson et al. 2004; Block et al. 2005; Rooker et al. 2008b; Rooker et al. 2008a; Arkhipkin et al. 2009). This study will assess the genetic stock structure and the levels of genetic variation of blackfin tuna in the Gulf of Mexico and Northwest Atlantic Ocean. Alvarado Bremer et al. (1997) characterized the levels of variation in the mtDNA control region for a few individuals with the purpose of determining its phylogenetic relatedness relative to other tunas. By contrast, this study will characterize variation of a large number of individuals at both the mitochondrial and nuclear genomes. The present study is unique in that it seeks to characterize the genetic population structure before the species suffers dramatic reductions in population size. Accordingly, the information generated could be used as baseline data to aid managers monitoring blackfin tuna populations and prevent future losses of genetic variation (Ward 2000).

The remainder of this thesis consists of three chapters. In Chapter II the historical demography of blackfin tuna is reconstructed by characterizing 322 base pairs (bp) of sequence of the mitochondrial DNA (mtDNA) control region I (CR-I), also known as the left domain of the d-loop region. These mtDNA CR-I sequences are used in Chapter III to test whether samples blackfin tuna from the Gulf of Mexico and the NW Atlantic regions correspond to the same population. In addition, six nuclear microsatellite loci used previously in yellowfin tuna (*T. albacares*) and Pacific bluefin

tuna (*T. orientalis*) (Grewe and Hampton 1998; Takagi et al. 1999; Appleyard et al. 2001). Microsatellites were used because they are assumed to evolve more rapidly than the mtDNA CR-I, and thus may better resolve the genetic population structure of blackfin tuna. Chapter IV estimates the genetic distance between two tuna sibling species by comparing mitochondrial and microsatellite data of blackfin tuna (this study) against the corresponding data of yellowfin tuna (Farnham 2003). These comparisons are used to estimate the mutation rates of the mtDNA CR-I and microsatellites by employing estimated divergence time of blackfin tuna and yellowfin tuna. Finally, the conclusions of this thesis are given in Chapter V.

CHAPTER II

HISTORICAL DEMOGRAPHY OF BLACKFIN TUNA

(*Thunnus atlanticus*)

Introduction

The gradual increase in the total catch (MT) of blackfin tuna during the past 25 years (see Chapter I) is likely to continue given the dramatic reductions in biomass of other tunas, and the increase in demand of fish products worldwide (Pauly et al. 2000). Most often 'new' or expanded fisheries are exploited without possessing relevant biological information necessary to implement sound management practices. For blackfin tuna in particular, factors affecting recruitment, current and historical levels of genetic variability and stock structure (see Chapter III) are all unresolved.

Information on the current levels of genetic variation within- and among-populations (population structure), and an understanding of the historical demographic factors that may explain the distribution of this variation, when analyzed together, could become valuable tools for conservation. Specifically, assessments of the current levels of genetic variation could be employed in the future as benchmark data to assess the impact of fisheries, particularly if the population experiences severe reductions in population size. Similarly, historical demography data may provide information on whether a population experienced recent or ancient bottlenecks or whether that population is at equilibrium or undergoing growth or decline (Slatkin and Hudson 1991; Rogers and Harpending 1992). Accordingly, these data could also be used to predict future rates of loss as a result of fluctuations in population size caused by natural or

anthropogenic activities, or the combination of both and could be used for future management purposes.

The analysis of mismatch distributions and neutrality tests are tools that can be used to give insight into a population's past. Mismatch distribution consists of the tabulation of the number of pairwise differences among all DNA sequences in a sample, with the shape of the distribution affected by the demographic history of a population (Rogers and Harpending 1992). Populations that have been stationary for a long time tend to have multimodal distributions, often described as ragged or erratic, whereas populations that experienced rapid expansion typically have smooth uni-modal Poisson-like distributions. Because mtDNA is not recombined any new mutations are assumed to lead to the increase in the number of pairwise differences. Larger more stable populations have a wider range in the number of pairwise differences that occur in more similar frequencies, generating the multimodal distribution. Populations having experienced rapid expansion have a smaller range with a number of pairwise differences occurring in a higher frequency with others varying around the mean, therefore creating the wave-like distribution. In addition, the smaller the initial population the steeper the leading face of the wave should be. The position of this mode, or τ, reflects the amount of time since expansion under the assumption that mutation rate is constant (Li 1977; Rogers and Harpending 1992; Harpending 1994). In some cases the distribution is indicative of an ancient bottleneck followed by a rapid expansion, whereas others, characterized by haplotypes separated by one or two steps, resulting in a star-like

phylogeny, indicate a more recent population expansion (Slatkin and Hudson 1991; Alvarado Bremer et al. 2005a).

Estimates of the demographic parameters θ_0 (effective population size before expansion event) and θ_1 (effective population size after expansion event) can help explain levels of genetic differentiation between sub-populations (Rogers and Harpending 1992). For instance, using mtDNA data Ely et al. (2005) found that the signal of genetic differentiation between Atlantic and Pacific samples of yellowfin tuna was extremely low and absent for skipjack tuna. The corresponding reduction and absence in the signal of genetic partitioning between oceans, was attributed to the extremely large female effective population size (N_{ef}) of yellowfin tuna, and the even larger N_{ef} of skipjack tuna, as indicated by their very high values of θ_1, as the effects of random genetic drift on the mtDNA genome would be minimized.

One limitation of employing mismatch distributions to reconstruct the demographic history of populations is that the associated tests are extremely conservative (Ramos-Onsins and Rozas 2002). Neutrality tests offer an alternative approach to infer such histories especially when studying neutral segments of DNA, such as the mtDNA control region (CR-I) (Tajima 1989; Harpending et al. 1993; Fu 1997; Ramos-Onsins and Rozas 2002). Ramos-Onsins and Rozas (2002) compared the statistical power of several neutrality test statistics and found that under a variety of scenarios Fu's F_s, which uses information from haplotype distribution, and Ramos-Onsins and Rozas R_2, which uses the difference between singleton sites and the average number of nucleotide differences, had the highest power to detect historical population

growth. They provide evidence that Fu's F_s test performs better with larger sample sizes, whereas their R_2 was far superior for small sample sizes, whereas Tajima's D (Tajima 1989) and the raggedness index (Harpending et al. 1993) performed at much lower levels.

The demographic history of the mtDNA CR has been characterized for several highly migratory pelagic fishes, including swordfish (*Xiphias gladius*), sailfish (*Istiophorus platypterus*), bluefin tuna (*Thunnus thynnus)*, skipjack tuna, and yellowfin tuna (Farnham 2003; Alvarado Bremer et al. 2005a; Ely et al. 2005; Bangma 2006). These studies contain useful comparative data for the interpretation of blackfin tuna demographic history. Data for Atlantic yellowfin tuna is particularly appropriate, as this species is very closely related to blackfin tuna (Sharp and Pirages 1978; Alvarado Bremer et al. 1997). The mismatch distribution of yellowfin tuna mtDNA CR-I generates a smooth bell-shaped and a statistically significant Fu's F_s test (Farnham 2003), both of which are indicative of population expansion. Because of the close phylogenetic relationship between blackfin tuna and yellowfin tuna, it could be expected to possess similar demographic signatures of expansion. However, these two species have distinct geographic distributions and environmental limitations, with yellowfin tuna being cosmopolitan and blackfin tuna restricted to the NW Atlantic and waters above 20^{o}C, and thus it is likely that these species experienced different paleoceanographic conditions. Accordingly, the Atlantic populations may have very distinct demographic histories and the timeline of expansion events may not coincide.

In this chapter, a 323 base pair sequence from the hypervariable mtDNA CR-I was used to reconstruct the historical demography of blackfin tuna. The data was used to test for evidence of past population expansion, and to estimate historical effective female population sizes. Results were compared against the demographic signal of yellowfin tuna and other highly migratory pelagic species.

Methods

In total 163 blackfin tuna specimens was collected in the Gulf of Mexico and the NW Atlantic. The Gulf of Mexico sample (GoM) consisted of adults (n=54) and larvae (n=74) collected between 2001-2007. The NW Atlantic sample consisted of adults (NW Atl, n=35) collected between 1994-1995 (Table 2-1). Tissue samples from Gulf of Mexico adults consisted of axial muscle that was initially frozen and then stored in a 70% ethanol solution. Tissue from the NW Atlantic consisted of heart or spleen preserved in SDS-Urea. Adult samples from the Gulf of Mexico and the NW Atlantic were collected through recreational fisheries. Larvae from several species of tuna were collected from 2005-2007 in the Gulf of Mexico using neuston nets (500μm and 1200μm mesh) towed just below the surface at 2.5 knots for 10 minutes at a time (Tidwell et al. 2008). Larvae were preserved in 70% ethanol and each individual was assigned a unique identification number (Appendix A). Seven tows, consisting of 20 individual larvae, were randomly selected and were forensically identified in the laboratory (see below for details).

Table 2-1. Sample data of blackfin tuna, grouped by region of capture from the NW Atlantic (NW Atl) and the Gulf of Mexico (GoM). (GoML)= Larval samples from the Gulf of Mexico. Location data for the Charleston sample could not be identified so region of capture was used.

Group	Sample	Year	n	Tissue Type	Location
NW Atl n=35	Charleston	1994	18	Adult	North Atlantic
	Islamorada	1994	16	Adult	26 N, 80 W
	Bermuda	1995	1	Adult	35 N, 65 W
GoM n=125	Freeport	2001	17	Adult	27 N, 94 W
		2002	1	Adult	27 N, 94 W
	Venice	2002	19	Adult	28 N, 94 W
		2003	18	Adult	28 N, 94 W
	GoML	2005	3	Larvae	27-28 N, 88-94 W
		2006	46	Larvae	27-28 N, 88-94 W
		2007	25	Larvae	27-28 N, 88-94 W

DNA extraction protocols for the various preservation methods followed those described by Farnham (2003) (Appendix B). A segment of the control region (CR-I) was amplified using fish specific primers CSBD-H and L15998 (Alvarado Bremer 1994). The DNA sequences of successful amplicons were then determined using an ABI Prism™ 310 Genetic Analyzer, and an ABI 3130™ Genetic Analyzer. Details of DNA extractions, PCR conditions, and sequencing methods are given in Appendix C. Multiple sequence alignments were performed using CLUSTAL (Thompson et al. 1994; Higgins et al. 1996) implemented in MEGA 4.0 (Tamura et al. 2007) followed by visual inspection for optimization. Previously characterized CR-I sequences of yellowfin tuna, longtail tuna (outgroups), and blackfin tuna (Alvarado Bremer et al. 1997) were used during the alignment process. Two yellowfin and two bluefin tuna larvae were identified, and removed from further analyses.

After additional optimizations of the mtDNA CR-I alignment without the outgroup, standard diversity indices including, number of segregating sites (S),

nucleotide diversity (π), and haplotypic diversity (h) were estimated with ARLEQUIN 3.11 (Excoffier et al. 2005). The best evolutionary model to account for the observed substitution pattern in blackfin tuna was estimated using MODELTEST 3.7 (Posada and Crandall 1998). The HKY+I+G distance model, with α= 0.70 was selected, and this gamma value was then used in ARLEQUIN to calculate demographic parameters (see below). Gene-trees were generated in MEGA 4.0 (Tamura et al. 2007) using neighbor-joining (NJ) (Saitou and Nei 1987) with the pair-wise deletion option and Tamura-Nei distances since MEGA 4.0 does not support the HKY+I+G distance model. Trees were then examined for pattern of phylogeographic association of blackfin tuna haplotypes. Only forensically identified blackfin tuna adults (n=89) and larvae (n=74) were included in this tree, which was rooted using yellowfin tuna and longtail tuna sequences as outgroups.

The demographic history of blackfin tuna population was reconstructed using two approaches. First, an un-rooted NJ tree was generated excluding outgroups to determine the shape of the tree topology, where a star phylogeny would be indicative of a recent population expansion; a second unrooted NJ tree was created using yellowfin tuna data (n=159) generated by Farnham (2003) and used in comparison with blackfin tuna. Second, a mismatch distribution and the population parameters τ, θ_0 and θ_1, were estimated using ARLEQUIN 3.11. Estimates of effective female population size (N_{ef}) prior and after expansion were estimated using θ_0 and θ_1, respectively. In addition, the theta estimates derived from segregating sites (θ_s), were calculated using MIGRATE 2.0 (Beerli 1997-2004; Beerli and Felsenstein 1999; Beerli and Felsenstein 2001) that

reports the maximum likelihood (ML) estimators of the posterior distribution of the effective population sizes, under the assumption that migration rates remained constant over time. The θ_s parameter was estimated using Markov chain Monte Carlo (MCMC) approaches. This approach tends to yield better results when the start parameters are close to the ML values; which can be accomplished by running several short chains and using the result of the last chain as a starting point (Beerli 1997-2004). Ten short chains with 500 recorded steps per chain and three long chains with 5,000 recorded steps per chain were run with a burn-in of 10,000 trees for each chain.

Time since expansion was estimated by substituting the estimated τ value in the formula $\tau=2\mu t$, where μ is the mutation rate per sequence per generation and t is time (Harpending 1994). In this study we employed a slow-paced molecular clock rate for scombrid fishes of about 1.6% per million years (Alvarado Bremer et al. 2005a) and the faster paced teleost rate of 4.9% Ma^{-1} (Donaldson and Wilson Jr. 1999; Tringali et al. 1999), and a mean generation time of 2 years, the age when 50% of the females are sexually mature.

Statistical tests of neutrality were carried out using Fu's F_s (Bailey et al. 1997) and Ramos-Onsins and Rozas' R_2 statistics (Ramos-Onsins and Rozas 2002) in DNASP 4.5 (Rozas et al. 2003). An excess of recent mutations yields negative F_s values, and therefore large negative values for a selectively neutral segment are likely the result of population expansion as opposed to selection (Bailey et al. 1997).

Results

Phylogenetic Analysis

A total of 323 base pairs of the mtDNA CR-I was characterized for 163 adult and larval blackfin tuna. Of the 155 blackfin tuna haplotypes identified, the majority occurred once and only eight repeated twice. Haplotype tree and frequency tables are found in Appendix A. Consequently, the values of gene or haplotypic diversity approached unity (h>0.995), and thus indicative of the high probability that any pair of haplotypes drawn randomly from any sample will be different (Table 2-2). In addition, all groups showed identical values of nucleotide diversity (π=0.047). Accordingly, any random pair of haplotypes differs by about 5%, or roughly 15 nucleotide differences between the pair ($k\approx$15). The neighbor joining (NJ) tree generated in MEGA 4.0 (see Appendix A) that reconstructed the phenetic relationship of lineages revealed no obvious pattern of phylogeographic association. Haplotypes from all regions were interspersed throughout the tree, however small pockets of haplotype clustering, mostly in the Gulf of Mexico sample, were observed; possibly attributing to any observed differentiation between the Gulf of Mexico and NW Atlantic samples (Chapter III). Accordingly, a test for isolation by distance (IBD) conducted for the regions of capture listed above (see Methods), was not significant (See Chapter III for details). Based on these results, samples were pooled within-region, resulting in two regional samples, namely GoM (n=128) and NW Atl (n= 35). However, when appropriate, analyses were conducted for the total pooled sample (n=163).

Table 2-2. Summary of diversity indices for blackfin tuna within region of capture for a 323 bp segment of the mitochondrial control region I. n= number of samples, M= number of haplotypes, S= number of segregating sites, *h*= haplotypic diversity, π= nucleotide diversity, *k*= mean number of pairwise differences. Values in parentheses are standard deviations.

Population	n	M	S	*h*	π	*k*
NW Atlantic	35	32	61	0.995 (0.008)	0.047 (0.02)	14.97 (6.86)
Gulf of Mexico	128	123	103	0.999 (0.001)	0.047 (0.02)	15.18 (6.83)

Mismatch Distribution and Population Expansion

Historical demographic parameters and neutrality tests were analyzed separately by region (Gulf of Mexico, and NW Atlantic). Separate analyses are justified since both mtDNA CR-I and microsatellites data show a shallow, but significant signal of genetic differentiation between regions (Chapter III). Values of Fu's F_s test were large and negative, providing statistically significant evidence of rapid population expansion for both samples (Table 2-3). The R_2 statistic failed to confirm these results, however, that test is more suitable for small sample sizes (n<20) and when the number of segregating sites is small (S<50). Estimates of sudden expansion are given in Table 2-4, and the mismatch distributions are shown in Fig. 2-1. In all cases, the curves are smooth and unimodal. The resulting NJ tree topology of the blackfin tuna CR-I haplotypes (Figure 2-2a) has characteristics of a species having undergone rapid population expansion, but is not a true star-phylogeny such as that of yellowfin tuna (Figure 2-2b). Furthermore, the distance between any two blackfin tuna haplotypes is nearly twice as long as the average distance between any two yellowfin tuna haplotypes.

Table 2-3. Estimates of selective neutrality tests as estimated in DnaSP with blackfin tuna CR-I sequences. Values in parentheses are p-values for the tests. Sample designations are the same as previously used. Both statistics test the null hypothesis of a population in neutrality.

	NW Atlantic	Gulf of Mexico
Fu's F_s	-16.530 (0.000)	-24.070 (0.000)
R_2	0.082 (0.154)	0.057 (0.098)

Table 2-4. Estimates of historical demographic parameters for blackfin tuna samples. τ= units of mutational time before the present, θ_0= diversity before expansion, and θ_1= diversity after expansion. 95% confidence intervals (CI) in parentheses.

Population	θ_0	θ_1	τ
NW Atlantic	0.000 (0.000, 1.860)	592.500 (181.410, Inf.)	12.436 (9.460, 13.380)
Gulf of Mexico	0.000 (0.000, 1.930)	741.250 (363.750, Inf.)	14.379 (12.250, 15.390)

(a)

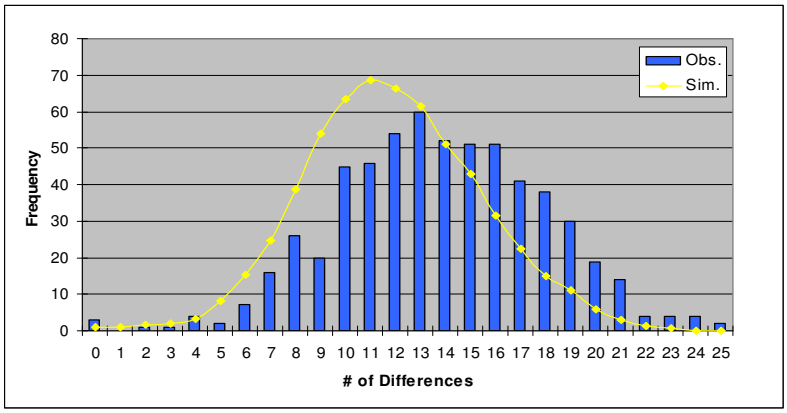


Figure 2-1. Mismatch distributions for blackfin tuna samples. (a) North West Atlantic samples, (b) Gulf of Mexico samples. Distributions generated using mtDNA CR-I data in ARLEQUIN with bars being the observed number of pairwise differences between sequences, and the yellow line the expected number of pairwise differences between the sequences within samples.

(b)

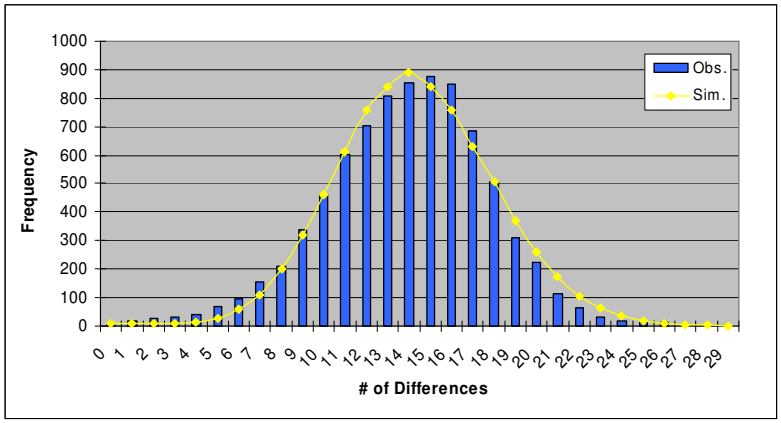


Figure 2-1. Continued

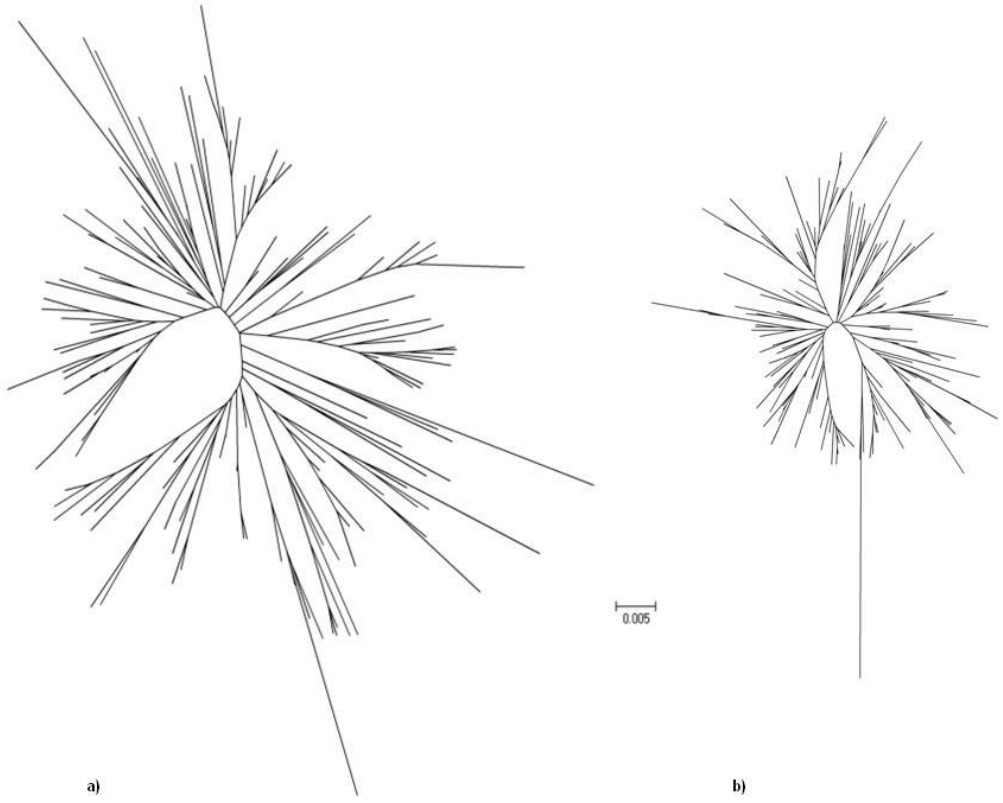


Figure 2-2. Unrooted NJ trees showing the phenetic relationship of control region I haplotypes (h) of a) blackfin tuna (n=163, h=155), and b) yellowfin tuna (n=159, h=155). Yellowfin tuna sequences are from Farnham (2003). Trees are drawn to the same scale.

Historical demography estimates from the sudden expansion model are listed in Table 2-4. Based on the corresponding values of τ and divergence rate for the mtDNA CR-I of about 1.6% Ma^{-1}, and a generation time of 2 years (see Materials and Methods), the Gulf of Mexico blackfin tuna population expanded at about 1.4 Ma. The estimate of expansion for the NW Atlantic is slightly more recent, at about 1.2 Ma. Using the faster teleost rate of 4.9% Ma^{-1} yielded proportionately younger expansion times, with expansion in the Gulf of Mexico at 788 ka and in the NW Atlantic at 600 ka. It should be noted that there is substantial overlap in the respective upper and lower limits of τ for the NW Atlantic and the Gulf of Mexico (Table 2-4). This difference in expansion times in between the Gulf of Mexico and the NW Atlantic are tentative at best. The estimates of female effective population size (N_{ef}) before expansion event (θ_0) ranged from zero to several thousands of fish. The estimates of N_{ef} after expansion event (θ_1) at the 1.6% rate are extremely large at about 72 million females for the Gulf of Mexico and 57 million females for the NW Atlantic. Again, the estimates using the faster rate of 4.9% are about one third those reported using the slower rate of 1.6%. N_{ef} values obtained with ML were substantially smaller at about 12.8 and 7.8 million females respectively for the Gulf of Mexico and the NW Atlantic, and a third of that size if the 4.9% Ma^{-1} rate is adopted (See Table 2-5). The samples used in this study are assumed to be representative of both the NW Atlantic and the Gulf of Mexico populations. At the level of blackfin mtDNA large amounts of genetic diversity were observed between the adults and the larval samples. Furthermore, both the NW Atlantic and Gulf of Mexico samples

yielded similarly large values of haplotypic diversity and nucleotide diversity regardless of sample size.

Table 2-5. Estimates of female effective population size (*Nef*) for the Gulf of Mexico and NW Atlantic samples using the 1.6% and 4.9% rates for θ_1, θ_s. Reported values are in millions of individual females.

Sample	Rate	θ_1	θ_s
NW Atlantic	1.60%	57 million	7.8 million
	4.90%	19 million	2.6 million
Gulf of Mexico	1.60%	72 million	12.8 million
	4.90%	24 million	4.3 million

Effects of Sample Size on Mismatch Distribution Analysis and Neutrality

Historical demography estimates and in particular neutrality tests are different between the Gulf of Mexico and the NW Atlantic samples (see tables 2-3, and 2-4). The Gulf of Mexico sample with its smooth, unimodal mismatch distribution conforms to the expectation of a population that expanded rapidly. By contrast, the NW Atlantic sample is characterized by a more ragged distribution (Figure 2-1). While this distribution may indeed represent the demographic signal of the NW Atlantic population, it is possible that the observed differences between regions are due to sampling error. To test the effect of small sample sizes on the shape of mismatch distributions, three replicate sets of 35 haplotypes were selected without replacement from the various cohorts from the Gulf of Mexico sample utilizing a random number generator. Mismatch distributions were estimated in ARLEQUIN, and neutrality tests were calculated for each replicate in DNASP. Results are given in Table 2-6. Mismatch graphs were less smooth in the replicate sets (Fig. 2-3) than for the entire sample from the Gulf of Mexico, and one of

the replicates (Replicate 1) yielded a statistically significant value for the R_2 statistic, in contrast with replicates 2 and 3 that yielded non-significant values. The τ estimates for replicates 1 and 3 were similar (τ_1= 14.836, and τ_3= 14.355), whereas the value for replicate 2 was lower (τ= 13.467) (Table 2-6). The results from the replicate sets indicate that the signal observed in the NW Atlantic sample could be generated by its small sample size.

Table 2-6. Neutrality test statistics and tau values of the Gulf of Mexico replicates as estimated in DnaSP. Values in parentheses are p-values

	Replicate 1	Replicate 2	Replicate 3
Fu's F_s	-23.330 (0.000)	-19.10 (0.000)	-23.837 (0.000)
R_2	0.070 (0.044)	0.081 (0.126)	0.078 (0.089)
τ	14.836	13.467	14.355

(a)

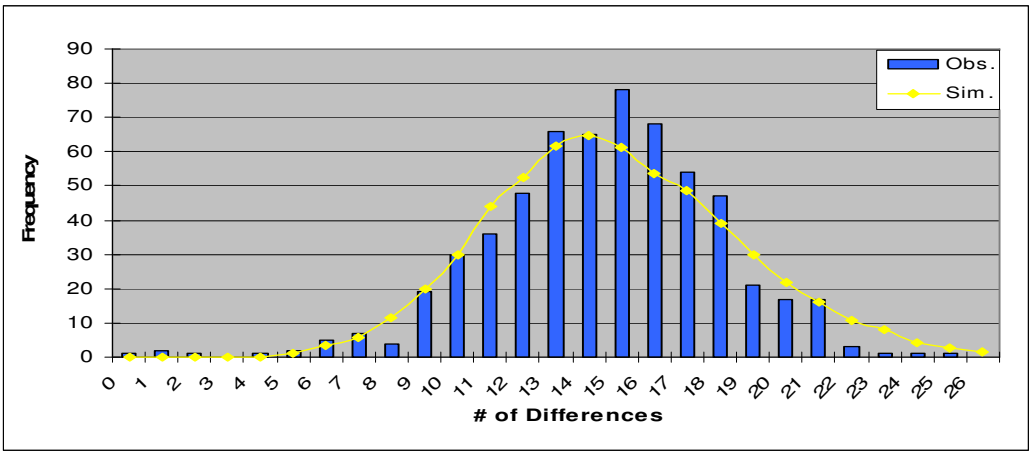
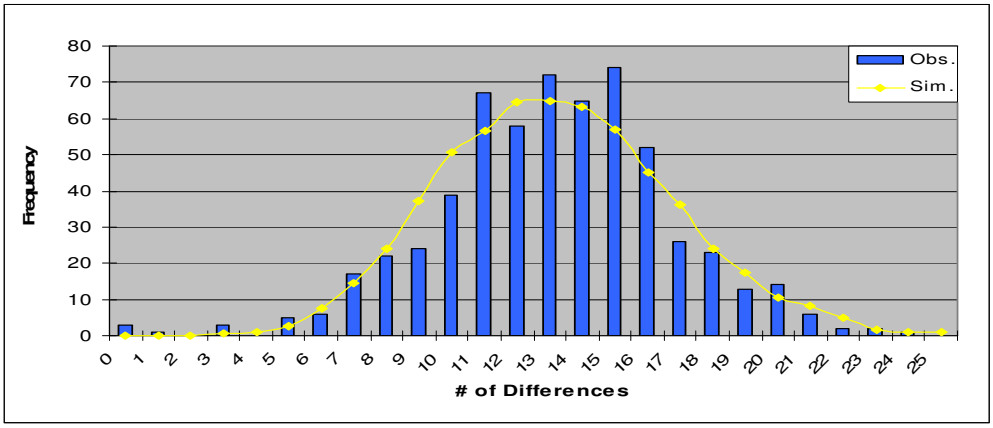


Figure 2-3. Mismatch distribution analysis of 3 random replicate sets, using the Gulf of Mexico sample. (a) Replicate 1, (b) Replicate 2, (c) Replicate 3. Distributions generated using mtDNA CR-I data in ARLEQUIN with bars being the observed number of pairwise differences between sequences and the line being the simulated number of pairwise differences between the sequences within samples.

(b)



(c)

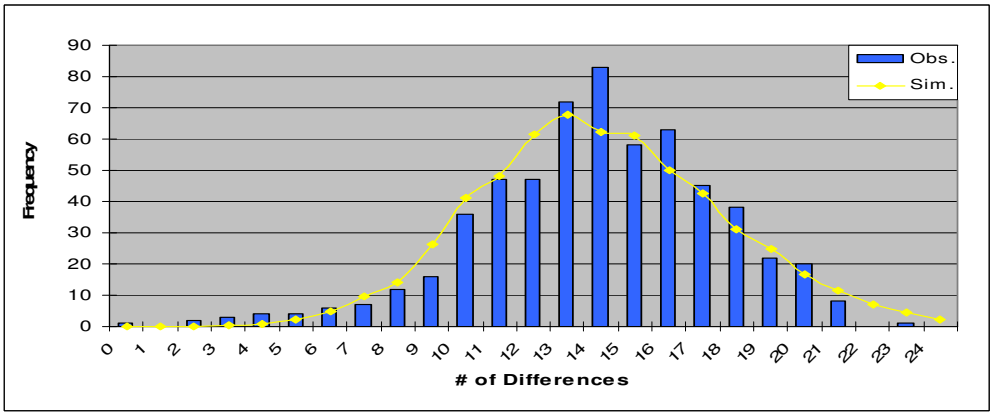


Figure 2-3. Continued

Discussion

Population Expansion

Blackfin tuna mtDNA CR-I contains extremely large values of diversity with mean nucleotide differences between individual haplotypes larger than any other tuna characterized so far and rivaled only by skipjack tuna (Table 2-2). Both the Gulf of Mexico and the NW Atlantic samples yielded similarly large values of haplotypic diversity and the same value of nucleotide diversity despite the size discrepancy between

the two samples, both sample sets. Thus, estimates of CR-I diversity are only marginally influenced by the sample sizes employed in this study.

Blackfin tuna in the Gulf of Mexico and the NW Atlantic appears to have experienced a major population expansion at about 1.4 Ma and 1.2 Ma, respectively. Support for a rapid population expansion, in both from a very small population, in both samples, is supported by the overall mismatch distribution of pairwise differences with the characteristic bell-shaped curve (Figures 2-1 and 2-3), by the θ_0 estimates (Table 2-4), and by statistically significant tests indicating departures from neutrality (Slatkin and Hudson 1991; Harpending et al. 1993; Harpending 1994; Fu 1997; Ramos-Onsins and Rozas 2002). While other forces, such as a selective sweep, could potentially generate similar distributions, however the mitochondrial control region is most likely neutral exhibiting very high levels of nucleotide diversity, making this alternative explanation unlikely (Farnham 2003). By contrast, the NW Atlantic sample mismatch distribution displays characteristics that are different than that of the Gulf of Mexico. A shift between the observed and simulated vales was observed. This shift is believed to be a function of small sample size, and not a function of the natural population. However, these results should be interpreted with caution because of the relatively small size of the NW Atlantic sample. In fact, randomly generated replicate sets of the Gulf of Mexico sample revealed that small samples have the potential to yield signals similar to that observed in the NW Atlantic. Furthermore, future studies of blackfin tuna in the NW Atlantic should employ larger sample sizes to verify whether or not the differences

between the Gulf of Mexico and the NW Atlantic are a function of the difference in sample size.

Population expansions similar to that of blackfin tuna have been documented in several highly migratory pelagic fishes in the Atlantic. For instance, Farnham (2003) employed a molecular clock rate for the CR-I of 5 and 10% Ma^{-1} and estimated that yellowfin tuna population in the Atlantic expanded at 40-80 ka. However, these dates were underestimated by more than 10-fold as the value of μ employed in that study was not corrected by the length of the segment of sequence, or by the generation time. Calculations based on the demographic parameters in Farnham (2003), and using the rates of 1.6% and 4.9% divergence per million years, yield expansion times occurring at 1.4 Ma and 487 ka, respectively. Remarkably similar expansion times have been reported for other highly migratory pelagic species (Table 2-7). Adopting the 1.6% Ma^{-1} rate in the ensuing discussion, we find that the Atlantic populations of bluefin tuna (*Thunnus thynnus*), swordfish (*Xiphias gladius*) and sailfish (*Istiophorus platypterus*) all expanded at about 1.4 Ma (Alvarado Bremer et al. 2005b; Alvarado Bremer et al. 2005a; Ely et al. 2005; Bangma 2006) that also coincide with the population expansion of blackfin tuna reported in here. In sharp contrast, highly abundant pelagic fishes like skipjack tuna (*Katsuwonus pelamis*) and albacore tuna (*T. alalunga*) display characteristics of populations that have remained at equilibrium for a long time (see Table 2-7). These species mtDNA CR-I have multi-modal mismatch distributions, tree topologies with deep branch lengths, and non-significant neutrality tests (Ely et al. 2005; Bangma 2006). Such historical demographic signatures are expected for ancient

populations, or for populations with extremely large effective population sizes, such as skipjack tuna and albacore tuna.

Table 2-7. Historical demographic parameters for the mtDNA CR-I of other highly migratory pelagic fish species. τ= units of mutational time before the present, θ_0= diversity before expansion, and θ_1= diversity after expansion. 95% confidence intervals (CI) in parentheses. T= time since expansion using the 1.6% rate in Ma.

Reference	Species	θ_0	θ_1	τ	T
Farnham (2003)	*Thunnus albacares*	0.030	682.813	8.475	1.400
Ely (2005)	*Katsuwonus pelamis*	13.500	6655.000	15.320	1.400
Alvarado (2005)	*Thunnus thynnus*	0.001	104.805	8.062	1.400
Alvarado (2005)	*Xiphias gladius* (Clade-I)	1.472	71.150	7.022	1.450
Bangma (2006)	*Istiophorus platypterus* (Clade-I)	1.527	13.026	4.838	1.400

The Atlantic Ocean underwent dramatic changes during the Pleistocene, including fluctuations in sea level, sea surface temperature (SST), and shifts in deep water circulation (Mix and Fairbanks 1985; Rampino and Self 1992; McManus et al. 1999). All of these paleoceanographic changes could have influenced the expansion events of many of these tunas and billfishes. However, further studies are needed resolve the cause of this expansion event.

CHAPTER III

THE GENETIC POPULATION STRUCTURE OF BLACKFIN TUNA (*Thunnus atlanticus*) IN THE GULF OF MEXICO AND NORTH WEST ATLANTIC

Introduction

Fisheries management has become necessary to ensure long-term sustainable exploitation of commercial fisheries and to assist in the recovery of depleted stocks (Ward 2000). In order to be effective, management requires the proper assessment of stock abundance and dynamics, as well as accurate productivity estimates (Gulland 1983; Cooper 2006). This entails also the characterization of the stock's spatial distribution, or stock structure (Deriso and Quinn 1998). In here, stock is considered as an intra-specific group of randomly mating individuals with temporal and spatial integrity (Ihssen et al. 1981), a definition closely tied to the biological reality of a population. Unfortunately, most genetic assessments of variation and population structure are conducted after the stocks have been severely overfished (Gold and Turner 2002; Farnham 2003; Clark et al. 2004; Bangma 2006). An assessment of genetic variation conducted before the stock is overfished could serve as a baseline data for future assessments, and as an aid to select among various conservation strategies that would allow long-term preservation of genetic variation (Ward 2000).

Studies have shown that determining the genetic stock structure of marine fishes is difficult because in general the levels of differentiation among populations are much lower than in freshwater fishes (Ward 2000). Such disparity is largely due to the geographic isolation of freshwater systems (i.e., lakes and drainages), the precursor of

genetic differentiation, but also because the effective population size (N_e) of marine fish populations is substantially larger, which minimizes the effects of random genetic drift (DeWoody and Avise 2000). Such disparity would be particularly accentuated in large highly migratory pelagic fishes because in addition to large N_e there is high dispersal potential for all life stages in a continuous and dynamic ocean environment (Ward et al. 1994; Feldheim et al. 2001). Surprisingly, significant and even pronounced differentiation among the populations of several pelagic fishes has been reported using both mitochondrial DNA (mtDNA) and nuclear DNA (nDNA). These examples include differentiation between Atlantic and Pacific populations of blue marlin (*Makaira nigricans*), sailfish (*Istiophorus platypterus*), swordfish (*Xiphias gladius*), albacore tuna (*T. alalunga*) and bigeye tuna (*T. obesus*) (Graves 1998). Furthermore, the genetic characterization of Atlantic bonito (*Sarda sarda*), a species with epineritic habits similar to blackfin tuna, revealed a pattern of genetic structure that conforms to isolation by distance (IBD) along the Mediterranean Sea (Vinãs et al. 2004).

All tuna species of the genus *Thunnus* are important for commercial fisheries (Takagi et al. 1999), and as other tuna stocks become severely overfished, heavier pressure may be placed upon blackfin tuna. However, there are no published studies on stock differentiation of blackfin tuna. Evidence of differentiation could be inferred from records of spawning activity, particularly if temporal-spatial discreetness exists. Around Florida the spawning season extends from April to November with a peak in May, whereas in the Gulf of Mexico it extends from June to September (Collette 1983). Blackfin tuna have also been observed spawning off northeastern Brazil (Freire et al.

2005). Unfortunately, there is very little independent evidence from either fisheries data or tagging experiments to infer levels of connectivity throughout the range of the species. The one exception is an ongoing tagging study of blackfin tuna in Bermuda waters characterized by a large proportion of tag recoveries (Luckhurst et al. 2001). It is assumed that blackfin tuna leave the feeding grounds around the Bermuda Seamount during the winter, head south towards tropical waters to reproduce, and return to the Bermuda Seamount the following summer. If site fidelity towards spawning grounds is also observed, and assuming that similar patterns are happening elsewhere, and then the possibility to detect genetic differentiation on a regional basis exists.

Studies of genetic differentiation of pelagic fishes have involved the characterization of variation in the mitochondrial and the nuclear genomes. Mitochondrial DNA is particularly well suited for studies of population differentiation because it is non-recombining, maternally inherited and has a high average rate of mutation (Parker et al. 1998). Specifically, the mtDNA control or d-loop region is appropriate because it is selectively neutral and displays a faster mutation rate than the rest of the mitochondrial molecule. In addition, the control region is highly conserved in length and contains taxon-specific sequence pattern, making it possible to forensically identify all species of the genus *Thunnus* (Alvarado Bremer et al. 1997; Farnham 2003). Furthermore, the analysis of sequence data of the first domain of the control region (CR-I) has revealed population differentiation among populations within species of tunas and bonitos (Alvarado Bremer 1998; Carlsson et al. 2004; Vinãs et al. 2004; Viñas et al. 2006; Carlsson et al. 2007).

Despite the utility of mtDNA CR-I data to unravel population substructure in pelagic fishes, this molecule lacks the power to account for population mixture or whether a local sample belongs to a single panmictic population (Takagi et al. 1999). These limitations can be resolved using nuclear DNA markers. In particular, nuclear microsatellite loci have been shown to resolve low levels of differentiation in a variety of pelagic fishes (Shaw et al. 1999; Nesbo et al. 2000; Appleyard et al. 2001; Wirth and Bernatchez 2001; Selkoe and Toonen 2006). Microsatellites are fast evolving, tandemly repeated, short sequences of DNA, usually with di-, tri-, tetra-, and penta-nucleotide patterns (Schlötterer 2000). Because of their faster mutation rate, microsatellites are also thought to have higher resolving power to identify population structure more accurately than allozymes or mitochondrial DNA (Bentzen et al. 1996; Blouin et al. 1996; DeWoody and Avise 2000; Gold and Turner 2002; Chistiakov et al. 2006; Selkoe and Toonen 2006).

In this chapter, a 323 base pair fragment from the hypervariable mitochondrial control region and six *Thunnus*-specific microsatellite markers were characterized. The mtDNA data was used to test for isolation-by-distance between the regions of capture. Both mtDNA and microsatellite data was used to test the null hypothesis of no differentiation among samples collected in the Gulf of Mexico and the North West (NW) Atlantic, as well as estimate the possible number of migrants between the two basins.

Methods

Adult and larval blackfin tuna muscle tissue from the Gulf of Mexico (mitochondrial DNA, n=128 and nuclear, n=76) and the NW Atlantic (mitochondrial, n=35 and nuclear, n=49) (Table 3-1) were used for this study; further details on sample collection data can be found in Chapter II and Appendix A. Protocols for tissue digestion, DNA extraction and isolation, polymerase chain reaction (PCR), sequencing, and microsatellite amplification and fragment analysis are described in detail in Appendices B, C and D respectively.

Table 3-1. Sample Data of Blackfin tuna, grouped by region of capture. (GoML)= Larval samples. Location data for the Charleston sample could not be identified so region of capture was used.

Group	Sample	Specimen ID	n	Tissue Type	Location
Gulf of Mexico	Freeport	Tatl 068-087	17	Adult	27 N, 94 W
	Venice	Tatl 030-064, 088-108	37	Adult	28 N, 94 W
	GoML	GoML 001-140	74	Larvae	Gulf of Mexico
NW Atlantic	Islamorada	Tatl 109-138	16	Adult	26 N, 80 W
	Charleston	Tatl 002-021	18	Adult	North Atlantic
	Bermuda	Tatl 028	1	Adult	35 N, 65 W

Mitochondrial Control Region Analysis

The control region (CR-I) was amplified using fish specific primers CSBD-H and L15998 (Alvarado Bremer 1994). Nucleotide sequences were aligned and determined in MEGA 4.0 (Tamura et al. 2007) as detailed in Chapter II, and were used to analyze the genetic population structure of blackfin tuna in the Gulf of Mexico and the NW Atlantic. The number of haplotypes (M), values of haplotypic diversity (h), nucleotide diversity (π), and mean number of pairwise differences (k) were calculated using ARLEQUIN

3.11 (Excoffier et al. 2005) (see Chapter II). The HKY+I+G distance, with α= 0.70 was identified as the optimal substitution model for blackfin tuna mitochondrial DNA CR-I using a hierarchical series of likelihood ratio tests implemented in MODELTEST 3.7 (Posada and Crandall 1998) (see Chapter II). This distance was used in PAUP 4.0 (Swofford 1993) to calculate a Neighbor Joining (NJ) tree, which was used to detect phylogeographic association of haplotypes. The same value of α was then used in ARLEQUIN to correct the Tamura-Nei distance matrix employed in the analyses of molecular variance (AMOVAs) (Excoffier et al. 1992) since ARLEQUIN does not allow the implementation of the HKY+I+G distance model. Isolation-by-distance (IBD) tests were carried by comparing pairwise F_{ST} values, generated in ARLEQUIN, between localities of capture for adult blackfin tuna (Table 3-1) against the geographic distance between localities, which was estimated to be the shortest marine route between samples, and was subjected to regression analysis. It has been shown that comparing these F_{ST} values produce less biased slopes when gene flow is expected to be high (Pogson et al. 2001).

Microsatellite Analysis

A total of six nuclear microsatellite loci were amplified using *Thunnus*-specific primers originally designed for yellowfin tuna (cmrTA-113, 125, 144, and 208) (Appleyard et al. 2001) and Pacific bluefin tuna (Ttho-1, and Ttho-4) (Takagi et al. 1999). Fragments were amplified for each sample using multiplex PCR. Each locus was fluorescently labeled with one of three dyes (FAM, HEX, or TET) and grouped

accordingly. More details on the multiplex microsatellite analysis can be found in Appendix D. Fragment sizes were directly read and analyzed using GENESCAN 3.7 (Applied Biosystems, Foster City, California). Allele frequencies, number of alleles per locus, test of Hardy-Weinberg equilibrium (HWE) (Heterozygote deficiency test), and the amount of genic (allele frequency) differentiation were calculated using GENEPOP 4.0 (Rousset 2008). Loci found to be out of HWE were checked with MICRO-CHECKER 2.2 (Van Oosterhout et al. 2004) for the presence of null alleles, stuttering and allelic dropouts. Loci with possible null alleles present were visually re-inspected and rescored by eye (Appendix D).

Both, global and locus-by-locus AMOVAs and corresponding F_{ST} values were conducted in ARLEQUIN. However, Wright's (1951) F_{ST} assumes a low mutation rate, and that the result of a mutation event is independent of the prior allelic state. Alternatively, the R_{ST} statistic has been proposed for microsatellite data because the index accounts for higher mutation rates and assumes a stepwise mutational model (Slatkin 1995). The R_{ST} test statistic assumes populations of equal size and equal variances across all loci which cannot be met by the data (Goodman 1997). Therefore, the genetic differentiation parameter R_{HO} will be calculated using RSTCALC (Goodman 1997) because it is an unbiased estimator of Slatkin's R_{ST} and accounts for the differences in variance between loci and differences in sample size between populations (Goodman 1997).

The number of possible migrants between the Gulf of Mexico and NW Atlantic data sets was estimated with MIGRATE 2.0 (Beerli 1997-2004; Beerli and Felsenstein

1999; Beerli and Felsenstein 2001) from both mitochondrial and microsatellite data. The number of migrants was estimated using Markov chain Monte Carlo (MCMC) approaches. This approach tends to yield better results when the start parameters are close to the ML values, which can be accomplished by running several short chains and using the result of the last chain as a starting point (Beerli 1997-2004). Ten short chains with 500 recorded steps per chain and three long chains with 5,000 recorded steps per chain were run with a burn-in of 10,000 trees for each chain.

Results

mtDNA Analysis

Blackfin tuna mtDNA CR-I contained 97 segregating sites, 92 of which were parsimony informative (sites that contains at least two types of nucleotides). Of the 155 blackfin tuna haplotypes identified, the majority occurred once and only eight repeated twice. Haplotype tree and frequency tables are found in Appendix A. The pooled sample had a haplotypic diversity of 0.999 ± .001, and a nucleotide diversity of 0.047 ± 0.02; other standard diversity indices can be found in Chapter II. The IBD test revealed a regression line no different from zero, with no correlation between locality of capture and geographic distance (Table 3-2 and Figure 3-1). Samples were pooled within region to increase sample sizes. An AMOVA of the pooled samples revealed that the majority of the variance (99%) was contained within samples, yet a small (1%) but significant ($P< 0.05$) proportion of the variance differentiates the Gulf of Mexico and the NW Atlantic samples (Table 3-3). The estimates of the number of migrants per generation

using CR-I data obtained with MIGRATE, suggests that in the NW Atlantic twice as many migrants per generation come from the Gulf of Mexico (M=346) than the reverse (M=150).

Table 3-2. Table of pairwise F_{ST} values generated in ARLEQUIN. The Tamura-Nei distance matrix was used along with Gamma α=0.70. P-values shown in parentheses.

	NW Atl.	Islamorada	Venice
Islamorada	-0.002 (.460)	0	
Venice	0.008 (.130)	0.011 (.190)	0
Freeport	0.004 (.350)	0.034 (.070)	0.007 (.250)

Table 3-3. Analysis of Molecular Variance (AMOVA), using nucleotide sequence data from the mitochondrial control region I comparing the Gulf of Mexico with the NW Atlantic. Results generated in ARLEQUIN with the Tamura-Nei distance matrix and a Gamma α=0.70.

Source of Variation	d.f.	Sum of Squares	Variance Components	Percentage of Variation
Among Populations	1	11.794	0.07682 Va	1.00
Within Populations	161	1218.863	7.57058 Vb	99.00
Total	162	1230.657		
Fixation Index	F_{ST}= 0.01005			
Va and F_{ST}	P=0.049 ± 0.008			

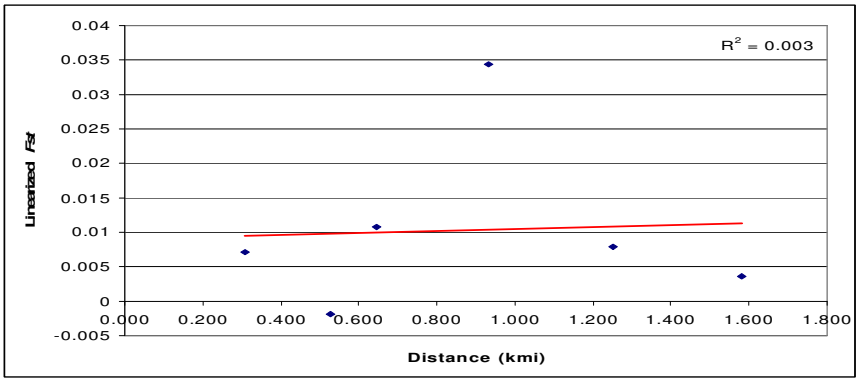


Figure 3-1. Correlation between geographic distance and linearized pairwise F_{ST}s generated in ARLEQUIN for blackfin tuna sample localities. Least-squares regression line in red.

Microsatellite Analysis (Larval)

A substantial amount of genetic heterogeneity (F_{ST}= 0.081, P=0.000) differentiated the GoM larval and the pooled GoM adult samples. Inspection of the allele frequency histograms for all six loci reveals that the larval sample contained a relatively large proportion of alleles outside the range observed in adults (Figure 3-2). These included a large proportion of smaller alleles at locus cmrTA-125 and of larger alleles at locus cmrTA-208. Re-inspection of the raw data revealed that these 'alleles' are more likely artifacts associated with failed amplifications, as in many individuals, peaks corresponding to the expected allele size for the corresponding locus could be observed. However, because the signal of the 'true' alleles was weak, re-scoring was not possible. Accordingly, and to prevent the introduction of a possible bias in the comparison of microsatellite data between the NW Atlantic and the Gulf of Mexico samples, the GoM larvae were excluded in subsequent analyses. Similar difficulties in scoring larval microsatellite samples have been reported in Farnham (2003) and Bangma (2006).

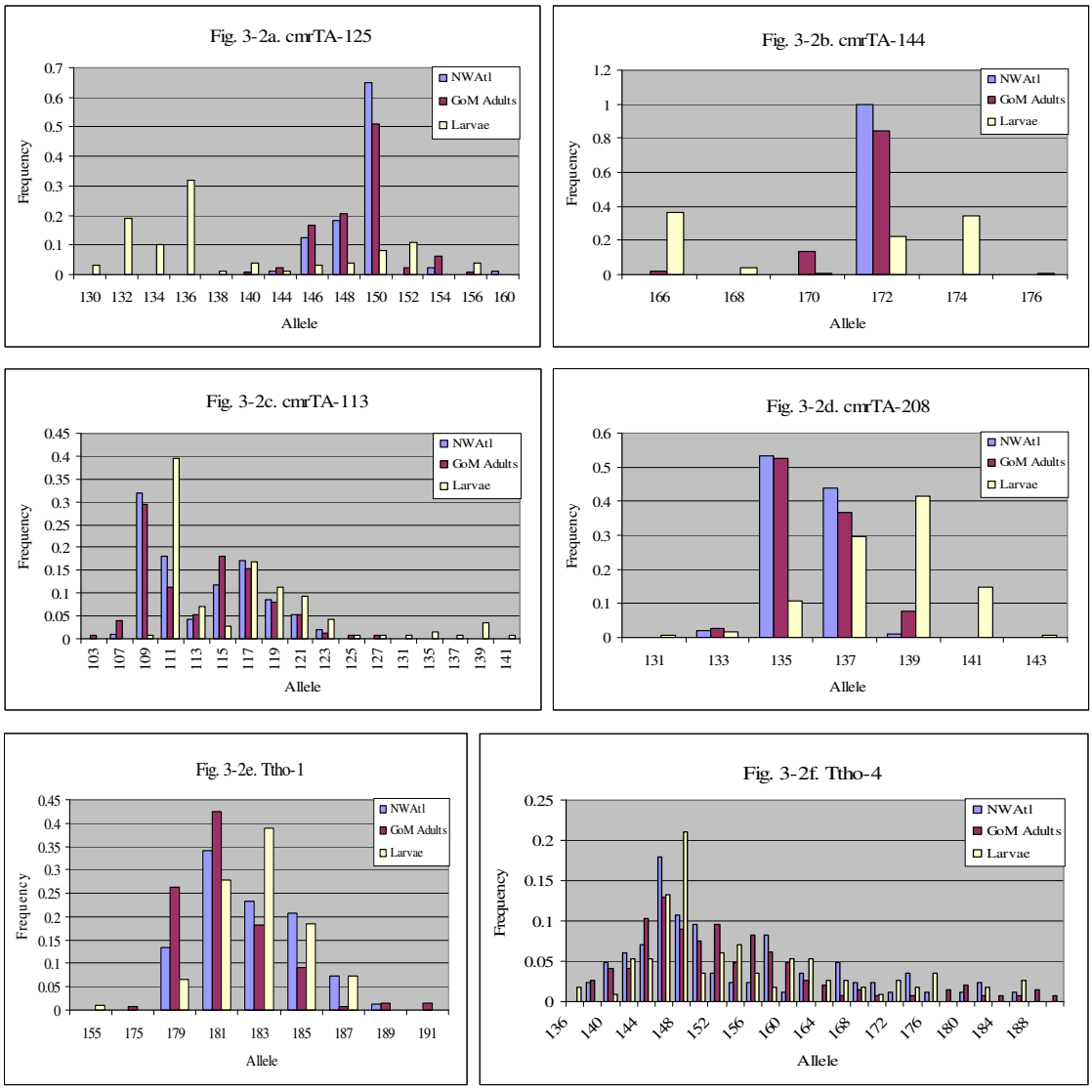


Figure 3-2(a-f). Allele frequency histogram for six microsatellite loci, comparing the NW Atlantic (NWAtl), Gulf of Mexico (GoM), and the Gulf of Mexico larval (Larvae) samples. Allele frequencies were generated with GENEPOP.

Microsatellite Analysis (Adult)

125 adult blackfin tuna (NWAtl, n=49 and GoM, n=76) were successfully amplified at all six dinucleotide microsatellite loci. All loci examined were found to be polymorphic, except locus 144 in the NW Atl sample, which was fixed for allele 174

(Figure 3-2). Allele tables for both sample groups for all six loci can be found in Appendix D. Summary data regarding the six microsatellite loci analyzed is in Table 3-4. The NW Atlantic had an average of 5.2 alleles/loci with an average gene diversity of 58.4%, whereas the Gulf of Mexico averaged 10.0 alleles/loci, with a slightly larger average gene diversity of 58.9%. The GoM sample had higher values of allelic richness (A_R) at most loci. However, the allelic richness was larger in the NW Atlantic at locus Ttho-4 (Table 3-4). The observed heterozygousity values (H_O) were similar to the expected heterozygousity values (H_E) for both samples at all loci (Table 3-4). With the exception of locus 144 that could not be tested in the NWAtl, all other loci were in HW equilibrium in both samples (Table 3-5).

Table 3-4. Summary statistics for the six microsatellite loci analyzed for the NW Atlantic (NWAtl) and the Gulf of Mexico (GoM) samples. Summary statistics are n (number of individuals), N_A (Number of alleles), A_R (Allelic Richness), R (range of alleles in bp), H_E (expected heterozygotes), H_O (observed number of heterozygotes), and F_{IS} (calculated in GENEPOP).

		Locus						
Location		cmrTA-125	cmrTA-144	cmrTA-113	cmrTA-208	Ttho-1	Ttho-4	Mean (all loci)
NW Atl	n	37	21	47	48	41	42	39.33
	N_A	6	1	9	4	6		5.2
	A_R	4.7	1.0	8.0	3.1	5.5	17.5	6.63
	R	146-162	174	109-125	135-141	181-191	140-188	-
	H_E	23.6	0	38.5	25.5	31.7	39.1	26.40
	H_O	26	0	44	25	30	41	27.67
	F_{IS}	-0.102	N/A	-0.144	0.02	0.054	-0.049	-0.044
GoM	n	68	44	75	76	72	73	68.00
	N_A	8	3	12	4	8	25	10.00
	A_R	6.0	2.7	9.0	3.7	5.6	16.7	7.30
	R	142-158	168-174	105-129	135-141	177-193	140-194	-
	H_E	45.9	12.2	62.8	44.4	51.5	68.2	47.50
	H_O	47	12	69	56	50	73	51.17
	F_{IS}	-0.025	0.015	-0.1	-0.264	0.029	-0.071	-0.069

Table 3-5. P-value (standard error) of Heterozygote deficiency test (HWE test) at each locus for the NW Atlantic (NWAtl) and Gulf of Mexico (GoM) as generated in GENEPOP. H_0= no evidence of heterozygote deficiency.

Locus	NWAtl	GoM
TA125	0.9446 (0.0038)	0.0802 (0.0046)
TA144	-	0.5841 (0.0000)
TA113	0.9902 (0.0016)	0.7416 (0.0137)
TA208	0.5022 (0.0000)	0.8000 (0.0000)
Ttho-1	0.4339 (0.0061)	0.1898 (0.0113)
Ttho-4	0.2842 (0.0285)	1.0000 (0.0000)

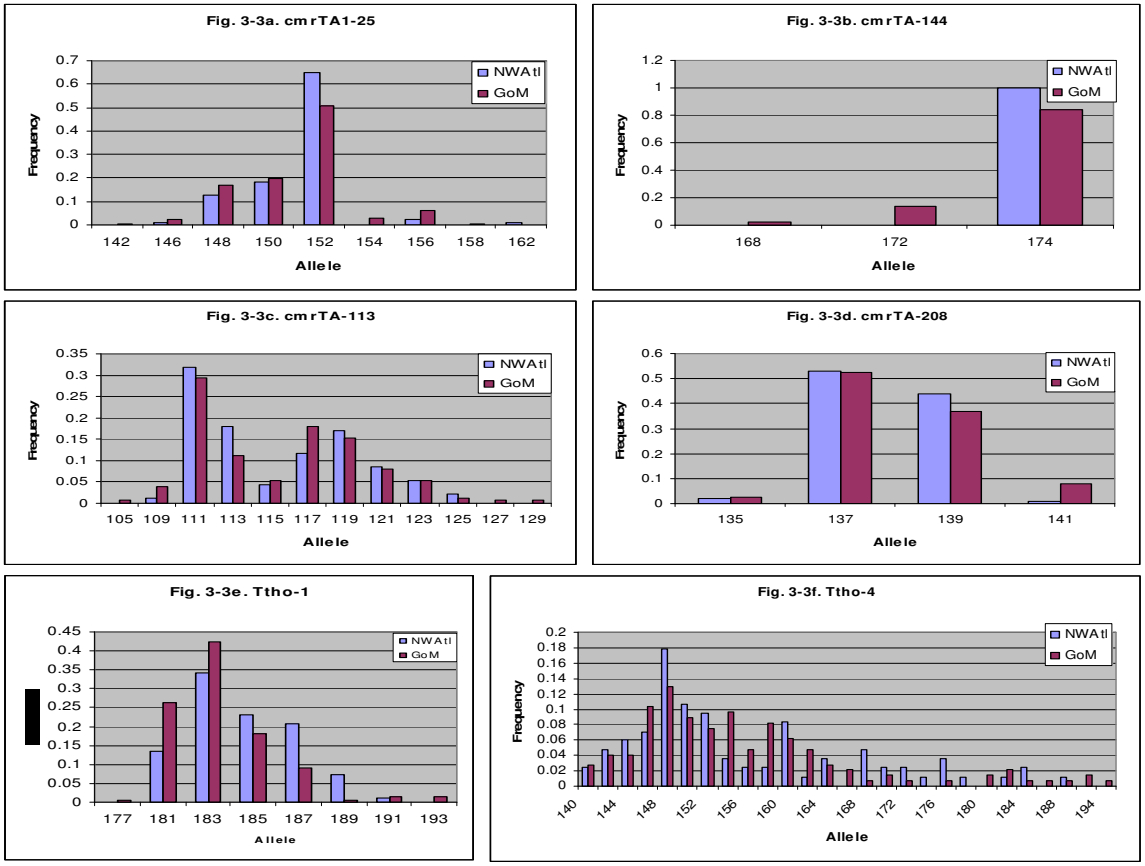


Figure 3-3(a-f). Allele frequency histogram of six microsatellite loci comparing the NW Atlantic (NWAtl), Gulf of Mexico (GoM) blackfin tuna samples. Allele frequencies were generated with GENEPOP 4.0.

The global AMOVA with microsatellite data between the NW Atlantic and Gulf of Mexico revealed that while most of the variance (99.0%) is contained within the samples, a statistically significant proportion of the variance differentiated samples from the two regions (Table 3-5). Evidence of differentiation between the Gulf of Mexico and the NW Atlantic is also supported with R_{HO} estimates (p= 0.000) (Table 3-6). The locus-by-locus AMOVA (Table 3-7), and the test of genic differentiation (Table 3-8), indicate that the observed heterogeneity between the Gulf of Mexico and NW Atlantic sample was explained by only two (cmrTA-144, and *Ttho*-1) of the six loci examined. Estimates of migration between the two regions from microsatellite data, generated in MIGRATE, indicates that NW Atlantic receives approximately four migrants per generation from the Gulf of Mexico, while the Gulf of Mexico receives on average seven individuals per generation from the NW Atlantic.

Table 3-6. Global AMOVA, using microsatellite data from six loci, comparing the Gulf of Mexico with the NW Atlantic, as generated in ARLEQUIN. Also, includes summary of the genetic differentiation R_{HO} calculations from RSTCALC. Comparisons are across all loci with 100 permutations. VC- value averaged over the variance component, Loci- value averaged over loci.

Source of Variation	Sum of Squares	Variance Components	Percentage of Variation
Among Populations	3.526	0.020 V_a	1.000
Within Populations	439.464	1.936 V_b	99.000
Total	442.990	1.955	
Fixation Index	F_{ST}= 0.010		R_{HO} (VC)= 0.02753 (0.000)
Va and F_{ST}	*P*=0.0056		R_{HO} (Loci)= 0.02940 (0.000)

Table 3-7. Results from the locus-by-locus AMOVAs from six microsatellite loci comparing the Gulf of Mexico with the NW Atlantic, as generated in ARLEQUIN.

Locus	% variation	F_{ST}	P-value
cmrTA-125	1.023	0.010	0.087
cmrTA-144	9.126	0.091	0.015
cmrTA-113	-0.223	-0.002	0.512
cmrTA-208	-0.005	-0.000	0.284
Ttho-1	2.028	0.020	0.032
Ttho-4	0.045	0.001	0.307

Table 3-8. Genic (allele frequency) differentiation between the NWAtl and GoM samples at each microsatellite locus as calculated in GENEPOP. Values are probabilities with standard errors. Across all loci the values are χ^2, (degrees of freedom), and p-value.

	125	144	113	208	*Ttho*-1	*Ttho*-4	χ^2 across loci
NWAtl:GoM	0.185 ±0.005	0.004 ±0.000	0.797 ±0.004	0.078 ± 0.002	0.005 ±0.0001	0.316 ±0.001	33.048 (12) 0.001

Discussion

Results from this study suggest that the mtDNA CR-I of blackfin tuna (n= 164) contains extremely high levels of genetic diversity. Specifically, nucleotide diversity for this locus was two times larger than in Atlantic yellowfin tuna (n= 187) (Farnham 2003). This result is particularly interesting, given that yellowfin tuna populations, which have a cosmopolitan distribution, and that in the Atlantic can be found in all regions, are reportedly much larger. However, assuming constancy in mutation rate between species (see Chapter II), it follows that the observed levels of variation fall within what would be expected for the shorter generation time of blackfin tuna. The high levels of diversity in the mtDNA CR-I are mirrored by considerable amount of variation found in five of the six microsatellite loci surveyed in this study. All loci, except cmrTA-144, had wide allele ranges for both the Gulf of Mexico and the NW Atlantic samples (Table 3-4).

Population Differentiation between the Gulf of Mexico and the NW Atlantic

The null hypothesis of a single panmictic population between the Gulf of Mexico and the NW Atlantic was rejected for this study. Both the mitochondrial CR-I and the global microsatellite AMOVA identified a statistically significant proportion of variance between the Gulf of Mexico and the NW Atlantic samples (Tables 3-3, 3-5). This differentiation is supported by the R_{ST} calculations, as the R_{HO} values were significant for both the sample and locus comparisons (Table 3-6). It should be noted that most of the microsatellite heterogeneity was driven by two of the six loci characterized in this study (Table 3-7). The locus-by-locus AMOVA indicates that locus 144 was responsible for explaining the highest proportion of among-group variation. The comparatively high amount of variation (9.1%) can be explained by the NW Atl sample being fixed for allele 174, and this allele is also the most common allele in the GoM sample.

The estimated number of migrants per generation from microsatellite data (M=11) was much smaller than that estimated from mtDNA CR-I (M=496). Mitochondrial data suggest that twice as many females per generation (M=346) migrate from the Gulf of Mexico into the NW Atlantic, than in the opposite direction (M=150). No similar asymmetry in the number of migrants was detected with microsatellite data. However, the number of migrants estimated with both markers is sufficiently large to prevent fixation haplotypes within region. Luckhurst et al. (2001) analyzed catch and tagging data for yellowfin tuna and blackfin tuna from Bermuda waters. They observed that blackfin tuna landings are the highest during the 3rd quarter of the year. They also had high recapture rates of tagged individuals for both species. However, all blackfin

tuna recaptures were local and give little indication of migratory patterns. Tagging studies conducted on other tunas, predominately bluefin tuna, indicate migration between the Gulf of Mexico and the NW Atlantic for spawning purposes (Block et al. 2001; Block et al. 2005; Wilson et al. 2005). However, inferring patterns of migration from one species to another is inappropriate. Tagging studies are needed to verify the levels of exchange of individuals in blackfin tuna between the Gulf of Mexico and the NW Atlantic.

Oceanographic Patterns and Population Connectivity

The role of oceanographic features both present and past appear to influence the contact between pelagic populations, and thus the degree of genetic structuring. For instance, uni-directional gene-flow from the Indo-Pacific into the South Atlantic appears to be facilitated by the Aguhlas in many cosmopolitan pelagic species (Finnerty and Block 1995; Graves and McDowell 1995; Alvarado Bremer 1998; Graves 1998; Alvarado Bremer et al. 2005a; Bangma 2006). The role of currents has been invoked to account for genetic differences between east and west populations of Atlantic bluefin tuna (*Thunnus thynnus thynnus*) in the Mediterranean (Carlsson et al. 2004). It is possible that circulating patterns effecting the Gulf of Mexico and western North Atlantic could be influential in the level of differentiation between blackfin tuna populations in these areas. The Gulf Stream enters the Gulf of Mexico from the Caribbean, via the Strait of the Yucatan, and forms the Loop Current that travels northwest, then south, then east, to finally exit into the NW Atlantic through the Straits

of Florida (Fig. 3-4). This strong unidirectional flow could potentially carry blackfin tuna eggs and larvae from the Gulf of Mexico into the NW Atlantic, while preventing larvae originating in the NW Atlantic to enter the Gulf of Mexico. This interpretation would be consistent with the larger proportion of migrants leaving the Gulf of Mexico into the NW Atlantic, as indicated by mtDNA CR-I data. The weak level of differentiation detected, could also be explained in part by this oceanographic feature that promotes connectivity unidirectionally. This interpretation would also include the possibility of gene-flow from the Caribbean into the Gulf of Mexico facilitated by the Yucatan Current entering from the south; however samples from the Caribbean are needed to assess this possibility.

This study provides baseline genetic data on the population structure of blackfin tuna between the Gulf of Mexico and the NW Atlantic that could be useful for management purposes. However, more intensive tagging and genetic assessments, utilizing samples representative of entire geographic distribution, are needed to further resolve the stock structure of the blackfin tuna

CHAPTER IV

PATTERNS OF MOLECULAR EVOLUTION OF MICROSATELLITES: INSIGHTS FROM THE COMPARISON OF TWO CLOSELY RELATED TUNA SPECIES

Introduction

Microsatellites, or Short Tandem Repeats (STRs), are highly variable co-dominant loci consisting of short repeating units (2-6 base pairs long) believed to be selectively neutral. They have gained wide acceptance in population studies because their typically high levels of polymorphism provide enough power to obtain robust estimates of migration, kinship, and effective population size (N_e). Because of their faster mutation rate, microsatellites are also thought to have higher resolving power to identify population structure more accurately than allozymes or mitochondrial DNA (Bentzen et al. 1996; Blouin et al. 1996; DeWoody and Avise 2000; Gold and Turner 2002; Chistiakov et al. 2006; Selkoe and Toonen 2006). Interestingly, the mutation rate of most microsatellite loci is not known, but it is always assumed high, ranging from 10^{-2} to 10^{-6} mutations per locus per generation with an average of $5x10^{-4}$ (Weber and Wong 1993; Balloux and Lugon-Moulin 2002; Selkoe and Toonen 2006). The most accurate method for estimating the mutation rate is direct observation of the number of mutation events that occur during a given number of generations (Heyer et al. 1997; Schlötterer 2000), but this approach is generally not feasible when studying wild populations. Theoretically, in a population in that has reached mutation drift equilibrium (MDE), mutation rate can also be calculated from N_e provided the variance in repeat number (V) and the mutational model are known parameters. Unfortunately, the mode of mutation

of microsatellites is a subject of controversy and direct measures of N_e (number of effective breeders) are difficult to obtain accurately in natural populations(Schlötterer 2000).

Because of the complications to estimate mutation rate, it has become common practice to adopt the rates from another species or taxonomic group when studying wild populations (Buchanan et al. 1994; DeLeon et al. 1998; Goodman 1998; Bagley et al. 1999; Turner et al. 2002; Ball and Chapman 2003; Poulsen et al. 2006). In some instances, rate constancy is assumed for all microsatellites and for all members of a taxonomic group (e.g., fishes). However, selecting a rate that is too fast or slow can lead to gross under/overestimates in certain population parameters for the species in question (Buonaccorsi et al. 2001; Selkoe and Toonen 2006), and may translate into ineffective management recommendations. A substantial degree of bias is a function of the potential large variance in mutation rate across loci. Mutation rate in microsatellites appears to depend on multiple factors, such as repeat type, base composition, nature of the flanking regions, and variance across taxonomic groups (Balloux and Lugon-Moulin 2002). Therefore, instead of adopting an 'average' mutation rate, alternative methods to estimate the mutation rates in microsatellites are needed.

The approach used in this study involves estimating the mutation rate using the genetic distance $(\delta\mu)^2$ (Goldstein et al. 1995) between two closely related species, the generation time (g) and a time of divergence (T_D) that is concordant with fossil record of tunas (Graham and Dickson 2004). Although this distance also assumes mutation drift equilibrium (MDE) which is rarely met, other parameters, such as variance in mutation

rate or N_e, are not required. Furthermore, since generation time and frequency data for the same loci for two sibling species is incorporated, a more realistic estimate of the microsatellite mutation rate can be expected, particularly when compared to adopting 'universal' molecular clock rates. Accordingly, the estimates of certain demographic parameters, such as N_e, should be more accurate.

Sibling Tuna Species

Phylogenetic analyses using mitochondrial DNA (mtDNA) control region (CR) sequence data confirms the very close relationship of the Yellowfin tuna (*Thunnus albacares*) and blackfin tuna (*Thunnus atlanticus*) (Alvarado Bremer et al. 1997). Population parameters using microsatellites have been estimated for these two closely related species in other studies that lends them to be used to test the method employed in this study.

The purpose of this study is to estimate the genetic distance between blackfin tuna and yellowfin tuna, and provide estimates of mutation rate using six microsatellite markers and a divergence time concordant with the fossil record (Graham and Dickson 2004). The mutation rates derived in such manner will be then compared with 'average' mutation rates for fishes and other vertebrates.

Methods

Data consisted of fragment sizes of six microsatellite (msat) loci amplified using primers originally designed for yellowfin tuna (cmrTA-113, 125, 144, 208) (Appleyard

et al. 2001) and Pacific bluefin tuna (Ttho-1 and Ttho-4) (Takagi et al. 1999). A total of 76 adult blackfin tuna were characterized with all six microsatellites. In addition, 322 bp of sequence of the mitochondrial control region I (mtDNA CR-I) were obtained for 73 adult blackfin tuna. Data (msats, n=49; mtDNA, n=73) for adult yellowfin tuna from the Gulf of Mexico was generated in Farnham (2003). Methods of DNA extraction, PCR, and characterization of fragment sizes and sequencing are included in Farnham (2003), and were optimized when necessary for use with blackfin tuna; and are given in detail in Appendices B-D.

Standard diversity measurements between the two species were estimated in ARLEQUIN 3.11 (Excoffier et al. 2005), for both mitochondrial and microsatellite data. Locus by locus AMOVAs using F_{ST} and R_{ST} estimates between blackfin tuna and yellowfin tuna were also generated in ARLEQUIN. The average genetic distance (D_a) (Nei et al. 1983), between the two species, was estimated in MEGA 4.0 (Tamura et al. 2007) for CR-I data, and using Microsatellite Analyzer (MSA) (Dieringer and Schlötterer 2003) for microsatellite data. The genetic distance measure $(\delta\mu)^2$ (Goldstein et al. 1995) was estimated in RSTCALC (Goodman 1997) because it determines the distance in mean microsatellite allele size between "populations". Goldstein's (1995) $(\delta\mu)^2$ is independent of population size (Goldstein et al. 1995) and is fairly robust to evaluations of MDE (Takezaki and Nei 1996); and should allow direct estimation of the rate when the divergence time is known. Allele frequencies, number of alleles per locus, the exact test of Hardy-Weinberg equilibrium (HWE), and the amount of genic (allele frequency) differentiation were estimated with GENEPOP 4.0 (Rousset 2008). Loci

found to be out of HW equilibrium were checked with MICRO-CHECKER 2.2 (Van Oosterhout et al. 2004) for the presence of null alleles, stuttering and allelic dropouts. Loci with possible null alleles present were visually re-inspected and rescored (Appendix D).

Ritz et al. (2000) used Equation 4-1 to estimate divergence time (T_D) between various species of the tribe Bovini; selecting Crawford and Cuthbertson's (1996) sheep mutation rate (β= 1.1 x10^{-4}) that incorporates Goldstein's (1995) $(\delta\mu)^2$ distance parameter, such that:

$$T_D = [(\delta\mu)^2/2\beta]g. \quad (4\text{-}1)$$

In Ritz et al. (2000), all of the species had the same generation (g) time (g= 7 years). In here, the microsatellite mutation rate was estimated by solving equation 4-1 for β, such that

$$\beta = (1/2*(\delta\mu)^2g)/T_D. \quad (4\text{-}2)$$

However, since blackfin tuna and yellowfin tuna differ in generation time, 2 and 3.5 years, respectively, the mean (g= 2.8) was used. The rise of the Isthmus of Panama, about 3 million years ago (Ma) (Bermingham et al. 1997), was used as the timeline of divergence between these currently parapatric species. It is assumed, that yellowfin tuna, longtail tuna and blackfin tuna, evolved in allopatry respectively in the Pacific, Indian

and Atlantic Ocean, and that the current cosmopolitan distribution of yellowfin tuna is the result of a population expansion during the Pleistocene (Farnham 2003).

Results

Mitochondrial DNA Data

There were 72 blackfin tuna CR-I haplotypes, with only one haplotype repeated twice. Farnham's (2003) yellowfin tuna sample contained 65 CR-I haplotypes, with eight haplotypes repeated more than once. Haplotypic diversity (h) approached unity in both species; however, nucleotide diversity (π) was nearly twice as large in blackfin tuna (π =0.047) than in yellowfin tuna (π= 0.027). The corrected genetic distance (D_a) between blackfin tuna and yellowfin tuna is 3.2%.

Microsatellite Data

All six microsatellite loci were polymorphic (Fig. 4-1) and in HWE for each of the tuna species (Table 4-2). Blackfin tuna had 148 haplotypes and an average gene diversity of 52.2%, where as the yellowfin tuna had 81 haplotypes and an average gene diversity of 58.9% (Table 4-1). The corrected average distance between the two species from microsatellite data was D_a= 4.0%, whereas the $(\delta\mu)^2$ was estimated to be 1.08. The overall mutation rate (β) was estimated to be 4.3×10^{-7} Ma^{-1} to 5.2×10^{-7} Ma^{-1} (Table 4-3).

Table 4-1. Diversity indices calculated using six microsatellite loci for blackfin tuna (BKF) and yellowfin tuna (YFT) from the Gulf of Mexico.

	h	n	Mean number of Pairwise Differences (S.D.)	Average Gene Diversity (S.D.)
BKF	148	76	3.132	0.5220 (0.3023)
YFT	81	49	3.534	0.5890 (0.3336)

Table 4-2. *P*-value (standard error) of Hardy-Weinberg Equilibrium heterozygote deficiency test at each locus for blackfin tuna (BKF) and yellowfin tuna (YFT). Results generated in GENEPOP, with the null hypothesis being no heterozygote deficiency.

Locus	BKF	YFT
TA125	0.0742 (0.0046)	0.1091 (0.0054)
TA144	0.5841 (0.0000)	1.0000 (0.0000)
TA113	0.7188 (0.0141)	0.1358 (0.0108)
TA208	0.8000 (0.0000)	0.5155 (0.0090)
Ttho-1	0.1844 (0.0105)	0.0973 (0.0055)
Ttho-4	1.0000 (0.0000)	0.9783 (0.0039)

Table 4-3. Reported microsatellite mutation rates for various species that have been re-estimated using Equation 4-*2*.

Species	T_D	g	$(\delta\mu)^2$	Reported (β)	Est. (β)
Human	30KY-100KY	27	2.07-6.47	$5.6\text{x}10^{-4}$	$6.0\text{x}10^{-4}$ to $9.3\text{x}10^{-4}$
Sheep	1.5KY	4	0.2*	$2.7\text{x}10^{-4}$	$2.7\text{x}10^{-4}$
Tuna	3.5 MY	2.8	1.08		$4.3\text{x}10^{-7}$ to $5.2\text{x}10^{-7}$

Note: * Nei's (1987) distance was used.

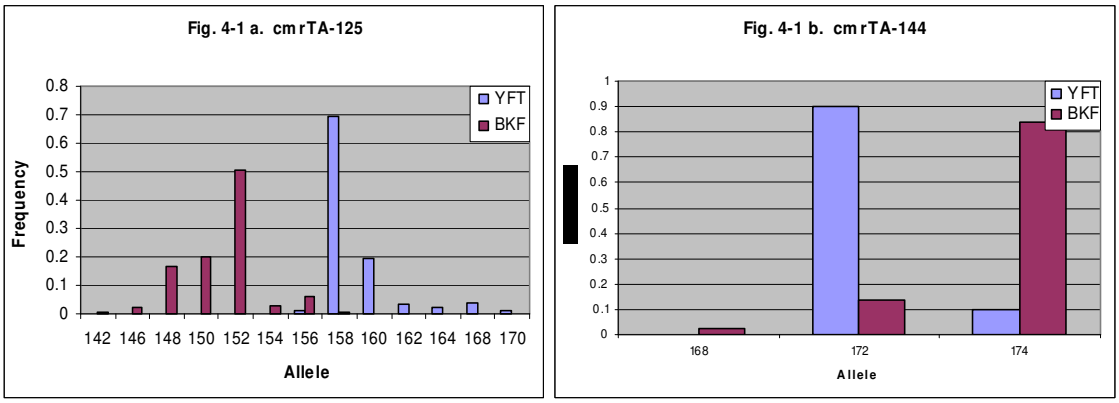


Figure 4-1 (a-f). Allele frequency histogram of six microsatellite loci for adult blackfin tuna (BKF) and yellowfin tuna (YFT) from the Gulf of Mexico.

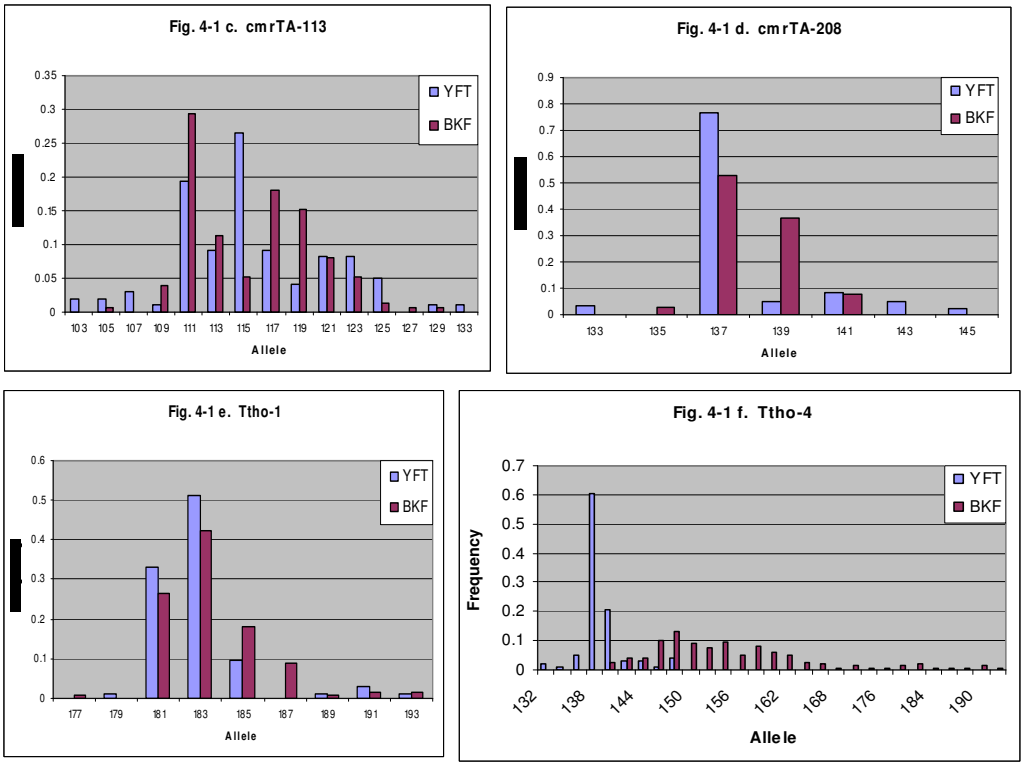


Figure 4-1(a-f). Continued

Discussion

All loci were in HWE for both blackfin tuna and yellowfin tuna (Table 4-2). Blackfin tuna microsatellite loci were amplified using primers designed for yellowfin tuna and for the more distantly related bluefin tuna, yet no evidence of null alleles or allelic drop-out was detected. This is relevant because in species with large *Ne* and fast mutation rate, such as insects and mollusks, a high occurrence of null alleles has been reported, as mutations affect the priming sites and thus amplification success (Chapuis and Estoup 2007). Yellowfin tuna and blackfin tuna also have large effective populations sizes (Ely et al. 2005; Chapter II), but the absence of null alleles can be

partially explained by a slow rate of mutation. A broader comparison involving additional cross-species amplification would be required to confirm this hypothesis.

Estimation of Mutation Rate

The estimated microsatellite mutation rate obtained by comparing the distance between blackfin and yellowfin tuna was $4.3x10^{-7}$ to $5.2x10^{-7}$ Ma^{-1}. This value is three orders of magnitude slower than the rates reported for other vertebrates, with an average rate of $1x10^{-4}$. To examine whether the estimates obtained using Equation 4-2 were reasonable, commonly used rates for humans and sheep were re-estimated using the corresponding data for humans (Goldstein et al. 1995) and sheep (Forbes et al. 1995) (Table 4-3). The estimated lower range for humans is slightly higher than average human rate of $5.6x10^{-4}$ Ma^{-1} reported by Goldstein's (1995), whereas the estimated mutation rate for sheep, at $2.7x10^{-4}$ Ma^{-1}, is the same as that in Forbes (1995). These results suggest that equation 4-2 provides reasonable estimates of average microsatellite mutation rate, provided, that comparative data from sister-taxa is available. Its use would significantly reduce the bias introduced when population demographic estimates are calculated using the mutation rate of microsatellites from another species.

Allele Size Homoplasy or Plesiomorphy? Insights from the Comparison of Microsatellite Alleles in Two Closely Related Tuna Species

One striking observation of this study is similarity in allele frequency distributions of yellowfin tuna and blackfin tuna at four of the six microsatellite loci

(Fig. 4-1). Loci cmrTA-125 and Ttho-4 had allele distributions significantly different for both the F_{ST} and R_{ST} estimates between the two tuna species (Table 4-4), with minimal overlap towards the edges (Fig 4-1 a, f). Locus cmrTA-144 also had substantial amounts of differentiation for both estimates (highest amount for F_{ST} estimate); however, it still had a very limited range of allele sizes. Loci cmrTA-113, 208 and Ttho-1 had levels of differentiation expected to be observed between intraspecific populations. Locus Ttho-1 had non-significant p-values for both estimates (P_{FST}= 0.086, P_{RST}=0.076), whereas cmrTA-113 and 208 had non-significant R_{ST} estimates only (P= 0.850 and 0.361, respectively). The extreme similarity in allele frequency distributions prompted us to compare these data with four cmrTA loci used to characterize bigeye tuna from the Philippines (Grewe and Hampton 1998).

Table 4-4. Locus by locus AMOVA F_{ST} and R_{ST} results between blackfin tuna (BKF) and yellowfin tuna (YFT) as calculated in ARLEQUIN. Values in italics had non significant p-values.

	BKF:YFT	
	F_{ST}	R_{ST}
cmrTA-125	0.411	0.848
cmrTA-144	0.710	0.554
cmrTA-113	0.038	*-0.008*
cmrTA-208	0.130	*0.000*
Ttho-1	*0.012*	*0.018*
Ttho-4	0.216	0.628
All loci	0.264	0.585

The allele distributions of Grewe and Hampton's (1998) bigeye tuna at loci cmrTA-113 and cmrTA-208 are extremely different from those of Farnham's (2003) yellowfin tuna and blackfin tuna. In addition, bigeye tuna is characterized by a wider

allele size range at these two loci (Fig. 4-2). However, the allele size range at locus cmrTA-144 among the three species is extremely similar, whereas at locus cmrTA125, the similarities in allele frequency between bigeye tuna and yellowfin tuna are striking, and very different from blackfin tuna (Fig 4-2a). But which mechanisms best account for the observed patterns? First, a constraint in the number of repeat copies clearly limits variability at microsatellite cmrTA-144. Constraints in the number of repeats can result from either natural selection (Kunkel 1993) or the mutation process (Walsh 1987). Because this locus together with all other microsatellites characterized in this study conform to HWE, the role of selection may not be as important to constrain the copy number compared to the mutation process operating in that locus.

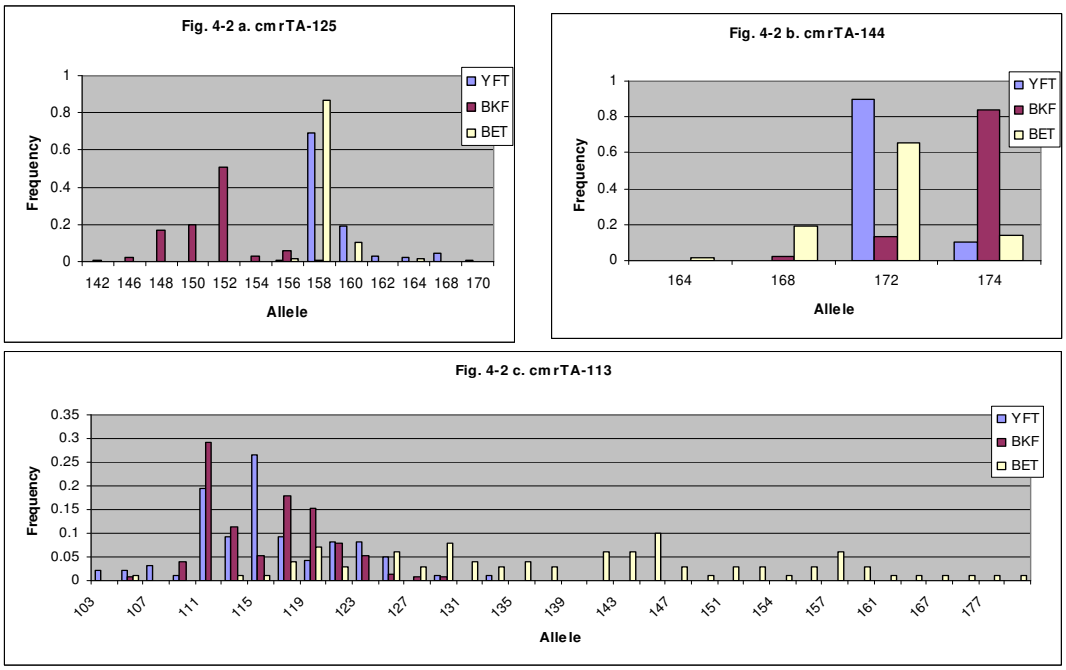


Figure 4-2 (a-d). Allele frequency histogram of six microsatellite loci comparing adult blackfin tuna (BKF) and yellowfin tuna (YFT) from the Gulf of Mexico and bigeye tuna (BET) from the Philippines.

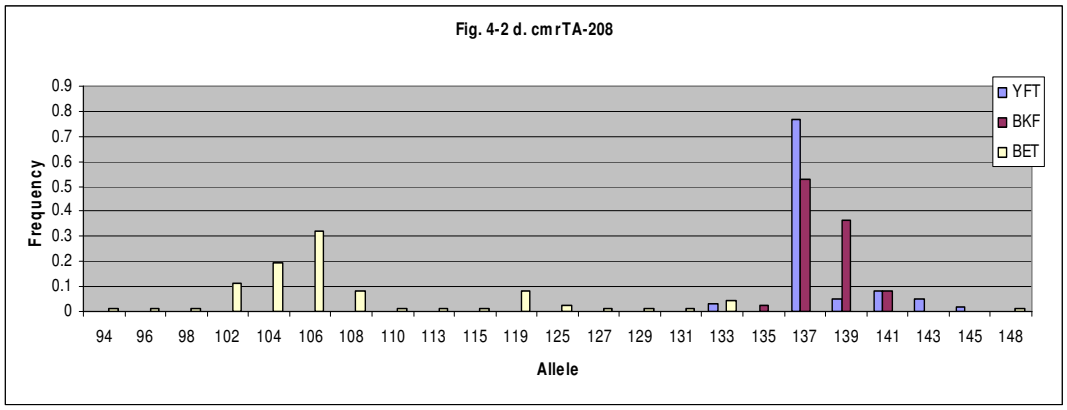


Figure 4-2 (a-d). Continued

Conversely, the similarities in allele size frequency distribution at microsatellite locus cmrTA125 between yellowfin tuna and bigeye tuna, and at loci cmrTA113, and cmrTA208 between yellowfin tuna and blackfin tuna, all appear to indicate that they are symplesiomorphic (shared by common descent) and *not* due to homoplasy (convergent evolution) in repeat number. Garza and Freimer (1996) reported similarities in homoplasy in allele size between human and chimpanzees, but the reported similarities consist of shared allele sizes where the tails of the respective allele frequency distribution overlap. Homoplasy in allele size between populations and among species has also been reported in felids (Culver et al. 2001; Driscoll et al. 2002). In fact, this is the first study that provides evidence of inter-specific microsatellite symplesiomorphy, and it is particularly significant given that the comparison included an outgroup (bigeye tuna). If mutation rates in microsatellites were as fast as those reported for other vertebrates, including fishes, then the allele distributions would differ in shape and or have minimal overlap in allele sizes. Instead, the low divergence level estimated between blackfin tuna and yellowfin tuna at both mtDNA and microsatellites, is similar

to that observed between populations of other vertebrate species, and therefore consistent with an extremely slow mutation rate in these fishes.

Blackfin tuna and yellowfin tuna have an estimated level of divergence of 3.2% for the 322bp fragment of mtDNA CR-I, and 4.0% divergence for the six nuclear microsatellites analyzed in this study. Low level interspecific divergence has been reported between the sister species of white marlin (*Tetrapterus albidus*) and striped marlin (*Tetrapterus audax*) (mtDNA= 2.3%) (Graves and McDowell 2003), and between clades of bigeye tuna (*Thunnus obesus*), swordfish (*Xiphias gladius*), blue marlin (*Makaira nigricans*) and sailfish (*Istiophorus platypterus*), all which have higher divergence values than those reported here between yellowfin tuna and blackfin tuna, ranging between 4%- 6% for mtDNA (Alvarado Bremer et al. 1996; Alvarado Bremer et al. 1997; Durand et al. 2005; Bangma 2006).

In addition, the estimated $(\delta\mu)^2$ between blackfin tuna and yellowfin tuna was 1.08 at T_D= 3.5 Ma. Unfortunately, there is no comparative data for other tuna or billfish species-pairs to evaluate whether this value is reasonable. However, the value is lower than the divergence between certain human populations. Goldstein et al. (1995) estimated genetic distances between groups of modern humans based on archaeological estimates, and found that the distance between Amerindians and the East Asian group from which they separated approximately 30 ka is $(\delta\mu)^2$= 2.07(Goldstein et al. 1995). Accordingly, the rate of molecular evolution of microsatellites in blackfin tuna and yellowfin tuna is roughly 200 times slower than in humans. Similarly, the estimated

mutation rate for tunas (β= $4.3x10^{-7}$- $5.2x10^{-7}$ Ma^{-1}) is two orders of magnitude slower than what has been used for other fishes of $1x10^{-5}$ (Turner et al. 2002).

Rico et al. (1996) estimated a mutation rate of the sequences flanking microsatellites at about 0.05% Ma^{-1} for various marine fish species, representing three major super orders, whereas the rate for cetaceans is slightly faster rate of 0.09-0.15% Ma^{-1} (Schlötterer et al. 1991). The rate of mutation of the flanking regions of the microsatellites characterized in this study is unknown, but can be assumed to be extremely slow. However, this can only be confirmed by generating sequence data for the three species of tuna compared in this study. These data would also help to confirm whether the reported similarities in allele sizes are plesiomorphic. While in general the rates of mutation of microsatellite in marine organisms are thought to be slower than terrestrial species (Fitzsimmons et al. 1995; Rico et al. 1996), the extreme slow pace of mutation reported here has no parallel. Such discrepancy raises questions whether our data truly reflects a slowed-down rate of molecular evolution in tunas, as opposed to a sampling artifact of the loci selected, or due to a bias or an error introduced in the calculation. Given that all the microsatellites characterized in this study are polymorphic both among-species and within-species, inclusive of being capable of distinguishing populations of blackfin tuna (Chapter III), and correspond to a variety of repeat types in both content and length (Appendix D), it is unlikely that these loci are not representative of tuna microsatellites. An important bias may have been introduced by selecting the rise of the Isthmus of Panama as the event that explains, and calibrates the distance between yellowfin tuna and blackfin tuna. However, to account for the reported

disparity, the speciation event that gave rise to these sibling species would have occurred only about 40ka. This date is not consistent with the fossil record (Graham and Dickson 2004) that places the origin of *Neothunnus* about 5Ma. A way of verifying whether the estimate of mutation rate of microsatellites calculated here is reasonable is to estimate the effective population size of blackfin tuna, and compare this value with the results obtained with mtDNA CR-I data (Chapter II). If we employ the accepted fish microsatellite rate of $1x10^{-5}$, the N_e for blackfin tuna in the NW Atlantic would consist of 2,250 individuals. By comparison, using the slowed-down rate from this study a more realistic estimate of 2.1 million breeding individuals is obtained, which is also more congruent with the 7.9 million individuals estimated using mtDNA data. Furthermore, the extremely high values of variability reported at the mtDNA CR-I (Chapter II) and microsatellite data (this study) could not be accounted for if the N_e in blackfin tuna consisted of a couple thousand individuals.

Finally, the reported disparities up to three order of magnitude different between census estimates and genetically derived N_e, have been hypothesized to be the result of extreme levels of reproductive variance in marine fishes (Turner et al. 2002), associated with a variance in reproductive success reported for marine invertebrates (Hedgecock et al. 1994). By contrast, the possibility that such disparity is due to substantial differences in mutation rates needs to be considered as it could have pronounced implications in term of management of wild population

CHAPTER V

CONCLUSIONS

In this thesis I examined the historical demography, the genetic population structure and the microsatellite mutation rate of the blackfin tuna from the Gulf of Mexico and the Northwest (NW) Atlantic. The following conclusions were arrived at. First, the analysis of the mitochondrial control region-I (CR-I) revealed high levels of genetic variation in blackfin tuna. Mitochondrial DNA analysis also revealed evidence of population expansion. Using a slow paced molecular clock rate of 1.6% to 4.9% Ma^{-1} and a generation time of 2 years, it is estimated that blackfin tuna in the Gulf of Mexico underwent expansion 1.4 Ma to 788 ka, whereas NW Atlantic blackfin tuna are slightly younger having undergone expansion at 1.2 Ma and 600 ka, however these estimates were not significantly different between the two samples. When compared to other highly migratory pelagic fish species it was found that bluefin tuna, yellowfin tuna, skipjack tuna, swordfish from clade-I, sailfish from clade-I, and blackfin tuna all have similar times since expansion (~1.4 Ma). However, more in-depth studies, utilizing larger sample sizes, are needed to resolve the cause of this expansion event. Historical demography analysis also revealed that sample size can have an effect on estimates of demographic parameters. Replicates of the Gulf of Mexico, utilizing smaller sample sizes (n=35), produced more "ragged" mismatch distribution graphs and different estimates of neutrality.

Second, data analyzed from the mitochondrial DNA CR-I and six microsatellite loci revealed evidence of significant population differentiation between blackfin tuna

from the Gulf of Mexico and the NW Atlantic. AMOVA revealed a significant 1% differentiation between the two samples for both the mtDNA CR-I and the microsatellite loci. It is argued that the Loop Current and Gulf Stream may play a role in influencing the pattern of genetic diversity detected in this study. As the current exits into the NW Atlantic it potentially prevents Atlantic blackfin tuna larvae from entering the Gulf of Mexico, while the Loop Current transports Gulf of Mexico larvae into the NW Atlantic. This theory is supported by the estimated number of migrants entering the NW Atlantic being larger than the estimates of individuals entering the Gulf of Mexico using the mitochondrial data. However, more studies utilizing larger sample sizes and samples encompassing the blackfin tuna's entire geographic distribution should be conducted to verify this weak differentiation.

Finally, a genetic distance and an overall microsatellite mutation rate (β) between blackfin tuna and yellowfin tuna were estimated using six microsatellite loci. The genetic distance between these two tuna species was estimated to be 3.2% for the mtDNA CR-I and 4.0% for microsatellite data. The genetic distance for the mtDNA CR-I is smaller than distances between sister-species of billfish and between clades of other pelagic fishes. Analysis of the microsatellite data revealed a mutation rate much slower in tunas than in other terrestrial organisms. Back-calculation of β revealed a slow mutation rate of 4.3×10^{-7}- 5.2×10^{-7} Ma^{-1} between blackfin and yellowfin microsatellites. This is supported by multiple pieces of evidence. The test for HWE revealed no heterozygous deficiencies or evidence of null alleles or allelic dropout at any of the loci used. This is relevant because species with large N_e and a fast mutation rate display null

alleles. A significant amount of similarity was observed in the allele frequencies of the species analyzed. Results also indicate the similarity in allele frequencies is symplesiomorphic and not due to size homoplasy. If tuna microsatellites were mutating at a fast rate it would be expected that the three species would have different frequency distributions. This is also the first study to note inter-specific symplesiomorphy in microsatellites.

The findings from this thesis provide baseline data that can be used for future management purposes to conserve pre-exploitation levels of variation in the blackfin tuna. Large amounts of genetic diversity and the extremely large effective population sizes indicate that blackfin tuna can sustain commercial pressure. This study also provides evidence of genetic population structure between blackfin tuna in the Gulf of Mexico and the NW Atlantic. The larval sample validates that blackfin tuna use the Gulf of Mexico as a spawning location. However, more genetic assessments and intensive tagging studies are needed to better understand stock structure throughout the blackfin tuna's entire geographic distribution to ensure long-term sustainability of this species.

Furthermore, population parameters estimated with microsatellite data (i.e. effective population size) should be carefully scrutinized as the mutation rate selected heavily influences these parameters. Effective population size estimates in various marine species from previous studies were found to be several orders of magnitude smaller than adult census size. These differences were generally attributed to reproductive variance. However, results from this study indicate that reproductive

variance may not be the only explanation for the large discrepancy between the two population size estimates. Using the method provided in this study should allow researchers to generate more accurate effective population size estimates, allowing for more effective management strategies.

REFERENCES

Adams D. 2004. Total mercury levels in tunas from offshore waters of the Florida Atlantic coast. Marine Pollution Bulletin 49**:** 659-663.

Alvarado Bremer JR. 1994. Assessment of morphological and genetic variation of the swordfish (*Xiphias gladius* Linnaeus): evolutionary implication of allometric growth and of the patterns of nucleotide substitution in the mitochondrial genome (Ph.D. thesis). Toronto, Canada: University of Toronto.

Alvarado Bremer JR, Mejuto J, Greig TW and Ely B. 1996. Global population structure of the swordfish (*Xiphias gladius* L.) as revealed by analysis of the mitochondrial DNA control region. Journal of Experimental Marine Biology and Ecology 197**:** 295-310.

Alvarado Bremer JR, Naseri, I., Ely, B. 1997. Orthodox and unorthodox phylogenetic relationships among tunas revealed by the nucleotide sequence analysis of the mitochondrial DNA control region. Journal of Fish Biology 50**:** 540-554.

Alvarado Bremer JR, B. Stequert, N. W. Robertson, B. Ely. 1998. Genetic evidence for inter-oceanic subdivision of bigeye tuna (*Thunnus obesus*) populations. Marine Biology 132**:** 547-557.

Alvarado Bremer JR, Vinãs J, Mejuto J, Ely B and Pla C. 2005a. Comparative phylogeography of Atlantic bluefin tuna and swordfish: the combined effects of vicariance, secondary contact, introgression, and population expansion on the regional phylogenies of two highly migratory pelagic fishes. Molecular Phylogenetics and Evolution 36**:** 169-187.

Alvarado Bremer JR, Mejuto J, Gomez-Marquez J, Boan F, Carpintero P, Rodriguez JM et al. 2005b. Hierarchical analyses of genetic variation of samples from breeding and feeding grounds confirm the genetic partitioning of northwest Atlantic and South Atlantic populations of swordfish (*Xiphias gladius* L.). Journal of Experimental Marine Biology and Ecology 327**:** 167-182.

Appleyard SA, Grewe PM, Innes BH and Ward RD. 2001. Population structure of yellowfin tuna (*Thunnus albacares*) in the western Pacific Ocean, inferred from microsatellite loci. Marine Biology 139**:** 383-393.

Arkhipkin AI, Schuchert PC and Danyushevsky L. 2009. Otolith chemistry reveals fine population structure and close affinity to the Pacific and Atlantic oceanic spawning grounds in the migratory southern blue whiting (*Micromesistius australis australis*). Fisheries Research 96**:** 188-194.

Bagley MJ, Lindquist DG and Geller JB. 1999. Microsatellite variation, effective population size, and population genetic structure of vermilion snapper, *Rhomboplites aurorubens*, off the southeastern USA. Marine Biology 134**:** 609-620.

Bailey KM, Stabeno PJ and Powers DA. 1997. The role of larval retention and transport features in mortality and potential gene flow of walleye pollock. Journal of Fish Biology 51**:** 135-154.

Ball AO and Chapman RW. 2003. Population genetic analysis of white shrimp, *Litopenaeus setiferus*, using microsatellite genetic markers. Molecular Ecology 12**:** 2319-2330.

Balloux F and Lugon-Moulin N. 2002. The estimation of population differentiation with microsatellite markers. Molecular Ecology 11**:** 155-165.

Bangma JL. 2006. An assessment of contemporary population structure, historical demography and effective population size of sailfish (*Istiophorus platypterus*) in the Atlantic Ocean (MS thesis). College Station (TX): Texas A&M University.

Beerli P. 1997-2004. Migrate: documentation and program, part of LAMARC. Version 2.0. (2008 July 02). Available from: http://evolution.gs.washington.edu/lamarc.html.

Beerli P and Felsenstein J. 1999. Maximum-likelihood estimation of migration rates and effective population numbers in two populations using a coalescent approach. Genetics 152**:** 763-773.

Beerli P and Felsenstein J. 2001. Maximum likelihood estimation of a migration matrix and effective population sizes in n subpopulations by using a coalescent approach. Proceedings of the National Academy of Sciences of the United States of America 98**:** 4563-4568.

Bentzen P, Taggart CT, Ruzzante DE and Cook D. 1996. Microsatellite polymorphism and the population structure of Atlantic cod (*Gadus morhua*) in the northwest Atlantic. Canadian Journal of Fisheries and Aquatic Sciences 53**:** 2706-2721.

Bermingham E, McCafferty SS and Martin AP (1997). Fish biogeography and molecular clocks: Perspectives from the Panamanian Isthmus. In: Stepien CA, editor, Molecular Systematics of Fishes. San Diego: Academic Press.

Block BA, Dewar H, Blackwell SB, Williams TD, Prince ED, Farwell CJ et al. 2001. Migratory movements, depth preferences, and thermal biology of Atlantic bluefin tuna. Science 293**:** 1310-1314.

Block BA, Teo SLH, Walli A, Boustany A, Stokesbury MJW, Farwell CJ et al. 2005. Electronic tagging and population structure of Atlantic bluefin tuna. Nature 434**:** 1121-1127

Blouin MS, Parsons M, Lacaille V and Lotz S. 1996. Use of microsatellite loci to classify individuals by relatedness. Molecular Ecology 5**:** 393-401.

Breen G, Sham P, Li T, Shaw D, Collier DA and St Clair D. 1999. Accuracy and sensitivity of DNA pooling with microsatellite repeats using capillary electrophoresis. Molecular and Cellular Probes 13**:** 359-365.

Buchanan FC, Adams LJ, Littlejohn RP, Maddox JF and Crawford AM. 1994. Determination of evolutionary relationships among sheep breeds using microsatellites. Genomics 22**:** 397-403.

Buonaccorsi VP, McDowell JR and Graves JE. 2001. Reconciling patterns of inter-ocean molecular variance from four classes of molecular markers in blue marlin (*Makaira nigricans*). Molecular Ecology 10**:** 1179-1196.

Carlsson J, McDowell JR, Diaz-Jaimes P, Carlsson JEL, Boles SB, Gold JR et al. 2004. Microsatellite and mitochondrial DNA analyses of Atlantic bluefin tuna (*Thunnus thynnus thynnus*) population structure in the Mediterranean Sea. Molecular Ecology 13**:** 3345-3356.

Chapuis MP and Estoup A. 2007. Microsatellite null alleles and estimation of population differentiation. Molecular Biology and Evolution 24**:** 621-631.

Chistiakov DA, Hellemans B and Volckaert FAM. 2006. Microsatellites and their genomic distribution, evolution, function and applications: A review with special reference to fish genetics. Aquaculture 255**:** 1-29.

Clark TB, Ma L, Saillant E and Gold JR. 2004. Microsatellite DNA markers for population-genetic studies of Atlantic bluefin tuna (*Thunnus thynnus thynnus*) and other species of genus *Thunnus*. Molecular Ecology Notes 4**:** 70-73.

Collette BB (1979). Adaptations and systematics of the mackerels and tunas. In: Sharp GD and Dizon A, editors, The Physiological Ecology of Tunas. New York: Academic Press.

Collette BBN, C.E. 1983. FAO Species Catalogue Vol. 2, Scombrids of the world: an annotated and illustrated catalogue of tunas, mackerels, bonitos and related species known to date. Rome: Food and Agriculture Organization.

Cooper AB. 2006. A guide to fisheries stock assessment: From data to recommendations. Durham NH: NH Sea Grant College Program.

Crawford AM and Cuthbertson RP. 1996. Mutations in sheep microsatellites. Genome Research 6**:** 876-879.

Culver M, Menotti-Raymond MA and O'Brien SJ. 2001. Patterns of size homoplasy at 10 microsatellite loci in pumas (*Puma concolor*). Molecular Biology and Evolution 18**:** 1151-1156.

DeLeon FJG, Canonne M, Quillet E, Bonhomme F and Chatain B. 1998. The application of microsatellite markers markers to breeding programmes in the sea bass, *Dicentrarchus labrax*. Aquaculture 159**:** 303-316.

Deriso R and Quinn T. 1998. Improving Fish Stock Assessments. Washington DC: National Academy Press.

DeWoody JA and Avise JC. 2000. Microsatellite variation in marine, freshwater and anadromous fishes compared with other animals. Journal of Fish Biology 56**:** 461-473.

Dieringer D and Schlötterer C. 2003. Microsatellite analyser (MSA): a platform independent analysis tool for large microsatellite data sets. Molecular Ecology Notes 3**:** 167-169.

Donaldson KA and Wilson Jr. RR. 1999. Amphi-panamic geminates of snook (Percoidei: Centropomidae) provide a calibration of the divergence rate in the mitochondrial DNA control region of fishes. Molecular Phylogenetics and Evolution 13**:** 208-213.

Driscoll CA, Menotti-Raymond M, Nelson G, Goldstein D and O'Brien SJ. 2002. Genomic Microsatellites as evolutionary chronometers: a test in wild cats. Genome Research 12**:** 414-423.

Durand JD, Collette A, Chow S, Guinand B and Borsa P. 2005. Nuclear and mitochondrial DNA markers indicate unidirectional gene flow of Indo-Pacific to Atlantic bigeye tuna (*Thunnus obesus*) populations, and their admixture off southern Africa. Marine Biology 147**:** 313-322.

Ely B, Vinas J, Bremer JRA, Black D, Lucas L, Covello K et al. 2005. Consequences of the historical demography on the global population structure of two highly migratory cosmopolitan marine fishes: the yellowfin tuna (Thunnus albacares) and the skipjack tuna (Katsuwonus pelamis). Bmc Evolutionary Biology 5**:** 9.

Excoffier L, Smouse PE and Quattro JM. 1992. Analysis of molecular variance inferred from metric distances among DNA haplotypes - Application to human mitochondrial-DNA restriction data. Genetics 131**:** 479-491.

Excoffier L, Laval G and Schneider S. 2005. Arlequin ver. 3.0: An integrated software package for population genetics data analysis. Evolutionary Bioinformatics Online 1**:** 47-50.

Farnham T. 2003. Genetic variation in Atlantic yellowfin tuna (*Thunnus albacares*) to assess stock structure and reproductive variance (MS thesis). College Station (TX): Texas A&M University.

Feldheim KA, Gruber SH and Ashley MV. 2001. Population genetic structure of the lemon shark (*Negaprion brevirostris*) in the western Atlantic: DNA microsatellite variation. Molecular Ecology 10**:** 295-303.

Finnerty JR and Block BA. 1995. Evolution of cytochrome-B in the Scombroidei (Teleostei) - Molecular insights into billfish (Istiophoridae and Xiphiidae) relationships. Fishery Bulletin 93**:** 78-96.

Fitzsimmons NN, Moritz C and Moore SS. 1995. Conservation and dynamics of microsatellite loci over 300-million years of marine turtle evolution. Molecular Biology and Evolution 12**:** 432-440.

Forbes SH, Hogg JT, Buchanan FC, Crawford AM and Allendorf FW. 1995. Microsatellite evolution in congeneric mammals: domestic and bighorn sheep. Molecular Biology and Evolution 12**:** 1106-1113.

Freire KMF, Lessa R and Lins-Oliveira JE. 2005. Fishery and biology of blackfin tuna, *Thunnus atlanticus*, off Northeastern Brazil. Gulf and Caribbean Research 17**:** 15-24.

Fu YX. 1997. Statistical tests of neutrality of mutations against population growth, hitchhiking and background selection. Genetics 147**:** 915-925.

Garza JC and Freimer NB. 1996. Homoplasy for size at microsatellite loci in humans and chimpanzees. Genome Research 6**:** 211-217.

Gibbs RH and Collette BB. 1967. Comparative anatomy and systematics of tunas genus *Thunnus*. United States Fish and Wildlife Service Fishery Bulletin 66**:** 65-130.

Gold JR and Turner TF. 2002. Population structure of red drum (*Sciaenops ocellatus*) in the northern Gulf of Mexico, as inferred from variation in nuclear-encoded microsatellites. Marine Biology 140**:** 249-265.

Goldstein DB, Ruiz Linares A, Cavalli-Sforza LL and Feldman MW. 1995. Genetic absolute dating based on microsatellites and the origin of modern humans. Proceedings of the National Academy of Sciences of the United States of America 92**:** 6723-6727.

Goodman SJ. 1997. R_{ST} Calc: a collection of computer programs for calculating estimates of genetic differentiation from microsatellite data and determining their significance. Molecular Ecology 6**:** 881-885.

Goodman SJ. 1998. Patterns of extensive genetic differentiation and variation among European harbor seals (*Phoca vitulina vitulina*) revealed using microsatellite DNA polymorphisms. Molecular Biology and Evolution 15**:** 104-118.

Gothreaux C. 2007. Know your tunas- The smaller tunas: Little tunny, skipjack and blackfin. Lagniappe 31**:** 2-4.

Graham JB and Dickson KA. 2004. Tuna comparative physiology. Journal of Experimental Biology 207**:** 4015-4024.

Graves JE and McDowell JR. 1995. Inter-ocean genetic-divergence of Istiophorid billfishes. Marine Biology 122**:** 193-203.

Graves JE. 1998. Molecular insights into the population structures of cosmopolitan marine fishes. Journal of Heredity 89**:** 427-437.

Graves JE and McDowell JR. 2003. Stock structure of the world's istiophorid billfishes: a genetic perspective. Marine and Freshwater Research 54**:** 287-298.

Grewe PM and Hampton J. 1998. An assessment of bigeye (*Thunnus obesus*) population structure in the Pacific Ocean, based on mitochondrial DNA and DNA microsatellite analysis. JIMAR Contribution**:** 98-320.

Gulland JA. 1983. Fish Stock Assessment: A Manual of Basic Methods, Vol 1. New York: Wiley-Interscience.

Harpending HC, Sherry ST, Rogers AR and Stoneking M. 1993. The genetic structure of ancient human populations. Current Anthropology 34**:** 483.

Harpending HC. 1994. Signature of ancient population-growth in a low-resolution mitochondrial-DNA mismatch distribution. Human Biology 66**:** 591-600.

Hedgecock D, Siri P and Strong DR. 1994. Conservation biology of endangered pacific salmonids - Introductory remarks. Conservation Biology 8**:** 863-864.

Heyer E, Puymirat J, Dieltjes P, Bakker E and deKnijff P. 1997. Estimating Y chromosome specific microsatellite mutation frequencies using deep rooting pedigrees. Human Molecular Genetics 6**:** 799-803.

Higgins DG, Thompson JD and Gibson TJ. 1996. Using CLUSTAL for multiple sequence alignments. Computer Methods for Macromolecular Sequence Analysis 266**:** 383-402.

International Commission for the Conservation of Atlantic Tunas. 2006. SMT- Table-1. Estimated landings (t) reported to ICCAT for small tunas species in 1980-2004, by region and flag. In: Report for biennial period, 2004-05 Part II (2005) 2. Madrid: ICCAT.

International Commission for the Conservation of Atlantic Tunas. 2008. Executive Summary: Small Tunas. In: Report for biennial period, 2006-07 Part II (2007) 2. Madrid: ICCAT.

Ihssen PE, Brooke HE, Casselman JM, McGlade JM, Payne NR and Utter FM. 1981. Stock identification: materials and methods. Canadian Journal of Fisheries and Aquatic Sciences 38**:** 1838-1855.

Kunkel TA. 1993. Slippery DNA and diseases. Nature 365**:** 207-208.

Laikre L, Palm S and Ryman N. 2005. Genetic population structure of fishes: Implications for coastal zone management. Ambio 34**:** 111-119.

Li WH. 1977. Distribution of nucleotide differences between 2 randomly chosen cistrons in a finite population. Genetics 85**:** 331-337.

Luckhurst BE, Trott T and Manuel S. 2001. Landings, seasonality, catch per unit effort, and tag-recapture results of yellowfin and blackfin tuna at Bermuda. American Fisheries Society Symposium 25**:** 225-234.

McManus JF, Oppo DW and Cullen JL. 1999. A 0.5-million-year record of millennial-scale climate variability in the North Atlantic. Science 283**:** 971-975.

Mix AC and Fairbanks RG. 1985. North Atlantic surface-ocean control of Pleistocene deep-ocean circulation. Earth and Planetary Science Letters 73**:** 231-243.

Myers RA and Worm B. 2003. Rapid worldwide depletion of predatory fish communities. Nature 423**:** 280-283.

Nei M. 1987. Molecular Evolutionary Genetics. New York: Columbia University Press.

Nei M, Tajima F and Tateno Y. 1983. Accuracy of estimated phylogenetic trees from molecular data. Journal of Molecular Evolution 19**:** 153-170.

Nesbo CL, Rueness EK, Iversen SA, Skagen DW and Jakobsen KS. 2000. Phylogeography and population history of Atlantic mackerel (*Scomber scombrus* L.): a genealogical approach reveals genetic structuring among the eastern Atlantic stocks. Proceedings: Biological Sciences 267**:** 281-292.

Parker PG, Snow AA, Schug MD, Booton GC and Fuerst PA. 1998. What molecules can tell us about populations: choosing and using a molecular marker. Ecology 79**:** 361-382.

Pauly D, Christensen V, Froese R and Palomares ML. 2000. Fishing down aquatic food webs. American Scientist 88**:** 46-51.

Platt AR, Woodhall RW and George AL. 2007. Improved DNA sequencing quality and efficiency using an optimized fast cycle sequencing protocol. Biotechniques 43**:** 58-62.

Pogson GH, Taggart CT, Mesa KA and Boutilier RG. 2001. Isolation by distance in the Atlantic cod, *Gadus morhua*, at large and small geographic scales. Evolution 55**:** 131-146.

Posada D and Crandall KA. 1998. Modeltest: Testing the model of DNA substitution. Bioinformatics 14**:** 817-818.

Poulsen NA, Nielsen EE, Schierup MH, Loeschcke V and Gronkjaer P. 2006. Long-term stability and effective population size in North Sea and Baltic Sea cod (*Gadus morhua*). Molecular Ecology 15**:** 321-331.

Ramos-Onsins SE and Rozas J. 2002. Statistical properties of new neutrality tests against population growth. Molecular Biology and Evolution 19**:** 2092-2100.

Rampino MR and Self S. 1992. Volcanic winter and accelerated glaciation following the Toba super-eruption. Nature 359**:** 50-52.

Rico C, Rico I and Hewitt G. 1996. 470 million years of conservation of microsatellite loci among fish species. Proceedings: Biological Sciences 263**:** 549-557.

Ritz LR, Glowatzki-Mullis ML, MacHugh DE and Gaillard C. 2000. Phylogenetic analysis of the tribe Bovini using microsatellites. Animal Genetics 31**:** 178-185.

Rogers AR and Harpending H. 1992. Population growth makes waves in the distribution of pairwise genetic-differences. Molecular Biology and Evolution 9**:** 552-569.

Rooker JR, Secor DH, DeMetrio G, Kaufman AJ, Rios AB and Ticina V. 2008a. Evidence of trans-Atlantic movement and natal homing of bluefin tuna from stable isotopes in otoliths. Marine Ecology-Progress Series 368**:** 231-239.

Rooker JR, Secor DH, De Metrio G, Schloesser R, Block BA and Neilson JD. 2008b. Natal homing and connectivity in Atlantic bluefin tuna populations. Science 322**:** 742-744.

Rousset F. 2008. Genepop'007: A complete re-implementation of the genepop software for Windows and Linux Molecular Ecology Resources 8**:** 103-106.

Rozas J, Sanchez-DelBarrio JC, Messeguer X and Rozas. R. 2003. DnaSP: DNA polymorphism analyses by the coalescent and other methods. Bioinformatics 19**:** 2496-2497.

Saitou N and Nei M. 1987. The neighbor-joining method - A new method for reconstructing phylogenetic trees. Molecular Biology and Evolution 4**:** 406-425.

Sambrook J, Fritsch EF and Maniatis T. 1989. Molecular Cloning: A Laboratory Manual. Cold Spring Harbor NY: Cold Spring Harbor Laboratory Press.

Schlötterer C. 2000. Evolutionary dynamics of microsatellite DNA. Chromosoma 109**:** 365-371.

Schlötterer C, Amos B and Tautz D. 1991. Conservation of polymorphic simple sequence loci in Cetacean species. Nature 354**:** 63-65.

Selkoe KA and Toonen RJ. 2006. Microsatellites for ecologists: a practical guide to using and evaluating microsatellite markers. Ecology Letters 9**:** 615-629.

Sharp GD and Pirages S (1978). The distribution of red and white swimming muscles, their biochemistry, and the biochemical phylogeny of selected scombrid fishes. In: Sharp GD and Dizon A, editors, The Physiological Ecology of Tunas. New York: Academic Press.

Shaw PW, Turan C, Wright JM, O'Connell M and Carvalho GR. 1999. Microsatellite DNA analysis of population structure in Atlantic herring (*Clupea harengus*), with direct comparison to allozyme and mtDNA RFLP analyses. Heredity 83**:** 490-499.

Simpson RJ, Wilding CS and Grahame J. 1999. Simple protocol for extracting nuclear DNA from single embryos of a marine snail. Biotechniques 26**:** 1050-1052.

Slatkin M and Hudson RR. 1991. Pairwise comparisons of mitochondrial-DNA sequences in stable and exponentially growing populations. Genetics 129**:** 555-562.

Slatkin M. 1995. A measure of population subdivision based on microsatellite allele frequencies. Genetics 139**:** 457-462.

Swofford DL. 1993. PAUP - A computer program for phylogenetic inference using maximum parsimony. Journal of General Physiology 102**:** A9-A9.

Tajima F. 1989. Statistical method for testing the neutral mutation hypothesis by DNA polymorphism. Genetics 123**:** 585-595.

Takagi M, Okamura T, Chow S and Taniguchi N. 1999. PCR primers for microsatellite loci in tuna species of the genus *Thunnus* and its application for population genetic study. Fisheries Science 65**:** 571-576.

Takezaki N and Nei M. 1996. Genetic distances and reconstruction of phylogenetic trees from microsatellite DNA. Genetics 144**:** 389-399.

Tamura K, Dudley J, Nei M and Kumar S. 2007. MEGA4: Molecular evolutionary genetics analysis (MEGA) software version 4.0. Molecular Biology and Evolution 24**:** 1596-1599.

Thompson JD, Higgins DG and Gibson TJ. 1994. CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. Nucleic Acids Res. 22**:** 4673-4680.

Tidwell MT, Holt S, Rooker JR and Holt GJ. 2008. The distribution and feeding ecology of larval billfish in the Northern Gulf of Mexico. In: Acosta A and Creswell RL, editors, 60th annual meeting of the Gulf and Caribbean Fisheries Institute; 2008 Nov 5-9, Punta Cana, Dominican Republic.Fort Pierce: Omnipress. p. 379-384.

Tringali MD, Berta TM, Seyouma S, Bermingham E and Bartolaccia D. 1999. Molecular phylogenetics and ecological diversification of the transisthmian fish genus Centropomus (Perciformes: Centropomidae). Molecular Phylogenetics and Evolution 13**:** 193-207.

Turner TF, Wares JP and Gold JR. 2002. Genetic effective size Is three orders of magnitude smaller than adult census size in an abundant, estuarine dependent marine fish (*Sciaenops ocellatus*). Genetics 162**:** 1329-1339.

Van Oosterhout C, Hutchinson WF, Wills DPM and Shipley P. 2004. MICRO-CHECKER: Software for identifying and correcting genotyping errors in microsatellite data. Molecular Ecology Notes 4**:** 535-538.

Vieira KR, Lins-Oliveira JE, Barbalho MC and Gracia Jr. J. 2005. Reproductive characteristics of blackfin tuna *Thunnus atlanticus* (Lesson 1831), in Northeastern Brazil. Collective Volume of Scientific Papers ICCAT 58**:** 1629-1634.

Vinãs J, Alvarado Bremer JR and Pla C. 2004. Phylogeography of the Atlantic bonito (*Sarda sarda*) in the northern Mediterranean: The combined effects of historical vicariance, population expansion, secondary invasion, and isolation by distance. Molecular Phylogenetics and Evolution 33**:** 32-42.

Walsh JB. 1987. Persistence of tandem arrays: implications for satellite and simple-sequence DNAs. Genetics 115**:** 553-567.

Ward RD, Elliott NG, Grewe PM and Smolenski AJ. 1994. Allozyme and mitochondrial-DNA variation in yellowfin tuna (*Thunnus albacares*) from the Pacific-Ocean. Marine Biology 118**:** 531-539.

Ward RD. 2000. Genetics in fisheries management. Hydrobiologia 420**:** 191-201.

Weber JL and Wong C. 1993. Mutation of human short tandem repeats. Human Molecular Genetics 2**:** 1123-1128.

White PS and Densmore III LD (1992). Mitochondrial DNA Isolation. In: Hoelzel AR, editor, Molecular Genetic Analysis of Populations, a Practical Approach. New York: Oxford University Press.

Wilson SG, Lutcavage ME, Brill RW, Genovese MP, Cooper AB and Everly AW. 2005. Movements of bluefin tuna (*Thunnus thynnus*) in the northwestern Atlantic Ocean recorded by pop-up satellite archival tags. Marine Biology 146**:** 409-423.

Wirth T and Bernatchez L. 2001. Genetic evidence against panmixia in the European eel. Nature 409**:** 1037-1040.

Wright S. 1951. The genetical structure of populations. Annals of Eugenics 15**:** 323-354.

APPENDIX A

Sampling of Tuna Larvae in the Gulf of Mexico

Ichthyoplankton surveys were conducted in shelf and slope waters of the northern Gulf of Mexico during the summers of 2005 through 2007. Surveys were focused in the region from 27 - 28° N and 88 - 94° W. Larval surveys were conducted with neuston nets (2m width x 1m height frame, one 500µm and one 1200µm mesh size) ending in a cod end collection bucket with equivalent mesh. Nets were towed through the upper meter of the water column at approximately 2.5 knots for 10 minutes. Paired tows were taken (port and starboard side of vessel) at each sampling station with 60-70 equidistant (8 nautical miles apart) stations per survey (Tidwell et al. 2008).

At each station, GPS start and stop points, water temperature (°C), salinity (ppt) and dissolved oxygen (mg/L) were recorded using a Sonde 6920 (YSI Incorporated). Fish larvae and associated invertebrates collected in the net's cod end were preserved, onboard, in 95% ethanol. Preserved samples were sorted in the lab with the use of Leica MZ stereomicroscopes and all tuna larvae were removed and stored in 70% ethanol (Tidwell et al. 2008).

Table A-1. Haplotype frequencies for NW Atlantic and Gulf of Mexico blackfin tuna mtDNA CR-I sequences. Haplotypes aligned by 323bp fragment. Specimen localities in Table 3-1.

Haplotype	Specimen ID	NW Atl	GoM	Haplotype	Specimen ID	NW Atl	GoM
h001	Tatl 047, GoML 040		2	h050	GoML 052		1
h002	Tatl 020, GoML 087	1	1	h051	Tatl 074		1
h003	Tatl 005, Tatl 010	2		h052	Tatl 034		1
h004	GoML 010, GoML 093		2	h053	Tatl 075		1
h005	Tatl 006, Tatl 113	2		h054	Tatl 095		1
h006	GoML 007, GoML 139		2	h055	GoML 001		1
h007	GoML 082, GoML 135		2	h056	Tatl 068		1
h008	Tatl 003, Tatl 110	2		h057	Tatl 089		1
h009	GoML 088		1	h058	Tatl 076		1
h010	GoML 099		1	h059	Tatl 132	1	
h011	Tatl 088		1	h060	Tatl 082		1
h012	GoML 120		1	h061	GoML 016		1
h013	GoML 137		1	h062	GoML 035		1
h014	GoML 103		1	h063	GoML 081		1
h015	GoML 029		1	h064	GoML 033		1
h016	Tatl 098		1	h065	Tatl 017	1	
h017	GoML 009		1	h066	Tatl 052		1
h018	Tatl 097		1	h067	GoML 023		1
h019	GoML 006		1	h068	Tatl 106		1
h020	Tatl 130	1		h069	GoML 053		1
h021	GoML 037		1	h070	Tatl 083		1
h022	Tatl 090		1	h071	Tatl 135	1	
h023	GoML 089		1	h072	Tatl 094		1
h024	Tatl 012	1		h073	Tatl 108		1
h025	Tatl 015	1		h074	Tatl 030		1
h026	GoML 018		1	h075	Tatl 008	1	
h027	Tatl 117	1		h076	GoML 039		1
h028	Tatl 060		1	h077	GoML 014		1
h029	Tatl 044		1	h078	Tatl 073		1
h030	GoML 095		1	h079	Tatl 087		1
h031	GoML 100		1	h080	GoML 008		1
h032	Tatl 056		1	h081	Tatl 009	1	
h033	GoML 028		1	h082	Tatl 002	1	
h034	Tatl 028	1		h083	Tatl 109	1	
h035	Tatl 084		1	h084	GoML 024		1
h036	GoML 078		1	h085	Tatl 093		1
h037	GoML 017		1	h086	GoML 026		1
h038	GoML 125		1	h087	Tatl 050		1
h039	Tatl 004	1		h088	Tatl 014	1	
h040	Tatl 111	1		h089	Tatl 055		1
h041	BLKFIN04	1		h090	Tatl 016	1	
h042	GoML 020		1	h091	BLKFIN27	1	
h043	GoML 084		1	h092	Tatl 081		1
h044	GoML 128		1	h093	GoML 019		1
h045	GoML 059		1	h094	Tatl 072		1
h046	Tatl 031		1	h095	GoML 036		1
h047	GoML 063		1	h096	Tatl 099		1
h048	Tatl 018	1		h097	BLKFIN26	1	
h049	Tatl 125	1		h098	GoML 031		1

Table A-1. Continued.

Haplotype	Specimen ID	NW Atl	GoM	Haplotype	Specimen ID	NW Atl	GoM
h099	GoML 005		1	h148	Tatl 107		1
h100	GoML 004		1	h149	GoML 025		1
h101	Tatl 021	1		h150	GoML 027		1
h102	GoML 011		1	h151	GoML 050		1
h103	Tatl 069		1	h152	GoML 098		1
h104	GoML 038		1	h153	Tatl 131	1	
h105	GoML 042		1	h154	Tatl 059		1
h106	GoML 090		1	h155	GoML 003		1
h107	GoML 030		1				
h108	GoML 034		1				
h109	GoML 131		1				
h110	Tatl 043		1				
h111	Tatl 100		1				
h112	Tatl 091		1				
h113	Tatl 071		1				
h114	Tatl 092		1				
h115	Tatl 070		1				
h116	Tatl 078		1				
h117	GoML 127		1				
h118	BLCKFIN	1					
h119	Tatl 103		1				
h120	Tatl 105		1				
h121	Tatl 104		1				
h122	GoML 068		1				
h123	GoML 021		1				
h124	Tatl 080		1				
h125	Tatl 096		1				
h126	Tatl 035		1				
h127	GoML 013		1				
h128	GoML 002		1				
h129	Tatl 048		1				
h130	Tatl 033		1				
h131	GoML 138		1				
h132	GoML 015		1				
h133	GoML 132		1				
h134	GoML 086		1				
h135	GoML 012		1				
h136	GoML 096		1				
h137	Tatl 102		1				
h138	Tatl 007	1					
h139	Tatl 114	1					
h140	Tatl 101		1				
h141	Tatl 085		1				
h142	Tatl 019	1					
h143	Tatl 126	1					
h144	GoML 094		1				
h145	GoML 091		1				
h146	GoML 107		1				
h147	Tatl 036		1				

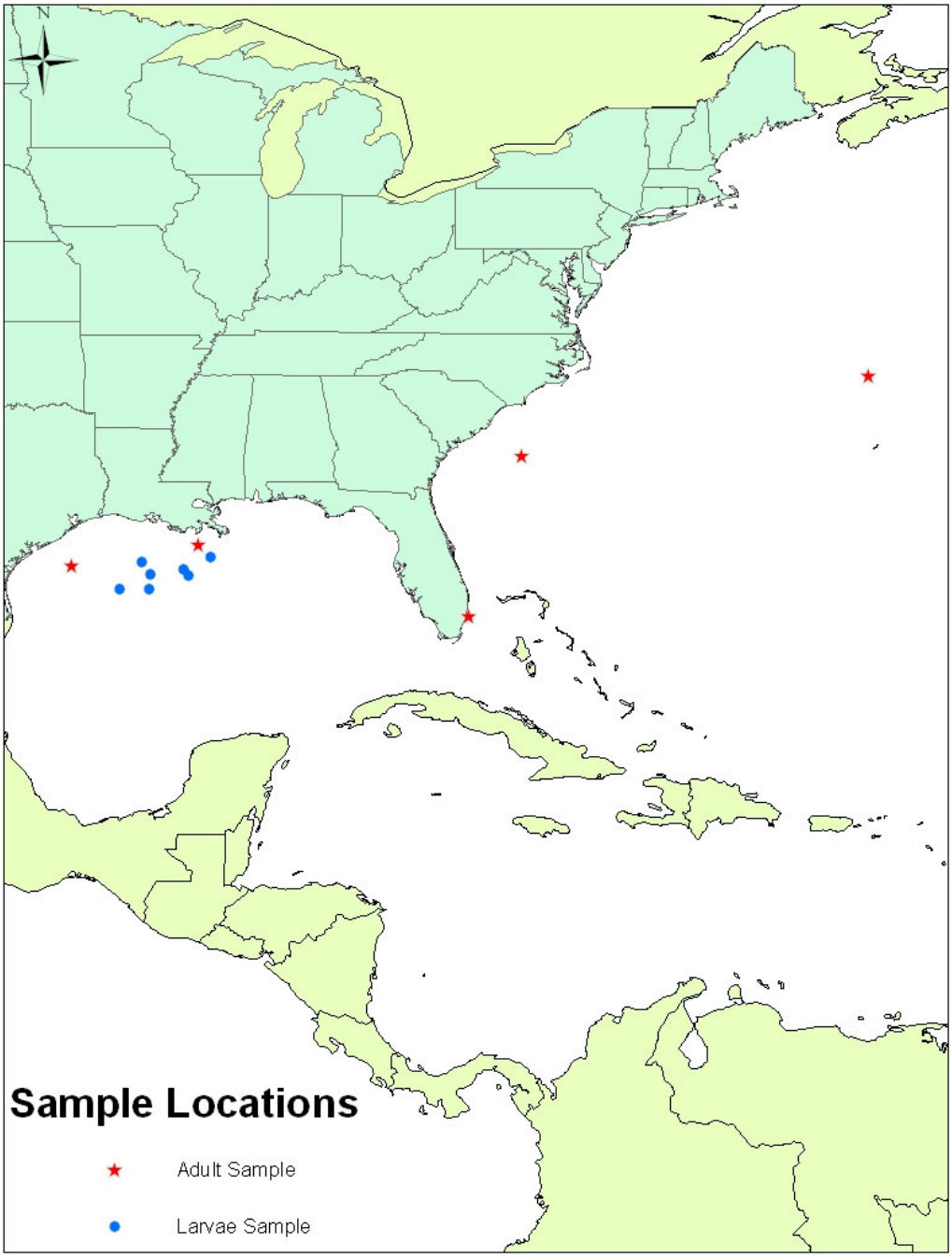


Figure A-1. Sample localities for both adult and larval blackfin tuna (*Thunnus atlanticus)* tissue samples.

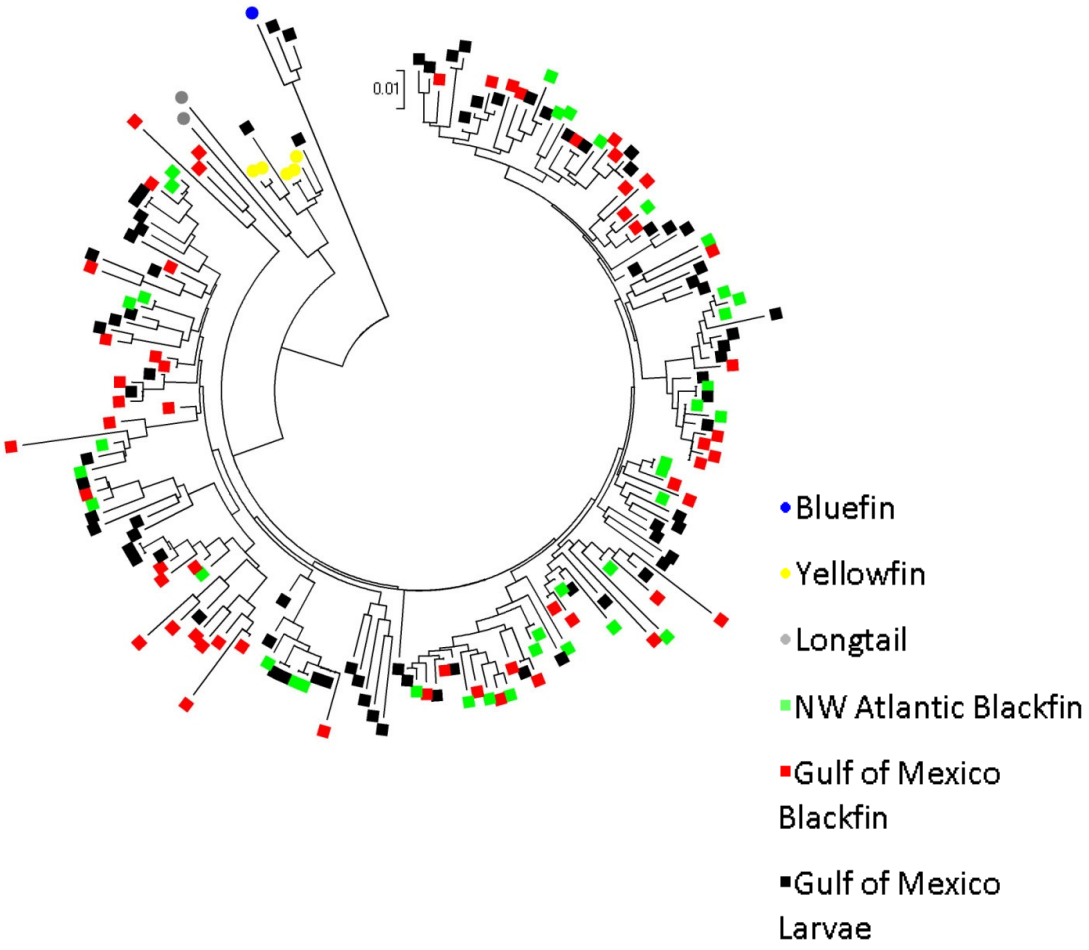


Figure A-2: Rooted phylogeny of 155 blackfin tuna control region I haplotypes, to test for phylogeographic distribution, constructed in PAUP 4.0 (Swofford 1993).

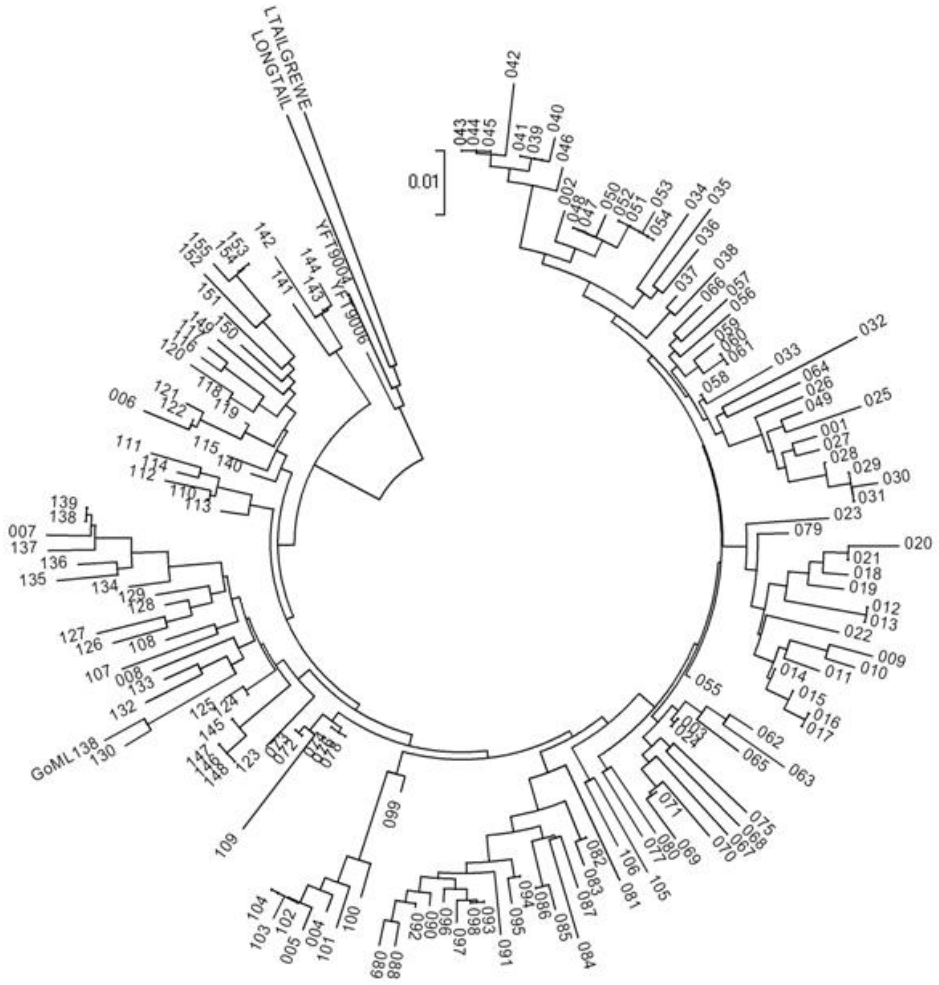


Figure A-3. Rooted circular NJ tree of 155 blackfin tuna mtDNA CR-I haplotypes characterized in this study using yellowfin tuna and longtail tuna as outgroups. Haplotype numbers for blackfin tuna are those given in Table A-1.

APPENDIX B

Extraction of Mitochondrial and Nuclear DNA

Larvae

Extraction of DNA followed the protocol described by Simpson et al. (1999) modified by Farnham (2003) and Bangma (2006). Briefly, a portion of each larva was placed in 30 microliters (µl) of extraction buffer (50mM KCl, 10mM Tris-HCl, pH 8.3, 2.5mM $MgCl_2$, 0.01% gelatin, 0.9% Tween® 20), 20µl of 10mg/ml proteinase K and 10 µl of 10mg/ml RNase in a 0.2 ml PCR tube. Samples were incubated for 60 minutes at 65°C and denatured for 15 minutes at 44°C. The supernatant was then used as template DNA. Cross contamination of sample DNA was avoided by using larval tail tissue or an eyeball so that gut contents were not accidentally extracted or amplified.

Adults

A small piece of muscle tissue, approximately 4µg, was clipped from each sample with sterilized scissors and forceps. Each sample was then placed in a 1.5 ml tube containing 200µl of TENS solution (50mM Tris-HCl, pH 8.0, 100mM NaCl, 100mM EDTA, and 1% SDS), 20µl of 10mg/ml proteinase K and 10µl of 10mg/ml RNase. Samples were incubated at 55°C, and occasionally inverted until digestion was complete.

DNA was precipitated by adding 20µl of 5M NaCl and two volumes of ice cold 95% ethanol to each sample and left to precipitate overnight at -20°C. Samples were centrifuged (Fisher Scientific accuSpin™) for 10 minutes at 13,000rpm and the resulting supernatant was decanted. DNA pellets were washed with 300µl of cold 70% ethanol,

and centrifuged once more. The supernatant was decanted and the tubes were left open to air dry over night to remove any remaining ethanol. DNA was re-suspended in 100µl of TE Buffer (10mM Tris-HCl, pH 8.0, and 1mM EDTA) and incubated at 55°C for 15 minutes.

Isolation of SDS-urea Preserved Samples

Mitochondrial and nuclear DNA from samples stored in SDS-urea was extracted following modified protocols from Sambrook et al. (1989) and White and Densmore III (1992). Samples were placed in 500µl of isolation buffer (50mM EDTA, 50mM Tris, 150mM NaCl, pH 8.0) and digested with 20µl of 10mg/ml proteinase K and 10µl of 10mg/ml RNase. Ten percent SDS was added and the samples were allowed to incubate at 37°C for at least 2hours to as long as overnight. After complete digestion, phenol: chloroform: isoamyl alcohol (25:24:1) was added to the samples and centrifuged at 13,000 rpm for 10 minutes at 4°C. The previous step was repeated once more using the aqueous layer. The aqueous layer was transferred into a new tube and mixed with equal volumes of chloroform: isoamyl alcohol (24:1). The samples were then centrifuged for 10mins at 4°C. The aqueous phase was transferred into a new tube, again, and the DNA was precipitated out by adding 2 volumes of cold ethanol. Samples were then placed in -20°C and allowed to precipitate for 2 hours or more. After precipitation, samples were centrifuged at 13,000rpm at 4°C for 10 minutes. The supernatant was decanted and the pellet was washed with 70% ethanol, and then centrifuged under the same conditions as above. The supernatant was decanted and the pellet was allowed to air-dry completely overnight. Template DNA was resuspended in 40µL of TE buffer (Sambrook et al.

1989; White and Densmore III 1992).

APPENDIX C

Mitochondrial DNA Amplification and Sequencing

Amplification

Amplification and sequencing protocols were modified by Farnham (2003) and Bangma (2006) and were optimized for use with blackfin tuna in the lab. Polymerase chain reactions (PCR) were prepared in 12.5µl volumes containing: 8.4µl ddH_2O, 1.25µl 10x Buffer, 0.5µl 2mM $MgCl_2$, 0.25µl dNTP's, 0.5µl of each primer (L15998, CSBD-H (Alvarado Bremer et al. 1996)), 0.1µl of *Taq* polymerase, and 1µl of isolated DNA template.

DNA amplification was carried out in an Eppendorf Mastercycler® Gradient thermal cycler. Initially, a denaturing step of 2 minutes at 94°C was included and followed by 36 cycles of strand denaturation for 30 seconds, primer annealing at 53°C for 45 seconds, and extension at 72°C for 1 minute. A final extension step of 72°C for 3 minutes was also included. Three microliters of each PCR product was loaded into a 1% agarose gel, pre-stained with 0.1 µg/ml of ethidium bromide, and run at 120V for 20 minutes, and viewed under ultra-violet transilluminator to determine the quality of the amplifications.

Sequencing and Forensic Identification

Excess primers and dNTP's were removed from amplified PCR products by adding 2µl of ExoSAP-IT™ (USB Corporation, Cleveland, Ohio) to 5µl of PCR product, following the manufacturer's recommendations. Alternatively, amplifications

were cleaned by adding 0.5µl exo-nuclease I, 0.5µl Shrimp Alkaline Phosphatase (SAP) and 0.7µl 10x SAP Buffer (USB Corporation, Cleveland, Ohio) and 0.3 µl of ddH_2O to 5 µl of PCR product. Big Dye™ Terminator v 1.0 Cycle Sequencing Ready Reaction Kit (Perkin-Elmer Corporation, Foster City, California) was used in the cycle sequencing reaction. The reaction involved combining 1µl of BigDye™ Terminator, 2µl of 5X Big Dye Buffer, 4µl of diluted L15998 primer at 3:1 concentration, 2µl of cleaned DNA, and 1µl of ddH_2O. The samples were pulse centrifuged and then loaded in the thermal cycler for cycle sequencing. Platt et al.'s (2007) stepped elongation time (ST_eP) cycle sequencing protocol was used to optimize the cycle sequencing reaction by increasing the extension time after 15 and 20 cycles, the program consists of an initial denaturation step of 95°C for 1 minute, followed by 14 cycles of denaturation at 95°C for 10 seconds, annealing at 50°C for 5 seconds, an extension period at 60°C for 1 minute 15 seconds. After the first 15 cycles the extension time was increased to 1 minute and 30 seconds for 4 cycles, at the same temperatures, and the extension time was increased once more to 2 minutes (Platt et al. 2007).

DNA samples sequenced on the older ABI 310 genetic analyzer (Perkin-Elmer Corporation, Foster City, California) were precipitated by adding 1µl of 7.5M ammonium acetate and 25µl of ice cold 95% ethanol to each 0.2ml tube. Samples were inverted and pulse centrifuged. After precipitating for 10 minutes at room temperature, the samples were placed into the Fisher Scientific accuSpin™ for 25 minutes at 2,000 rpm, and decanted by inversion. 150µl of 70% ethanol was added to each tube and mixed by inverting. Samples were centrifuged again for 10 minutes at 13,000 rpm.

Resulting supernatant was discarded and samples were allowed to completely air dry. In preparation for sequencing in the ABI 310 genetic analyzer (Perkin-Elmer Corporation, Foster City, California), 25µl of formamide was added to each sample, vortexed for 10 seconds and pulse centrifuged. Samples were denatured in the thermal cycler at 95^{o}C for 2 minutes, and kept on ice until loaded into the sequencer.

DNA samples sequenced on the new ABI 3130 genetic analyzer (Perkin-Elmer Corporation, Foster City, California) underwent the same cleaning processes as previously mentioned, but the BigDye^{TM} XTerminator Purification kit (Applied Biosystems, Foster City, California) was used in place of ethanol precipitation. The manufacturer's recommendations were as follows: add 45µl of SAM^{TM} solution and 10µl of BigDye^{TM} Xterminator solution to cleaned DNA samples. Place samples on Scientific Instruments^{TM} Vortex Genie-2 digital plate shaker and agitate for 30 minutes at 1900 rpms, centrifuge at 1,000rpm for 2 minutes. Samples were then loaded onto the genetic analyzer.

APPENDIX D

Microsatellite DNA Amplification and Fragment Analysis

Four dinucleotide microsatellite loci, primers which were developed for yellowfin tuna, *Thunnus albacares*, by Appleyard et al. (2001), were analyzed and included loci cmrTa-113, cmrTa-125, cmrTA-144, and cmrTA-208 (referred in the text as 113, 125, 144, 161, and 208). The forward primer of each primer pair was end-labeled with one of three fluorescent tags, FAM, HEX, and TET. Multiplex PCR was set up in 12µl reactions with the following final concentrations: 10mM Tris-HCl (pH 8.3), 50mM KCl, 2.5mM $MgCl_2$, 100µM dNTP's, and 0.8µM for each forward and reverse primer (Farnham 2003). Each reaction also contained 1µl of template DNA and 0.5 units of *Taq* polymerase. The PCR conditions followed that of Farnham (2003) as: 93^{o}C for 10 minutes, 55^{o}C for 15 seconds, 72^{o} C for 2 minutes, and an additional 50 minutes at 72^{o}C to allow extension to come to completion, to overcome the phenomenon of plus-A addition (Breen et al. 1999; Takagi et al. 1999; Farnham 2003). Multiplex PCR primer groups can be found below in Table D-1.

Two other dinucleotide microsatellite loci, isolated from Pacific northern bluefin tuna, *Thunnus thynnus orientalis*, that have been shown to amplify in yellowfin tuna (Takagi et al. 1999) were also analyzed. The two loci used were Ttho-1, and Ttho-4. The forward primer of each primer pair was also fluorescently end-labeled with FAM or HEX tags. Multiplex PCR was setup in 12µl reactions with the following final concentrations: 10mM Tris-HCl (pH 8.3), 50mM KCl, 1mM $MgCl_2$, 0.01% gelatin, 100µM dNTP's, and 0.8µM for each forward and reverse primer (Farnham 2003). Each

reaction also contained 1µl of template DNA and 0.5 units of *Taq* polymerase. PCR conditions followed that as described by Takagi et al (1999) and modified by Farnham (2003) as: 7 cycles of 94°C for 1 minute, 52°C for 30 seconds, and 72°C for 30 seconds, followed by 33 cycles of 90°C for 30 seconds, 52°C for 30 seconds, and 72°C for 30 seconds. A final 72°C incubation step of 50 minutes was also added to account for plus-A addition (Breen et al. 1999).

Following PCR, the amplicons were prepared for analysis following Farnham's (2003) protocols and modified in the lab as follows: all amplicons were diluted 1:10. Five microliters of the diluted samples were cleaned with 0.5µl of exonuclease-I and 1.5µl of ddH_2O to remove any excess primers or unused dNTP's. Samples were loaded onto the thermal cycler; PCR conditions followed that of the ExoSAP-IT™ (USB Corporation, Cleveland, Ohio) treatment for cleaning mitochondrial DNA (Appendix B). For each reaction, 1µl of a GeneScan™-500 size standard, either TAMRA™ or ROX™ (ABI Prism, Applied Biosystems, Warrington, UK), was mixed with 22µl of formamide. Twenty-two microliters of this cocktail was mixed with 1µl of the cleaned DNA, denatured for 2 minutes at 95°C and placed directly on ice. The products were run on the ABI 310 genetic analyzer and analyzed with the GENESCAN 3.7 program (Applied Biosystems, Foster City, CA).

After analysis with GENESCAN 3.7 was completed all loci were checked with MICRO-CHECKER 2.2 (Van Oosterhout et al. 2004) for the presence of null alleles. Affected loci were visually re-inspected and scored by eye. A common cause of wrong allele calls were incorrect scoring of the standard peaks. After readjusting the standard

scores (correct peak with correct base-pair size) shifts of some original scores occurred by as much as 10bp. Another cause was the inability of auto-binning to score a peak if it fell on the limits for that particular bin. Occasionally, GENESCAN 3.7 would call a heterozygous individual homozygous, even if the second peak was obvious. These types of scoring errors are possible causes of initial heterozygous deficiencies. After, re-inspection the loci were checked with MICRO-CHECKER 2.2 once more. This cleaning process allowed most loci to fall within Hardy-Weinberg equilibrium.

Table D-1. Microsatellite multiplex PCR groups.

Author	Locus	Dye	Motif	Anneal Temp
Appleyard (2001)	125	F-Tet	(CA)	
cmrTA	144	F-Fam	(CA)	55
	161	F-Hex	(CA)	
Appleyard (2001)	208	F-Hex	(CA)	55
cmrTA	117	F-Fam	(CA)	
Takagi (1999)	Ttho-1	F-Fam	(GT)	50
	Ttho-4	F-Hex	(CA)	

Table D-2. Microsatellite genotypes for the NW Atlantic (NWAtl) and Gulf of Mexico (G0M) blackfin tuna samples for all six loci. Specimen localities in Table 3-1.

NW Atl	125	144	113	208	Ttho-1	Ttho-4
Tatl002	152,152	174,174	113,125	137,139	185,185	140,170
Tatl003	148,150	174,174	121,123	135,139	?	?
Tatl004	150,152	174,174	113,119	137,137	183,183	148,168
Tatl005	148,152	174,174	111,113	137,137	183,185	158,162
Tatl006	148,150	174,174	113,113	137,139	185,189	142,150
Tatl007	152,152	174,174	113,121	137,137	187,189	168,176
Tatl008	146,148	174,174	113,125	135,139	183,187	146,148
Tatl009	152,152	?	113,121	137,139	185,187	146,148
Tatl010	?	?	?	139,139	?	?
Tatl012	152,162	174,174	111,113	137,137	?	?
Tatl014	150,152	?	113,119	137,137	185,187	142,146
Tatl015	150,152	?	113,117	139,139	183,187	152,164
Tatl016	148,150	?	117,119	137,139	183,187	150,152
Tatl017	148,152	174,174	117,119	139,139	183,187	144,148
Tatl018	150,152	?	113,123	139,139	183,183	144,148
Tatl019	150,152	?	113,123	139,139	?	?
Tatl020	?	?	?	?	?	148,156
Tatl021	?	?	113,119	137,139	?	?
Tatl028	152,156	174,174	111,113	137,139	181,183	176,178
Tatl109	150,152	174,174	111,123	137,139	185,185	140,170
Tatl110	148,150	174,174	119,121	137,137	185,187	148,150
Tatl111	152,152	174,174	111,117	137,137	183,183	148,168
Tatl112	152,152	?	111,111	137,137	181,183	158,160
Tatl113	150,152	?	109,111	137,139	185,189	142,150
Tatl114	150,152	?	111,119	137,137	185,189	144,152
Tatl115	148,152	?	111,123	137,137	185,189	168,176
Tatl116	150,152	?	111,119	137,137	183,187	146,148
Tatl117	148,152	174,174	119,121	139,139	185,187	146,148
Tatl118	152,152	?	111,113	137,139	183,185	160,164
Tatl119	152,152	174,174	111,113	137,137	187,189	172,172
Tatl120	152,152	?	115,117	139,139	181,183	174,182
Tatl121	152,156	?	111,117	137,137	185,187	142,146
Tatl122	150,152	?	111,115	139,139	181,185	152,164
Tatl123	148,152	174,174	115,117	137,139	181,187	150,152
Tatl124	152,152	?	115,117	137,139	181,187	144,148
Tatl125	150,152	174,174	111,121	137,139	183,183	144,148
Tatl126	150,152	174,174	111,121	139,139	183,183	154,188
Tatl127	152,152	174,174	111,111	137,141	183,183	148,156
Tatl128	152,152	174,174	111,117	137,139	?	?
Tatl129	152,152	?	111,117	137,139	?	?
Tatl130	152,152	?	111,119	137,139	181,181	150,160
Tatl131	152,152	?	111,119	137,139	183,185	148,150
Tatl132	?	?	111,121	137,139	181,187	152,154
Tatl133	?	?	111,119	137,137	183,183	160,184
Tatl134	148,152	?	111,119	137,139	183,183	152,160
Tatl135	152,152	174,174	111,117	137,139	181,191	160,184
Tatl136	152,152	?	111,119	137,139	185,187	150,154
Tatl137	152,152	?	111,119	137,139	181,183	148,150
Tatl138	152,152	?	111,119	137,139	185,187	152,160
GoM Sample						
Tatl030	152,152	174,174	109,109	137,139	183,185	146,148
Tatl031	152,156	?	113,117	137,139	183,187	146,180
Tatl032	?	?	119,121	137,139	183,187	148,154
Tatl033	?	174,174	111,113	137,139	181,183	150,154
Tatl034	?	?	113,119	137,139	181,185	146,160
Tatl035	152,152	174,174	117,119	137,141	181,183	154,190
Tatl036	148,150	?	119,121	135,137	183,183	148,156
Tatl037	148,152	?	111,129	137,139	181,183	148,158
Tatl038	148,152	174,174	111,121	137,137	?	146,154
Tatl039	152,152	174,174	105,111	137,139	183,183	158,182
Tatl040	150,152	?	113,119	135,139	181,183	150,158
Tatl041	152,152	174,174	111,121	137,139	183,183	146,148
Tatl042	152,154	?	115,117	139,139	183,193	146,152
Tatl043	150,156	?	113,113	137,139	181,183	150,152
Tatl044	?	?	111,113	137,139	181,183	146,154
Tatl045	152,152	174,174	113,113	137,139	181,183	150,154
Tatl046	148,152	?	113,121	137,139	183,183	152,162
Tatl047	148,152	?	115,119	137,139	181,183	164,166
Tatl048	148,152	?	111,115	135,137	183,183	142,144
Tatl049	148,152	174,174	115,121	137,141	181,193	140,160

Table D-2.Continued.

GoM	125	144	113	208	Ttho-1	Ttho-4	GoM	125	144	113	208	Ttho-1	Ttho-4
Tatl050	152,152	168,174	115,125	137,139	181,183	152,160	Tatl088	148,152	174,174	111,123	137,139	177,187	148,150
Tatl051	148,150	?	111,113	137,139	183,185	160,162	Tatl089	152,152	174,174	111,111	137,137	181,185	148,160
Tatl052	152,156	174,174	117,121	135,137	181,181	160,168	Tatl090	152,152	168,174	111,119	139,139	181,185	146,156
Tatl053	150,150	174,174	111,113	137,139	183,183	146,148	Tatl091	148,150	174,174	111,119	137,139	181,183	154,166
Tatl054	148,152	172,174	119,123	137,139	185,185	148,158	Tatl092	152,152	174,174	111,117	137,137	181,183	156,158
Tatl055	150,152	?	117,119	137,139	181,185	148,164	Tatl093	152,156	?	111,127	137,139	183,191	150,152
Tatl056	150,152	172,174	119,125	137,139	181,187	150,152	Tatl094	146,148	?	117,119	137,139	183,183	150,170
Tatl057	148,150	172,174	119,121	137,139	181,181	140,164	Tatl095	148,152	174,174	111,123	137,139	181,183	148,162
Tatl058	?	?	113,121	137,139	185,185	142,152	Tatl096	150,152	172,174	111,119	137,137	183,187	146,158
Tatl059	148,152	172,174	111,113	139,141	183,185	148,154	Tatl097	150,152	?	111,115	137,137	185,189	148,194
Tatl060	?	?	?	139,141	181,181	186,188	Tatl098	152,156	174,174	111,117	137,139	187,187	146,172
Tatl061	?	?	117,119	137,139	181,183	150,160	Tatl099	152,156	?	111,117	137,137	183,183	140,152
Tatl062	148,152	?	111,117	137,139	183,183	148,164	Tatl100	150,152	?	111,117	137,139	183,187	142,144
Tatl063	150,150	174,174	111,117	137,139	181,183	148,154	Tatl101	148,152	?	111,117	141,141	183,187	146,162
Tatl064	148,150	172,174	119,123	137,139	183,185	146,158	Tatl102	148,152	174,174	109,111	137,137	183,183	142,160
Tatl068	?	?	111,113	137,139	181,181	154,156	Tatl103	148,148	174,174	111,117	141,141	183,185	152,154
Tatl069	152,152	174,174	111,119	137,141	181,181	148,156	Tatl104	150,152	172,174	111,121	137,139	185,185	154,158
Tatl070	150,150	174,174	111,117	137,137	181,183	170,182	Tatl105	152,156	172,174	117,119	139,139	?	?
Tatl071	152,158	174,174	117,119	137,139	183,185	182,184	Tatl106	150,152	172,174	121,123	137,137	?	?
Tatl072	150,152	?	117,119	137,141	183,185	150,156	Tatl107	148,152	?	111,119	137,139	?	?
Tatl073	152,152	172,172	109,111	137,139	181,187	146,158	Tatl108	150,152	172,174	111,117	137,137	183,183	142,150
Tatl074	146,152	174,174	113,123	137,137	181,185	158,162	GoML002	132,136	?	111,111	?	179,183	146,148
Tatl075	146,152	174,174	113,123	139,139	181,185	158,162	GoML003	148,152	168,174	117,117	137,139	183,185	146,148
Tatl076	152,154	174,174	117,117	137,139	183,185	148,166	GoML004	132,136	?	117,119	137,141	181,185	146,148
Tatl077	150,150	174,174	117,119	137,141	181,183	144,150	GoML005	132,136	166,174	117,121	137,139	183,187	148,150
Tatl078	152,152	174,174	111,115	137,139	183,185	152,190	GoML006	?	166,174	111,111	?	181,183	148,160
Tatl079	142,152	174,174	111,119	139,141	185,185	158,160	GoML007	132,136	166,174	111,131	139,139	181,183	152,162
Tatl080	152,152	174,174	117,121	137,139	181,183	154,180	GoML008	132,136	166,174	117,119	137,139	181,183	148,154
Tatl081	154,154	174,174	111,115	137,137	181,183	146,152	GoML009	130,134	?	111,121	137,137	?	154,158
Tatl082	148,150	174,174	109,111	137,137	181,187	144,176	GoML010	132,136	168,174	111,117	139,139	179,179	152,152
Tatl083	152,156	?	117,123	137,139	183,187	140,148	GoML011	?	166,174	111,117	135,137	?	?
Tatl084	152,152	?	109,111	137,139	183,183	144,148	GoML012	130,134	?	121,123	137,139	183,185	148,154
Tatl085	152,152	?	111,117	137,139	183,191	150,162	GoML014	132,136	?	111,111	135,139	181,183	148,150
Tatl086	150,152	?	111,117	137,139	185,187	142,144	GoML016	?	?	115,135	135,137	?	?
Tatl087	150,152	?	111,111	137,137	183,185	154,156	GoML017	?	?	111,119	139,141	181,183	148,160

Table D-2. Continued.

GoM	125	144	113	208	Ttho-1	Ttho-4
GoML019	132,136	166,172	111,117	139,143	179,181	150,160
GoML020	?	166,172	113,121	?	?	?
GoML021	134,136	?	111,119	133,137	181,183	144,146
GoML022	?	?	111,111	137,139	179,181	144,176
GoML023	132,136	166,174	111,117	139,141	183,185	152,154
GoML024	136,150	166,172	111,121	137,141	183,183	152,154
GoML025	136,140	166,172	111,119	137,139	181,185	148,154
GoML026	134,136	166,174	109,111	137,139	?	142,142
GoML027	132,138	166,168	111,111	135,137	183,183	154,156
GoML028	136,150	166,174	111,119	135,139	181,185	166,168
GoML029	134,136	166,172	111,111	135,137	181,181	140,142
GoML030	132,136	174,174	111,111	137,139	185,185	148,160
GoML031	134,136	?	111,139	135,137	181,183	146,148
GoML033	?	166,172	113,123	137,141	?	144,162
GoML034	136,140	?	121,123	137,139	?	?
GoML035	132,136	166,172	113,113	?	183,187	148,176
GoML036	132,136	166,172	111,137	?	183,187	142,148
GoML037	136,136	166,172	111,121	137,139	183,185	164,186
GoML038	132,136	172,174	111,115	137,139	183,185	142,176
GoML039	134,136	?	117,119	135,139	183,185	146,150
GoML040	134,136	166,174	111,111	135,139	155,181	172,174
GoML042	132,136	166,172	113,121	135,139	?	?
GoML050	?	?	111,119	?	?	?
GoML052	?	?	113,141	?	?	?
GoML053	?	?	117,139	135,137	181,181	148,148
GoML054	?	?	121,139	135,139	?	136,138
GoML059	?	174,176	113,115	131,133	183,183	148,154
GoML063	?	172,174	117,119	?	?	?
GoML068	?	166,172	113,119	139,141	179,185	136,136
GoML078	?	166,174	111,119	139,141	183,185	146,152
GoML081	?	166,174	111,111	139,141	?	148,156
GoML082	?	166,174	117,119	137,139	183,187	?
GoML084	150,152	?	117,119	137,139	?	?
GoML086	132,136	166,174	117,139	137,139	?	146,148
GoML087	146,152	168,174	117,123	139,141	185,187	144,176

GoM	125	144	113	208	Ttho-1	Ttho-4
GoML088	148,156	172,174	111,111	139,141	183,185	146,162
GoML089	?	172,174	115,117	137,137	181,183	142,166
GoML090	?	174,174	111,121	139,141	181,183	144,148
GoML091	?	166,172	113,123	?	181,183	160,186
GoML092	?	166,174	?	137,139	?	158,160
GoML094	132,136	166,174	119,119	137,139	?	172,186
GoML095	136,150	166,174	117,139	135,139	179,185	146,148
GoML096	?	?	?	139,139	?	?
GoML098	134,136	166,174	111,125	139,141	181,183	144,146
GoML099	146,148	166,174	111,117	139,141	181,183	144,148
GoML100	?	166,174	111,111	139,139	?	?
GoML103	132,136	170,172	111,119	137,139	183,183	162,164
GoML107	140,156	?	111,111	139,141	181,183	146,164
GoML120	136,150	166,172	111,111	137,139	181,183	148,174
GoML125	144,152	?	111,111	137,139	185,187	148,156
GoML127	148,152	172,172	111,113	139,141	181,185	156,162
GoML128	134,140	?	121,127	139,141	181,183	166,182
GoML131	132,152	166,172	117,123	137,139	183,183	146,148
GoML132	152,156	?	111,117	137,139	181,183	152,168
GoML135	130,146	?	117,121	139,141	181,183	172,182
GoML137	150,152	166,172	111,117	141,141	183,187	146,148
GoML138	150,152	?	111,121	137,139	181,185	156,170
GoML139	150,152	166,174	111,111	135,137	183,187	158,160
GoML140	152,156	174,174	117,135	137,139	181,185	152,162

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Objective

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Education

2006-2009	M.Sc., Wildlife and Fisheries Sciences Texas A&M University College Station/Galveston, TX
2000-2004	B.Sc., Marine Biology Texas A&M University Galveston Galveston, TX

Laboratory Skills

- Familiar with DNA isolation of adult and larval fish tissues, PCR and Q-PCR related techniques, sequencing reactions, and gel electrophoresis
- Competent with the use and care of ABI 310, and 3130 Genetic Analyzers and associated programs for DNA sequencing and fragment analysis
- Extensive knowledge of contemporary genetic analysis software
- Familiar with programming various thermal cyclers and autoclaves

Publications

Saxton, Brandon L., and Jaime Alvarado Bremer. Genetic characterization of Blackfin Tuna (*Thunnus atlanticus*): Historical Demography and Population Structure. 60th Annual Conference of the Gulf and Caribbean Fisheries Institute. 5-9 November of 2007. Punta Cana, Dominican Republic. (Poster Session).

Alvarado Bremer, J. R. Jessica L. Bangma, Tiffany Talley-Farnham, J. Rooker, B. Stequert and **B. Saxton**. Beyond species ID of billfishes and tunas. 2006. The advantages of DNA sequence data. Poster Session. Large Pelagic Fishes in the Caribbean Sea and the Gulf of Mexico: Current Status and Integrated Management. A symposium convened at the 59thAnnual Conference of the Gulf and Caribbean Fisheries Institute. 6-11 November of 2006, Belize City, Belize.