

GENETIC ANALYSIS OF WHITE MARLIN (*TETRAPTURUS ALBIDUS*) STOCK STRUCTURE

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ABSTRACT

The genetic basis of stock structure of white marlin (*Tetrapturus albidus* Poey, 1860) was inferred from analyses of five tetranucleotide repeat microsatellite loci ($n = 214$) and the mitochondrial (mt)DNA control region ($n = 99$) of white marlin from four geographic regions in the Atlantic Ocean. Considerable genetic variation was present in all collections for all molecular markers. Analysis of replicate collections taken in different years from three regions revealed no significant differences in the distribution of allele frequencies among years within regions. The value of global F-statistics for both multilocus microsatellite data and mtDNA control region sequences approached significance (0.0022, $P = 0.057$, and 0.0163, $P = 0.069$, respectively). Pairwise comparisons of regional collections based on microsatellite data revealed one significant comparison (western North Atlantic vs western South Atlantic), as did pairwise comparisons of mtDNA control region values (western North Atlantic vs Caribbean), and inferences from analysis of molecular variance (AMOVA) differed between mitochondrial and nuclear markers. However, there was a general trend for increased genetic divergence with increased geographic separation for both sets of markers. While these results are consistent with current management of white marlin as a single stock, the observation of significant heterogeneity warrants further analysis.

White marlin (*Tetrapturus albidus* Poey, 1860) are distributed throughout the tropical and subtropical waters of the Atlantic Ocean and adjacent seas, occurring between 45°N and 45°S (Nakamura, 1985). The species supports a directed recreational fishery throughout its range and is also targeted by localized artisanal fisheries; however, the majority of white marlin fishing mortality results from interactions with the pelagic longline fishery that targets tunas and swordfish. While white marlin represent a minor by-catch of this fishery, the sheer number of longline hooks deployed on an annual basis results in substantial white marlin mortality (ICCAT, 2003).

White marlin catches increased dramatically with the inception of the high seas pelagic longline fishery in the early 1960s, peaking at 4911 metric tons (mt) in 1965 (ICCAT, 2005). Over the next few years, catches of white marlin rapidly declined to about 2000 mt, and between 1970 and 1995, the reported annual catches of white marlin fluctuated between 1000 and 2000 mt. Since the mid 1990s, reported catches of white marlin have gradually decreased to about 600 mt annually.

White marlin are managed by the member countries of the International Commission for the Conservation of Atlantic Tunas (ICCAT). At the 2002 white marlin stock assessment workshop, ICCAT's Standing Committee for Research and Statistics (SCRS) used a variety of model and data set combinations to estimate population biomass for the species. As expected, these methods provided a wide range of results regarding stock status, but the majority of analyses indicated the stock was seriously overexploited. The continuity case assessment, which employed a model and data set combination similar to those used in previous assessments, indicated the current

(2000) biomass to be 12% of that needed to produce maximum sustainable yield (ICCAT, 2003).

Despite the economic importance of the white marlin resource and the current depleted status of the stock, little is known about the stock structure of the species. Historically, ICCAT's SCRS considered two different stock models for white marlin: a single Atlantic stock, and distinct North Atlantic and South Atlantic stocks separated at 5°N. The two-stock model was supported by the distribution of catches of white marlin in the early years of the fishery in areas north and south of the equator (Uozumi and Nakano, 1994), the occurrence of seasonally displaced spawning in the northern and southern hemispheres, and a lack of trans-equatorial movements by tagged fish (Nakamura, 1985; Scott et al., 1990). A dividing line of 5°N was chosen as it coincided with ICCAT statistical areas. However, the validity of the two-stock model has been questioned as expansion of the pelagic longline fishery within the equatorial region demonstrated the presence of white marlin in this area throughout the year. In addition, analysis of Japanese longline data does not indicate a break in catch per unit effort values in this region (Uozumi and Nakano, 1994). Furthermore, long distance recaptures of white marlin tagged in the western North Atlantic demonstrate movement across the proposed stock boundary at 5°N as well as trans-Atlantic movements (Scott et al., 1990; Jaen and Jaen, 1994; Ortiz et al., 2003).

There have been few genetic analyses of white marlin stock structure. Edmunds (1972) analyzed variation of seven blood and tissue proteins from more than 100 white marlin collected in the mid-Atlantic Bight, Gulf of Mexico, and Caribbean Sea. On the basis of the distribution of alleles among samples, the null hypothesis that white marlin comprise a common gene pool could not be rejected. More recently, Graves and McDowell (2001, 2003) employed restriction fragment length polymorphism (RFLP) analysis of mitochondrial (mt)DNA to evaluate stock structure within white marlin. Analysis of 226 individuals from four geographically distant locations in the Atlantic revealed much more variation than the earlier analysis of blood and tissue proteins; however, the RFLP variation was distributed homogeneously across the sampling locations. This result contrasted sharply with the significant heterogeneity demonstrated by the same technique among geographically distant samples of the closely related striped marlin, *Tetrapturus audax* (Philippi, 1887), within the Pacific Ocean (Graves and McDowell, 1994).

While RFLP analysis of mtDNA reveals considerable variation within white marlin, there are several other classes of molecular markers with higher levels of genetic resolution that can be used to test for stock structure. Recently, analyses of hypervariable gene regions such as nuclear microsatellite loci and mtDNA control region sequences have been used to evaluate stock structure of istiophorid billfishes (Buonacorsi et al., 2001; McDowell, 2002; Graves and McDowell, 2003) as well as a host of other large pelagic fishes including bluefin tuna [*Thunnus thynnus* (Linnaeus, 1758)] (Broughton and Gold, 1997; Alvarado Bremer et al., 1999; Takagi et al., 1999; Ely et al., 2002; Carlsson et al., 2004), yellowfin tuna [*Thunnus albacares* (Bonnaterre, 1788)] (Appleyard et al., 2001), bigeye tuna [*Thunnus obesus* (Lowe, 1839)] (Alvarado Bremer et al., 1998; Martínez et al., 2006), albacore [*Thunnus alalunga* (Bonnaterre, 1788)] (Chow et al. 2000; Viñas et al. 2004) and swordfish [*Xiphias gladius* Linnaeus, 1758] (Alvarado Bremer et al., 1996; Rosel and Block, 1996; Reeb et al., 2000). In the present study, we surveyed genetic variation at five tetranucleotide repeat microsatellite loci and the mtDNA control region within collections of white marlin from

four geographically distant regions in the Atlantic Ocean, as well as from collections of animals taken in the same region in different years, to evaluate the null hypothesis that white marlin comprise a single, genetically homogeneous stock within the Atlantic Ocean.

MATERIALS AND METHODS

White marlin were collected from four major geographic regions: the western North Atlantic (WNA; U.S. mid-Atlantic), the Caribbean Sea (CAR; Dominican Republic and Venezuela), the western South Atlantic (WSA; southern Brazil) and the eastern Atlantic (EA; Morocco) between 1992 and 2003 (Table 1). Samples consisted of either heart tissue removed after capture, cooled on ice, and stored at -80°C , or white muscle preserved in 0.25mM EDTA pH 8.0, 20% DMSO, and saturated NaCl (Seutin et al., 1991) at room temperature.

DNA was isolated using either a phenol-chloroform (Sambrook and Russel, 2001), or a proteinase K-chelex extraction (Estoup et al., 1996). Five microsatellite loci originally developed for use in the blue marlin, *Makaira nigricans* (Lacépède, 1802); *Mn01*, *Mn08*, *Mn10*, *Mn60*, and *Mn90*, were amplified using the parameters outlined in Buonaccorsi and Graves (2000). Microsatellite loci were analyzed on a Li-Cor 4200 Global IR² automated sequencer (Li-Cor, Lincoln, NE). A 50-350 base pair (bp) size standard (Li-Cor, Lincoln, NE) was loaded onto each end and the center of each gel to determine allele sizes. To ensure identical scoring of alleles at a locus across gels, between four and eight lanes of each run consisted of samples for which allele sizes were known. Approximately 20% of the samples were re-run to verify that alleles could be consistently scored. Alleles were scored using the GenImagIR 4.05 software (Scanalytics Inc., Fairfax, VA).

An 834 bp fragment of the mtDNA control region was amplified from subsets of 20 or more individuals from each of the four sampling locations using the Pro-5 and 12SAR-3 primers (Palumbi, 1996). Primers were modified to include M13 tails. Amplified products were sequenced on a Li-Cor 4200 Global IR² system using IRD-800-labelled forward primer M13F

Table 1. White marlin (*Tetrapturus albidus*) collection information, including the number of individuals assayed for variation at five microsatellite loci (microsatellites) and the mitochondrial DNA control region sequence (mtDNA).

	Microsatellites		mtDNA
Western North Atlantic			
Cape May, NJ	1992	14	8
Cape May, NJ	1993	20	5
Cape May, NJ	1994	22	5
Cape May, NJ	1995	18	0
Cape May, NJ	2003	0	2
Total:		74	20
Caribbean			
Dominican Republic	1992	16	16
Cumaná, Venezuela	1996	25	20
Total:		41	36
Western South Atlantic			
Santos, Brazil	1993	45	9
Santos, Brazil	1995	18	11
Total:		63	20
Eastern Atlantic			
Morocco	1995	36	23
Total		214	99

(-29), IRD-700-labelled reverse primer M13R (both from Li-Cor, Lincoln, NE), and an internal reverse primer designed specifically for the teleost control region (CCA TCT TAA CAT CTT CAG TG; S. Boles, Virginia Institute of Marine Science, unpubl.). Standard chromatographic curves of forward and reverse sequences were imported into the program Sequencher 4.2.2 (Gene Codes Corp., Ann Arbor, MI) aligned, and edited. A consensus of forward and reverse sequences was created and exported to the program MacVector 7.2.3 (Oxford Molecular LTD, Madison, WI). An alignment was created using ClustalW (Thompson et al., 1994), and adjusted by eye.

The program Arlequin 3.0 (Excoffier et al., 2005) was used to calculate haplotype diversity (h) and nucleotide diversity (π) for the mtDNA control region sequence data, and PAUP* 4.0 (Swofford, 2000) was used to generate a UPGMA tree based on the Tamura-Nei (1993) distance. DNASP 4.10 (Rozas et al., 2003) was used to estimate the nearest-neighbor statistic, S_{nn} (Hudson, 2000) for the mtDNA control region sequences using 10,000 permutations with gaps excluded. The S_{nn} statistic measures how often the nearest neighbors in sequence space are from the same locality in geographical space and is particularly appropriate when haplotype diversity is large and sample sizes are small (Hudson, 2000).

MICRO-CHECKER 2.2.3 (Van Oosterhout et al., 2004) was used to check for the presence of null alleles and evidence of scoring errors due to stuttering and large allele drop-out in the microsatellite data. GENEPOP 3.1b (Raymond and Rousset, 1995) was used to perform exact tests (10,000 iterations; Guo and Thompson, 1992) for deviations of genotypic distributions from the expectations of Hardy-Weinberg equilibrium, to calculate observed (H_o) and expected (H_e) heterozygosities, and to test for heterozygote excess and deficiencies. To control for Type I error due to replicated testing, significance of single locus Hardy-Weinberg values was corrected using the sequential Bonferroni method (Rice, 1989). The program FSTAT 2.9.3 (Goudet, 1995) was used to estimate allelic richness per locus and sample (R_s) to allow comparison of the number of alleles independent of sample size for microsatellite data. A principal component analysis (PCA) was performed on the multilocus microsatellite data using the program PCAGEN 1.2 (J. Goudet, unpubl., <http://unil.ch/izea/software/pcagen.html>). Populations were ordinated according to the first and second axes. The percent inertia of each PCA axis and its respective P value were assessed by performing 10,000 randomizations of genotypes.

The Arlequin 3.0 software package (Excoffier et al., 2005) was used to estimate Weir and Cockerham's (1984) unbiased estimator of Wright's F-statistics (F_{ST}) and Φ_{ST} (an mtDNA analogue for F_{ST} ; Excoffier et al., 1992) and for hierarchical F_{ST} and Φ_{ST} analyses (AMOVA, 10,100 permutations) on both microsatellite and mtDNA sequence data. The Φ_{ST} analyses were performed using a matrix of Tamura and Nei (1993) distances. Variance was partitioned to individuals within temporal samples, temporal samples within geographical areas, and among geographical areas. Arlequin was also used to determine whether increased pairwise genetic differentiation between samples was correlated with geographic distance using Mantel tests. Spatial analysis of molecular variance (SAMOVA; Doupanloup et al., 2002) was used to verify that we had identified the grouping that maximized F_{CT} , setting the number of groups of populations to identify (k) = 2 and 3 for the microsatellite data.

RESULTS

MICROSATELLITE LOCI.— All five microsatellite loci revealed considerable variation. For the combined data set of 214 individuals the number of alleles ranged from 14 (*Mn01*) to 31 (*Mn60*), and mean collection heterozygosities ranged from 0.838 (EA) to 0.892 (WSA) (Table 2). The distribution of genotypes was consistent with the expectations of Hardy-Weinberg equilibrium for each of the five loci in all nine collections (data not shown) and there was no evidence for null alleles or scoring errors at any locus. When temporal collections within the four regions were combined, 19

Table 2. Summary statistics for five microsatellite loci among white marlin collections from western north Atlantic (WNA), Caribbean (CAR), Western South Atlantic (WSA), and Eastern Atlantic (EA). N is the number of individuals; A is the number of alleles; Rs is allelic richness per locus and sample; RPT range is size range in number of repeats; H_E is expected heterozygosity; H_O is observed heterozygosity; HW is probability of concordance with Hardy-Weinberg expectations. Values in bold are significant after correction for multiple tests (initial $\alpha = 0.05 / 5 = 0.01$).

Sample	Locus				
	MN01	MN08	MN10	MN60	MN90
WNA					
N	74	74	74	72	71
A	11	22	13	19	18
Rs	9.51	20.02	10.13	15.47	14.3
RPT range	12–26	10–34	13–35	11–62	47–90
H_E	0.805	0.944	0.836	0.897	0.901
H_O	0.783	0.971	0.855	0.797	0.927
HW	0.239	0.418	0.803	0.035	0.526
CAR					
N	41	41	41	41	39
A	10	20	12	11	13
Rs	9.75	19.59	11.59	10.86	12.84
RPT range	12–23	10–31	13–25	15–25	48–76
H_E	0.840	0.948	0.820	0.890	0.902
H_O	0.875	0.900	0.825	0.900	0.923
HW	0.899	0.199	0.896	0.109	0.353
WSA					
N	63	63	63	63	62
A	12	26	12	25	19
Rs	11.13	23.34	10.51	19.93	15.85
RPT range	12–27	4–32	13–38	13–74	18–98
H_E	0.844	0.948	0.813	0.902	0.910
H_O	0.758	0.968	0.871	0.910	0.952
HW	0.006	0.577	0.791	0.273	0.898
EA					
N	36	36	36	36	36
A	8	18	11	16	14
Rs	8.00	18.00	11.00	16.00	14.00
RPT range	15–22	10–43	10–21	12–30	48–90
H_E	0.412	0.468	0.421	0.450	0.442
H_O	0.361	0.430	0.389	0.472	0.444
HW	0.107	0.101	0.326	0.700	0.133

of 20 locus/collection combinations had genotypic distributions consistent with the expectations of Hardy-Weinberg equilibrium. A significant departure (heterozygote deficiency) was noted for *Mn01* in the WSA collection. Genotypic data for all loci were included in subsequent analyses.

Replicate collections of white marlin were obtained from three regions: WNA, CAR, and WSA. None of the pairwise F_{ST} values between the four temporal collections from the WNA, two temporal samples from the CAR, or two temporal samples

Table 3. Genetic relationships of white marlin collections. The lower matrix consists of pairwise F_{ST} values based on microsatellite data and the upper matrix of pairwise Φ_{ST} values for mtDNA sequences based on the Tamura-Nei (1993) distances. All temporal samples combined. Significance values (in parentheses) are based on 10,000 permutations.

	WNA	CAR	WSA	EA
WNA	-----	0.040 (0.045)	0.032 (0.081)	-0.012 (0.802)
CAR	0.0023 (0.097)	-----	0.011 (0.225)	0.005 (0.243)
WSA	0.0041 (0.017)	0.0018 (0.179)	-----	0.02 (0.081)
EA	0.0043 (0.059)	0.0023 (0.228)	(0.0034) (0.059)	-----

from the WSA was significantly different from 0 ($P = 0.55$, $P = 0.75$, and $P = 0.11$, respectively). Based on the observed homogeneity among annual collections within a region, temporal samples were combined to increase the power of subsequent spatial analyses.

Variation across the four regional collections was consistently high and there was a trend for slightly higher variation in the WSA than the other three geographic samples. For example, the average allelic richness in the WSA collection was 16.15, compared to 13.89 in the WNA, 12.93 in the CAR, and 13.40 in the EA. This trend was also evident for each locus individually (Table 2).

The mean number of pairwise repeat differences between randomly selected alleles in each collection ranged from 4.35 (CAR) to 4.41 (WSA). These values were about the same magnitude as the mean number of pairwise repeat differences between alleles randomly selected from different collections. As a result, the net (corrected) pairwise differences between collections were negligible, ranging from 0 (WNA/CAR) to 0.019 (WNA/EA). F_{ST} values for the combined five microsatellite loci between collections ranged from 0 (WNA/CAR) to 0.004 (WNA/WSA and WNA/EA). Of the six comparisons, only the value between the WNA and WSA was significant ($P = 0.017$; Table 3). Global F_{ST} values for individual microsatellite loci were all nonsignificant while the multilocus global F_{ST} (all loci over all collections) of 0.0022 approached significance ($P = 0.057$).

Hierarchical analysis of molecular variance was used in an exploratory manner to evaluate population structuring among the four collection locations for the combined microsatellite loci. These included comparisons of North Atlantic (WNA, CAR, and EA) vs South Atlantic (WSA), and western Atlantic (WNA, CAR, and WSA) vs eastern Atlantic (EA). In both cases, the component of variance attributable to between regional groups was minimal ($\leq 0.2227\%$; Table 4). Similarly, the F_{ST} value between groups was extremely small (< 0.003), although it was significantly different from 0 ($P < 0.001$) for the North Atlantic vs South Atlantic comparison. AMOVAs involving three regional groupings (WNA/CAR vs WSA vs EA; WNA vs CAR/WSA vs EA; and WNA vs CAR vs WSA/EA) also revealed low levels of between-region variance although the value for the WNA/CAR vs WSA vs EA was significantly different from 0. Inspection

of AMOVAs run for each microsatellite locus individually (data not shown) produced qualitatively similar results.

The SAMOVA analysis indicated that the North Atlantic vs South Atlantic comparison maximized microsatellite allele genetic divergence between groups, as noted in the AMOVA analysis described above. When the a priori number of groups was set to three in the SAMOVA analysis F_{CT} was maximized at 0.015 ($P = 0.078$) for the WNA/CAR vs WSA vs EA comparison. This value was lower than that calculated for the North Atlantic vs South Atlantic comparison, suggesting that a two-group scenario is more appropriate.

We used a Mantel test to evaluate the correlation of pairwise genetic differences between the four geographic samples with geographic distance. The correlation approached significance ($P = 0.085$), a result that would be consistent with an isolation-by-distance model. PCA analysis of the microsatellite data indicated a lack of genetic structuring among the four regional collections. The first principal component explained 22.77% of the variance among the collections and was not significant ($P = 0.49$).

MTDNA CONTROL REGION.—The mtDNA control region was sequenced from 99 individuals chosen arbitrarily to include representatives from temporal replicates within a collection location (Table 1). The 834 bp gene region had 758 sites excluding gaps, and 234 variable sites. A total of 90 haplotypes (Genbank accession numbers DQ835191–DQ835281) was represented by the 99 individuals (haplotypic diversity = 0.9979, nucleotide diversity = 0.0320).

Diversity was high in all collections and the mean number of pairwise differences between individuals randomly drawn from a collection ranged from 21.9 (WNA) to 27.4 (WSA). These values were similar to those between randomly selected individuals from different collections. Consequently, the corrected mean pairwise differences between collection values were relatively small, ranging from 0 (WNA/EA) to 1.07 (WNA/CAR). The global Φ_{ST} based on Tamura-Nei (1993) distances was 0.0163, a value that approached significance ($P = 0.069$). Pairwise Φ_{ST} values ranged from 0 (WNA/EA) to 0.039 (WNA/CAR), and only the WNA/CAR was significantly different from 0 (Table 3). Visual inspection of a UPGMA clustering of the 90 different mtDNA control region sequences (Fig. 1) did not indicate an association between similar haplotypes and collection location, nor did the nearest-neighbor statistic (S_{nn}) reveal a significant non-random association between mtDNA sequence similarity and geographic location; $S_{nn} = 0.314$, $P = 0.149$.

As with the microsatellite data, exploratory analyses of molecular variance were performed to gain insight into population structuring. Comparison of North Atlantic vs South Atlantic collections and western Atlantic vs eastern Atlantic collections did not reveal a significant component of variance attributable to between group differences. Similarly, none of comparisons involving three groups (WNA/CAR vs WSA vs EA; WNA vs CAR/WSA vs EA; and WNA vs CAR vs WSA/EA) yielded a significant between group component of variance (Table 4).

The SAMOVA analysis indicated that a three group comparison maximized genetic divergence. The highest F_{CT} (0.013, $P = 0.007$) was observed in the comparison of WNA vs EA/CAR vs WSA.

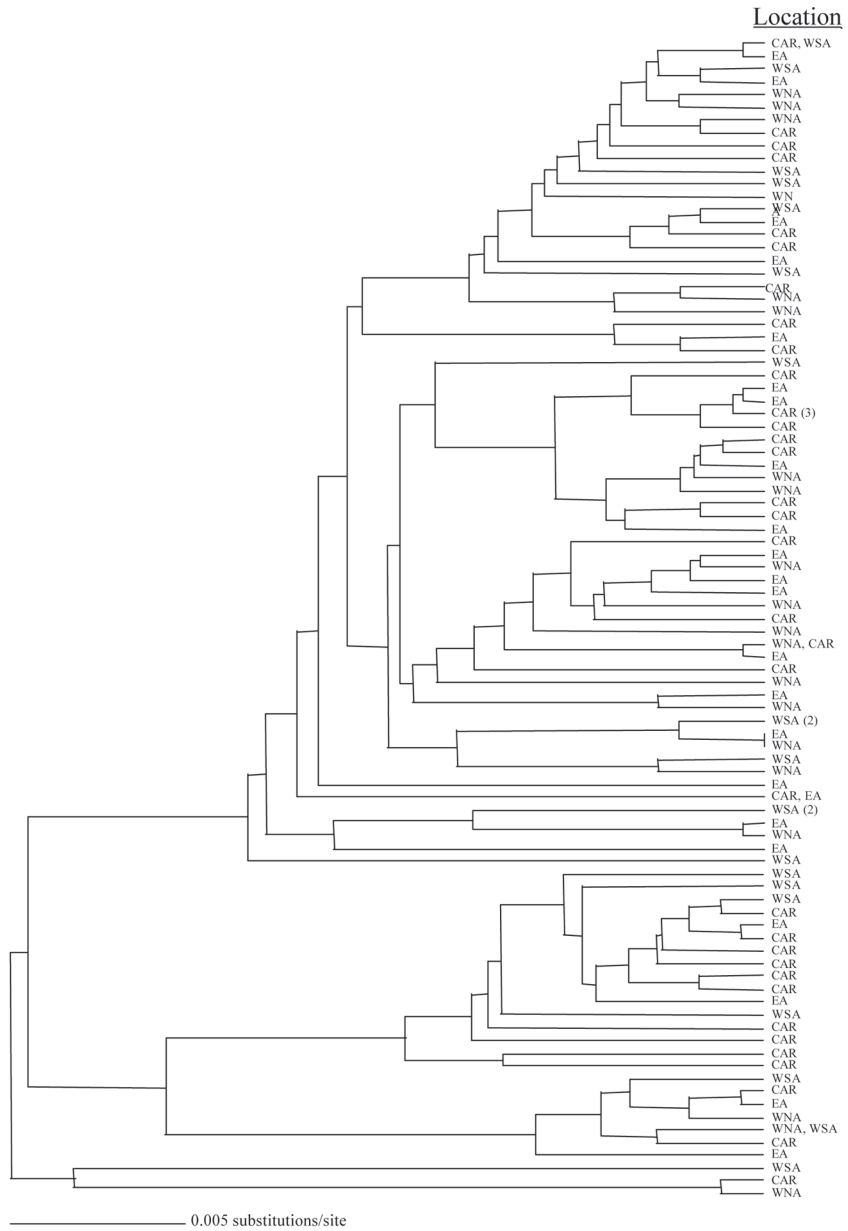


Figure 1. UPGMA clustering of white marlin mtDNA control region sequences based on Tamura-Nei (1993) distances.

DISCUSSION

To effectively test the null hypothesis that white marlin comprise a single, homogeneous gene pool within the Atlantic Ocean, the molecular characters surveyed should be sufficiently variable to allow a rigorous analysis of the spatial and temporal

Table 4. Analysis of molecular variance (AMOVA) among regions and collections within regions (temporal samples combined) for white marlin based on the distribution of alleles (F_{ST}) for microsatellite data and Tamura-Nei (1993) distances for DNA sequence data. Significance was assessed using 10,100 permutations. WNA = western North Atlantic, CAR = Caribbean, WSA = western South Atlantic, EA = eastern Atlantic, N = North, and S = South.

Microsatellites	Source of variation (F_{ST})	% Variation	Fixation index	Significance (P)
N vs S	Among regions	0.27	0.00274	< 0.0001
	Among colls/regions	0.06	0.0006	0.0035
E vs W	Among regions	0.22	0.0022	0.2536
	Among colls/regions	0.13	0.00131	0.0288
WNA/CAR	Among regions	0.7	0.00698	< 0.0001
vs WSA vs EA	Among colls/regions	-0.36	-0.00361	0.0155
WNA vs EA	Among regions	0.04	0.00043	0.1659
vs CAR/WSA	Among colls/regions	0.18	0.00181	0.2476
WNA vs CAR	Among regions	-0.15	-0.00149	0.4956
vs WSA/EA	Among colls/regions	0.34	0.00344	0.2502
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mtDNA	Source of variation (F_{ST})	% Variation	Fixation index	Significance (P)
N vs S	Among regions	0.69	0.00691	0.7511
	Among colls/regions	1.32	0.01333	0.0691
E vs W	Among regions	-2.44	-0.02437	0.75396
	Among colls/regions	2.87	0.02803	0.02495
WNA/CAR	Among regions	-2.83	0.02826	0.83584
vs WSA vs EA	Among colls/regions	3.93	0.03824	0.08931
WNA vs EA	Among regions	0.14	0.00136	0.33515
vs CAR/WSA	Among colls/regions	1.55	0.01556	0.03752
WNA vs CAR	Among regions	-0.51	-0.0051	0.50376
vs WSA/EA	Among colls/regions	2.11	0.02099	0.11267

partitioning of genetic variation. In the present study, the five nuclear microsatellite loci and the mtDNA control gene region exhibited high levels of variation. In fact, using these molecular markers, no two fish shared the same genotypes at all six loci. This level of variation is considerably greater than that reported in previous genetic analyses of white marlin stock structure, which surveyed proteins (Edmunds, 1972) and mtDNA restriction fragments (Graves and McDowell, 2001, 2003). However, with increased levels of variation, larger sample sizes are required to determine allele frequencies with the same degree of precision. Thus the power of some analyses in the present study, especially those involving the smaller temporal samples, is constrained by limited sample sizes.

The temporal stability of genetic variation was evaluated in three of the four sampling regions, including four collections from the WNA, two from the CAR, and three from the WSA. Significant heterogeneity was not observed in the distribution of microsatellite allele frequencies among the temporal collections at any of the three locations; however, as noted above, the small sample sizes of most temporal replicates limited the power of the analyses to reveal differences among annual collections. A previous analysis of white marlin stock structure using a less variable molecular marker, found no significant heterogeneity of mtDNA/RFLP haplotypes between fairly robust annual collections ($n = 28$ and 35) from southern Brazil (Graves and McDowell, 2001). As significant differences were not found among temporal replicates within a region in the present study, the annual collections were combined

to increase the statistical power of subsequent analyses of the spatial distribution of genetic variation.

Together, the various analyses of the microsatellite and mtDNA control region data reveal some significant spatial heterogeneity among geographically distant collections of white marlin, but there is a lack of concordance among the two classes of molecular markers and different types of genetic analyses. One of the six pairwise comparisons of the combined five microsatellite loci resulted in a significant F_{ST} value (WNA vs WSA) and one of the six mtDNA control region sequences comparisons resulted in a significant Φ_{ST} value (WNA vs CAR). Neither the global microsatellite F_{ST} nor global mtDNA control region Φ_{ST} values were significantly different from 0, although both values approached significance. Exploratory clustering of regional collections resulted in two significant AMOVAs for the microsatellite data, the North Atlantic collections (WNA, CAR, and EA) vs the South Atlantic (WSA) and WNA/CAR vs WSA vs EA. In contrast, the same groupings for the mtDNA control region data resulted in non-significant AMOVAs ($P = 0.75$, $P = 0.84$, respectively). The SAMOVA analysis indicated that of the possible groupings, microsatellite allele genetic divergence was maximized in the North Atlantic vs South Atlantic comparison. In contrast, mtDNA control region genetic divergence was maximized in a three group comparison involving the WNA vs CAR/EA vs WSA. Together, these results suggest that if population structuring exists, it is relatively weak and difficult to discern with the current sample sizes. O'Reilly et al. (2004) noted a negative correlation between microsatellite allele variation and F_{ST} values. Thus the high levels of microsatellite polymorphism in this study coupled with relatively small sample sizes may have hindered detection of significant differences. Similarly, the power of the SAMOVA analysis to retrieve the appropriate number of groups is decreased when the differences between groups are weak (Douponloup et al., 2002). Whether the levels of heterogeneity observed in this study are the result of sampling error, isolation by distance, or discrete stocks that were mixed at the time of sampling, cannot be determined at this time. Analysis of larger sample sizes is clearly warranted.

The observed level of genetically based stock structure within the white marlin contrasts with the high levels of heterogeneity observed among collections of striped marlin in the Pacific Ocean. RFLP analysis of mtDNA demonstrated significant spatial partitioning of genetic variation among geographically distant collections of striped marlin (Graves and McDowell, 1994), but not in white marlin (Graves and McDowell, 2001, 2003). More recently, analysis of striped marlin microsatellite loci and mtDNA control region sequences demonstrated significant heterogeneity between collections from throughout the Pacific Ocean for both classes of molecular markers (McDowell and Graves, in press). This included highly significant global F_{ST} and Φ_{ST} values, and consistent differences for both classes of molecular markers in many of the pairwise comparisons. While principal components analysis of microsatellite data did not indicate any discernable population structure in white marlin, a similar analysis revealed considerable structuring in striped marlin. In white marlin, the first principal component explained 22.77% of the genetic diversity and was not significant. Whereas in striped marlin, the first and second principal components were significant and explained 29.81% and 20.86% of the variance, respectively. White marlin and striped marlin are very closely related genetically (Finnerty and Block, 1995; Graves and McDowell, 1995) and a large difference in levels of population structuring within each species was not expected. Whether the difference in

levels of population structuring results from a much greater range for striped marlin in the Pacific Ocean relative to the white marlin in the Atlantic Ocean, or behavioral differences between the two species, cannot be determined at this time. Additional information on the movements and spawning site fidelity of these two species is needed to understand the reasons for differences in stock structure.

The results of this study and previous studies of white marlin stock structure (Edmunds, 1972; Graves and McDowell 2001, 2003) are not inconsistent with the null hypothesis that white marlin exhibit sufficient gene flow throughout their range to prevent the accumulation of significant genetic divergence. However, as one cannot prove the null hypothesis, it is possible that genetically based stock structure exists and as a result of sampling error, uninformed sample design, or a lack of statistical power due to limited sample sizes, we were unable to detect those differences. The occurrence of significant heterogeneity in some of these present analyses, and the near significance of some others, indicates that further investigation is warranted.

White marlin are not abundant in most areas of the Atlantic, and obtaining even modest sample sizes requires considerable effort. However, as noted above, larger sample sizes are required to increase the power of genetic analyses, and this is especially important for studies using hypervariable molecular markers. Increased numbers of microsatellite loci will also enhance the power of future analyses. In addition, a greater consideration should be given to sample design. If discrete stocks of white marlin exist, it is likely that they are separated (either spatially or temporally) at the time of spawning. Therefore, to have the greatest chance of elucidating stock structure, if it exists, it would be prudent to collect animals in spawning condition or larvae from known spawning areas (Graves et al., 1996; Carlsson et al., 2004, Bowen et al., 2005).

The results of the present study of white marlin stock structure, as well as previous aforementioned studies, are consistent with the hypothesis that there is sufficient gene flow throughout the Atlantic to prevent the accumulation of significant genetic differences. White marlin are highly migratory animals, and as such, are capable of interacting with conspecifics over a large geographic range, a behavior that would promote gene flow. Conventional tagging of white marlin, conducted primarily in the western North Atlantic, has demonstrated trans-Atlantic movements of some individuals, but also a strong pattern of seasonal site fidelity for others. In addition, considerable connectivity has been shown between white marlin in the Gulf of Mexico, the U.S. mid-Atlantic and Venezuela, as well as between Venezuela and Brazil (Scott et al., 1990; Jaen and Jaen, 1994; Ortiz et al., 2003). Tagging of white marlin captured outside of the western North Atlantic will be necessary to elucidate movements among other geographic locations within the Atlantic, but the vagility of this species provides a means to facilitate genetic connectivity throughout the Atlantic Ocean.

Understanding the stock structure of white marlin is necessary for proper assessment and management of the species. In previous assessments, ICCAT has considered both two stock and single stock models for white marlin. The results from highly variable microsatellite loci and mtDNA control region sequences surveyed in the present study, as well as proteins (Edmunds, 1972) and mtDNA restriction fragments (Graves and McDowell 2001, 2003) surveyed in previous studies, are most consistent with a single, Atlantic-wide stock of white marlin. The genetic inference is supported by the geographical distribution of the species over time (Uozumi and Na-

kano, 1994), its high vagility (Ortiz et al., 2003), and the fact that spawning is broadly distributed in both space and time (Nakamura, 1985). Thus, at the present time, the biological and genetic data suggest that a single, Atlantic-wide stock of white marlin is most appropriate for the purposes of management. This is contribution number 2782 of the Virginia Institute of Marine Science.

LITERATURE CITED

- Alvarado Bremer, J. R., I. Naseri, and B. Ely. 1999. A provisional study of northern bluefin tuna populations. Coll. Vol. Sci. Papers ICCAT 49: 127–129.
- _____, J. R. Mejuto, T. W. Grieg, and B. Ely. 1996. Global population structure of the swordfish (*Xiphias gladius* L.) as revealed by analysis of the mitochondrial DNA control region. J. Exp. Mar. Biol. Ecol. 197: 295–310.
- _____, B. Stequert, W. Robertson, and B. Ely. 1998. Genetic evidence for inter-oceanic subdivision of bigeye tuna (*Thunnus obesus*) populations. Mar. Biol. 132: 547–557.
- Appleyard, S. A., P. M. Grewe, B. H. Innes, and R. D. Ward. 2001. Population structure of yellowfin tuna (*Thunnus albacares*) in the western Pacific Ocean inferred from microsatellite loci. Mar. Biol. 139: 383–393.
- Bowen, B. W., A. L. Bass, L. Soares, and R. J. Toonen. 2005. Conservation implications of complex population structure: lessons from the loggerhead turtle (*Caretta caretta*). Mol. Ecol. 14: 2389–2402.
- Broughton, R. E. and J. R. Gold. 1997. Microsatellite development and survey of variation in northern bluefin tuna (*Thunnus thynnus*). Mol. Mar. Biol. Biotech. 6: 308–314.
- Buonaccorsi, V. P. and J. E. Graves. 2000. Isolation and characterization of novel polymorphic tetra-nucleotide microsatellite markers from the blue marlin, *Makaira nigricans*. Mol. Ecol. 9: 820–821.
- _____, J. R. McDowell, and J. E. Graves. 2001. Reconciling patterns of inter-oceanic molecular variance from four classes of molecular markers in blue marlin (*Makaira nigricans*). Mol. Ecol. 10: 1179–1196.
- Carlsson, J., J. R. McDowell, P. Diaz-Jaimes, J. E. L. Carlsson, S. B. Boles, J. R. Gold, and J. E. Graves. 2004. Microsatellite and mitochondrial DNA analyses of Atlantic bluefin tuna (*Thunnus thynnus thynnus*) population structure in the Mediterranean Sea. Mol. Ecol. 13: 3345–3356.
- Doupanloup, I., S. Schneider, and L. Excoffier. 2002. A simulated annealing approach to define the genetic structure of populations. Mol. Ecol. 11: 2571–2581.
- Ely, B., D. S. Stoner, J. R. Alvarado Bremer, J. M. Dean, P. Addis, A. Cau, E. J. Thelen, W. J. Jones, D. E. Black, L. Smith, K. Scott, I. Naseri, and J. M. Quattro. 2002. Analyses of nuclear IdhA gene and mtDNA control region sequences of Atlantic Northern bluefin tuna populations. Mar. Biotech. 4: 583–588.
- Edmunds, P. H. 1972. Genic polymorphism of blood proteins from white marlin. NMFS, NOAA Res. Rep. 77, 15 p.
- Estoup, A., C. R. Largiader, E. Perrot, and D. Chourrout. 1996. Rapid one-tube DNA extraction for reliable PCR detection of fish polymorphic markers and transgenes. Mol. Mar. Biol. Biotech. 5: 295–298.
- Excoffier, L., P. E. Smouse, and J. M. Quattro. 1992. Analysis of molecular variance inferred from metric distances among DNA haplotypes: application of human mitochondrial DNA restriction data. Genetics 147: 915–925.
- _____, G. Laval, and S. Schneider. 2005. Arlequin ver. 3.0: An integrated software package for population genetics data analysis. Evol. Bioinformatics Online 1: 47–50.
- Finnerty, J. R. and B. A. Block. 1995. Evolution of cytochrome b in the Scombroidei (Teleostei): molecular insights into billfish (Istiophoridae and Xiphiidae) relationships. Fish. Bull. 93: 78–96.

- Goudet, J. 1995. FSTAT (vers. 1.2): a computer program to calculate F-statistics. *J. Hered.* 86: 485–486.
- Graves, J. E. and J. R. McDowell. 1994. Genetic analysis of striped marlin *Tetrapturus audax* population structure in the Pacific Ocean. *Can. J. Fish. Aquat. Sci.* 51: 1762–1768.
- _____ and _____. 1995. Inter-ocean genetic divergence of istiophorid billfishes. *Mar. Biol.* 122: 193–203.
- _____ and _____. 2001. A genetic perspective on the stock structures of blue marlin and white marlin in the Atlantic Ocean. *Coll. Vol. Sci. Papers ICCAT* 53: 180–187.
- _____ and _____. 2003. Stock structure of the world's istiophorid billfishes: a genetic perspective. *Mar. Freshw. Res.* 54: 1–11.
- _____, J. R. Gold, B. Ely, J. M. Quattro, C. Woodley, and J. M. Dean. 1996. Population genetic structure of bluefin tuna in the North Atlantic Ocean. I. Identification of variable genetic markers. *International Commission for the Conservation of Atlantic Tunas, Col. Vol. Sci. Papers* 45: 155–157.
- Guo, S. W. and E. A. Thompson. 1992. Performing the exact test for Hardy-Weinberg proportion for multiple alleles. *Biometrics* 48: 361–372.
- Hudson, R. R. 2000. A new statistic of detecting genetic differentiation. *Genetics* 155: 2001–2014.
- ICCAT. 2003. Report of the ICCAT 2002 white marlin stock assessment meeting. *Coll. Vol. Sci. Papers ICCAT* 55: 350–452.
- _____. 2005. Report of the Standing Committee on Research and Statistics, 2004. ICCAT. Madrid. 188 p.
- Jaen, R. C. and M. H. Jaen. 1994. Migraciones de los istioforidos en el Caribe. *Cromotip, Caracas*.
- Martínez, P., E. G. González, R. Castilho, and R. Zardoya. 2006. Genetic diversity and historical demography of Atlantic bigeye tuna (*Thunnus obesus*). *Mol. Phylogenetics Evol.* 39: 404–416.
- McDowell, J. R. 2002. Genetic stock structure of the sailfish, *Istiophorus platypterus*, based on nuclear and mitochondrial. Ph.D. Diss. College of William and Mary: Virginia Institute of Marine Science. Gloucester Point. 231 p.
- _____ and J. E. Graves. Microsatellite and mitochondrial DNA analyses of striped marlin (*Tetrapturus audax*) population structure in the Pacific Ocean. *Can. J. Fish. Sci.* In press.
- Nakamura, I. 1985. FAO species catalogue. Vol. 5: Billfishes of the world: an annotated and illustrated catalogue of marlins, sailfishes, spearfishes, and swordfishes known to date. FAO Fish. Synop. 125, 65 p.
- O'Reilly, P. T., M. F. Canino, K. M. Bailey, and P. Bentzen. 2004. Inverse relationship between F_{ST} and microsatellite polymorphism in the marine fish, walleye Pollock (*Theragra chalcogramma*): implications for resolving weak population structure. *Mol. Ecol.* 13: 1799–1814.
- Ortiz, M., E. D. Prince, J. E. Serafy, D. B. Holts, K. B. Davy, J. G. Pepperell, M. B. Lowry, and J. C. Holdsworth. 2003. Global overview of the major constituent-based billfish tagging programs and their results since 1954. *Mar. Freshw. Res.* 54: 489–508.
- Palumbi, S. R. 1996. Nucleic acids II. the polymerase chain reaction. *Molecular systematics*. 2nd ed. Pages 205–247 in D. M. Hillis, C. Moritz, and B. Mable, eds. Sinauer Associates, Sunderland.
- Raymond, M. and F. Rousset. 1995. GENEPOP (version 1.2) population genetics software for exact test and ecumenicism. *J. Hered.* 86: 248–249.
- Reeb, C. A., L. Arcangeli, and B. A. Block. 2000. Structure and migration corridors in Pacific populations of the swordfish *Xiphias gladius*, as inferred through analyses of mitochondrial DNA. *Mar. Biol.* 136: 1123–1131.
- Rice, W. R. 1989. Analyzing tables of statistical tests. *Evolution* 43: 223–225.
- Rosel, P. and B. A. Block. 1996. Mitochondrial control region variability and global population structure in the swordfish, *Xiphias gladius*. *Mar. Biol.* 125: 11–22.

- Rozas, J., J. C. Sánchez-DelBarrio, X. Messeguer, and R. Rozas. 2003. DnaSP, DNA polymorphism analyses by the coalescent and other methods. *Bioinformatics* 19: 2496–2497.
- Sambrook, J. and D. W. Russel. 2001. *Molecular cloning: a laboratory manual*. 3rd ed. Cold Spring Harbor Laboratory Press, Cold Spring Harbor.
- Scott, E. L., E. D. Prince, and C. D. Goodyear. 1990. History of the cooperative game fish tagging program in the Atlantic Ocean, Gulf of Mexico, and Caribbean Sea, 1954–1987. *Amer. Fish. Soc. Symp.* 7: 841–853.
- Seutin, G., B. N. White, and P. T. Boag. 1991. Preservation of avian blood and tissue samples for DNA analysis. *Can. J. Zool.* 69: 82–90.
- Swofford, D. L. 2000. PAUP*. Phylogenetic analysis using parsimony (*and other methods), version 4. Sinauer Associates, Sunderland.
- Takagi, M., T. Okamura, S. Chow, and N. Taniguchi. 1999. PCR primers for microsatellite loci in tuna species of the genus *Thunnus* and its application for population genetic study. *Fish. Sci.* 65: 571–576.
- Tamura, K. and M. Nei. 1993. Estimation of the number of nucleotide substitutions in the control region of mitochondrial DNA in humans and chimpanzees. *Mol. Biol. Evol.* 10: 512–526.
- Thompson, J. D., D. G. Higgins, and T. J. Gibson. 1994. ClustalW: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nuc. Acids Res.* 22: 4673–4680.
- Uozumi, Y. and H. Nakano. 1994. A historical review of Japanese longline fishery and billfish catches in the Atlantic Ocean. *Coll. Vol. Sci. Papers ICCAT* 41: 233–243.
- Van Oosterhout, C., W. F. Hutchinson, D. P. M. Wills, and P. Shipley. 2004. MICRO-CHECKER: software for identifying and correcting genotyping errors in microsatellite data. *Mol. Ecol. Notes* 4: 535–538.
- Viñas, J., J. R. Alvarado Bremer, and C. Pla. 2004. Inter-oceanic genetic differentiation among albacore (*Thunnus alalunga*) populations. *Mar. Biol.* 145: 225–232.
- Weir, B. S. and C. C. Cockerham. 1984. Estimating F-statistics for the analysis of population structure. *Evolution* 38: 1358–1370.

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