POPULATION GENETIC STRUCTURE OF ATLANTIC ISTIOPHORID BILLFISHES

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SUMMARY

Population structure was investigated in the white marlin, blue marlin, and sailfish within the Atlantic Ocean using restriction fragment length polymorphism (RFLP) analysis of mitochondrial DNA (mtDNA). For each species, genetic variation was compared among samples from geographically distant locations, as well as among samples taken from the same location over two or more years. Analysis of 209 white marlin from the United States, Dominican Republic, Brazil, and Morocco revealed considerable genetic variation within each sample, but the distribution of mtDNA haplotypes was similar within and among locations and the null hypothesis that white marlin comprise a single genetic stock could not be rejected. Analysis of 235 blue marlin from United States, Caribbean, and Brazilian waters revealed higher levels of genetic variation than were found in white marlin. Distributions of haplotypes at most locations were temporally stable, but significant genetic heterogeneity was noted among samples taken in Jamaica over five years. No significant heterogeneity was found among samples within the northwest Atlantic Ocean, a result consistent with a single genetic stock within that region. Sailfish exhibited a level of genetic variation comparable to that observed in white marlin. Analysis of 69 specimens from the United States and Brazil demonstrated temporal stability of mtDNA haplotype distributions between samples collected in Brazil during consecutive years, and no significant differences were found in the distribution of haplotypes between samples from Florida and Brazil. Based on these data we could not reject the null hypothesis that sailfish within the western Atlantic Ocean comprise a single genetic stock.

RÉSUMÉ

On a étudié la structure des populations de makaire blanc, de makaire bleu, et de voilier dans l'Océan Atlantique en utilisant l'analyse restrictive du polymorphisme de la longueur fragmentaire (RFLP) de l'ADN mitochondrial (ADNmt). On a comparé pour chaque espèce la variation génétique existant entre des échantillons provenant aussi bien de lieux géographiques différents que d'échantillons ayant été relevés au même endroit au cours d'une période de deux ans ou plus. L'analyse de 209 makaires blancs provenant des Etats-Unis, de la République Dominicaine, du Brésil et du Maroc a révélé une variation génétique considérable à l'intérieur de chaque échantillon, mais la répartition des haplotypes ADNmt à l'intérieur de chaque échantillon et entre échantillons provenant de différents endroits était simillaire, de sorte que l'hypothèse comme quoi le makaire blanc constitue un seul stock génétique ne pouvait pas être écartée. L'analyse de 235 makaires bleus provenant des Etats-Unis, des Caraïbes et du Brésil a révélé des niveaux de variation génétique plus élevés que ceux qui avaient été trouvés pour le makaire blanc. Les répartitions d'haplotypes dans la plupart des endroits étaient temporellement stables, mais une hétérogénéité génétique importante a été relevée parmi les échantillons prélevés depuis 5 ans. On n'a pas trouvé d'hétérogénéité importante entre les échantillons dans l'Océan Atlantique nord-ouest, ce qui est un résultat convergent avec l'idée d'un stock génétique unique dans cette région. Le voilier a montré un niveau de variation génétique comparable à celui observé pour le makaire blanc. L'analyse de 69 specimens en provenance des Etats-Unis et du Brésil a démontré une stabilité temporelle de répartition d'haplotype ADNtm entre les échantillons collectés au Brésil au cours d'années consécutives, et il n'a pas été trouvé de différences significatives en ce qui concerne la répartition des haplotypes entre les échantillons de Floride et ceux du Brésil. Sur la base de ces données nous ne pouvions pas écarter l'hypothèse comme quoi le voilier dans l'Atlantique Ouest constitue un seul stock génétique.

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RESUMEN

Se investigó la estructura de población de la aguja blanca, aguja azull y pez vela en el océano Atlántico utilizando análisis de RFLP (restriction fragment length polymorphism) *de ADN mitocondrial (ADNmt). Se comparó, para cada especie, la variación genética entre muestras de lugares geográficamente distantes, así como entre muestras tomadas del mismo lugar durante dos o más años. El análisis de 209 ejemplares de aguja blanca de Estados Unidos, República Dominicana, Brasil y Marruecos reveló una considerable variación genética en cada muestra, pero la distribución de los haplotipos de ADNmt era similar dentro y entre lugares y no se pudo rechazar la hipótesis nula de que la aguja blanca comprende un stock genético único. El análisis de 235 ejemplares de aguja azul de aguas de Estados Unidos, Caribe y Brasil reveló niveles de variación genética superiores a los que se encuentran en la aguja blanca. Las distribuciones de los haplotipos en la mayor parte de los lugares eran temporalmente estables, pero se observó una importante heterogeneidad genética entre las muestras tomadas en Jamaica durante cinco años. No se encontró una heterogeneidad significativa entre las muestras del océano Atlántico noroeste, resultado que es coherente con un stock de genética única comprendido en esa región. El pez vela mostraba un nivel de variación genética comparable al que se observó en la aguja blanca. El análisis de 69 especímenes de Estados Unidos y Brasil demostró una estabilidad temporal de la distribución de haplotipos de ADNmt entre las muestras recolectadas en Brasil durante años consecutivos, y se no se encontraron diferencias importantes en la distribución de haplotipos entre las muestras de Florida y Brasil. Basándose en estos datos no se pudo rechazar la hipótesis nula de que el pez vela en el océano Atlántico oeste conforma un stock genético único.*

1. INTRODUCTION

Istiophorid billfishes represent an important commercial and recreational fishery resource within the Atlantic Ocean. Despite the economic importance of these animals, surprisingly little is known of the population (stock) structure or basic biology of the various species. Inferences about the population structure of istiophorid billfishes have been gained from analyses of morphological, catch per unit effort, and mark and recapture data. However, while contributing valuable insights into the natural history of these species, each of the above methodologies has limitations in defining genetic stock structure.

Until recently there have been few genetic analyses of the population structure of istiophorid billfishes. Allozyme (protein) electrophoresis, which provided great insight into the population structure of many marine fishes (Allendorf *et al.* 1987), was not extensively applied to istiophorid billfishes. Edmunds (1972) used allozyme analysis to evaluate genetic heterogeneity among white marlin from the east and Gulf coasts of the United States, and the Caribbean, and found no differences in the distribution of alleles at the few polymorphic loci. Shaklee *et al.* (1983) reported that based on the level of variation found within a sample of blue marlin from Kona, Hawaii, that allozyme analysis had the potential to reveal population structure within Pacific blue marlin, if it exists. However, the technique was not applied to blue marlin from other locations.

Over the past five years there has been an increased interest in the population structure of istiophorid billfishes, resulting in several studies employing analyses of DNA. Finnerty and Block (1992) amplified and sequenced the cytochrome *b* gene of the mitochondrial (mt) DNA genome of a total of 14 Atlantic and Pacific blue marlin and reported a striking difference in the frequency of mtDNA haplotypes (alleles) between ocean samples. Graves and McDowell (1994a, 1995), employed restriction fragment length polymorphism (RFLP) analysis of the entire mtDNA genome of approximately 50 individuals each of Atlantic and Pacific blue marlin, sailfish, and white/striped marlin. They reported highly significant differences in the distribution of mtDNA haplotypes between conspecific samples from the two oceans. In each case, several haplotypes were common to individuals from each ocean, but the frequency of the haplotypes were quite different. For both blue marlin and sailfish, a genetically distinct clade of mtDNA haplotypes was present in a large fraction of Atlantic individuals that was not found within the Pacific samples. RFLP analysis of mtDNA was also used by Graves and McDowell (1994b) to reveal significant population structuring among geographically distant populations of striped marlin within the Pacific Ocean.

The selection of an appropriate molecular marker is critical to the success of any analysis of population structure (Avise 1994). Molecular markers that are extremely variable require the analysis of enormous numbers of individuals to adequately sample variation at each location. At the other extreme, one cannot investigate the spatial and temporal partitioning of variation if a molecular marker exhibits little variation. Based on prior studies in our laboratory, we have shown that RFLP analysis of mtDNA reveals moderate levels of genetic variation that allow analyses of population structure. Over the past five years we have accumulated genetic data on more than 500 individuals of Atlantic istiophorid

billfishes. This paper presents a summary of our current knowledge of the population structure of white marlin, blue marlin and sailfish based on these collections, and outlines areas where further information is needed.

2. MATERIALS AND METHODS

Tissue samples were collected from istiophorid billfishes taken in both commercial and recreational fisheries throughout the Atlantic Ocean. The dates and locations of sample collections are presented in Table 1. Hearts were removed from individuals, frozen on location, and transported to the laboratory on dry ice or commercial ice substitutes. For those tissues which arrived in good condition, mtDNA was purified using the cesium chloride density gradient ultracentrifugation protocols of Lansman *et al.* 1981. Following dialysis, mtDNA was analyzed with 9-12 restriction endonucleases, endlabeled with ^{35}S nucleotide triphosphates using the Klenow fragment (Sambrook *et al.* 1989). MtDNA fragments were separated on horizontal agarose gels and visualized by autoradiography as described in Graves and McDowell (1994b). For those samples which were not immediately frozen upon capture, or thawed before arriving at the laboratory, mtDNA-enriched genomic DNA was isolated following the protocols of Chapman and Powers (1984) and digested with 9-12 restriction endonucleases. Following electrophoresis, DNA was transferred to nylon filter by capillary transfer (Sambrook *et al.* 1989). Filters were subsequently hybridized with biotin-labeled probe (cloned fragments of yellowfin tuna mtDNA) as described in Graves and McDowell (1994b). After stringency washes filters were developed using the protocols of the BRL BluGene Non-Radioactive Nucleic Acid Detection Kit.

Composite mtDNA haplotypes, consisting of a letter designation for the fragment pattern produced by each restriction endonuclease, were developed for each individual. Similar letters indicate identical restriction patterns within a species, but not between species. The number of restriction site changes (site gains or losses) between haplotypes was inferred from completely additive changes in fragment patterns. The restriction site approach of Nei and Miller (1990) was used to estimate the nucleotide sequence divergence between composite haplotypes. Haplotype diversity (the probability that different haplotypes will be encountered in replicate draws from a sample) and mean nucleotide sequence diversity (the mean nucleotide sequence divergence between haplotypes encountered in replicate draws from a sample) were determined following the methods of Nei (1987). The above statistics were calculated using the restriction enzyme analysis package REAP (McElroy *et al.* 1991). Chi-square analysis was used to evaluate homogeneity of haplotype frequencies among samples without combining rare genotypes using the Monte Carlo approach of Roff and Bentzen (1989) with 1000 randomizations.

3. RESULTS

3.1. White marlin

Analysis of mtDNA from 209 white marlin with 11 restriction endonucleases resulted in 37 composite haplotypes, 16 of which were represented by a single individual (Table 2). The most common haplotype (#1, Table 2) occurred in approximately one-half (103) of the individuals, and two other haplotypes (#s 2 & 12) occurred at frequencies above 0.1 (21 and 23, respectively).

Collections of white marlin taken during the summer months over a period of four years in New Jersey, USA and over three years in southern Brazil were compared to assess the temporal stability of haplotype distributions at a particular location. Although the New Jersey samples varied in size from 13 to 23 individuals, the three most common haplotypes (#s 1, 2, & 12, Table 3) were present in all four samples. Levels of genetic variation were similar across the New Jersey samples with haplotype diversities ranging from 0.74 to 0.83, and nucleotide sequence diversities varying from 0.13% to 0.19%. Results of a Monte Carlo chi-square analysis of homogeneity of haplotype distributions suggested that there were not significant differences in haplotype distributions over the four years in New Jersey. The probability of exceeding the chi-square value observed among temporal samples by chance alone was $p = 0.565$. The samples were therefore combined for subsequent geographic (spatial) analyses of homogeneity.

Temporal analysis of white marlin haplotype distributions among samples from southern Brazil collected over three years also demonstrated a high degree of genetic homogeneity. Levels of variation were similar among samples, with haplotype diversities varying between 0.58 and 0.76, and nucleotide sequence diversities ranging from 0.07% to 0.16%. Homogeneity chi-square analysis of haplotype distributions among yearly collections was consistent with the hypothesis of a temporally stable gene pool as 822 of the 1000 simulations ($p = 0.822$) resulted in chi-square values higher than the observed. The three samples from Brazil were subsequently combined to increase the power of the spatial analyses of homogeneity.

Differences in the distribution of haplotypes among collections from different geographic locations were evaluated with homogeneity chi-square analysis. No significant differences were found between the small (n = 10) Panama City, FL (Gulf of Mexico) sample of white marlin and the much larger combined New Jersey sample (n = 70) and they were subsequently combined into a single U.S. sample. Haplotype distributions among samples from the United States, Dominican Republic, Brazil and Morocco were surprisingly similar. Haplotype #1 occurred at a frequency of approximately 0.5 in each sample, and even less frequent genotypes also appeared to be homogeneously distributed. For example, haplotype # 27, which was represented in 7 of the 209 individuals, occurred in each of the four geographically distant collections. Chi-square analysis of homogeneity resulted in 530 of the randomized chi-square values exceeding the observed value (p = 0.530), a finding consistent with a single, genetically homogeneous stock of white marlin in the Atlantic Ocean.

Because the power of the homogeneity analysis was limited by the size of the smallest sample (Dominican Republic, n = 18), we decided to address specific alternate hypotheses with greater power by only considering the larger samples. The ICCAT currently assumes two possible stock models for white marlin: a single (total) Atlantic stock, and separate North and South Atlantic stocks. To address the latter hypothesis we compared haplotype distributions of the combined New Jersey samples (n = 70) and the combined Brazilian samples (n = 75). This resulted in 122 of the 1000 randomizations with chi-square values larger than the observed (p = 0.122). Including the relatively large eastern Atlantic (Morocco) sample (n = 36) in a three location analysis of homogeneity, resulted in 80 randomizations exceeding the observed chi-square value (p = 0.080).

3.2. Blue marlin

Analysis of 235 blue marlin with 11 restriction endonucleases resulted in more than 110 mtDNA haplotypes, and haplotype diversity values within samples exceeded 0.95. Levels of variation were so high that if a haplotype occurred in a sample, it was typically present in a single individual. Even for the Jamaican samples (n = 137) collected over five years, an analysis of the last year of sampling resulted in many new haplotypes, indicating that even large samples were not representative of the variation present at that location. As it was impossible to go back in time and increase sample sizes, we decided to reduce overall variation by removing the two most variable enzymes (*Ban* I and *Nci* I) from the analysis. This change resulted in a total of 60 haplotypes, and haplotype diversities approaching 0.90. Mean nucleotide sequence diversities, which were not greatly affected by the reduction of restriction endonucleases, averaged about 0.5%, considerably higher than in the white marlin.

Collections of blue marlin at two locations, New Jersey and Jamaica, were adequate for temporal analyses of homogeneity. The three small (n = 5-20) samples from New Jersey were relatively homogeneous (p = 0.232). In contrast, analysis of the five larger samples from Jamaica (n = 20-49) revealed significant temporal heterogeneity; only 27 of the 1000 randomizations resulted in chi-square values larger than the observed (p = 0.027). This result was somewhat surprising as the collections were made at the same location (Port Antonio) in the same month (October) during each of the five years.

Recognizing that the heterogeneity among the Jamaican samples invalidated pooling, analyses of spatial genetic homogeneity with the combined samples were undertaken simply to observe the magnitude of spatial heterogeneity relative to that observed temporally. Comparison of the three largest samples (Puerto Rico, Jamaica and the United States) resulted in 224 of the chi-square randomizations exceeding the observed value (p = 0.224). Including the small Brazilian sample (n = 9) in the analysis resulted in 100 randomizations exceeding the observed value (p = 0.100).

2.3 Sailfish

Analysis of the mtDNA purified from 69 sailfish with 12 restriction endonucleases revealed 14 composite haplotypes (Table 6). Eight of the 14 haplotypes occurred only once in the pooled sample, while the most common haplotype (#6, Table 6) was found in just more than one-half of all the sailfish.

Replicate samples of sailfish were obtained from Brazil over a two year period. Levels of within-sample variation were similar between the two collections, with haplotype diversities of 0.63 and 0.72 for the 1992 and 1993, respectively, and the nucleotide sequence diversity was 0.24% for each sample. Results of the chi-square analysis of homogeneity between the two Brazilian samples were consistent with a lack of significant temporal variation, as 399 of the 1000 simulations had chi-square values exceeding the observed value (p = 0.399). Consequently, the two Brazilian samples were combined for analyses of geographic population structure.

Comparison of sailfish samples from Florida and southern Brazil revealed similar levels of within-sample variation at both locations with haplotype diversities of 0.74 and 0.69 and nucleotide sequence diversities of 0.20% and 0.24%, respectively. Haplotype frequencies were also similar between the two samples. The most common haplotype occurred at a frequency of approximately 0.5 in each sample, and of the six haplotypes occurring in more than one individual, only

one (#21, Table 6) was restricted to a geographic location. Monte Carlo chi-square analysis of homogeneity resulted in 497 of the 1000 randomizations with higher chi-square values than that observed between the two samples.

3. DISCUSSION

Recent developments in biotechnology have provided a number of molecular markers that reveal considerable intraspecific genetic variation. While increased variation improves the resolution of genetic studies, larger numbers of individuals must be surveyed to rigorously test hypotheses of population structure. In this study, RFLP analysis of mtDNA revealed substantial variation within the white marlin and sailfish (haplotype diversity levels around 0.7), and a very high level of variation within the blue marlin, even with a reduced number of restriction enzymes (haplotype diversities approached 0.9). As a result, large sample sizes were required at each location to adequately assess genetic heterogeneity.

Because billfish catches are typically rare events, it is often difficult to obtain large numbers of individuals from a location over a short period of time. Consequently, most of the samples employed in this study were relatively small (<20 per time/location). To increase sample sizes (and statistical power) for analyses of spatial homogeneity, it was necessary to pool collections taken in different years at the same location. For the moderately variable white marlin and sailfish, several haplotypes were common to samples from the same location, and analyses of homogeneity were not significant. This was not the case for the blue marlin which harbored much higher levels of variation. Although no significant heterogeneity was revealed among small ($n= 5$-20) temporal samples from New Jersey, five large ($n= 20$-49) annual collections of blue marlin taken during the same month of the year from the same location off Jamaica were highly heterogeneous. The heterogeneity observed among the larger samples suggests that lack of heterogeneity observed among the smaller samples may have been an artifact of sample size. With a high haplotype diversity, there is a strong likelihood that most of the haplotypes in smaller samples will be unique, and test of heterogeneity will have little, if any, power to reveal differences in haplotype distributions. This same problem extends to analyses of spatial homogeneity as well.

The distributions of haplotypes among collections of white marlin from throughout the Atlantic Ocean were relatively homogeneous, a finding consistent with the null hypothesis of a single genetic stock. However, as discussed above, analysis of homogeneity with small sample sizes may not provide much information. Rigorous testing of the North Atlantic/South Atlantic hypothesis with temporal collections of 70 or more individuals from New Jersey and southern Brazil revealed no significant heterogeneity ($p = 0.122$). The results were still not significant ($p = 0.080$) when the relatively large sample ($n = 36$) from the eastern Atlantic (Morocco) was included.

One cannot prove the null hypothesis (genetic homogeneity of white marlin within the Atlantic Ocean) in this, or any, population genetic study, and it is possible that increased sample sizes from each location may ultimately lead to the observation of significant heterogeneity among geographically distant collections. However, the results of this study can be used to directly compare levels of population structuring among closely related species analyzed with the same techniques. White marlin demonstrate much lower levels of mtDNA differentiation among geographically distant locations than was found among widespread collections of the striped marlin within the Pacific Ocean (Graves and McDowell 1994b). Striped marlin demonstrated significant differences in the frequencies of several haplotypes among collections of approximately 40 individuals each from Mexico, Ecuador, Hawaii, and Australia, and none of the Monte Carlo randomizations produced chi-square values exceeding the observed value ($p < 0.001$). These data suggest that if there are barriers to gene flow for the white marlin in the Atlantic Ocean, they are not as formidable as those among striped marlin from geographically distant areas within the Pacific Ocean.

Blue marlin exhibited high levels of variation in the Atlantic, and as a result, necessitated very large sample sizes to adequately test hypotheses of population structure. Analyses of spatial homogeneity using small samples revealed no significant heterogeneity, but the significance of the results were limited by the power of the analysis. Sample sizes could not be increased at a location by pooling temporal samples because, as discussed above, the five relatively large annual samples from Jamaica were significantly heterogeneous. Whether the observed heterogeneity represents sampling error or has a biological basis requires investigation, but the result underscores the need for larger sample sizes for both temporal and spatial analyses of homogeneity. We are currently in the process of collecting large numbers of blue marlin from Brazil to effectively test the North Atlantic/South Atlantic stock hypothesis.

Sailfish, like white marlin, harbored substantial genetic variation as revealed by RFLP analysis of mtDNA. Temporal samples from Brazil were relatively homogeneous, and the haplotype distribution of the combined samples from Brazil was not significantly different from Florida, USA sailfish. These results are consistent with the hypothesis of genetic homogeneity of sailfish within the western Atlantic, although larger sample sizes are required to test the hypothesis with adequate power. In addition to collecting samples from the western North Atlantic, we are currently arranging for a large sample of sailfish from Africa to evaluate the east/west Atlantic stock hypothesis.

The levels of intraspecific genetic divergence among Atlantic samples of the three species analyzed in this study were all much smaller than the levels reported between Atlantic and Pacific populations of blue marlin, sailfish and white/striped marlin (Graves and McDowell 1995). This suggests that if within-ocean population structure exists, it is far less than is observed between ocean populations, and is consistent with at least a minimal amount of genetic exchange across the Atlantic within each species .

The potential for within-ocean gene flow of white marlin, blue marlin, and sailfish is also consistent with results from other types of analyses. Tag and recapture studies have demonstrated extensive movements for all three species including trans-oceanic, trans-Atlantic, and trans-equatorial movement for blue marlin; trans-Atlantic movement and movement to within 150 km of the equator for white marlin; and movement greater than 3,200 km for sailfish. (Scott *et al.* 1990, SCRS/96/96). Furthermore, quarterly analyses of Japanese longline catch data for more than thirty years indicate that the distribution of all three species is continuous across the tropical Atlantic Ocean in both space and time (Third ICCAT Billfish Workshop Report, this volume). Together these data are consistent with the null hypothesis of a single Atlantic stock for white marlin, blue marlin, and sailfish.

The results of the genetic analyses presented here are not definitive, and increased sample sizes are especially needed for rigorous testing of blue marlin and sailfish stock hypotheses. Until such sample sizes have been collected, analyzed, and the data applied to evaluate various biologically meaningful stock hypotheses, it would be prudent for ICCAT to take a precautionary approach and consider the effect of alternate stock hypotheses on population assessments of these species.

4. LITERATURE CITED

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Table 1. Billfish collection information

Species	Location	Date	Number
White Marlin	Cape May, NJ USA	1992	16
		1993	18
		1994	23
		1995	13
	Panama City, FL USA	1993	10
	Cabeza del Toro, Dominican Republic	1991	18
	Santos/Vitoria, Brazil	1993	28
		1994	35
		1995	12
	Casablanca, Morocco	1995	36
Blue Marlin	Cape May, NJ USA	1992	20
		1993	6
		1994	5
	Panama City, FL USA	1993	6
	San Juan, Puerto Rico	1991/2	31
	Port Antonio, Jamaica	1991	31
		1992	49
		1993	18
		1994	40
		1995	20
	Santos/Vitoria, Brazil	1992	9
Sailfish	Islamorada, FL USA	1991	23
	Santos, Brazil	1991	13
	Rio de Janeiro, Brazil	1992	33

Table 2. White marlin composite mtDNA haplotypes. Letters represent digestion with the following restriction enzymes (in order): *Apa* I, *Ava* I, *Ava* II, *Ban* I, *Bcl* I, *Bgl* I, *Bst*E II, *Dra* I, *Hae* III, *Hind* III, and *Nci* I.

No.	Haplotype	United States	Dominican Republic	Brazil	Morocco	Total
1	AABAAAAAABA	32	11	40	20	103
2	AABAAACAABA	12	0	8	1	21
3	AABCAAAAABA	1	0	2	0	3
4	AABAAACAANA	0	0	1	0	1
5	AABAAAAAABE	0	0	1	0	1
6	AAEAAAAAABA	0	0	1	0	1
7	AABCAAAAANA	0	0	1	0	1
8	AAFAAAAAABA	0	0	1	0	1
9	AABAAAAAABF	0	0	1	1	2
10	AABAAAAAAJA	1	0	1	0	2
11	AABAAABAAMA	3	1	2	1	7
12	AABAAABAABA	15	3	4	1	23
13	AABABAAAABA	2	1	3	1	7
14	CABAAABAABA	0	0	1	0	1
15	AACAAAAAABA	0	0	2	0	2
16	AABAADAAABA	0	0	1	0	1
17	AABACAAAABA	0	0	1	0	1
18	AABAAAAAAOA	0	0	1	0	1
19	AABACABAABA	1	0	1	0	2
20	AABAAAADAQA	0	0	1	0	1
21	AABAAAACABA	1	0	0	0	1
22	AABAAACAALA	1	0	0	0	1
23	AABAAAAAANA	2	0	0	0	2
24	AABAAACAAKA	1	0	0	0	1
25	AAEAAAAAAJA	1	0	0	0	1
26	AADAAACAABA	1	0	0	0	1
27	DABAAAAAABA	2	0	0	0	2
28	AABAAABAABD	1	0	0	1	2
29	AABAAAAABBA	1	2	1	1	5
30	FABAAAAAABA	0	0	0	1	1
31	AAHAAAAAABA	1	0	0	1	2
32	AABADAAAABA	0	0	0	1	1
33	EABAAABAABA	1	0	0	2	3
34	AABAAADAABA	0	0	0	1	1
35	AAGAAAAAABA	0	0	0	1	1
36	AABABADAABA	0	0	0	1	1
37	AABAAEAAABA	0	0	0	1	1
	Total	**80**	**18**	**75**	**36**	**209**
Haplotype Diversity		0.81	0.61	0.75	0.70	0.77
Nucleotide Sequence Diversity		0.17%	0.10%	0.14%	0.15%	0.17%

Table 3. Distribution of white marlin composite mtDNA haplotypes among collections at the same geographic locations during different years. Haplotype numbers correspond to composite haplotypes listed in Table 2 (NJ = New Jersey, USA; BR = southern Brazil; HD = haplotype diversity; NSD = nucleotide sequence diversity).

Haplotype	NJ92	NJ93	NJ94	NJ95	NJTotal	BR92	BR93	BR95	BRTotal
1	7	6	9	5	27	15	17	8	40
2	2	5	3	1	11	4	3	1	8
3	0	0	1	0	1	2	0	0	2
4	0	0	0	0	0	1	0	0	1
5	0	0	0	0	0	1	0	0	1
6	0	0	0	0	0	1	0	0	1
7	0	0	0	0	0	1	0	0	1
8	0	0	0	0	0	1	0	0	1
9	0	0	0	0	0	0	1	0	1
10	0	1	0	0	1	0	1	0	1
11	1	2	0	0	3	0	2	0	2
12	2	2	3	5	12	0	3	1	4
13	1	0	0	0	1	0	2	1	3
14	0	0	0	0	0	0	1	0	1
15	0	0	0	0	0	1	1	0	2
16	0	0	0	0	0	0	1	0	1
17	0	0	0	0	0	0	1	0	1
18	0	0	0	0	0	0	1	0	1
19	0	0	1	0	1	0	1	0	1
20	0	0	0	0	0	0	0	1	1
21	1	0	0	0	1	0	0	0	0
22	1	0	0	0	1	0	0	0	0
23	1	1	0	0	2	0	0	0	0
24	0	1	0	0	1	0	0	0	0
25	0	0	1	0	1	0	0	0	0
26	0	0	1	0	1	0	0	0	0
27	0	0	2	0	2	0	0	0	0
28	0	0	1	0	1	0	0	0	0
29	0	0	1	0	1	1	0	0	1
31	0	0	0	1	1	0	0	0	0
33	0	0	0	1	1	0	0	0	0
Total	**16**	**18**	**23**	**13**	**70**	**28**	**35**	**12**	**75**
HD	0.81	0.82	0.83	0.74	0.81	0.70	0.76	0.58	0.70
NSD	0.17%	0.18%	0.19%	0.13%	0.17%	0.13%	0.16%	0.07%	0.14%

Table 4. Blue marlinc omposite mtDNA haplotypes. Letters represent digestion with the followingr estrictionenzymes (inorder): *Ava* I, *Ava* II, *Bc* II, *Bst*E II, *Hae* II, *Hind* III, *Bg* II, *Apa* I, and *Hinc* II.

No.	Haplotype	United States	Puerto Rico	Jamaica	Brazil	Total
1	AAAAAAAAA	11	8	46	0	65
2	AABAAAAAA	3	3	7	0	13
3	ACAAAAAAA	3	4	12	1	20
4	AABABAAAA	0	0	0	1	1
6	ABAAAAAAA	0	1	1	0	2
8	ACBAAAABA	1	1	16	0	18
9	ACBBABABA	0	1	2	0	3
10	ACBBCAACA	0	1	0	0	1
11	AAAAABAAA	1	1	4	1	7
12	ACABCAABA	1	2	2	2	7
13	BCAAAAABA	0	1	1	0	2
14	ACABAAABA	2	1	7	2	12
15	AAABAAAAA	0	1	6	0	7
17	AAAACAABA	0	2	0	0	2
21	ACCACAABA	0	1	0	0	1
22	ACBABAABA	0	1	0	0	1
26	AACAAAAAA	0	0	3	0	3
32	ACAACAABA	4	0	4	0	8
33	ACBBCAAAA	0	0	1	0	1
34	AECAAAAAA	0	0	1	0	1
35	ACDACAABA	0	0	1	0	1
39	ACABCBABA	1	0	0	0	1
42	ACABAAAAA	1	0	1	1	3
43	ACBBAAAAA	1	0	0	0	1
44	ADBAAAABA	1	0	0	0	1
46	AAAACAAAA	1	0	1	0	2
48	ACBAABABA	0	0	3	0	3
51	ABAAAAAAC	0	0	1	0	1
52	ACBAABAAA	0	0	1	0	1
53	ACBBCAABA	0	0	4	0	4
54	ACBBAAABA	0	0	3	0	3
56	AAABDAABA	0	0	1	0	1
57	ACABCAAAA	0	0	1	0	1
58	ACAABAAAA	0	0	1	0	1
60	AAABAAABA	0	0	2	0	2
61	AAABABABA	0	0	1	0	1
62	ACAACAAAA	1	0	1	0	2
63	ACBCAAAAA	0	0	1	0	1
64	AABCAAAAA	0	0	1	0	1
65	AABAAAABA	0	1	1	0	2
69	AEAAAAAAB	1	0	0	0	1
75	AADAAAAAA	1	0	1	0	2
76	ADADAAAAA	1	0	0	0	1
77	AAAABAABA	0	0	0	1	1
80	ACABBAAAA	0	1	0	0	1
94	AEAACAAEA	1	0	0	0	1
96	AGAAAAAAA	0	0	1	0	1
98	ACBBAAAFA	1	0	0	0	1
99	ACBAAAAAA	0	0	2	0	2
100	ACEAAAABA	0	0	2	0	2
102	AAABBAAAA	0	0	1	0	1
106	ACAAEAABA	0	0	1	0	1
107	AAAABAAAA	0	0	3	0	3
112	AGAAAAABA	0	0	1	0	1
116	ACAAAAABA	0	0	3	0	3
117	ACAACBABA	0	0	1	0	1
119	ACAAABABA	0	0	1	0	1
120	AHBAAAABA	0	0	1	0	1
124	ACAAAAAAC	0	0	1	0	1
125	ACADAAABA	0	0	1	0	1
Total		**37**	**31**	**158**	**9**	**235**
Haplotype Diversity		0.91	0.92	0.89	0.94	0.90
Nucleotide Seq. Diversity		0.50%	0.49%	0.47%	0.54%0	0.48%

Table5. Distribution of blue marlin composite mtDNA haplotypes among collections at the same geographic locations during different years. Haplotype numbers correspond to composite haplotypes listed in Table 4 (NJ = New Jersey, USA; JA = Jamaica; HD = haplotyped iversity; NSD = nucleotide equence iversity).

No.	NJ92	NJ93	NJ94	NJTotal	JA91	JA92	JA93	JA94	JA95	JATotal
1	5	1	3	10	10	16	4	11	5	46
2	3	0	0	3	1	4	0	2	0	7
3	2	0	0	2	5	1	4	2	0	12
6	0	0	0	0	0	1	0	0	0	1
8	0	1	0	1	5	6	1	3	1	16
9	0	0	0	0	0	2	0	0	0	2
11	0	0	0	0	1	3	0	0	0	4
12	0	0	1	1	1	0	1	0	0	2
13	0	0	0	0	0	1	0	0	0	1
14	0	1	0	1	1	1	2	3	0	7
15	0	0	0	0	0	0	1	1	4	6
26	0	0	0	0	1	0	0	1	1	3
31	0	0	0	0	1	0	0	0	0	1
32	2	1	0	3	2	0	0	0	2	4
33	0	0	0	0	1	0	0	0	0	1
34	0	0	0	0	1	0	0	0	0	1
35	0	0	0	0	1	0	0	0	0	1
39	1	0	0	1	0	0	0	0	0	0
42	1	0	0	1	0	1	0	0	0	1
43	1	0	0	1	0	0	0	0	0	0
44	1	0	0	1	0	0	0	0	0	0
46	1	0	0	1	0	0	0	1	0	1
48	0	0	0	0	0	1	0	0	2	3
51	0	0	0	0	0	1	0	0	0	1
52	0	0	0	0	0	1	0	0	0	1
53	0	0	0	0	0	1	0	3	0	4
54	0	0	0	0	0	1	1	1	0	3
56	0	0	0	0	0	1	0	0	0	1
57	0	0	0	0	0	1	0	0	0	1
58	0	0	0	0	0	1	0	0	0	1
60	0	0	0	0	0	1	0	1	0	2
61	0	0	0	0	0	1	0	0	0	1
62	1	0	0	1	0	1	0	0	0	1
63	0	0	0	0	0	1	0	0	0	1
64	0	0	0	0	0	1	0	0	0	1
65	0	0	0	0	1	0	0	0	0	1
75	1	0	0	1	0	0	0	1	0	1
76	1	0	0	1	0	0	0	0	0	0
94	0	0	1	1	0	0	0	0	0	0
96	0	0	0	0	0	0	0	1	0	1
98	0	1	0	1	0	0	0	0	0	0
99	0	1	0	1	0	0	0	2	0	2
100	0	0	0	0	0	0	0	0	2	2
102	0	0	0	0	0	0	0	0	1	1
106	0	0	0	0	0	0	0	0	1	1
107	0	0	0	0	0	0	1	1	1	3
112	0	0	0	0	0	0	0	1	0	1
116	0	0	0	0	0	0	1	2	0	3
117	0	0	0	0	0	0	0	1	0	1
119	0	0	0	0	0	0	0	1	0	1
120	0	0	0	0	0	0	0	1	0	1
124	0	0	0	0	0	0	1	0	0	1
125	0	0	0	0	0	0	1	0	0	1
Total	**20**	**6**	**1**	**31**	**49**	**18**	**40**	**20**	**15**	**158**
HD	0.91	1.00	0.70	0.91	0.86	0.88	0.92	0.91	0.89	0.89
NSD	0.47%	0.46%	0.57%	0.50%	0.41%	0.49%	0.46%	0.46%	0.49%	0.47%

Table 6. Distribution of sailfish mtDNA composite haplotypes among collections from Florida, USA and southern Brazil. Letters represent fragment patterns produced by the following restriction endonucleases (inorder): *Ava* I, *Ava* II, *Bcl* I, *Bst* EI, *Hae* II, *Hin*d III, *Nci* I, *Bgl* I, *Ban* II, *Apa* I, *Hin*c II, and *Sca* I.

No.	Haplotype	Florida	Brazil 1991	Brazil 1992	Brazil Total	Total
3	AAAAAAAAAAAA	7	1	5	6	13
4	AAABAABAAAAA	1	0	0	0	1
5	AABAAAAAAAAA	2	0	1	1	3
6	AAABAAAAAAAA	10	8	17	25	35
7	BAABAACAAAAA	1	0	2	2	3
8	CABBAACAAAAB	1	0	0	0	1
9	BBABAACAAAAB	0	1	0	1	1
10	BGABAACAAAAB	0	1	0	1	1
18	AAAAAAABAAAA	0	0	1	1	1
19	AAABAAABAAAA	0	0	1	1	1
20	AAABAAAAAAAD	0	0	1	1	1
21	EGABAACAAAAB	0	0	3	3	3
22	AABBAAAAAAAA	0	0	1	1	1
	Total	**23**	**13**	**33**	**46**	**69**
Haplotype Diversity		0.73	0.63	0.72	0.69	0.71
Nucleotide Sequence Diversity		0.20%	0.24%	0.24%	0.24%	0.23%