GENETIC ANALYSIS OF BILLFISH POPULATION STRUCTURE

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SUMMARY

Restriction fragment length polymorphism analysis of purified mitochondrial DNA (mtDNA) was used to obtain preliminary estimates of the magnitude of intraspecific genetic differentiation within striped marlin, blue marlin and sailfish. Composite mtDNA genotypes were generated for each individual using 11 - 13 informative restriction enzymes. Significant heterogeneity was observed in the distribution of composite mtDNA genotypes among samples of approximately 40 striped marlin each from Mexico, Ecuador, Australia, and Hawaii, indicating reduced gene flow among these sites. Greater genetic differentiation was found between samples of 20 - 25 Atlantic and Pacific blue marlin and sailfish. Each species possessed two distinct mtDNA clonal types which differed by several restriction site changes. For both blue marlin and sailfish, only one clonal type was found in Pacific individuals, while both clonal types were found in the Atlantic. These results indicate limited gene flow between oceans and contrast sharply with those reported for several species of tuna.

RESUME

L'analyse du polymorphisme de la longueur des fragments de restriction du DNA mitochondrial (mtDNA) purifié a été utilisée pour obtenir des estimations préliminaires de la magnitude de la différentiation génétique intra-spécifique du Tetrapturus audax du makaire bleu et du voilier. Des génotypes composites de mtDNA ont été créés pour chaque poisson au moyen de 11-13 enzymes de restriction marqueurs. Un degré significatif d'hétérogénéité a été observé dans la distribution des génotypes composites de mtDNA entre les échantillons d'environ 40 Tetrapturus audax en provenance du Mexique, de l'Ecuador, de l'Australie et d'Hawaï, ce qui signale un flux génétique réduit entre ces locations. Une différentiation génétique plus accusée a été observée entre les échantillons de 20-25 makaires bleus et voiliers de l'Atlantique et du Pacifique. Chaque espèce présentait deux types clonaux différents de DNA, qui différaient selon diverses altérations des locations de restriction. Pour le makaire bleu comme pour le voilier, un seul type clonal a été observé chez les poissons du Pacifique, alors que les deux types ont été observés chez ceux de l'Atlantique. Ces résultats indiquent un flux génétique réduit entre les océans, et montrent un contraste accusé avec ceux qui sont signalés pour plusieurs espèces de thonidés.

RESUMEN

Se llevó a cabo un estudio sobre el polimorfismo de los fragmentos de restricción del ADN mitocondrial purificado (mtADN) para obtener estimaciones provisionales del grado de diferenciación genética entre las especies pez aguja, aguja blanca y pez vela. Se generó el genotipo compuesto de mtADN para cada individuo, empleando de 11 a 13 enzimas de restricción específicas. Se observó una apreciable heterogeneidad en la distribución de los genotipos compuestos de mtADN entre las muestras, cada una de aproximadamente 40 peces aguja, procedentes de México, Ecuador, Australia y Hawai, lo que indicaba un flujo genético reducido entre estas zonas. Una mayor heterogeneidad genética se observó entre grupos de 20-25 agujas azules y peces vela procedentes del Atlántico y el Pacífico. En cada una de estas dos especies pueden distinguirse dos clonotipos diferentes de mtADN que

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diferían en varias alteraciones en los lugares de restricción. Tanto en el caso de la aguja azul como del pez vela, sólo se encontró uno de estos clonotipos en los individuos procedentes del Pacífico, mientras que en los individuos procedentes del Atlántico se encontraron ambos clonotipos. Estos resultados indican un flujo genético reducido entre océanos, lo que contrasta claramente con los resultados presentados anteriormente para varias especies de túnidos.

1. INTRODUCTION

The billfishes of the family Istiophoridae, including the marlins, sprearfishes and sailfish, support large commercial and recreational fisheries throughout the world's subtropical and tropical oceans. While much is known about the distribution and general biology of these large, pelagic vertebrates, relatively little information exists about the genetic basis of stock structure of any of the species. In fact, even the taxonomic status of Atlantic and Indo-Pacific populations of several billfishes is problematic.

What is known about billfish population structure has been inferred from several different types of analyses. Traditionally, morphological differentiation has been used to describe population structure of many fishes, although many morphological characters in fishes have been demonstrated to be environmentally influenced (Barlow 1961). Morphological analyses of billfishes have reported differences in lateral line morphology between Atlantic and Indo-Pacific blue marlin, and Nakamura (1985) has suggested that two species of blue marlin be recognized on the basis of these differences. Similarly, differences in the relative length of the pectoral fin between Atlantic and Indo-Pacific sailfish have been the basis to question the validity of a single, circumtropical species (Nakamura 1985).

Insights into billfish population structure have also been gained from tag and recapture studies. Although tag return rates are extremely low for billfishes relative to many shorefishes, there have been a sufficient number of recaptures for most billfish species to provide some information about their movements. Clearly, some billfish do travel long distances. Individual blue marlin, striped marlin, white marlin and sailfish have all been recaptured over 1000 km from the point of release (Scott et al. 1990). However, the majority of fish are recaptured in the general vicinity of their point of release, even after several years at liberty (Scott et al. 1990). It is not known whether long distance movements of billfish result in gene flow (interbreeding) among fish from distant areas, but the opportunity appears to exist.

Over the last 30 years fisheries biologists have applied a variety of biochemical genetic techniques to elucidate population structure of a wide variety of fishes, employing analyses of both proteins and DNA. Surprisingly, little effort has been made to apply these techniques to survey billfish population structure, possibly due to the difficulty of collecting large sample sizes over a broad geographic range. Edmunds (1972) analyzed variation of seven blood and tissue proteins from a total of more than 100 white marlin from the mid-Atlantic Bight, Gulf of Mexico, and Caribbean Sea, but was unable to disprove the null hypothesis that the animals shared the same gene pool (comprised a single genetic stock). Shaklee et al. (1983) surveyed allozyme variation at more than 35 loci within 95 blue marlin from Hawaii and reported that there was sufficient variation for a large geographic analysis of population structure; however, such an investigation was not completed.

Over the past 3 years our laboratory has used both allozyme analysis of proteins and restriction fragment length polymorphism (RFLP) analysis of mitochondrial DNA (mtDNA) to study the population structure of several billfishes. Specifically, we have investigated the stock structure of the striped marlin (*Tetrapturus audax*) within the Pacific Ocean, and estimated interocean genetic differentiation between Atlantic and Pacific blue marlin (*Makaira nigricans*) and sailfish (*Istiophorus platyptenus*), as well as the interspecific relationship between striped and white marlin (*T. albidus*). Of the two techniques, the RFLP analysis of mtDNA provided a more detailed resolution of population structure. This report summarizes the mtDNA findings, and the results of the allozyme analysis are presented in Morgan (1992).

2. MATERIALS AND METHODS

2.1 Billfish Collections

Billfish tissue samples were collected from sportfish tournaments and artisinal fisheries to ensure accurate date and area of capture data for each specimen. Table 1 presents collection records for all fish used in this study. Fish were typically brought to the dock within 8 hours of capture where hearts were removed and immediately chilled on ice. Tissue samples were frozen in a standard freezer (-10 to -20°C) within a few hours of dissection. Shipments were transported from collection locations to the laboratory with pre-chilled ice packs, or on dry ice. Tissues were maintained in the laboratory at -70°C until processing.

2.2 mtDNA Analysis

Depending on the quality of the billfish samples, one of two techniques was used to isolate and analyze the mtDNA. For those specimens which were still frozen when they arrived at the laboratory, mtDNA was purified using the equilibrium density gradient centrifugation protocols of Lansman et al. (1981). Aliquots of mtDNA were individually digested with 11 - 13 restriction endonucleases following the manufacturer's instructions. The resultant fragments were endlabeled with ^{35}S nucleotide triphosphates, separated electrophoretically, and visualized autoradiographically (Sambrook et al. 1989).

Purified mtDNA could not be obtained from one shipment of billfish hearts that thawed before they arrived at the lab (20 striped marlin from Kona, Hawaii). For these samples, the upper band in the cesium chloride gradient containing both nuclear DNA and relaxed mtDNA was saved and dialyzed as described for mtDNA bands in Lansman et al. (1981), or genomic DNA enriched for mtDNA was isolated following the Chapman and Powers (1984) protocol. Aliquots of these samples were digested with restriction endonucleases as above and the fragments separated electrophoretically. Gels were transferred to a solid support and hybridized following the protocols of Sambrook et al. (1989). Highly purified white marlin mtDNA, nick translated with biotin-7-dATP, was used as a probe for mtDNA fragments. Hybridization filters were visualized after stringency washes following the procedures of the BRL BluGene Gene Detection Kit.

The different restriction fragment patterns produced by each restriction endonuclease were assigned a letter, and a composite mtDNA genotype, consisting of 11 - 13 letters representing the fragment patterns generated by each of the restriction endonucleases, was constructed for each individual. The genotypic diversity (h) was caluclated following Nei (1987):

$$h = [n/(n-1)][1-\Sigma(f_i)^2] \quad (1)$$

where f_i is the frequency of genotype i, in a sample of size n. Genotypic diversities were calculated for each sample and for the pooled samples. The nucleotide sequence divergence (p_{ij}) among mtDNA genotypes was estimated by the site approach of Nei and Li (1979). The mean nucleotide sequence diversity within a sample and mean nucleotide sequence divergence between samples were calculated following Nei (1987):

$$p = (n/n-1)\Sigma f_i f_j \, p_{ij} \quad (2)$$

where f_i and f_j are the frequencies of genotypes i and j in a sample of size n, and p_{ij} is the estimated nucleotide sequence divergence between genotypes i and j. Mean nucleotide sequence divergence values were corrected for within-group diversity following the method of Nei (1987):

$$p_{corrected} = p - 0.5(p_{popA} + p_{popB}) \quad (3)$$

The distributions of genotypic frequencies were evaluated for homogeneity among collections using the G-test (Sokal and Rohlf 1981).

3. RESULTS

3.1 Striped Marlin

Striped marlin were sampled from four sites within the Pacific Ocean (Mexico, Ecuador, Hawaii, and Australia), and the distribution of mtDNA genotypes among these samples, based on analysis with 11 restriction enzymes is presented in Table 2. Considerable mtDNA variation was found within each collection, as evidenced by genotypic diversities ranging from 0.55 (Ecuador) to 0.81 (Australia and Mexico). The mean nucleotide sequence diversity (Table 3), which provides a measure of the mean distance between genotypes drawn at random from each sample, ranged from 0.14% (Ecuador) to 0.82% (Australia).

The distribution of mtDNA variation among the striped marlin samples was not homogeneous (Table 2). For example, genotype SM-2 was found at a high frequency in Hawaiian and Australian striped marlin (0.22 and 0.21, respectively), but in low frequencies in fish from Mexico and Ecuador (0.03 and 0.05, respectively). In contrast, genotype SM-3 was found in low frequencies in the western Pacific (0.02 and 0.00), but in high frequency in the eastern Pacific (0.16 and 0.24). The results of a G-test indicate that the probability that the observed distribution of genotypes resulted from a single, randomly mating population is less than 0.001 (G_H = 75, 40 D.F.).

3.2 Blue Marlin

Substantial mtDNA variation was found within the pooled Atlantic and Pacific blue marlin samples (Table 4), but there was a striking difference in the level of mtDNA variation between the Atlantic and Pacific blue marlin. Pacific blue marlin demonstrated a relatively low level of genotypic variation. Four mtDNA genotypes were found in 25 Pacific individuals, with the common genotype occurring in 15 fish. The three alternate genotypes differed from the common genotype by no more than 2 restriction site changes. The overall genotypic diversity for the Pacific sample was 0.45, and the mean nucleotide sequence diversity was 0.33% (Table 3).

The level of variation within the Atlantic blue marlin sample was almost an order of magnitude higher than that in the Pacific sample. A total of 22 genotypes was found in the sample of 26 fish, resulting in a genotypic diversity of 0.98. The large degree of variation and the presence of two distinct families of mtDNA genotypes (clonal types) in the Atlantic sample resulted in a nucleotide sequence diversity of 2.92% (Table 3).

Three of the 4 genotypes found within Pacific blue marlin were also found within Atlantic blue marlin. In addition, 9 mtDNA genotypes found within the Atlantic sample were closely related to the common Pacific genotype, differing by no more than 3 restriction site changes. Ten of the 26 Atlantic fish had a genotype that was very different from the common Pacific genotype, differing by 5 - 8 restriction site changes. The majority of these genotypes differed from the common Pacific genotype by consistent restriction site changes produced by the enzymes ApaI, AvaII, BanI, BclI, NciI, and ScaI, and appear to represent a distinct clonal type.

The distribution of blue marlin genotypes between the Atlantic and Pacific samples was highly heterogeneous (G_H = 14.2, $p < 0.001$). The corrected mean nucleotide sequence divergence between the two samples was 0.68%, considerably less than the within-sample diversity of the Atlantic sample alone. This value reflects the fact that approximately 60% of the Atlantic blue marlin had mtDNA genotypes quite similar to those found in the Pacific sample. A much larger difference was calculated between the "Atlantic-like" and "Pacific-like" clonal types of mtDNA genotypes, with a corrected mean nucleotide sequence divergence of 3.30%.

3.3 Sailfish

Sailfish, like blue marlin, exhibited considerable genetic differentiation between Atlantic and Pacific samples, although variation within both sailfish samples was lower than in the blue marlin. All 20 Pacific sailfish shared a common mtDNA genotype (Table 5), resulting in a genotypic diversity and mean nucleotide sequence diversity of 0.00 (Table 3). A total of 6 genotypes was found among the 23 Atlantic

sailfish, with 17 individuals sharing a common genotype, causing low genotypic diversity (0.46) and nucleotide sequence diversity (0.45%) values.

The distribution of genotypes between Atlantic and Pacific sailfish was significantly different. The genotype found in all 20 Pacific sailfish was found in only 1 of 23 individuals within the Atlantic. Two other Atlantic sailfish had mtDNA genotypes that differed from the Pacific genotype by 1 restriction site change. The remaining 20 Atlantic sailfish mtDNA genotypes differed from the Pacific type by 3 consistent restriction site changes. The corrected mean nucleotide sequence divergence between the Atlantic and Pacific samples was 1.29% and between the "Atlantic-like" and "Pacific-like" genotypes was 1.57%.

3.4 White Marlin

The single sample of white marlin exhibited substantial mtDNA genotypic variation. The analysis of 17 fish with 13 restriction enzymes revealed 5 genotypes, a genotypic diversity of 0.70 and mean nucleotide sequence diversity of 0.35% (Table 3). Several restriction fragment length polymorphisms were common to both white marlin and striped marlin. This resulted in a corrected mean nucleotide sequence divergence of 0.49% between the Atlantic white marlin sample (n = 17) and the pooled Pacific sample of striped marlin (n = 160).

4. DISCUSSION

4.1 Intraspecific Variation

Genetic analyses of population structure require some degree of intraspecific variation, as population structuring will only be evident from a heterogeneous spatial/temporal distribution of the variation. All four species of billfishes surveyed in this analysis displayed ample mtDNA genotypic variation for analyses of population structure.

Two measures of intraspecific variation were calculated in this study, the genotypic diversity and the mean nucleotide sequence diversity. Comparing genotypic diversities between studies is difficult as the parameter is quite sensitive to the number of restriction sites surveyed (number of enzymes employed), but the results from this study can be compared to other studies employing about the same number of enzymes. Overall genotypic diversities for each species ranged from 0.62 in the sailfish to 0.86 in the blue marlin. These values fall within the range of 0.473 - 0.998 reported by Avise et al. (1989) for other fishes analyzed with about the same number of enzymes. While moderate nucleotide diversities were demonstrated by each of the billfish species, there were significant differences between populations within some species. For example, no mtDNA genotypic variation was detected within the Pacific sailfish sample, while the Atlantic sample had a genotypic diversity of 0.46. Similarly, the genotypic diversity value of 0.98 for the Atlantic blue marlin, which is among the highest reported for any organism (Avise et al. 1989), is much greater than the Pacific sample value of 0.45.

These same trends were apparent in the distribution of mean nucleotide sequence diversity, a measure of variation that can be more easily compared to other studies. Overall mean nucleotide sequence diversities ranged from 0.35% in the white marlin to 1.99% in the blue marlin. The values fall within the range of values reported in other studies of other marine fishes (Ovenden 1990).

A large difference in mean nucleotide sequence diversity was noted between samples of blue marlin from the Atlantic and Pacific Oceans. Atlantic blue marlin mtDNA genotypes differed from one another by an average value of 2.92%, while Pacific blue marlin mtDNA genotypes differed by only 0.33%. This represents almost an order of magnitude difference in the level of sequence variation maintained within the two populations. The presence of two distinct clonal types in the Atlantic blue marlin contributed significantly to the observed difference in levels of variation between the two populations. A similar difference in levels of variation has been reported for bluefish (*Pomatomus saltatrix*) from the U.S. mid-Atlantic coast, and the southeast coast of Australia (Graves et al. 1992). Differences in levels of mtDNA variation maintained within and between species have been attributed to several factors including different effective population sizes and different periods of isolation (Nei 1987, Avise et al. 1988, Chapman 1990,

Bowen and Avise 1990). However, neither of these explanations seems particularly appropriate for the blue marlin.

4.2 Intraspecific Differentiation

The major objective of this study was to elucidate the population structure of the striped marlin within the Pacific Ocean. At the onset, we did not expect to find much differentiation within the ocean basin, as previous studies had resolved little genetic differentiation between Atlantic and Pacific populations of skipjack and albacore tuna (Graves et al. 1984, Graves and Dizon 1989). However, our results suggest that tuna and billfish maintain very different levels of population structuring.

To provide a reference for the level of genetic differentiation within the striped marlin, we have included results from a similar mtDNA analysis of yellowfin tuna within the Pacific Ocean (Table 7, Scoles and Graves ms). Samples ($n = 20$) of yellowfin tuna were collected from the same areas as the striped marlin and analyzed with 12 restriction enzymes. The yellowfin tuna displayed slightly higher levels of mtDNA genotypic variation, but the distribution of major genotypes was homogeneous among the four yellowfin tuna samples ($G_H = 33$, 40 D.F. $p > 0.750$). This is in contrast to the heterogeneous distribution of genotypes among the striped marlin ($G_H = 75$, 21 D.F. $p < 0.001$), and strongly suggests that there are significant differences in the level of population structuring between the yellowfin tuna and striped marlin.

Although tunas possess many similarities with the billfish, they may not provide an appropriate group for comparing levels of population structure. To provide another reference, we analyzed the level of intraspecific differentiation between blue marlin and sailfish from the Atlantic and Pacific Ocean. As expected, interocean comparisons revealed even greater levels of genetic differentiation than the intraocean analysis of striped marlin population structure, although no fixed restriction site differences were found to discriminate Atlantic and Pacific populations of either blue marlin or sailfish populations.

The specific status of Atlantic and Pacific populations of blue marlin and sailfish has recently been questioned (Nakamura 1985). For the blue marlin, 3 of the genotypes found within the Pacific sample were also found within the Atlantic sample, and almost 60% of the Atlantic sample had a genotype similar to the Pacific genotypes (differing by no more than 3 restriction site changes) Similarly, one Atlantic sailfish possessed the same genotype found in all 20 Pacific sailfish, and two other Atlantic sailfish had genotypes that differed from the Pacific type by a single site change. The fact that mtDNA genotypes were shared between Atlantic and Pacific populations of blue marlin and sailfish is indicative of gene flow or recent isolation, and suggests that the Atlantic and Pacific populations of both species are conspecific.

While there was sufficient similarity among the genotypes to indicate that blue marlin and sailfish each comprise a single, circumtropical species, the results show that exchange between the oceans is very limited. Both species demonstrated significant heterogeneity in the distribution of genotypes between the Atlantic and Pacific, a result which contrasts with the homogeneity reported for skipjack, albacore, and yellowfin tunas (Graves et al. 1984, Graves and Dizon 1989, Scoles and Graves ms). Again, there appears to be a fundamental difference in the nature of population structure between the billfishes and tunas, with the billfishes exhibiting much higher levels of stock structure.

Two different clonal types of mtDNA were found within the Atlantic populations of both blue marlin and sailfish, while the Pacific sample of both species was characterized by a single clonal type that was also in the Atlantic at a lower frequency. The presence of two different clonal types suggests some form of isolation between conspecific populations, but the occurrence of both types in the Atlantic suggests recent gene flow from the Pacific to the Atlantic. These results are supported by a recent study of nucleotide sequence variation of the mtDNA cytochrome B gene (Finnerty and Block 1992). Their direct sequence analysis of 630 base pairs from a total of 25 blue marlin revealed a similar distribution of mtDNA genotypes between the Atlantic and Pacific Oceans.

The distribution of mtDNA genotypes within the blue marlin has direct management benefits. The current U.S. management plan for the blue marlin allows for the sale of Pacific blue marlin, but prohibits the sale of Atlantic blue marlin. However, enforcement of the management plan has been hindered by the inability to discriminate the individuals from the two oceans. Restriction fragment profiles produced

by the enzymes ApaI, AvaII, BanI, BclI, NciI, and ScaI, can unambiguously identify 38% of all Atlantic blue marlin without misclassifying any Pacific blue marlin.

Recently, the results of the tuna studies were questioned as direct sequencing of regions of tuna mtDNA amplified by the polymerase chain reaction (PCR) revealed more variation that RFLP analyses of the entire molecule (Bartlett and Davidson 1991). However, our current results suggest that billfish species maintain much greater levels of population structure than tunas, and that RFLP analyses work equally well to elucidate billfish population structure.

In summary, RFLP analysis of mtDNA has proved to be an extremely useful technique to elucidate the genetic basis of population structure of billfishes. Our results indicate very limited exchange of striped marlin among sampling locations throughout the Pacific, as well as significant genetic differentiation between Atlantic and Pacific populations of blue marlin and sailfish. Thus billfishes possess much greater intraspecific genetic structuring than the tunas. These data clearly demonstrate a genetic basis for stock structure within the billfish. Future studies will be required to determine the extent of exchange within ocean basins, but the data presented here indicate the presence of several stocks within an ocean basin. To prevent the loss of unique genetic variation, billfish management must be consider the genetic identity and relative independence of these fishery units.

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Table 1. Collection information for billfish samples. Individual data (weight, sex, etc.) are available from the authors upon request.

SPECIES	LOCATION	NUMBER	DATE	METHOD
Striped Marlin				
	Cabo San Lucas, Mexico	38	6/91	Sportfish
	Manta, Ecuador	42	4-10/90	Artisinal Fishery
	Kona, Hawaii	41	6-11/91	Sportfish
	Port Stephens, N.S.W., Australia	39	2/91, 2/92	Sportfish
Blue Marlin				
	Kona, Hawaii	25	8/90	Sportfish
	San Juan, Puerto Rico	12	9/90	Sportfish
	Arecibo, Puerto Rico	14	6/91	Sportfish
Sailfish				
	Islamorada, Florida	23	2/91	Sportfish
	Cabo San Lucas, Mexico	5	6/91	Sportfish
	Mazatlan, Mexico	15	6/91	Sportfish
White Marlin				
	Cabeza del Toro, Dominican Republic	17	6/90	Sportfish

Table 2. Distribution of striped marlin (Tetrapturus audax) mtDNA genotypes among four Pacific sampling locations. Composite genotypes reflect digestions with the following restriction endonucleases (left to right): AvaI, BanI, BclI, BglI, BstEII, HaeII, HindIII, SstII, ScaI, ApaI, and NciI.

STRIPED MARLIN mtDNA GENOTYPES

Genotype	Mexico	Ecuador	Hawaii	Australia
SM-1 AAAAAAAAAAA	15	26	23	13
SM-2 AAAAAABAAAA	1	2	9	8
SM-3 AAAAAAAAAAB	6	9	1	0
SM-4 BAAAAAAABAA	0	1	0	8
SM-5 BAAAAAAAAAA	0	1	2	4
SM-6 ABAAAAAAAAA	5	0	0	1
SM-7 AAAAAAAAAAF	4	0	0	0
OTHERS	7	3	6	5
	38	42	41	39

Table 3. Summary statistics of billfish population structure. Results from a similar study of yellowfin tuna population structure (Scoles and Graves, ms) are included for comparative purposes.

	N	Genotypic Diversity	Mean Nucleotide Sequence Diversity	Corrected Mean Nucleotide Sequence Divergence	
				Intraocean	Interocean
STRIPED MARLIN	160	0.74	0.54%	0.08%	-----
Mexico	38	0.81	0.64%	-----	-----
Ecuador	42	0.55	0.14%	-----	-----
Hawaii	41	0.65	0.44%	-----	-----
Australia	39	0.81	0.82%	-----	-----
BLUE MARLIN	51	0.86	1.99%	-----	0.68%
Atlantic	26	0.98	2.92%	-----	-----
Pacific	25	0.45	0.33%	-----	-----
SAILFISH	43	0.62	0.87%	-----	1.29%
Atlantic	23	0.46	0.45%	-----	-----
Pacific	20	0.00	0.00%	-----	-----
WHITE MARLIN	17	0.70	0.035%	-----	-----
YELLOWFIN TUNA	80	0.85	0.92%	0.07%	-----
Mexico	20	0.86	0.96%	-----	-----
Ecuador	20	0.86	1.04%	-----	-----
Hawaii	20	0.87	0.80%	-----	-----
Australia	20	0.81	0.91%	-----	-----

Table 4. Distribution of blue marlin (Makaira nigricans) mtDNA genotypes between the Atlantic Ocean (Puerto Rico and Jamaica) and Pacific Ocean (Kona, Hawaii). Composite genotypes reflect digestions with the following restriction endonucleases (left to right): AvaI, AvaII, BclI, BstEII, HaeII, HindIII, NciI, BglI, BanI, ApaI, HincII, and ScaI. Δ indicates the number of restriction site changes between each genotype and the common Pacific genotype (P1).

		BLUE MARLIN mtDNA GENOTYPES		
GENO-TYPE	COMPOSITE GENOPTYPE	Δ	ATLANTIC	PACIFIC
P1	AAAAAAAAAAAA	0	2	15
P2	AAABAAAAAAAA	1	1	5
P3	ACAAAAAAAAAA	1	3	4
P4	AABABAAAAAAA	2	0	1
P5	AAAAAAAACAAA	1	1	0
P6	ABAAAAAAAAAA	1	1	0
P7	AABAAAAAAAAA	1	2	0
P8	AAAAAAAAAAAB	1	1	0
P9	AAAAAABABAAB	3	1	0
P10	AAAAAAAAFAAA	1	1	0
P11	AAAAAAEAAAAA	1	1	0
P12	AAABAADAAAAA	2	1	0
P13	ACABAABAAAAA	3	1	0
A1	ACBAAABABBAB	6	1	0
A2	ACBBABCABBAB	8	1	0
A3	ACBBCABADCAB	8	1	0
A4	ACABCABADBAB	7	1	0
A5	BCAAAABAEBAB	7	1	0
A6	ACABAABAEBAB	6	1	0
A7	ACABCABAABAB	6	1	0
A8	AAABCAAADBAB	5	1	0
A9	ACCACABADBAB	7	1	0
A10	ACAABABAGBAB	6	1	0
			26	25

Table 5. Distribution of sailfish (Istiophorus platypterus) mtDNA genotypes between the Atlantic Ocean (Islamorada, Florida) and Pacific Ocean (Mazatlan and Cabo San Lucas, Mexico). Composite genotypes reflect digestions with the following restriction endonucleases (left to right): AvaI, AvaII, BclI, BstEII, HaeII, HindIII, NciI, BglI, BanI, ApaI, HincII, and ScaI. Δ indicates the number of restriction site changes between each genotype and the common Pacific genotype (P1).

		SAILFISH mtDNA GENOTYPES		
HAPLO-TYPE	COMPOSITE GENOPTYPE	Δ	ATLANTIC	PACIFIC
P1	BAAAACAAAAAB	0	1	20
P2	BAAAACAAAAAA	1	1	0
P3	CABAACAAAAAB	1	1	0
A1	AAAAAAAAAAAA	3	17	0
A2	AAAAABAAAAAA	4	1	0
A3	AABAAAAAAAAA	4	2	0
			23	20

Table 6. Distribution of white marlin (Tetrapturus albidus) mtDNA genotypes within a single sample from the Dominican Republic. Composite genotypes reflect digestions with the following restriction endonucleases (left to right): AvaI, BglI, HaeII, BanI, BclI, ApaI, HindIII, AvaII, BstEII, DraI, StyI, NciI, and ScaI. Δ indicates the number of restriction site changes between each genotype and the common genotype (WM-1). RFLP designations are not consistent for white marlin and striped marlin genotypes, although many are common to both species.

		WHITE MARLIN mtDNA GENOTYPES	
HAPLO-TYPE	COMPOSITE GENOPTYPE	Δ	NUMBER
WM-1	AAAAAAAAAAAA	0	9
WM-2	AAAAAAAAABAA	1	3
WM-3	AAAAAABAAAAA	1	2
WM-4	AAAAAAAAABAA	1	2
WM-5	AAAAAAAAABABA	2	1
			17

Table 7. Comparison of population structure of the striped marlin and yellowfin tuna within the Pacific Ocean. Although both species display about the same level of mtDNA variation, the spatial partitioning of the striped marlin variation indicates a high degree of population structuring while the yellowfin tuna data do not.

DISTRIBUTION OF YELLOWFIN TUNA AND STRIPED MARLIN mtDNA GENOTYPES				
Genotype	Mexico	Ecuador	Hawaii	Australia
		STRIPED MARLIN mtDNA GENOTYPES		
SM-1	15	26	23	13
SM-2	1	2	9	8
SM-3	6	9	1	0
SM-4	0	1	0	8
SM-5	0	1	2	4
SM-6	5	0	0	1
SM-7	4	0	0	0
OTHERS	7	3	6	5
	38	42	41	39
		YELLOWFIN TUNA mtDNA GENOTYPES		
YFT-1	6	7	7	8
YFT-2	5	2	3	4
YFT-3	0	3	1	0
YFT-4	0	0	2	0
YFT-5	1	2	0	0
YFT-6	2	0	0	1
YFT-7	1	1	0	2
OTHERS	5	5	7	5
	20	20	20	20

G-Test of Heterogeneity Among Collection Locations

Yellowfin Tuna	G = 33, 40 D.F. $p > 0.750$
Striped Marlin	G = 75, 21 D.F. $p < 0.001$**