Resolving the genetic structure of striped marlin, *Kajikia audax*, in the Pacific Ocean through spatial and temporal sampling of adult and immature fish

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Abstract: Spatial genetic structure in the highly migratory striped marlin (*Kajikia audax*) was examined using nuclear (microsatellite) and mitochondrial (control region sequences) DNA markers. While previous studies on striped marlin were limited by sampling design and sample size, this study employed a multiyear concurrent sampling scheme to collect tissue from seven locations representative of the species' range in the Pacific: Japan, Hawaii, Southern California, Mexico, Central America, New Zealand, and Australia. Mature and immature specimens were analyzed separately to evaluate life-stage-specific population structure and movements. Microsatellite and sequence results revealed small but significant overall spatial subdivision (F_{ST} = 0.0145 and K_{ST} = 0.06995, respectively). Pairwise microsatellite analyses (n = 1199) revealed four groups: (1) Japan – Immature Hawaii – Southern California, (2) Mature Hawaii, (3) Mexico – Central America, and (4) Australia – New Zealand. Mitochondrial sequence analysis (n = 451) showed similar patterns; however, no significant differentiation was found between groups 1 and 2. This enhanced resolution of geographic genetic structure is important for understanding the complex migration patterns in this species. Moreover, the consistency among independent genetic studies on striped marlin provides strong support for management of at least three clearly delineated Pacific stocks.

Résumé : Des marqueurs nucléaires (microsatellites) et mitochondriaux (séquences de la région de contrôle) d'ADN nous ont servi à déterminer la structure génétique spatiale du makaire strié (*Kajikia audax*), une espèce fortement migratrice. Alors que les études antérieures sur le makaire strié ont été limitées par leur plan d'échantillonnage et la taille des échantillons, notre étude utilise une méthode d'échantillonnage concurrent sur plusieurs années pour récolter des tissus dans sept régions représentatives de l'aire de répartition de l'espèce dans le Pacifique, soit le Japon, Hawaii, le sud de la Californie, le Mexique, l'Amérique Centrale, la Nouvelle-Zélande et l'Australie. Nous avons analysé les spécimens matures et immatures séparément de manière à évaluer la structure génétique et les déplacements spécifiques au stade de vie. Les résultats des analyses des microsatellites et des séquences indiquent globalement une subdivision faible mais significative (respectivement, F_{ST} = 0,0145 et K_{ST} = 0,06995). Des analyses appariées des microsatellites (n = 1199) révèlent l'existence de quatre groupes : (1) Japon – immatures d'Hawaii – sud de la Californie, (2) matures d'Hawaii, (3) Mexique – Amérique Centrale et (4) Australie – Nouvelle-Zélande. L'analyse des séquences mitochondriales (n = 451) montre des patrons similaires; il n'existe cependant pas de différenciation significative entre les groupes 1 et 2. Cette différenciation améliorée de la structure génétique géographique est importante pour comprendre les patrons complexes de migration de l'espèce. De plus, la concordance entre les études génétiques du makaire strié appuient fortement une gestion basée sur au moins trois stocks bien définis dans le Pacifique.

[Traduit par la Rédaction]

Introduction

Large pelagic species such as tuna and billfish roam the world's oceans, free of any obvious physical barriers. It has generally been thought that the highly migratory lifestyles of these marine fishes would lead to genetic homogeneity among conspecific populations, yet molecular data show that this is not always true. While some of these free-roaming fish show no genetic structure between or within oceans (e.g., skipjack tuna (*Katsuwonus pelamis*) and wahoo (*Acanthocybium solandri*) (Ely et al. 2005; Theisen et al. 2008), other species exhibit varying levels of genetic subdivision, such as blue marlin (*Makaira nigricans*) (Finnerty and Block 1992; Buonaccorsi and Graves 2000; Buonaccorsi et al. 2001) and bigeye tuna (*Thunnus obesus*) (Durand et al. 2005), that show genetic differentiation between Atlantic and Pacific Ocean populations. Genetic subdivision has also been seen within ocean basins; swordfish (*Xiphias gladius*) (Block and Reeb 2000), Pacific sailfish (*Istiophorus platypterus*) (Graves and McDowell 1995), and striped marlin (*Kajikia audax*) (Graves and McDowell 1994; McDowell and Graves 2008) all revealed subdivision within the Pacific Ocean.

While generally considered a bycatch species, striped marlin are sold commercially in Japan, Taiwan, Australia, and Hawaii, with smaller targeted fisheries in several locations. Striped marlin are also important to recreational fisheries around the Pacific, particularly in Hawaii, New Zealand, Aus-

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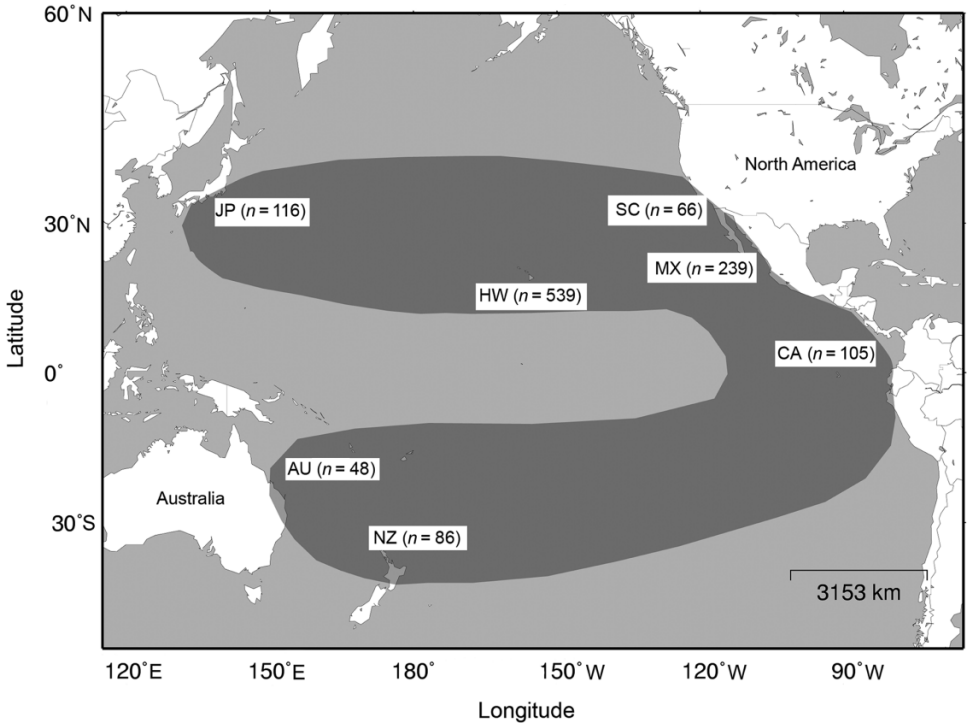
Fig. 1. The Pacific Ocean range of striped marlin (*Kajikia audax*) (shaded area), sampling locations, and number of specimens collected in those locations.



tralia, Southern California, and Mexico. These recreational fisheries contribute greatly to coastal economies through sportfishing and sportfishing-related tourism (Bromhead et al. 2004). Given this species' economical importance and the signs of fishing-related population strain already occurring (Bromhead et al. 2004; Worm et al. 2005), it is important to understand its spatial distribution to develop effective management strategies. This study focuses on examining the spatial genetic structure of striped marlin in the Pacific Ocean.

Previous work

Striped marlin occurs in the Pacific and Indian oceans and is considered the most abundant and widely distributed billfish (Nakamura 1985). In the Pacific, its distribution creates a horseshoe-shaped pattern across the ocean basin (Fig. 1), occurring in tropical, subtropical, and temperate regions. Individual striped marlin are capable of moving throughout their range as evidenced by tagging studies; however, the northernmost and southernmost extensions of their range are seasonal, as the waters become too cool during winter months (Squire 1972).

Several stock scenarios have been proposed for striped marlin: a single panmictic population within the Pacific (Shomura 1980), eastern–western Pacific stocks (Morrow 1957), and northern–southern Pacific stocks (Kamimura and Honma 1958). Alternatively, a regional stock hypothesis is bolstered by spatial and temporal partitioning of spawning events and may also fit with the results of Graves and McDowell (1994), who used mitochondrial restriction fragment length polymorphisms (RFLPs) in four Pacific locations to find shallow but significant levels of genetic subdivision, with 4/6 significant pairwise comparisons. These findings were again supported by a study using five microsatellites and mitochondrial control region sequences (McDowell and Graves 2008); the results suggested four regional stocks: Southwest Pacific (Australia), North Pacific (Japan–Taiwan–Hawaii–California), Mexico, and Southeast Pacific (Ecuador).

Despite previous investigations into the striped marlin's population structure, questions remained regarding the applicability of these studies to management issues because of sampling design, temporal replicates, and number of specimens. McDowell and Graves (2008) attempted to address these issues from their first study (Graves and McDowell 1994); however, with the complexities associated with sampling large pelagic fish, some of these difficulties persisted. Sample size, for instance, may have continued to be a limitation to the 2008 study; while 371 specimens were screened with microsatellites, only 85 specimens (from six of the seven locations) were sequenced at the mitochondrial control region. Pairwise mtDNA estimates did not detect genetic subdivision, possibly because of small sample size. These sampling limitations make it difficult to apply results of previous studies to striped marlin management issues.

In this project, sampling was conducted over seven locations representative of the striped marlin's range in the Pacific, with concurrently sampled temporal replicates in six of the locations. Unlike previous billfish studies, to better assess where breeding populations occur, initial analyses kept mature and immature individuals (in applicable locations) separate to determine if they were significantly different. To increase resolution power, two classes of molecular markers were used: microsatellites (12 loci) and mitochondrial DNA control region sequences. Concordance among markers adds confidence in the findings; however, differences among markers can also be informative and can help distinguish genetic signals due to selection or other forces.

Given that genetic variability in striped marlin is high and that sample size can greatly influence the patterns of genetic subdivision, a total of 1199 specimens were screened with microsatellites and 451 specimens were sequenced at the mtDNA control region. A greater number of loci, larger sample sizes, and a concurrent sampling scheme may more conclusively resolve population subdivision than previous studies and increase the usefulness of these results to managing agencies. Additionally, temporal analyses of the same data set (Purcell 2009; C. Purcell and S. Edmands, unpublished data) show that the spatial subdivision patterns reported here are largely robust to temporal variance.

While genetic subdivision is difficult to detect in migratory species, as even a few migrants would erase patterns of subdivision, the variation observed in striped marlin suggests differences in life-history traits and (or) population demographics between this species and the other migratory species not exhibiting genetic structure. Trying to understand the cause of these differences is crucial in developing effective management strategies to preserve this fishery.

Materials and methods

Sampling strategy

From 2001 to 2007, samples were collected from seven locations chosen to be representative of the species' range in the Pacific: Japan (2003), Hawaii (2003, 2004, 2005), Southern California (2001, 2002, 2004, 2005, 2006, 2007), Mexico (2003, 2004, 2005, 2006, 2007), Central America (2003, 2004, 2005, 2006), New Zealand (2004, 2006), and Australia (2004, 2005) (Fig. 1). The samples were provided through commercial and recreational fishing efforts. Commercial samples were collected through sponsored observer programs in collaboration with the National Marine Fisheries Service (NMFS), the Inter-American Tropical Tuna Commission (IATTC), the National Research Institute of the Far Seas Fisheries (NRIFSF, Japan), and the Secretariat for the Pacific Community (SPC, New Caledonia). Recreational samples were collected through independent environmental research firms, such as the Pfleger Institute of Environmental Research (Oceanside, California), Marine Conservation Science Institute (Fallbrook, California), Pepperell Research and Consulting (New South Wales, Australia), and Nelson Resources Consulting (Miami, Florida), and scientists working with Interdisciplinario de Ciencias Marinas (CICIMAR, Mexico). Recreational fishers also assisted in providing samples from kill tournaments and from live fish on catch-and-release trips with biopsy darts mounted on tagging poles. Sample numbers varied by location because of the overall abundance of striped marlin in the area, the type of fishing used to obtain samples, and the participation of contacts in those areas. Fin or muscle tissue was preserved in ethanol or 20% dimethyl sulfoxide buffer saturated with sodium chloride (Seutin et al. 1991). Some samples were not used in genetic analyses because of tissue degradation.

Sample preparation

For higher quality tissue, genomic DNA was extracted from small amounts of tissue using Chelex (BioRad) or lysis reactions (Edmands et al. 2005). In the lysis extraction, a few muscle tissue fibers or a small amount of fin skin were added to 50 μL lysis buffer at 65 °C for 1 h followed by 100 °C for 15 min. For degraded samples, an overnight cetyltrimethylammonium bromide – proteinase K incubation was followed by a standard phenol–chloroform extraction, with an ethanol precipitation, and if necessary a lithium chloride wash.

Determination of maturity

Samples were divided into reproductively immature and mature individuals using several methods because of variable biological information collected from the sample sources. Based on fish in the Coral Sea (Hanamoto 1977), 29 kg was used as the mass at first maturity and 143 cm was used as eye fork length at first maturity. A factor of 1.2 (dressed mass × 1.2 = round mass) was used to convert dressed masses into round masses based on published International Convention for the Conservation of Atlantic Tunas (ICCAT) estimates for billfish (Mejuto et al. 2002). Lower jaw fork lengths were converted into masses based on Kopf et al. (2005).

Microsatellite assays

Twelve microsatellite loci were used in this study: ten developed specifically for this project as described by Purcell et al. (2009) and two developed by Buonaccorsi and Graves (2000). Polymerase chain reaction (PCR) amplification conditions varied between microsatellite primer sets. Five microsatellites were amplified using specific fluorescently labeled forward primers; the other seven microsatellites were amplified with a modified nonlabeled forward primer containing a 25 bp zip-code tag (Chen et al. 2000). Fluorescent complementary primers for the zip-code tags were used for amplification of those modified microsatellites, with the resulting fragment sizes 25 bp longer. Both sets of fluorescent primers used Beckman WellRED D2, D3, or D4 dyes. PCR was conducted on a MJ Research PTC-200 DNA Engine and an Applied Biosystems GeneAmp PCR System 9700 with the following conditions: 15 ng template DNA, 0.25–1.0 μmol·L^{-1} primers, 1.5–3.5 mmol·L^{-1} $MgCl_2$, 0.25 mmol·L^{-1} dNTPs, 10 mmol·L^{-1} Tris-HCl, 50 mmol·L^{-1} KCl, and 0.3 U *Taq* polymerase in 12 μL total volume. Two cycling conditions were used: one for specifically labeled forward primers and another for labeled zip-code tags, described in Purcell et al. (2009). PCR products were analyzed using the fragment analysis on a Beckman–Coulter CEQ 8000 Capillary Sequencer and scored visually. Approximately 7% of samples were rerun for consistency in PCR amplification and fragment analysis on the sequencer. For scoring consistency of microsatellite fragments, approximately 20% of samples were rescored.

Mitochondrial control region assays

The mitochondrial control region was amplified for 451 striped marlin specimens from the locations listed above, with five specimens from Ecuador used in two of the analyses. PCR was conducted using three universal primers (K: 5′-AGCTCAGCGCCAGAGCGCCGGTCTTGTAAA-3′) (Lee et al. 1995), (L19: 5′-CCACTAGCTCCCAAAGCTA-3′) (Bernatchez et al. 1992), and (12 SAR-H: 5′-ATAGTGGGGTATCTAATCCCAGTT-3′) (Palumbi et al. 1991) to amplify approximately 1000 bp of this region. PCR was conducted with the following conditions: 25 ng template DNA,

1 μmol·L^{-1} forward and reverse primers, 0.25 mmol·L^{-1} dNTPs, 2.0–2.5 mmol·L^{-1} $MgCl_2$, 10 mmol·L^{-1} Tris–HCl, 50 mmol·L^{-1} KCl, and 1 U *Taq* polymerase in a 34 μL total volume. The cycling conditions consisted of the following: initial denaturation at 93 °C for 5 min, 35 cycles of denaturation at 93 °C for 1 min, primer annealing at 54 °C for 30 s, followed by extension at 72 °C for 1 min 15 s. After 35 cycles, a 5 min final extension at 72 °C was used, followed by a hold at 8 °C. After checking for amplification on 1.2% agarose gels, successful amplifications were submitted to High-Throughput Sequencing Solutions operated by the University of Washington, Department of Genome Sciences (Seattle, Washington) for ExoSAP PCR clean-up and sequencing using the primers from the original amplification. The entire control region was sequenced in both directions using those primers. Sequences were examined and aligned using SEQUENCHER 4.6 (Gene Codes Corporation).

Microsatellite data analyses

To explore spatial patterns in striped marlin, the 1199 specimens were first arranged by collection location and then further separated into mature and immature fish within each location (where applicable). For each population, observed (H_o) and expected (H_e) levels of heterozygosity were calculated using ARLEQUIN 3.1 (Excoffier et al. 2005). Deviations from Hardy–Weinberg equilibrium (HWE) were detected using F_{IS}, and genotypic disequilibrium was calculated for each locus-pair in each population using GENEPOP 1.2 (Raymond and Rousset 1995) with 10 000 dememorization steps, 1000 batches, and 10 000 iterations. The program ML-NullFreq (Kalinowski et al. 2006) was used to check for null allele frequency in each locus and population. The original data set was corrected for null alleles based on estimated frequencies found using ML-NullFreq, and the expected number of null homozygotes and heterozygotes were calculated for all loci within each population. The expected numbers of null homozygotes were added to individuals with missing data using the null allele "999". Null heterozygotes were incorporated by randomly adding the null allele to existing non-null allele homozygotes. The data incorporating the null alleles were permutated to mimic a round of sexual reproduction, thereby randomly mixing the null alleles throughout the population using GENETIX 4.04 (Belkir et al. 2000).

Using null-corrected data, the genetic structure of mature and immature groups within locations was determined. If no significant differences were found between mature and immature samples in a location, they were combined for the remaining analyses. However, if groups showed significant structure, then they remained as separate groups for the rest of the analyses.

Because the null allele correction method permutated data within locations to shuffle the null alleles, the original (non-corrected) data from the sample groups determined above were used to estimate observed and expected heterozygosity, F_{IS}, and genotypic disequilibrium using the programs and parameters mentioned above. Genetic variability among–within striped marlin populations, measured by allelic richness (A_R) and the number of alleles (N_A), was also calculated with the non-null-corrected data using FSTAT 2.9.3 (Goudet 1995).

Weir and Cockerham's overall F_{ST} and pairwise F_{ST} values were calculated for all sample groups using 10 000 permutations in GENETIX 4.04 (Belkir et al. 2000), followed by a straight Bonferroni correction of the pairwise estimates. For comparison, this was conducted for null-corrected and non-corrected data. Hedrick's G'_{ST} (2005) was calculated from estimates of Nei and Chesser's (1983) G_{ST} and H_S given in GENETIX (Belkir et al. 2000) for the null-corrected data. The program ISOLDE within GENEPOP was used to test the correlation between geographic distance, measured as the average distance among sampling locations in kilometres (km) and population structure, as estimated with F_{ST}. Correlation significance between genetic structure and geographical distance was assessed using the Mantel test in GENEPOP with 1000 permutations.

The model-based Bayesian clustering program STRUCTURE 2.2 (Pritchard et al. 2000) was also used to examine population structure. Five replicates were run with a burn-in of 100 000 steps and 500 000 Markov chain Monte Carlo (MCMC) steps for K from 1 to 11, using the admixture model and the assumption that allele frequencies are correlated among populations.

Mitochondrial data analyses

The number of haplotypes (h), haplotype diversity (h_d), and average number of differences between sequences (K) were calculated for sequences in each location using DNASP 4.50.3 (Rozas et al. 2003). The haplotype-based statistic (H_{ST}) and nucleotide sequence-based statistic (K_{ST}) were calculated in DNASP. Population pairwise comparisons were conducted for the average number of pairwise nucleotide differences (K_{xy}) and for the nearest-neighbor statistic, S_{nn}, which is a measure of how often the most similar sequences are from the same location (Hudson 2000) in DNASP. Also in DNASP, pairwise genetic subdivision estimates were calculated using the sequence-based statistic (K_{ST}) according to Hudson et al. (1992). A neighbor-joining tree was created using ClustalX 2.0.10 (Larkin et al. 2007) and MEGA 4 (Tamura et al. 2007) using a bootstrap test with 5000 replicates. A hierarchical structuring of the mitochondrial sequences using an analysis of molecular variance (AMOVA) within ARLEQUIN was used to assess the relative contribution of variance among groups, within groups, and within populations using the distance matrix of pairwise differences among sequences.

Results

Microsatellite analyses

During the microsatellite analysis, initial comparisons of observed and expected heterozygosities, F_{IS} values, and null allele frequency estimates indicated that a null allele correction should be incorporated into the data set (Supplemental Table S1[1], available online). Null frequencies ranged from 0% to 33.6% of homozygotes for a few locus–location combinations; however, it is important to note that even higher estimates may have reflected only one or two homozygous

[1]Supplementary data are available with the article through the journal Web site at http://nrcresearchpress.com/doi/suppl/10.1139/f2011-104.

individuals. Following this correction, the data were used to compare the mature and immature specimens within the locations. Based on F_{ST} estimates, immature and mature Mexican specimens and immature and mature Central American specimens were grouped together for the remaining analyses. However, significant structure was detected between immature and mature Hawaiian specimens, and they were therefore maintained as separate groups.

Genetic variability (microsatellites)

Because the null allele correction permuted data within locations to randomly distribute null alleles among samples, the original data (not corrected) was used in all summary statistics. Genetic variability (Table 1) within striped marlin populations varied widely among loci; allelic richness ranged from 2.4 (locus *164*) to 7 (locus *Mn08*), both in Japan, but no significant differences were found among locations in allelic richness ($p = 0.99$, using a two-tailed test implemented in FSTAT with 1000 permutations). The number of alleles varied widely depending on the locus, from 2 alleles in Australia and New Zealand (locus *218*) to 49 alleles (locus *Mn08*) in Mature Hawaii and Mexico, but again was not significantly different among locations ($p = 0.54$, using a two-tailed test implemented in FSTAT with 1000 permutations).

Observed levels of heterozygosity (H_o) also varied greatly among microsatellite loci (Table 1); however, average heterozygosity across all loci for each location fell within a narrow range. Lowest average H_o (0.652) was in Southern California, and the highest averages (0.727 and 0.696) were in Australia and New Zealand, respectively; neither H_o nor H_e among locations were significantly different ($p = 0.98$ and $p = 0.99$, respectively). Significant heterozygote deficits were seen in Mature Hawaii (seven loci), Immature Hawaii (five loci), Australia (four loci), Mexico (three loci), New Zealand (two loci), and Southern California and Central America (one locus each). Japan showed no significant differences.

F_{IS} was also used to investigate deviations from HWE (Table 1), and tests were conducted for the eight population groups, including the two groups from Hawaii. Numbers ranged from positive values, indicating a deficit of heterozygotes, to negative values, indicating an excess of heterozygotes. Significant deviations were found in Japan (three loci), Mature Hawaii (nine loci), Immature Hawaii (four loci), Southern California (four loci), Mexico (four loci), Central America (three loci), New Zealand (one locus), and Australia (three loci) (Table 1). F_{IS} values were predominately positive, and 32 of the 33 significant values were positive, indicating a heterozygote deficit.

Genotypic disequilibrium for all locus-pairs was calculated for the eight sample groups (Supplemental Table S2[1]) using the log likelihood ratio statistic (G test) and the Markov chain method with the parameters 10 000 dememorization steps, 1000 batches, and 10 000 iterations in GENEPOP. Following Bonferroni correction, significant disequilibrium was detected in 37 locus-pairs in Australia and one locus-pair in Mature Hawaii (Supplemental Table S2[1]). In total, 56% of locus-pairs in Australia showed significant genotypic disequilibrium.

Genetic structure (microsatellites)

Spatial structure was calculated using Wright's F_{ST} according to Weir and Cockerham (1984) and Hedrick's standardized genetic differentiation estimate, G'_{ST}, at all loci for each population using GENETIX. Significance levels were assessed using 10 000 permutations. These estimates were conducted for both null-corrected and original data to examine effects of null alleles. Although F_{ST} values were small, they were significant for the null-corrected and original data sets, 0.0145 and 0.0123, respectively. As expected, Hedrick's G'_{ST} estimates for both groups were much larger than the F_{ST} estimates, 0.0802 (null-corrected data) and 0.0559 (original data).

Pairwise F_{ST} comparisons for the null-corrected data (Table 2) detected significant subdivision in 23 of 28 comparisons. The five sample pairs without significant differentiation were Japan – Immature Hawaii, Japan – Southern California, Immature Hawaii – Southern California, Mexico – Central America, and New Zealand – Australia. The values and range of values in pairwise comparisons were larger using G'_{ST} rather than F_{ST} estimates, with the largest value of G'_{ST}, 0.1345, found between Southern California and Australia, and the smallest, 0.0050, found between Japan and Southern California (Table 2). This is compared with the largest F_{ST} estimate, 0.0374, between Mexico and Australia, and the smallest, 0.0013, between Southern California and Japan (Table 2). Despite the spatial heterogeneity detected using the estimates above, analyses using STRUCTURE showed that a single population ($K = 1$) provided the best fit to the data with the largest log-likelihood estimate of –41 364.2.

Pairwise F_{ST} comparisons of the original data revealed similar patterns to the null-corrected analysis, but with fewer significant relationships (Table 2). Key differences were that the original data showed no significant pairwise differences between Mature Hawaii and the Immature Hawaii, Southern California, and New Zealand sample groups. The smallest F_{ST} value, 0.0002, was found between Japan and Immature Hawaii, and the largest, 0.0340, between Central America and New Zealand. Hedrick's G'_{ST} showed a wider range in estimates, from 0.0004 (Japan – Immature Hawaii) to 0.0771 (Mexico–Australia). A significant positive correlation was found between geographic distance (average distance among sampling locations) and population structure (F_{ST}) for striped marlin specimens from different locations (one-sided Mantel test with 1000 permutations, $p = 0.0087$, $R = 0.3559$). The scatterplot of the average distance among sampling locations (km) and F_{ST} is shown (Fig. 2).

Genetic variability (mitochondrial sequences)

A total of 451 individuals were sequenced (GenBank accession Nos. JF755428–JF755878) from eight locations: Japan, Hawaii, Southern California, Mexico, Ecuador ($n = 5$, not shown in Table 3), Central America, New Zealand, and Australia. Out of 451 control region sequences, 351 were unique haplotypes. Relative to the number of sequences, the number of haplotypes in each location was very high, ranging from 18 (Central America) to 91 (Mexico) (Table 3). Not surprisingly, haplotype diversity was very high among the locations (Table 3). The average number of differences between sequences (K) ranged from a low in Australia (26) to a high in Central America (42).

Genetic structure (mitochondrial sequences)

The overall genetic differentiation based on the mitochon-

Table 1. For each location, data is shown for the number of specimens analyzed with each microsatellite locus (n), allelic richness (A_R), number of alleles per locus (N_A), observed (H_o) and expected (H_e) levels of heterozygosity (H_o/H_e), and F_{IS} values used to detect deviation from the Hardy–Weinberg equilibrium.

	Microsatellite locus											
	24	*157*	*162*	*164*	*218*	*235*	*105*	*149*	*155*	*193*	*Mn01*	*Mn08*
JP (n = 119)												
n	110	110	108	111	108	108	61	76	68	85	43	50
A_R	5.5	3	5.1	2.4	2.5	5.4	6.2	2.8	3.6	6.3	5.2	7.2
N_A	19	5	17	8	4	20	19	5	8	26	13	34
H_o/H_e	0.882/0.881	0.491/0.558	0.759/0.817	0.396/0.383	0.481/0.510	0.870/0.869	0.902/0.923	0.400/0.443	0.448/0.599	0.906/0.925	0.744/0.821	0.898/0.968
F_{IS}	–0.001	0.121	0.071	–0.036	0.057**	–0.002*	0.023	0.098	0.254	0.021**	0.094	0.073
MT HW (n = 312)												
n	282	279	275	279	222	274	100	125	166	180	98	193
A_R	5.6	3.1	5.2	3.2	3	5.8	6.3	3.1	3	6.4	5.3	7
N_A	25	6	21	10	7	27	25	6	6	30	15	49
H_o/H_e	0.876/0.889	0.513/0.560***	0.763/0.832***	0.446/0.510	0.489/0.547***	0.813/0.888***	0.788/0.920*	0.333/0.403	0.333/0.423***	0.817/0.927	0.786/0.844	0.932/0.958***
F_{IS}	0.015	0.085**	0.084***	0.126	0.107***	0.084***	0.145***	0.173	0.212***	0.119***	0.069***	0.027*
IM HW (n = 227)												
n	196	197	201	207	172	204	128	144	120	157	76	125
A_R	5.5	2.6	5.3	3.3	2.6	5.4	6.4	2.2	3.5	6.6	5.3	7.1
N_A	26	6	19	9	5	21	30	4	8	41	15	40
H_o/H_e	0.857/0.880	0.538/0.552	0.735/0.826***	0.398/0.489	0.444/0.504*	0.853/0.874	0.754/0.927***	0.486/0.472	0.431/0.566***	0.821/0.935**	0.803/0.844	0.920/0.960
F_{IS}	0.026	0.026	0.110***	0.186	0.118**	0.024	0.187***	–0.03	0.240***	0.123***	0.049**	0.042
SC (n = 66)												
n	58	61	59	59	61	63	29	44	37	38	22	37
A_R	6.1	2.6	4.6	2.3	2.4	5.5	6.1	2.9	3.3	6.6	5.4	7
N_A	19	4	16	7	4	16	17	5	6	22	9	30
H_o/H_e	0.862/0.911	0.508/0.535	0.746/0.751	0.356/0.353	0.574/0.517	0.857/0.875	0.862/0.910	0.349/0.436	0.286/0.462	0.868/0.934	0.773/0.855	0.779/0.966*
F_{IS}	0.054*	0.051	0.007	–0.01	–0.111	0.021*	0.053	0.202	0.385	0.071*	0.098	0.197***
MX (n = 239)												
n	218	218	214	224	183	211	84	106	117	188	79	174
A_R	5.5	4.6	2.1	3	6.3	3.2	6.6	2.8	5.4	2.3	4.5	7
N_A	19	7	16	6	6	22	24	5	7	35	13	49
H_o/H_e	0.858/0.879	0.564/0.581	0.687/0.760	0.339/0.330	0.404/0.509***	0.806/0.856***	0.845/0.921	0.500/0.465	0.624/0.617	0.855/0.934	0.744/0.774	0.920/0.957*
F_{IS}	0.024	0.029	0.096	–0.027	0.205**	0.059***	0.083**	–0.076	–0.011	0.085**	0.039	0.04
CA (n = 105)												
n	100	99	104	103	93	102	31	60	48	68	37	64
A_R	5.6	4.9	2.3	3.4	5.9	3.9	6.5	2.3	5.2	2.9	4.7	7
N_A	17	6	16	7	3	16	16	7	9	28	9	37
H_o/H_e	0.890/0.884	0.485/0.567	0.680/0.783	0.427/0.383	0.462/0.503	0.824/0.847	0.667/0.907	0.417/0.458	0.604/0.674	0.897/0.933	0.794/0.782	0.794/0.963*
F_{IS}	–0.007	0.145	0.132	–0.115	0.081	0.027	0.269**	0.091	0.105	0.039*	–0.015	0.177***
NZ (n = 86)												
n	76	71	78	79	80	79	56	29	67	58	56	67
A_R	5.7	2.8	5.3	3.7	2	5.6	6.4	3.2	2.8	6.3	5	6.9
N_A	17	6	13	7	2	15	23	5	6	23	12	33
H_o/H_e	0.895/0.893	0.535/0.542	0.808/0.860	0.582/0.653	0.538/0.499	0.899/0.892	0.746/0.933*	0.517/0.542	0.349/0.403	0.772/0.920*	0.804/0.827	0.911/0.951
F_{IS}	–0.002	0.012	0.062	0.109	–0.077	–0.008	0.203***	0.047	0.137	0.162	0.029	0.043

Table 1 (*concluded*).

	Microsatellite locus											
	24	*157*	*162*	*164*	*218*	*235*	*105*	*149*	*155*	*193*	*Mn01*	*Mn08*
AU (*n* = 45)												
n	42	44	41	45	43	45	42	44	44	44	42	42
A_R	5.1	2.5	5.4	4	2	4.9	6.3	2.1	3.4	6.4	4.5	6.6
N_A	14	4	13	9	2	9	21	3	6	20	8	22
H_O/H_e	0.857/0.838	0.636/0.556	0.675/0.852*	0.711/0.731	0.512/0.499	0.822/0.845	0.786/0.920*	0.545/0.484	0.614/0.656	0.750/0.926***	0.881/0.802	0.929/0.944**
F_{IS}	–0.023	–0.146	0.210*	0.027	–0.026	0.027	0.147*	–0.129	0.066	0.192**	–0.1	0.017

Note: Significant p values are denoted as follows: *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$. JP, Japan; MT HW, Mature Hawaii; IM HW, Immature Hawaii; SC, Southern California; MX, Mexico; CA, Central America; NZ, New Zealand; AU, Australia.

drial sequences was calculated by two methods, H_{ST} and K_{ST}, that use the χ^2 statistical approach proposed by Hudson et al. (1992, eq. 2 and eq. 9, respectively). Both methods showed significant differentiation, with H_{ST}, the haplotype-based statistic estimated at 0.00493 ($p < 0.001$) and $K_{ST,}$ the nucleotide sequence-based statistic estimated at 0.06995 ($p < 0.001$). Population comparisons conducted for the average number of pairwise nucleotide differences (K_{xy}) (Table 3) showed the lowest estimate of K_{xy} was 27.9 (Australia – New Zealand) and the highest was 44.3 (Central America – Southern California). For the nearest-neighbor statistic (S_{nn}; Table 3), values were lowest between Immature Hawaii and Japan (0.55) and highest between Australia and Mexico (0.95).

Pairwise genetic subdivision patterns were explored using the sequence-based statistic K_{ST} (Table 3). Because sequence-based methods utilize information not only based on the frequency of haplotypes, but also on the numbers of differences between haplotypes, they are powerful in detecting structure in longer sequences or within smaller sample sizes (Hudson et al. 1992; Hudson 2000). With the K_{ST} estimates, no significant differentiation was detected between any pairwise combination of the Japan, Mature Hawaii, Immature Hawaii, or Southern California locations. Also no population genetic structure was detected between Mexico and Central America or between New Zealand and Australia (Table 3). The K_{ST} estimates ranged from –0.0026 (Japan – Immature Hawaii) to 0.1172 (Australia – Central America).

A neighbor-joining haplotype tree (Fig. 3) was generated for the mtDNA control region sequences. For values over 50%, replicate trees where individuals clustered together in the bootstrap test (5000 replicates) are shown next to the branches. However, branches where separations were reproduced less than 50% of the time were collapsed. This tree showed some of the same spatial patterns revealed by the pairwise K_{ST} values, particularly clusters of individuals from Mexico, Central America, and Ecuador. However, other areas of the tree showed mixed regions of sequences from different locations, reflecting the overall low values of genetic differentiation.

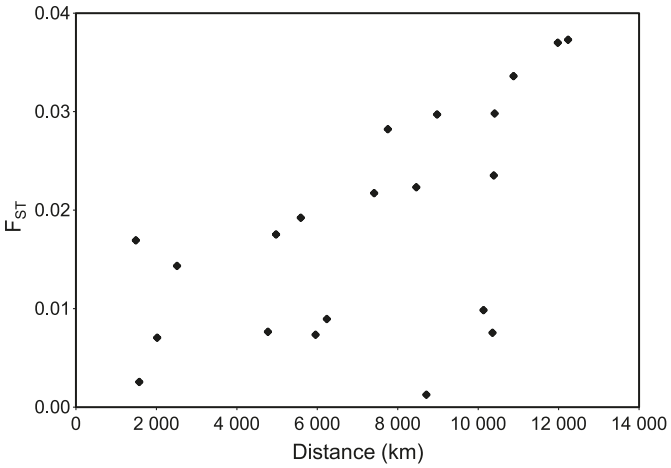
An AMOVA of spatial variation was computed by the distance matrix using pairwise distances in ARLEQUIN (10 000 permutations) for different groupings (Table 4), including several previous striped marlin stock models: northern vs. southern Pacific, western vs. eastern Pacific, and regional (Central America and Ecuador were grouped together because of the small sample size from Ecuador). Although, the majority of variation was contained within populations for all comparisons, those particular groupings did not minimize variation among populations within groups. The model that most minimized within-group variance, "Best fit" (Table 4), showed that although most variation (approximately 90%) occurred among samples within populations, about 10% (10.55%) of the variation was contained among groups, with effectively no variation among populations within groups. This model contained three groups: Mature Hawaii – Immature Hawaii – Japan – Southern California, New Zealand – Australia, and Mexico – Ecuador – Central America. Two alternate models were also provided for comparison. One was similar to the Best fit model except that Mature Hawaii was moved into a separate group, and while the AMOVA results were similar, slightly less variance was distributed among groups (9.77%). The second alternative moved Southern Cal-

Table 2. Weir and Cockerham's pairwise F_{ST} values (above diagonal) and Hedrick's G'_{ST} (below diagonal) for (*i*) null-corrected microsatellite data and (*ii*) original microsatellite data.

Population	JP	MT HW	IM HW	SC	MX	CA	NZ	AU
Null-corrected data								
JP		0.0074***	0.0035	0.0013	0.0099***	0.0076***	0.0224***	0.0284***
MT HW	0.0289		0.0066***	0.0077**	0.0193***	0.0176***	0.0090***	0.0219***
IM HW	0.0135	0.0263		0.0054	0.0154***	0.0131***	0.0121***	0.0173***
SC	0.005	0.0302	0.0196		0.0170***	0.0144***	0.0236***	0.0371***
MX	0.0337	0.0714	0.0539	0.0567		0.0026	0.0299***	0.0374***
CA	0.0273	0.0686	0.0483	0.0508	0.0087		0.0298***	0.0337***
NZ	0.0845	0.0358	0.0457	0.0862	0.1041	0.1103		0.0071
AU	0.1064	0.0879	0.0655	0.1345	0.1298	0.1233	0.027	
Original data								
JP		0.0081***	0.0002	0.0016	0.0070***	0.0077***	0.0216***	0.0263***
MT HW	0.0161		0.0029	0.003	0.0165***	0.0219***	0.0047	0.0183***
IM HW	0.0004	0.006		0.0003	0.0120***	0.0135***	0.0092***	0.0155***
SC	0.0031	0.0057	0.0008		0.0111***	0.0172***	0.0151***	0.0301***
MX	0.0135	0.032	0.0236	0.0206		0.0004	0.0308***	0.0377***
CA	0.0149	0.0432	0.0272	0.0332	0.0008		0.0340***	0.0335***
NZ	0.044	0.0094	0.0191	0.03	0.0615	0.0699		0.0083
AU	0.0549	0.0379	0.0327	0.0614	0.0771	0.0702	0.0177	

Note: Significant p values are denoted as follows: **, $p < 0.01$; ***, $p < 0.001$.

Fig. 2. Correlation between geographic distance (km) and population structure (F_{ST}) for striped marlin specimens ($R = 0.3559$), with significance determined by one-sided Mantel tests ($p = 0.0087$).



ifornia into the group with the rest of the eastern Pacific; however, results revealed this model was not nearly as good of a fit as the previous two groupings.

Discussion

Microsatellite analyses

Allelic richness and number of alleles were variable, depending on the locus and location. Heterozygosity levels were high but similar to what has been reported for striped marlin in a previous study (McDowell and Graves 2008) and in other pelagic fish such as Atlantic bluefin tuna (Carlsson et al. 2004) and Atlantic bigeye tuna (Gonzalez et al. 2008). Null alleles, a common cause of discrepancies between observed and expected heterozygosities, are known to be problematic and prevalent in microsatellite markers (Dakin and Avise 2004; Hedgecock et al. 2004; Kalinowski et al. 2006). In this study, null alleles were detected in a subset of loci and populations. While often mentioned as a caveat of population genetic data, usually no further treatment of the null alleles is attempted (Dakin and Avise 2004). However, given the detection of null alleles and the number of significant deviations in the heterozygosity and F_{IS} estimates, it was important to correct for null alleles in this data set, as it did change the number of significant pairwise relationships.

Overall F_{ST} and G'_{ST} values were low using microsatellites, but significant and in the range reported for other pelagic marine species (the reported median in Ward et al. 1994; O'Reilly et al. 2004; Rooker et al. 2007). Analysis of the eight groups of null-corrected samples revealed that striped marlin form four significantly different populations. The first group located in the southwest Pacific contained Australia and New Zealand. Australia had unusually high levels of genotypic disequilibrium, with 56% of locus-pairs showing significant disequilibrium, compared with only one other locus-pair (Mature Hawaii) in all other populations. One possible explanation was misidentification of other billfish species within that region's collection; however, all Australian samples had a striped marlin maternal background after sequencing the mitochondrial control region. Mexico and Central America in the eastern Pacific formed the second group; however, this group was significantly different from Southern California, despite also being in the eastern Pacific. The genetic heterogeneity found between Southern California and Mexico (primarily the Baja California region of Mexico) is surprising because of the close physical proximity between those two locations; yet it is also supported in the findings of McDowell and Graves (2008). Interesting spatial patterns were found in the northern Pacific. No significant differences were found between Japan and Southern California, but both were significantly different from Mature Hawaii. Interestingly, juvenile striped marlin caught around Hawaii were sig-

Table 3. Based on mitochondrial control region sequence analyses, (*i*) diversity estimates; (*ii*) K_{xy}; (*iii*) S_{nn}; and (*iv*) pairwise K_{ST} estimates.

Location	JP	MT HW	IM HW	SC	MX	CA	NZ	AU
Diversity estimates								
n_s	43	92	34	25	131	20	53	48
h	38	86	33	23	91	18	48	34
h_d	0.993	0.999	0.998	0.99	0.99	0.989	0.996	0.981
K	33	34	29	30	39	42	30	26
Pairwise K_{xy}								
MT HW	33.9							
IM HW	31.1	31.9						
SC	31.3	32.4	29.5					
MX	39.2	40.2	39.1	39.4				
CA	43.2	45.3	43.9	44.3	40.8			
NZ	33.2	33.5	30.6	32.2	39.9	44.1		
AU	31.7	32	28.8	30.9	38.9	43.5	27.9	
Pairwise S_{nn}								
MT HW	0.69**							
IM HW	0.55	0.62						
SC	0.57	0.69	0.61					
MX	0.92***	0.86***	0.89***	0.81***				
CA	0.85***	0.87***	0.83***	0.74**	0.79			
NZ	0.83***	0.69***	0.74**	0.83***	0.92***	0.81**		
AU	0.89***	0.77***	0.79***	0.89***	0.95***	0.94***	0.62*	
Pairwise K_{ST}								
MT HW	0.0025							
IM HW	–0.0026	0.0004						
SC	–0.0025	0.0048	–0.0006					
MX	0.0320***	0.0478***	0.0436***	0.0374***				
CA	0.0642***	0.0587***	0.1029***	0.1133***	0.0025			
NZ	0.0272**	0.0183***	0.0139*	0.0392***	0.0587***	0.0859***		
AU	0.0462***	0.0352***	0.0278**	0.0646***	0.0742***	0.1172***	–0.001	

Note: Diversity estimates include the number of sequences (n_s), number of haplotypes (h), haplotype diversity (h_d), and average number of differences between sequences (K); K_{xy} is the average number of pairwise nucleotide differences among sequences; S_{nn} is the nearest-neighbor statistic; and pairwise K_{ST} estimates are based on the sequence-based statistics of Hudson et al. (1992). Significant p values are denoted as follows: *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$.

nificantly different from mature fish in that area, but not significantly different from Japan and Southern California fish. Thus, according to the null-corrected data, striped marlin from Japan, California, and Immature Hawaiian fish form the third population, and Mature Hawaiian striped marlin constitute the fourth group.

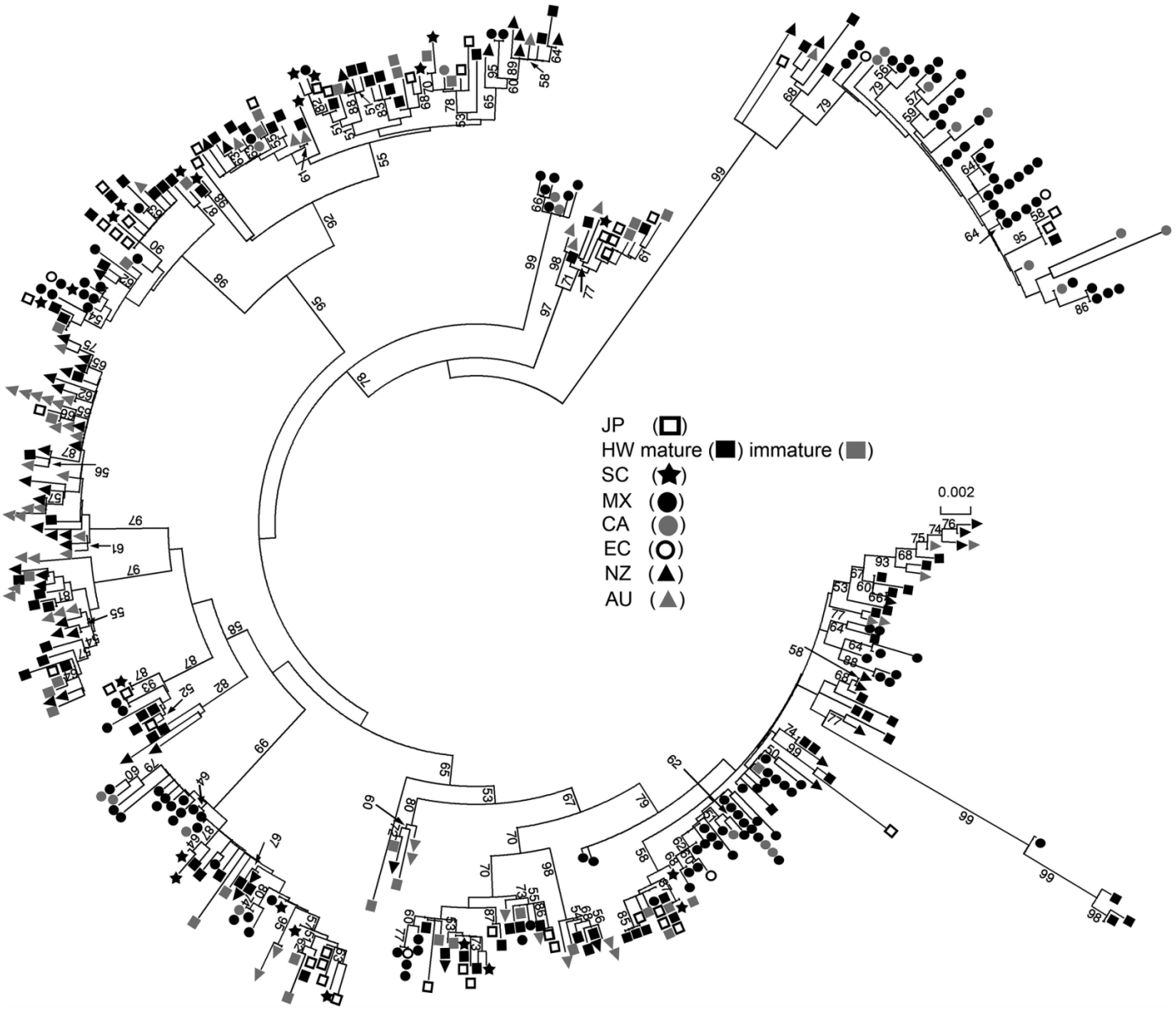
Despite the migratory behaviors that likely underlie the detected genetic patterns, there is still significant correlation between geographic distance and population structure, suggesting isolation by distance. Although some of these behaviors indicate trans-Pacific movement (Japan – Southern California), while others suggest limited mixing despite close proximity (Mexico – Southern California), in general, it appears that distance does limit genetic mixing, albeit at Pacific-wide scales. In contrast with pairwise analyses, the program STRUCTURE did not support more than one population of striped marlin in the Pacific. However, assignment methods have limited power when genetic differentiation is low among populations (Manel et al. 2005; Saenz-Agudelo et al. 2009).

When null allele frequencies were not incorporated into the data, overall F_{ST} and G'_{ST} estimates were lower, but many pairwise relationships remained the same. The primary difference was that Mature Hawaii showed fewer significant pairwise relationships; compared with null-corrected data no significant differences were detected with Southern California, Immature Hawaii, or New Zealand. For the original data, the pairwise F_{ST} values that changed in significance had some of the smallest significant values in the null-corrected data set. Thus, in this study, the null allele correction provided additional power to detect significant relationships rather than change the trends of those relationships. Interestingly, changes in significant pairwise groupings all involved Mature Hawaii, a group that contained the highest number of deviations in observed heterozygosity and F_{IS} estimates. This indicates that null allele correction was important to spatial heterogeneity analyses.

Mitochondrial DNA sequence analyses

Mitochondrial control region sequences contained a large number of haplotypes, and the haplotype diversity (h_d) was high but similar to other marine fish (Hauser et al. 2001;

Fig. 3. Neighbor-joining haplotype tree of mitochondrial control region sequences. Branches corresponding to separations reproduced less than 50% of the time are collapsed. The percentages of replicate trees where individuals clustered together in the bootstrap test (5000 replicates) are shown next to the branches for values over 50%. Symbols represent individual sequences.



Carlsson et al. 2004; Alvarado-Bremer et al. 2005). The eastern Pacific locations, Mexico and Central America, share the characteristics of having both the highest average numbers of differences between sequences (K), whereas Australia was lowest, which is perplexing given the unusual genotypic disequilibrium patterns in Australia mentioned above.

The overall genetic structure was significant using H_{ST} and K_{ST} estimates, and pairwise analyses showed patterns similar to the microsatellite analyses, with the same groups in the eastern Pacific (Mexico – Central America) and the southwest Pacific (Australia – New Zealand); however, there was a change in the North Pacific group. Sequence analyses did not reveal significant structure among Mature Hawaii and Japan, Immature Hawaii, or Southern California, and thus these specimens formed one group in the North Pacific. The nearest-neighbor statistic, S_{nn}, supported the K_{ST} results.

AMOVA comparisons also supported the mitochondrial pairwise results, with the best fit resulting in three groupings: (1) Australia and New Zealand, (2) Japan, Immature Hawaii, Mature Hawaii, and Southern California, and (3) Mexico, Central America, and Ecuador. Interestingly, the northern–southern and eastern–western stock model groupings proved to be a poor fit with the sequence data. The similarly poor fit of Southern California with the rest of the eastern Pacific sequences supports the pattern detected in the microsatellite analysis.

Overall spatial structure

Following the analysis of nuclear microsatellites and mitochondrial control region sequences, the spatial distribution and underlying migration patterns of striped marlin in the Pacific are starting to be understood. Large numbers of deviations in heterozygosity and F_{IS} values in mature Hawaiian specimens were at least partially explained by the presence of null alleles, but may also be indicative of a Wahlund effect,

Table 4. Analysis of molecular variance (AMOVA) of spatial variation in striped marlin control region sequences.

Structure tested	Variance	% Total	*F* statistics	*p*
West–east (MX, EC, CA, SC) vs. (JP, NZ, AU) vs. (MT HW and IM HW)				
Among groups	0.792 Va	4.23	F_{CT} = 0.0423	0.102
Among populations within groups	0.951 Vb	5.08	F_{SC} = 0.0530	<0.001
Within populations	16.988 Vc	90.69	F_{ST} = 0.0931	<0.001
North–south (EC, AU, NZ) vs. (JP, SC, MA HW, IM HW, MX, CA)				
Among groups	0.084 Va	0.45	F_{CT} = 0.0045	0.39
Among populations within groups	1.562 Vb	8.38	F_{SC} = 0.0842	<0.001
Within populations	16.988 Vc	91.16	F_{ST} = 0.0884	<0.001
Separate populations (IM HW and MT HW) vs. (CA, EC) vs. (JP) vs. (AU) vs. (SC) vs. (NZ) vs. (MX)				
Among groups	2.338 Va	12.57	F_{CT} = 0.1257	0.037
Among populations within groups	–0.730 Vb	–3.92	F_{SC} = –0.0449	<0.001
Within populations	16.988 Vc	91.35	F_{ST} = 0.0865	<0.001
All populations together (AU, MT HW, IM HW, JP, MX, NZ, SC, CA, EC)				
Among groups	1.605 Va	8.63	F_{CT} = N/A	NA
Among populations within groups	N/A	N/A	F_{SC} = N/A	NA
Within populations	16.988 Vb	91.37	F_{ST} = 0.0863	<0.001
Best fit (MT HW, IM HW, JP, SC) vs. (AU, NZ) vs. (MX, EC, CA)				
Among groups	2.001 Va	10.55	F_{CT} = 0.1055	<0.001
Among populations within groups	–0.013 Vb	–0.07	F_{SC} = –0.0008	<0.001
Within populations	16.988 Vc	89.52	F_{ST} = 0.1048	<0.001
Alternate group 1 (IM HW, JP, SC) vs. (MT HW) vs. (AU, NZ) vs. (MX, EC, CA)				
Among groups	1.831 Va	9.77	F_{CT} = 0.0977	<0.001
Among populations within groups	–0.085 Vb	–0.45	F_{SC} = –0.0050	<0.001
Within populations	16.989 Vc	90.68	F_{ST} = 0.0932	<0.001
Alternate group 2 (MT HW, IM HW, JP) vs. (MX, SC, EC, CA) vs. (AU, NZ)				
Among groups	1.266 Va	6.72	F_{CT} = 0.0672	0.003
Among populations within groups	0.576 Vb	3.06	F_{SC} = 0.0328	<0.001
Within populations	16.988 Vc	90.22	F_{ST} = 0.0979	<0.001

Note: Computed by the distance matrix using pairwise differences in Arlequin (10 000 permutations). AU, Australia; CA, Central America; EC, Ecuador; JP, Japan; IM HW, Immature Hawaii; MT HW, Mature Hawaii; MX, Mexico; NZ, New Zealand; SC, Southern California.

where sampling occurred across cohorts of juveniles or subgroups (Johnson and Black 1984; Lenfant and Planes 2002), especially as this location may serve as a stepping stone or feeding area for non-local striped marlin. There was disagreement between microsatellite and mitochondrial analyses; one possible reason may be lower resolution power with the fewer analyzed mtDNA sequences compared with the number of specimens analyzed with microsatellites. Alternatively, microsatellites may be more sensitive than mtDNA sequences, reflecting very low levels of gene flow between locations (Feulner et al. 2004; Keeney et al. 2005). It is also possible that Japanese juveniles migrating to (or through) Hawaii may be reflected in samples collected in that location, and mixing of these groups could impact analyses for both mature and immature Hawaiian samples. Japanese juveniles around Hawaii may grow and be included in analyses of mature Hawaiian fish while still not reproductively contributing to this location. Similarly, although spawning in striped marlin is now confirmed in Hawaii (Hyde et al. 2006), collections of Hawaiian juveniles likely have Japanese fish mixed in. Movement of juvenile striped marlin into Hawaiian waters is supported by previous reports (Squire and Suzuki 1990), where the fish likely use the area as a feeding ground before moving on to spawning locations (Matsumoto and Kazama 1974).

Overall, the North Pacific shows very little spatial subdivision among the Japan, Immature Hawaii, Mature Hawaii, and Southern California locations. The seasonal Southern California location does not have a known spawning location, and striped marlin in this area likely originate in Japan (Nishikawa et al. 1978). Fish from Japan may move eastward with the Kuroshiro Current to feeding grounds near the Hawaiian Islands; when currents or environmental conditions are right, some fish may continue eastward until they reach Southern California. The seasonal population in Southern California is not large, but their movement into the region is a regular occurrence, although the timing and number of fish each year varies. While it is uncertain how long the fish remain in this region, they may eventually utilize the North Equatorial Current to move back across the Pacific to their spawning grounds in Japan, or they may represent a population sink, not reproductively contributing to any population.

Based on the genetic analyses, Mexican and Central Amer-

ican striped marlin appear to form one stock that is differentiated from other Pacific populations. Despite the genetic exclusion of Southern Californian striped marlin from the rest of the eastern Pacific, tagging data indicate that striped marlin caught in Southern California move south into Baja California, Mexico, corresponding to cooling water temperatures off California (Domeier 2006). However, the Southern California fish do not appear in representative samples from Mexico; this may happen for a couple of reasons. First, their behavior in Mexico may reduce their catchability to the recreational fishery, which was the primary sample source in that region. This is plausible because the spawning season for the Mexican population coincides with the southward movement of Southern California fish, and evidence suggests that local striped marlin shift locations in preparation for spawning (Armas et al. 1999; Domeier 2006). Tournament catch rates indicate that the Mexican striped marlin population is far larger than the Southern California population. It would seem logical for recreational and commercial fishers to shift their effort to follow the larger, year-round population of Mexican fish to their spawning grounds. As the striped marlin from Southern California do not appear to be spawning in Mexico, they may largely escape the fishing pressure by not moving to the spawning locations. Alternatively, the Southern Californian striped marlin may not stay long in the waters off of Mexico, but rather move through Mexico to follow currents back across the Pacific. Through these (and possibly other) behavioral modifications, and with the relative size of the Southern California population compared with the Mexico population, it is not surprising that they are not significantly sampled within this region.

In the southwest Pacific, Australia and New Zealand form another independent stock. Australia displayed unusual genetic variability patterns, with high numbers of locus-pairs in genotypic disequilibrium and heterozygosity deviations. One possibility is gene flow occurring with Indian Ocean striped marlin on a semiregular basis, given the right environmental conditions (Bromhead et al. 2004), but with little genetic work done on Indian Ocean striped marlin, it is difficult to determine if mixing is driving these differences. If Pacific–Indian Ocean mixing was occurring, sequence variation would be expected to be higher here than in other locations, and the results do not support this hypothesis. However, if gene flow from the Indian Ocean was primarily male-mediated, then mitochondrial sequence variation would not be greatly impacted. Hybridization with other regional billfish species is another possibility. All maternal backgrounds were striped marlin, but a unidirectional hybrid cross is possible, though this has not been reported in billfish previously. Like Southern California, striped marlin in New Zealand are seasonal. The New Zealand fish likely originate from Australia and perhaps from other unsampled areas in the Central–South Pacific (Bromhead et al. 2004); they are noted for relatively high dispersal distances, likely correlated with changing water temperatures, in contrast with Australian striped marlin that make much shorter distance coastal shifts (Kopf et al. 2005; Domeier 2006).

The presumed large-scale movements underlying the genetic subdivision patterns of striped marlin during different portions of their life history, particularly in the North Pacific, demonstrate the need for a concurrent, temporally, and spatially representative sampling approach. While results of this study were similar to McDowell and Graves (2008), they did not analyze immature and mature fish separately; therefore no structure was detected between those groups in Hawaii. McDowell and Graves (2008) also found structure between Mexico and Ecuador, while in this study Mexico and Central America comprised a single stock. This difference is difficult to explain, as the larger numbers of markers and samples used in this study should increase resolution. One possible explanation is that the more southern location, Ecuador, showed stronger differentiation than Central America in this study; however, the collecting range for specimens from these two locations were relatively close geographically. Alternatively, this may represent sampling artifact in McDowell and Graves (2008), caused by relatively small, single collections in those locations (Mexico: $n = 32$, 1994; Ecuador: $n = 39$, 1995). Despite these differences, overall values of F_{ST} were similar (using microsatellites) between this study and the McDowell and Graves 2008 study (0.0145 and 0.0130, respectively). This study also showed concordance between the two molecular markers, including the same significant pairwise relationships (with the exception of mature Hawaiian specimens). This suggests that small sample size was likely the reason that McDowell and Graves (2008) did not detect significant subdivision in pairwise mtDNA estimates.

Conservation implications

While it is not easy to subdivide migratory species into distinctly manageable units, there are patterns from this study that can be applied at a management level. Based on all specimens, the north Pacific (Japan, Mature Hawaii, Immature Hawaii, and Southern California) showed little genetic differentiation among locations. However, subtle population structure between mature Hawaiian specimens and the other locations in this group indicates that effective migration (migration with reproductive contributions) between Hawaii and other North Pacific locations is probably limited. Therefore, it may be more accurate to consider them as separate stocks even though fishing efforts in Hawaii can impact both Hawaiian and Japanese striped marlin populations. The Hawaiian location is clearly important to several populations within the North Pacific, and because of this connection, temporal analyses may be very informative in managing this area. Next, Mexico and Central America appear differentiated from the other Pacific locations. Despite the movement of Southern Californian fish through that region, the eastern Pacific should be managed as a separate stock. Finally, the Southwest Pacific (Australia and New Zealand) form another distinctive stock. Australia, in particular, may be impacted by migration or hybridization in ways that are different from other regions in the Pacific.

Based on the analysis of over 1000 specimens collected from seven locations, using two classes of molecular markers, striped marlin populations showed shallow but significant overall genetic subdivision with evidence for at least three (Australia – New Zealand, Japan – Southern California – Hawaii, Mexico – Central America) and possibly four (Mature Hawaii) putative stocks in the Pacific. What may be more complicated is the level of independence of these groups, particularly with the connections in the North Pacific and possibly the Australian – New Zealand group with poten-

tial ties to the Indian Ocean. However, for a pelagic species capable of long migrations, striped marlin show considerable spatial variation throughout their range in the Pacific.

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References

Alvarado Bremer, J.R., Vinas, J., Mejuto, J., Ely, B., and Pla, C. 2005. Comparative phylogeography of Atlantic bluefin tuna and swordfish: the combined effects of vicariance, secondary contact, introgression, and population expansion on the regional phylogenies of two highly migratory pelagic fishes. Mol. Phylogenet. Evol. **36**(1): 169–187. doi:10.1016/j.ympev.2004.12.011. PMID: 15904864.

Armas, R.G., Sosa-Nishizaki, O., Rodríquez, R.F., and Perez, V.A.L. 1999. Confirmation of the spawning area of the striped marlin, *Tetrapturus audax*, in the so-called core area of the eastern tropical Pacific off Mexico. Fish. Oceanogr. **8**(3): 238–242. doi:10.1046/j.1365-2419.1999.00102.x.

Belkir, K., Borsa, P., Chickhi, L., Raufaste, N., and Bonhomme, F. 2000. GENETIX 4.04 Logiciel sous Windows TM, pour la Génétique des Populations. Laboratoire Génome, Populations, Interactions, Universite de Montpellier II, Montpellier, France.

Bernatchez, L., Guyomard, R., and Bonhomme, F. 1992. DNA sequence variation of the mitochondrial control region among geographically and morphologically remote European brown trout, *Salmo trutta*, populations. Mol. Ecol. **1**(3): 161–173. doi:10.1111/j.1365-294X.1992.tb00172.x. PMID:1344992.

Block, B., and Reeb, C. 2000. Genetic analysis of population structure in Pacific swordfish (*Xiphias gladius*) using microsatellite DNA techniques. Annual Report for Fiscal Year 2000. Joint Institute for Marine and Atmospheric Research, Pelagic Fisheries Research Division, Honolulu, Hawaii.

Bromhead, D., Pepperell, J., Wise, B., and Findlay, J. 2004. Striped marlin: biology and fisheries. Bureau of Rural Sciences, Canberra, Australia. ISBN: 0642475938.

Buonaccorsi, V., and Graves, J. 2000. Isolation and characterization of novel polymorphic tetra-nucleotide microsatellite markers from the blue marlin, *Makaira nigricans*. Mol. Ecol. **9**(6): 820–821. doi:10.1046/j.1365-294x.2000.00915-2.x. PMID:10849299.

Buonaccorsi, V.P., McDowell, J.R., and Graves, J.E. 2001. Reconciling patterns of inter-ocean molecular variance from four classes of molecular markers in blue marlin (*Makaira nigricans*). Mol. Ecol. **10**(5): 1179–1196. doi:10.1046/j.1365-294X.2001.01270.x. PMID:11380876.

Carlsson, J., McDowell, J.R., Diaz-Jaimes, P., Carlsson, J.E., Boles, S.B., Gold, J.R., and Graves, J.E. 2004. Microsatellite and mitochondrial DNA analyses of Atlantic bluefin tuna (*Thunnus thynnus thynnus*) population structure in the Mediterranean Sea. Mol. Ecol. **13**(11): 3345–3356. doi:10.1111/j.1365-294X.2004.02336.x. PMID:15487994.

Chen, J., Iannone, M.A., Li, M.S., Taylor, J.D., Rivers, P., Nelsen, A.J., Slentz-Kesler, K.A., Roses, A., and Weiner, M.P. 2000. A microsphere-based assay for multiplexed single nucleotide polymorphism analysis using single base chain extension. Genome Res. **10**(4): 549–557. doi:10.1101/gr.10.4.549. PMID:10779497.

Dakin, E.E., and Avise, J.C. 2004. Microsatellite null alleles in parentage analysis. Heredity, **93**(5): 504–509. doi:10.1038/sj.hdy.6800545. PMID:15292911.

Domeier, M.L. 2006. An analysis of Pacific striped marlin (*Tetrapturus audax*) horizontal movement patterns using pop-up satellite archival tags. Bull. Mar. Sci. **79**(3): 811–825.

Durand, J., Collet, A., Chow, S., Guinand, B., and Borsa, P. 2005. Nuclear and mitochondrial DNA markers indicate unidirectional gene flow of Indo-Pacific to Atlantic bigeye tuna (*Thunnus obesus*) populations, and their admixture off southern Africa. Mar. Biol. **147**(2): 313–322. doi:10.1007/s00227-005-1564-2.

Edmands, S., Feaman, H.V., Harrison, J.S., and Timmerman, C.C. 2005. Genetic consequences of many generations of hybridization between divergent copepod populations. J. Hered. **96**(2): 114–123. doi:10.1093/jhered/esi014. PMID:15618307.

Ely, B., Vinas, J., Alvarado Bremer, J.R., Black, D., Lucas, L., Covello, K., Labrie, A.V., and Thelen, E. 2005. Consequences of the historical demography on the global population structure of two highly migratory cosmopolitan marine fishes: the yellowfin tuna (*Thunnus albacares*) and the skipjack tuna (*Katsuwonus pelamis*). BMC Evol. Biol. **5**(1): 19. doi:10.1186/1471-2148-5-19. PMID:15725349.

Excoffier, L., Laval, G., and Schneider, S. 2005. Arlequin ver. 3.0: an integrated software package for population genetics data analysis. Evol. Bioinform. Online, **1**: 47–50.

Feulner, P.G.D., Bielfeldt, W., Zachos, F.E., Bradvarovic, J., Eckert, I., and Hartl, G.B. 2004. Mitochondrial DNA and microsatellite analyses of the genetic status of the presumed subspecies *Cervus elaphus montanus* (Carpathian red deer). Heredity, **93**(3): 299–306. doi:10.1038/sj.hdy.6800504. PMID:15241451.

Finnerty, J., and Block, B. 1992. Direct sequencing of mitochondrial DNA detects highly divergent haplotypes in blue marlin (*Makaira nigricans*). Mol. Mar. Biol. Biotechnol. **1**(3): 206–214. PMID: 1308204.

Gonzalez, E.G., Beerli, P., and Zardoya, R. 2008. Genetic structuring and migration patterns of Atlantic bigeye tuna, *Thunnus obesus* (Lowe, 1839). BMC Evol. Biol. **8**(1): 252. doi:10.1186/1471-2148-8-252. PMID:18798987.

Goudet, J. 1995. FSTAT version 1.2: a computer program to calculate F statistics. J. Hered. **86**(6): 485–486.

Graves, J.E., and McDowell, J.R. 1994. Genetic analysis of striped marlin (*Tetrapturus audax*) population structure in the Pacific. Can. J. Fish. Aquat. Sci. **51**(8): 1762–1768. doi:10.1139/f94-177.

Graves, J.E., and McDowell, J.R. 1995. Inter-ocean genetic divergence of istiophorid billfishes. Mar. Biol. **122**(2): 193–203.

Hanamoto, E. 1977. Fishery oceanography of striped marlin. Part 2: Spawning activity of the fish in the Southern Coral Sea. SO-Nippon Suisan Gakkaishi, Bull. Jpn. Soc. Sci. Fish. **43**: 1279–1286.

Hauser, L., Turan, C., and Carvalho, G.R. 2001. Haplotype frequency distribution and discriminatory power of two mtDNA fragments in a marine pelagic teleost (Atlantic herring, *Clupea harengus*). Heredity, **87**(6): 621–630. doi:10.1046/j.1365-2540.2001.00956.x. PMID:11903557.

Hedgecock, D., Li, G., Hubert, S., Bucklin, K., and Ribes, V. 2004. Widespread null alleles and poor cross-species amplification of microsatellite DNA loci cloned from the Pacific Oyster, *Crassostrea gigas*. J. Shellfish Res. **23**(2): 379–385.

Hedrick, P.W. 2005. A standardized genetic differentiation measure. Evolution, **59**(8): 1633–1638. PMID:16329237.

Hudson, R.R. 2000. A new statistic for detecting genetic differentiation. Genetics, **155**(4): 2011–2014. PMID:10924493.

Hudson, R.R., Boos, D.D., and Kaplan, N.L. 1992. A statistical test

for detecting geographic subdivision. Mol. Biol. Evol. **9**(1): 138–151. PMID:1552836.

Hyde, J.R., Humphreys, R., Musyl, M., Lynn, E., and Vetter, R. 2006. A central north Pacific spawning ground for striped marlin, *Tetrapturus audax*. Bull. Mar. Sci. **79**(3): 683–690.

Johnson, M.S., and Black, R. 1984. The Wahlund effect and the geographical scale of variation in the intertidal limpet *Siphonaria* sp. Mar. Biol. **79**(3): 295–302. doi:10.1007/BF00393261.

Kalinowski, S., Wagner, A., and Taper, M. 2006. ML-Relate: a computer program for maximum likelihood estimation of relatedness and relationship. Mol. Ecol. Notes, **6**(2): 576–579. doi:10.1111/j.1471-8286.2006.01256.x.

Kamimura, T., and Honma, M. 1958. A population study of the so-called *makajiki* (striped marlin) of both northern and southern hemispheres of the Pacific. Part I: Comparison of external characters. Rep. Nankai Fish. Res. Lab. **8**: 1–11. [Translated by I. Nakamura.]

Keeney, D.B., Heupel, M.R., Hueter, R.E., and Heist, E.J. 2005. Microsatellite and mitochondrial DNA analyses of the genetic structure of blacktip shark (*Carcharhinus limbatus*) nurseries in the northwestern Atlantic, Gulf of Mexico, and Caribbean Sea. Mol. Ecol. **14**(7): 1911–1923. doi:10.1111/j.1365-294X.2005.02549.x. PMID:15910315.

Kopf, R.K., Davie, P.S., and Holdsworth, J.C. 2005. Size trends and population characteristics of striped marlin, *Tetrapturus audax*, caught in the New Zealand recreational fishery. NZ J. Mar. Freshw. Res. **39**(5): 1145–1156. doi:10.1080/00288330.2005.9517381.

Larkin, M.A., Blackshields, G., Brown, N.P., Chenna, R., McGettigan, P.A., McWilliam, H., Valentin, F., Wallace, I.M., Wilm, A., Lopez, R., Thompson, J.D., Gibson, T.J., and Higgins, D.G. 2007. Clustal W and Clustal X version 2.0. Bioinformatics, **23**(21): 2947–2948. doi:10.1093/bioinformatics/btm404. PMID:17846036.

Lee, W., Conroy, J., Howell, W., and Kocher, T. 1995. Structure and evolution of teleost mitochondrial control regions. J. Mol. Evol. **41**(1): 54–66. doi:10.1007/BF00174041. PMID:7608989.

Lenfant, P., and Planes, S. 2002. Temporal genetic changes between cohorts in a natural population of a marine fish, *Diplodus sargus*. Biol. J. Linn. Soc. **76**(1): 9–20. doi:10.1111/j.1095-8312.2002.tb01710.x.

Manel, S., Gaggiotti, O.E., and Waples, R.S. 2005. Assignment methods: matching biological questions with appropriate techniques. Trends Ecol. Evol. **20**(3): 136–142. doi:10.1016/j.tree.2004.12.004. PMID:16701357.

Matsumoto, W.M., and Kazama, T.K. 1974. Occurrence of young billfishes in the central Pacific Ocean. *In* Proceedings of the International Billfish Symposium Kailua-Kona, Hawaii, 9–12 August 1972. Part 2. *Edited by* R.S. Shomura and F. Williams. National Marine Fisheries Service, Seattle, Wash. NOAA Tech. Rep. NMFS SSRF-675. pp. 238–247.

McDowell, J.R., and Graves, J.E. 2008. Population structure of striped marlin (*Kajikia audax*) in the Pacific Ocean based on analysis of microsatellite and mitochondrial DNA. Can. J. Fish. Aquat. Sci. **65**(7): 1307–1320. doi:10.1139/F08-054.

Mejuto, J., Garcia-Cortes, B., and De La Serna, J.M. 2002. Preliminary scientific estimations of billfish (Family Istiophoridae) landed by the Spanish surface longline fleet targeting swordfish in the Atlantic Ocean and Mediterranean Sea: years 1999–2000. Col. Vol. Sci. Pap. ICCAT, **54**: 826–833.

Morrow, J.E. 1957. Shore and pelagic fishes from Peru, with new records and the description of a new species of *Sphoeroides*. Bull. Bingham Oceanogr. Collect. **16**: 5–55.

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.

Nei, M., and Chesser, R.K. 1983. Estimation of fixation indices and gene diversities. Ann. Hum. Genet. **47**(3): 253–259. doi:10.1111/j.1469-1809.1983.tb00993.x. PMID:6614868.

Nishikawa, Y., Kikawa, S., Honma, M., and Ueyanagi, S. 1978. Distribution atlas of larval tunas, billfishes and related species: results of larval surveys by R/V *Shunyo Maru*, and *Shoyo Maru*, 1956–1975. Far Seas Fisheries Research Laboratory, Shimizu, Japan. pp. 1–100.

O'Reilly, P.T., Canino, M.F., Bailey, K.M., and Bentzen, P. 2004. Inverse relationship between F_{ST} and microsatellite polymorphism in the marine fish, walleye pollack (*Theragra chalcogramma*): implications for resolving weak population structure. Mol. Ecol. **13**(7): 1799–1814. doi:10.1111/j.1365-294X.2004.02214.x. PMID:15189204.

Palumbi, S.R., Martin, A.P., Romano, S., McMillan, W.O., Stice, L., and Grabowski, G. 1991. The simple fool's guide to PCR. Spec. Publ. Department of Zoology, University of Hawaii, Honolulu, Hawaii.

Pritchard, J.K., Stephens, M., and Donnelly, P. 2000. Inference of population structure using multilocus genotype data. Genetics, **155**(2): 945–959. PMID:10835412.

Purcell, C.M. 2009. Genetic analysis of population structure in striped marlin, *Tetrapturus audax*, in the Pacific Ocean. Ph.D. dissertation, Department of Biological Sciences, University of Southern California, Los Angeles, Calif.

Purcell, C.M., Harrison, J.S., and Edmands, S. 2009. Isolation and characterization of 10 polymorphic microsatellite markers from striped marlin, *Tetrapturus audax*. Mol. Ecol. Resour. **9**(6): 1556–1559. doi:10.1111/j.1755-0998.2009.02709.x. PMID:21564958.

Raymond, M., and Rousset, F. 1995. GENEPOP 3.3: population genetic software for exact test and ecumenism. J. Hered. **86**: 248–249.

Rooker, J.R., Alvarado Bremer, J.R., Block, B.A., Dewar, H., De Metrio, G., Corriero, A., Kraus, R.T., Prince, E.D., Rodriguez-Marin, E., and Secor, D.H. 2007. Life history and stock structure of Atlantic bluefin tuna (*Thunnus thynnus*). Rev. Fish. Sci. **15**(4): 265–310. doi:10.1080/10641260701484135.

Rozas, J., Sánchez-Delbarrio, J.C., Messeguer, X., and Rozas, R. 2003. DnaSP, DNA polymorphism analyses by the coalescent and other methods. Bioinformatics, **19**(18): 2496–2497. doi:10.1093/bioinformatics/btg359. PMID:14668244.

Saenz-Agudelo, P., Jones, G.P., Thorrold, S.R., and Planes, S. 2009. Estimating connectivity in marine populations: an empirical evaluation of assignment tests and parentage analysis under different gene flow scenarios. Mol. Ecol. **18**(8): 1765–1776. doi:10.1111/j.1365-294X.2009.04109.x. PMID:19243510.

Seutin, G., White, B.N., and Boag, P.T. 1991. Preservation of avian blood and tissue samples for DNA analysis. Can. J. Zool. **69**(1): 82–90. doi:10.1139/z91-013.

Shomura, R.S. 1980. Summary report of the billfish stock assessment workshop Pacific resources. US Dept. Comm. NOAA-TM-NMFS-SWFC-5.

Squire, J. 1972. Catch distribution and related sea surface temperature for striped marlin (*Tetrapturus audax*) caught off San Diego, California. Proceedings of the International Billfish Symposium 1972. NOAA Tech. Rep. NMFS SSRF-675 pp. 188–193.

Squire, J., and Suzuki, Z. 1990. Migration trends of striped marlin (*Tetrapturus audax*) in the Pacific Ocean. Planning the future of billfish research in the 90s and beyond. Part II. Contributed papers. *Edited by* R.H. Stroud. National Coalition for Marine Conservation, Savannah, Ga. pp. 67–80.

Tamura, K., Dudley, J., Nei, M., and Kumar, S. 2007. MEGA4: Molecular Evolutionary Genetics Analysis (MEGA) software

version 4.0. Mol. Biol. Evol. **24**(8): 1596–1599. doi:10.1093/molbev/msm092. PMID:17488738.

Theisen, T.C., Bowen, B.W., Lanier, W., and Baldwin, J.D. 2008. High connectivity on a global scale in the pelagic wahoo, *Acanthocybium solandri* (tuna family Scombridae). Mol. Ecol. **17**(19): 4233–4247. doi:10.1111/j.1365-294X.2008.03913.x. PMID:19378403.

Ward, R.D., Woodwark, M., and Skibinski, D.O.F. 1994. A comparison of genetic diversity levels in marine, freshwater and anadromous fishes. J. Fish Biol. **44**(2): 213–232. doi:10.1111/j.1095-8649.1994.tb01200.x.

Weir, B.S., and Cockerham, C.C. 1984. Estimating F-statistics for the analysis of population structure. Evolution, **38**(6): 1358–1370. doi:10.2307/2408641.

Worm, B., Sandow, M., Oschlies, A., Lotze, H.K., and Myers, R.A. 2005. Global patterns of predator diversity in the open oceans. Science, **309**(5739): 1365–1369. doi:10.1126/science.1113399. PMID:16051749.