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# Genetic Identity of YOY Bluefin Tuna from the Eastern and Western Atlantic Spawning Areas

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## Abstract

We used 320 young-of-the-year (YOY) specimens of the highly migratory and overfished Atlantic bluefin tuna, *Thunnus thynnus*, Linnaeus 1758, to evaluate the hypothesis that Atlantic bluefin tuna comprises 2 stocks with spawning grounds in the Gulf of Mexico and in the Mediterranean Sea. Significant genetic differentiation at 8 nuclear microsatellite loci ( $F_{ST} = 0.0059$ ,  $P = 0.0005$ ) and at the mitochondrial control region ( $\Phi_{ST} = 0.0129$ ,  $P = 0.0139$ ) was detected among YOY Atlantic bluefin tuna captured on spawning grounds in the Gulf of Mexico ( $n = 40$ ) versus the western ( $n = 255$ ) and eastern ( $n = 25$ ) basins of the Mediterranean Sea. The genetic divergence among spawning populations, combined with the extensive trans-Atlantic movements reported for juvenile and adult Atlantic bluefin tuna, indicates a high degree of spawning site fidelity. Recognition of genetically distinct populations necessitates independent management of Atlantic bluefin tuna on spawning grounds and warrants evaluation of the level of mixing of populations on feeding grounds. The genetic pattern might not have been detected unless juvenile specimens or actively spawning adults had been sampled.

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The Atlantic bluefin tuna, *Thunnus thynnus*, Linnaeus 1758, is a highly migratory species that supports a fishery throughout the North Atlantic Ocean and Mediterranean Sea. Increasing catches over the last several decades have resulted in severe overfishing and a rapid decline in abundance (ICCAT 2003). The Atlantic bluefin tuna is now considered to be one of the most depleted tuna species (Safina 1993; Magnuson et al. 1994) and was listed as endangered at the 8th Convention on International Trade in Endangered Species of Wild Fauna and Flora held in Kyoto (c.f., Taniguchi 2003). The International Commission for the Conservation of Atlantic Tunas (ICCAT) manages Atlantic bluefin tuna as separate western and eastern stocks, separated by the 45° W meridian. The stocks were originally recognized based on, among other observations, discontinuities in catches across the North Atlantic and the presence of 2 spawning areas, the Gulf of Mexico in the west and the Mediterranean Sea in the east (Mather et al. 1995; Fromentin and Powers 2005).

Tagging and microchemistry studies indicate that Atlantic bluefin tuna undertake transoceanic movements and that fish from the 2 putative stocks are mixed on the feeding grounds throughout the North Atlantic (Mather et al. 1995; Block et al. 2001, 2005; Rooker et al. 2003). Results of genetic studies employing a variety of molecular markers have not been

consistent. Broughton and Gold (1997) used microsatellites and found indications of Atlantic-wide genetic population structure in Atlantic bluefin tuna, but due to low sample sizes and age composition of samples, they were reluctant to draw explicit conclusions. Pujolar et al. (2003) found significant differences in allele frequencies at one allozyme locus between samples from the western Atlantic and the Mediterranean Sea, but this difference disappeared when more loci were included. Hence, to date, no study has unambiguously demonstrated population genetic structure between the putative eastern and western Atlantic bluefin tuna stocks (e.g., Edmunds and Sammons 1973; Takagi et al. 1999; Ely et al. 2002; Alvarado Bremer et al. 2005). It should be noted that all previous studies have included subadult and adult specimens that may have undertaken trans-Atlantic movements, and thus, the natal origin of these fish remains uncertain (Graves et al. 1996; Carlsson et al. 2004).

The extensive trans-Atlantic movements and the lack of significant genetic differences between the putative populations of Atlantic bluefin tuna have led researchers to question the validity of the 2-stock management approach. Information about population structure is critical for conservation efforts, and the absence of these data is impeding effective management of the highly migratory Atlantic bluefin tuna

as the current management strategy employed by ICCAT is based on the unconfirmed existence of 2 stocks.

The present study is based on a new sample consisting of Atlantic bluefin tuna larvae caught in the Gulf of Mexico. The pronounced migratory capacity of Atlantic bluefin tuna makes it crucial that population genetic studies are based on fish of known natal origin. Thus, previously analyzed young-of-the-year (YOY) Atlantic bluefin tuna from the Mediterranean Sea (Carlsson et al. 2004) were compared with YOY collected from the Gulf of Mexico to rigorously evaluate the genetic basis of the 2-stock hypotheses currently used to manage the species.

In this study, we tested the null hypothesis that there are no genetic differences between (YOY) Atlantic bluefin tuna caught at spawning grounds in the Mediterranean Sea and the Gulf of Mexico.

## Materials and Methods

### Biological Materials

YOY Atlantic bluefin tuna were collected from the known spawning grounds in the Gulf of Mexico and the Mediterranean Sea by commercial and scientific operations. Larvae were sampled in 2003 from the Gulf of Mexico (GM,  $n = 40$ ) and larger YOY (not exceeding 35 cm in total length and weighing less than 2 kg) were sampled in 1998 through 2002 from the western (WM,  $n = 255$ ) and the eastern (EM,  $n = 25$ ) basins of the Mediterranean Sea (Carlsson et al. 2004). Total genomic DNA was isolated and screened for genetic variability at 8 microsatellite loci as in Carlsson et al. (2004); *Tth5*, *Tth8*, *Tth10*, *Tth21*, and *Tth34* (McDowell et al. 2002) and *Ttho-1*, *Ttho-4*, and *Ttho-7* (Takagi et al. 1999). Approximately 20% of the individuals were rerun to ensure consistency in microsatellite allele scoring. In addition, a subset of individuals (GM,  $n = 40$ ; WM,  $n = 83$ ; and EM,  $n = 24$ ) were screened for nucleotide sequence variation within the mtDNA control region (847 bp) as in Carlsson et al. (2004).

### Statistical Analyses

To rule out the possibility that technical artifacts affected our results, we analyzed the microsatellite data set with MICRO-CHECKER 2.2.1 (van Oosterhout et al. 2004) (1000 randomizations) and found no evidence of stutter, large allele dropout, or null alleles. The ARLEQUIN 2.000 software (Schneider et al. 1997) was used to estimate observed and expected heterozygosities, deviations from Hardy–Weinberg equilibrium (HWE; 100,000 Markov Chain Steps and 1000 dememorisation steps), estimations of  $F_{ST}$  (10,000 permutations),  $\Phi_{ST}$  (10,000 permutations), nucleotide diversity ( $\pi$ ), and haplotype diversity ( $h$ ). The  $\Phi_{ST}$  analyses were performed using a matrix of Tamura and Nei (1993) distances. DNASP 4.0 (Rozas et al. 2003) was used to estimate the nearest-neighbor statistic,  $S_{nn}$  (Hudson 2000), for the mtDNA control region sequences (10,000 permutations).  $S_{nn}$  is a measure of how often the “nearest neighbors” (in sequence space) occur in the same locality in geographic space, and it is particularly suitable when haplotype diversity is large and sample

sizes are small (Hudson 2000). PAUP\* 4.0 (Swofford 2002) was used to draw an unweighted pair group method with arithmetic mean tree of the relationship between haplotypes.

Previous analyses of molecular variance including the temporal replicate samples from the Mediterranean Sea by Carlsson et al. (2004) indicated temporal stability of allele frequencies ( $F_{SC} = -0.0020$ ,  $P = 0.883$ ). However, as we lacked temporal replicate samples for the Gulf of Mexico, we could not use this approach to test for temporal stability in Gulf of Mexico. Hence, the maximum likelihood approach implemented in the COLONY 1.2 software (Wang 2004) was used on the microsatellite data to partition the Gulf of Mexico sample into full-sibling families. The software can take in to account both mistyping and allelic dropouts. We did not suspect that the data were suffering from allele dropout. We allowed, however, for 2% mistyping. We incorporated the variation in the number of families by repeating the analysis 10 times with each run having a new seed for the random number generator. The results presented here are, therefore, the average number of full-sibling families detected by COLONY 1.2 over 10 runs.

The sequential Bonferroni technique (Rice 1989) was used to adjust  $\alpha$ -levels in cases with multiple tests as indicated. The mtDNA control region sequences can be viewed in GenBank (accession numbers AY699888–AY699146 and DQ087532–DQ087594).

## Results

### Genetic Variability

All 8 microsatellite loci were variable in all collections, and the number of alleles per sample ranged from 2 to 20 [average = 9.13, standard error (SE) = 2.24, Table 1]. Observed and expected heterozygosities ranged from 0.35 to 0.92 (average = 0.66, SE = 0.03, Table 1) and from 0.43 to 0.88 per locus and sample (average = 0.66, SE = 0.03, Table 1), respectively. Probability tests of genotype frequencies for each microsatellite locus in each sample revealed a significant departure from HWE only at *Tth5* and *Tth10* both prior to and after Bonferroni correction ( $\alpha = 0.05$ ,  $k = 8$ ). The departures were not consistent across populations and only occurred in the WM sample. The mtDNA control region sequences were highly variable in all samples. Seven sequences found in the Mediterranean sample (Carlsson et al. 2004; this study) grouped with albacore, *Thunnus alalunga* (Bonnaterre, 1788), and were subsequently removed from further mtDNA analyses. It is unlikely that these individuals were misidentified as albacore as they were morphologically confirmed to be Atlantic bluefin tuna at the time of capture. Nor did these individuals exhibit rare or private alleles (data not shown), and hence, they were included in the microsatellite analyses. The number of haplotypes, and thus the minimum number of females contributing to each collection, was 38 in GM, 62 in WM, and 21 in EM (Table 2). The number of unique haplotypes was 37 in GM, 57 in WM, and 15 in EM (Table 2). Haplotype ( $h$ ) and nucleotide diversity ( $\pi$ ) per sample ranged from 0.947 to 0.998 and 0.012 to 0.023, respectively

**Table 1.** Summary statistics for 8 microsatellite loci among Atlantic bluefin tuna collections. Number of individuals ( $n$ ), number of alleles ( $a$ ), allele size range in base pairs ( $as$ ), expected heterozygosity ( $H_E$ ) and observed heterozygosity ( $H_O$ ), and probability values of concordance with Hardy–Weinberg Expectations (HWE). Values in bold face represent significant probability estimates after correction for multiple tests ( $\alpha = 0.05$ ,  $k = 8$ )

Sample	Locus								Average across loci
	<i>Tth5</i>	<i>Tth8</i>	<i>Tth10</i>	<i>Tth21</i>	<i>Tth34</i>	<i>Ttho-1</i>	<i>Ttho-4</i>	<i>Ttho-7</i>	
GM									
$n$	40	40	40	40	40	40	40	40	
$a$	5	11	3	3	15	7	10	16	8.750
$as$	117–133	296–346	114–122	125–133	101–169	181–195	136–166	182–226	
$H_E$	0.509	0.779	0.428	0.547	0.714	0.661	0.734	0.862	0.654
$H_O$	0.600	0.775	0.350	0.450	0.800	0.700	0.700	0.800	0.647
HWE	0.164	0.260	0.561	0.354	0.400	0.410	0.138	0.082	
WM									
$n$	255	255	255	255	255	255	255	255	
$a$	8	16	3	4	20	10	16	17	11.750
$as$	125–189	294–346	118–124	117–133	101–185	181–197	128–188	180–224	
$H_E$	0.510	0.789	0.440	0.478	0.803	0.625	0.796	0.855	0.662
$H_O$	0.478	0.776	0.522	0.486	0.820	0.616	0.796	0.871	0.670
HWE	<b>0.001</b>	0.254	<b>0.001</b>	0.595	0.632	0.642	0.489	0.822	
EM									
$n$	25	25	25	25	25	25	25	25	
$a$	3	9	2	2	14	7	7	11	6.875
$as$	125–133	298–348	118–122	125–129	101–165	179–195	138–164	198–224	
$H_E$	0.489	0.811	0.510	0.513	0.831	0.549	0.744	0.876	0.666
$H_O$	0.520	0.880	0.520	0.480	0.920	0.440	0.760	0.800	0.665
HWE	1.000	0.073	1.000	1.000	0.532	0.142	0.663	0.638	

(Table 2). The average number of full-sibling families represented in the Gulf of Mexico sample was estimated with the COLONY 1.2 software to be 28.7 (average family size over 10 replicate runs = 1.41 and average SE over 10 runs = 0.13).

### Population Structure

The distribution of nuclear microsatellite genotypes ( $n = 320$ ) and mitochondrial (mtDNA) control region sequences ( $n = 147$ ) among collections of YOY Atlantic bluefin tuna from GM, WM, and EM refuted the null hypothesis of no genetic differences. Analyses of microsatellite allele frequencies indicated population genetic structure (global multilocus  $F_{ST} = 0.0059$ ,  $P = 0.0005$ ), and the pairwise multilocus  $F_{ST}$  estimates indicated significant differentiation between WM and GM ( $F_{ST} = 0.0048$ ,  $P = 0.0260$ ), WM and EM ( $F_{ST} = 0.0067$ ,  $P = 0.0279$ ), and GM and EM ( $F_{ST} = 0.0117$ ,  $P = 0.0236$ ).

Similarly, the global  $\Phi_{ST}$  (mtDNA control region sequences) was significantly different from zero ( $\Phi_{ST} = 0.0129$ ,  $P = 0.0139$ ). Pairwise  $\Phi_{ST}$  analyses demonstrated that WM and GM were significantly differentiated ( $\Phi_{ST} = 0.0104$ ,  $P = 0.0359$ ), and WM was differentiated from EM ( $\Phi_{ST} = 0.0174$ ,  $P = 0.0482$ ), while no significant difference was found between GM and EM ( $\Phi_{ST} = 0.0134$ ,  $P = 0.1105$ ). The small sample size from EM lowered the power of the statistical analysis and likely contributed to the lack of significant  $\Phi_{ST}$  between GM and EM ( $n = 25$ ). To further test for evidence of population differentiation at the mtDNA locus, we calculated the nearest-neighbor statistic (Hudson 2000),

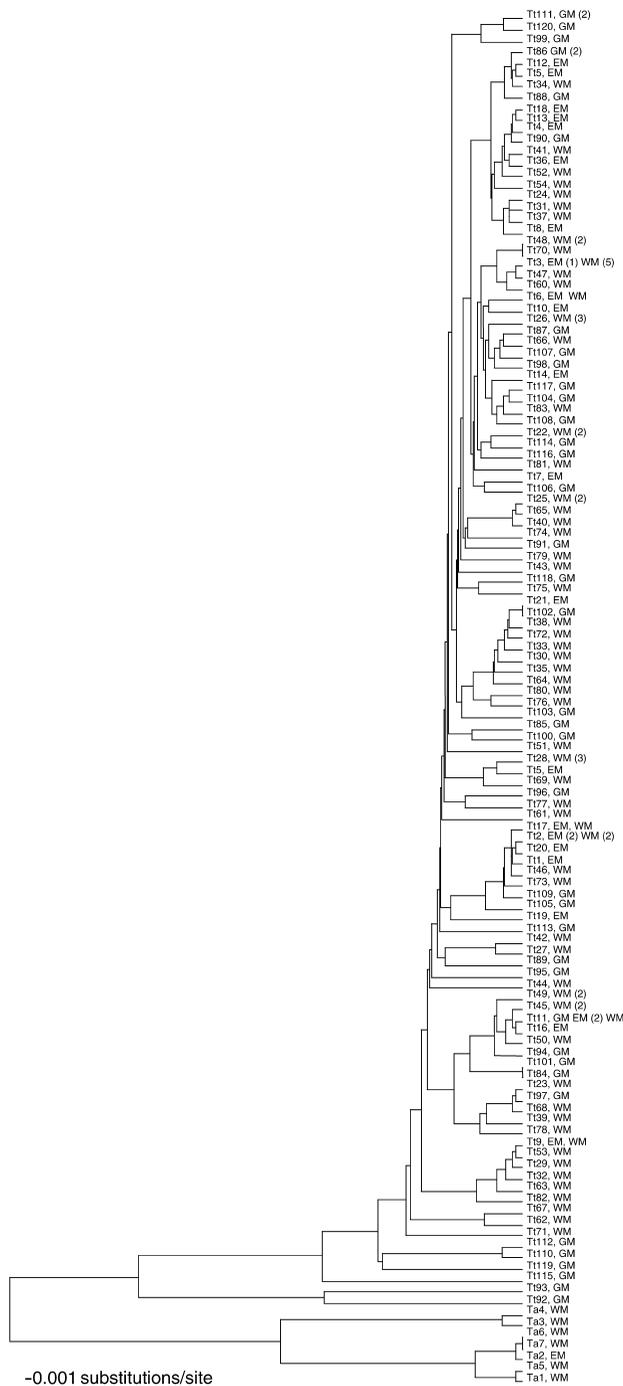
which revealed a significant association between sequence similarity and geographic location ( $S_{nn} = 0.564$ ,  $P < 0.001$ ). The association between the haplotypes is shown in Figure 1.

### Discussion

The finding of significant Atlantic-wide population structure based on both nuclear loci and mtDNA for YOY Atlantic bluefin tuna is remarkable in light of the absence of pronounced barriers that might limit gene flow among populations and the high trans-Atlantic migration rates demonstrated by tagging studies, as an exchange of even a small number of spawners is likely to rapidly degrade genetic population structure (e.g., Wright 1951). Population genetic structure has been studied in other highly migratory pelagic species, including white marlin, *Tetrapturus albidus* Poey, 1860; blue marlin, *Makaira nigricans* Lacepède, 1802; sailfish, *Istiophorus platypterus* Shaw and Nodder, 1791; and swordfish *Xiphias*

**Table 2.** MtDNA sequence variability in the control region for Atlantic bluefin tuna from the Gulf of Mexico and the Mediterranean Sea. Number of individuals ( $n$ ), number of haplotypes ( $nh$ ), number of unique haplotypes ( $nub$ ), haplotype diversity ( $h$ ), and nucleotide diversity ( $\pi$ )

Collection	$N$	$nh$	$nub$	$h$	$\pi$
GM	40	38	37	0.997	0.024
WM	77	62	57	0.992	0.015
EM	23	21	15	0.949	0.012



**Figure 1.** Unweighted pair group method with arithmetic mean tree of Atlantic bluefin tuna (Tt) and albacore-like (Ta) mitochondrial control region sequences. The Gulf of Mexico collection is denoted with GM, western Mediterranean Sea with WM, and the eastern Mediterranean Sea with EM.

*gladius* Linnaeus, 1758. These studies have used restriction fragment length polymorphism analysis of whole-molecule mtDNA, sequencing of the mitochondrial control region, and analysis of nuclear microsatellite loci (Alvarado-

Bremer et al. 1995; Reeb et al. 2000; Buonacorsi et al. 2001; McDowell 2002; Graves and McDowell forthcoming). Blue marlin and sailfish have been found to exhibit structure between ocean basins, and structure was found within Pacific sailfish and Pacific swordfish samples. However, with the exception of swordfish, none of these analyses found evidence of population genetic structure within the Atlantic Ocean. This has been attributed to the relatively small size of the Atlantic basin (Graves and McDowell 2003).

One major concern with the use of YOY specimens for population genetic studies of marine species is how representative they are of their source populations. Genetic structures could be caused by sweepstake recruitment where only a few families are represented in the samples and not be representative of their population of origin (Li and Hedgecock 1998). Additionally, in situations with slight, though statistically significant, population genetic structures, it is important to assess if the structures are also biologically meaningful (Waples 1998). One solution to detect sweepstake recruitment is to establish if the observed structures are temporally stable (i.e., comparing samples from different years or cohorts). A second approach is to assess how many families are represented in the sample. The observation of similar allele frequencies across years in the collections from the Mediterranean Sea indicates temporal stability (Carlsson et al. 2004) and makes it unlikely that our samples were not representative of their source populations. The difficulties associated with obtaining rare Atlantic bluefin tuna larvae from the Gulf of Mexico precluded temporal sampling from this spawning area. Hence, we used the second approach and estimated the number of families in this sample to assess if it was severely affected by sweepstake recruitment or family effects. The COLONY analysis indicated that the Gulf of Mexico sample comprised some 29 full-sibling families produced by at least 38 females. The larger number of families detected by mtDNA haplotypes compared with that detected by nuclear microsatellites was probably caused by the limited number of microsatellite loci used. Inclusion of additional microsatellite loci may have increased the number of families. Combining the observations from COLONY and the number of unique mtDNA control region sequences makes it unlikely that our data were nonrepresentative, influenced by family effects or sweepstake recruitment. In addition, the exclusive use of YOY, which are unlikely to have undertaken long distance movements, ensures that the samples were representative of their respective spawning populations (Graves et al. 1996; Bowen et al. 2005).

The findings from the microsatellite markers were corroborated by inferences based on the mtDNA analysis, reinforcing the validity of the observed population structure. Introgression between Atlantic bluefin tuna and albacore has previously only been documented in the Mediterranean Sea (e.g., Viñas et al. 2003). The 7 albacore-like haplotypes encountered in this study were restricted to the samples from the Mediterranean Sea. The lack of albacore-like mtDNA in YOY Atlantic bluefin tuna from the Gulf of Mexico is consistent with the observed population structure inferred from microsatellite and mtDNA variability and warrants further study.

Nemerson et al. (2000) argued that differences in age at spawning between Atlantic bluefin tuna in the Gulf of Mexico and Mediterranean Sea, with fish in the Gulf of Mexico maturing later and at larger sizes than fish in the Mediterranean Sea, could be under genetic control. Thus, the suggestion that the low, but significant, population genetic structure we observed is biologically significant gains support from the observations of different size and age at spawning. Similarly, low but significant, genetic population structure of herring (*Clupea harengus* Linnaeus, 1758) and cod (*Gadus morhua* Linnaeus, 1758) have been observed to correlate with differences in environmental conditions and behavioral traits (Imsland and Jónsdóttir 2003; Jørgensen et al. 2005).

The discrepancies between our study and earlier studies of the Atlantic-wide population genetic structure of Atlantic bluefin tuna (e.g., Edmunds and Sammons 1973; Takagi et al. 1999; Ely et al. 2002; Alvarado Bremer et al. 2005) are best explained by our explicit use of YOY animals sampled at the spawning grounds (Graves et al. 1996). It is not certain that the genetic structure would have been detected if adults had been used as Atlantic bluefin tuna are known to perform trans-Atlantic migrations to feeding and breeding grounds (Mather et al. 1995; Block et al. 2001, 2005; Rooker et al. 2003). The present study represents the first investigation of Atlantic-wide population structure in Atlantic bluefin tuna that is exclusively based on YOY individuals. Strikingly, it is also the first study to demonstrate the independence of the Mediterranean Sea and Gulf of Mexico spawning areas. Our study emphasizes the advantage of basing studies of highly migratory animals with large population ranges on young specimens (i.e., captured prior to performing long-range migrations). The findings presented here also lend support to conclusions made by Bowen et al. (2005) who convincingly demonstrated that genetic studies of highly migratory species based on samples derived from individuals on or near feeding grounds can be misleading as they can fail detect fine-scaled population structure.

Here we present data suggesting that YOY Atlantic bluefin tuna in the Gulf of Mexico and in the Mediterranean Sea are the products of genetically differentiated spawning populations. Although, the present study analyzed data based on Atlantic bluefin tuna from the Mediterranean Sea and the Gulf of Mexico, it does not preclude the presence of additional spawning areas (Fromentin and Powers 2005). Observations of Atlantic bluefin larvae in other regions of the North Atlantic would warrant further genetic analyses. We propose that the genetic structure is maintained by natal homing, even though individuals tagged in different regions of the Atlantic are known to be mixed on feeding grounds (Mather et al. 1995; Block et al. 2001, 2005; Rooker et al. 2003). These results substantiate the results of tagging studies which suggest that Atlantic bluefin tuna show spawning site fidelity and that the Mediterranean Sea and Gulf of Mexico populations do not overlap on spawning grounds (Block et al. 2005). Further, our observations indicate genetic differentiation at purportedly selectively neutral loci between YOY Atlantic bluefin tuna from the Gulf of Mexico and the Mediterranean Sea and confirm that there could be a genetic basis

for the differences in age and size for maturity (Nemerson et al. 2000). Combining results from genetic analyses with those from tagging (e.g., Block et al. 2005) and microchemistry studies (Rooker et al. 2003) would be an effective approach for quantifying the extent of exchange between stocks, the levels of spawning at the different spawning grounds, age at which individuals commence long-range migrations, and in particular, the extent of mixing on feeding grounds—information critical for the effective management of the severely overfished Atlantic bluefin tuna.

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