

Assessment of the Genetic Structure of Yellowfin and Blackfin Tuna in the Atlantic Ocean

Evaluación de la Estructura Genética del Atún de Aleta Amarilla y Aleta Azul en el Océano Atlántico

Etude de la Structure Génétique des Populations de Thons Jaunes et Noirs dans L'océan Atlantique

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ABSTRACT

The Yellowfin Tuna, *Thunnus albacares*, and the Blackfin Tuna, *Thunnus atlanticus*, are two tropical tunas commonly found in waters of the Gulf of Mexico and Caribbean Sea. Yellowfin Tunas are harvested by major commercial and recreational fisheries in both the East and West Atlantic. Blackfin Tuna occupy offshore waters of the west Atlantic where they are increasingly targeted by recreational fisheries and by commercial fisheries in the Caribbean region and South America. Information on stock structure is essential in order to develop sustainable management plans for these two important fish. In this work, robust panels of homologous microsatellite markers were developed and used to perform a first assessment of genetic stock structure of the two species in the Atlantic. After optimization and removal of error-prone markers, 14 and 13 polymorphic microsatellites were available to study population structure in the yellowfin and Blackfin Tuna respectively. To date, Yellowfin Tuna markers have been genotyped in a total of 752 samples collected over 3 sampling years from 3 regions in the West Atlantic and 2 regions in the East Atlantic. Divergence among samples was low ($F_{ST} = 0.002$) and no clear geographic pattern of population structure was evidenced. A total of 471 Blackfin Tunas from 7 regional populations were genotyped. Divergence among samples was also low ($F_{ST} = 0.0008$) and results to date suggest occurrence of a weak pattern of isolation by distance. Further study incorporating high density genome scans is in progress.

KEYWORDS: *Thunnus albacares*, *Thunnus atlanticus*, population structure

INTRODUCTION

The Yellowfin Tuna (*Thunnus albacares*, Bonnaterre, 1788) and the Blackfin Tuna (*Thunnus atlanticus*, Lesson, 1830) are two tropical tunas common in waters of the Gulf of Mexico and Caribbean regions. The two species support commercial and recreational fisheries throughout their range yet essential data needed to develop effective management plans in the West Atlantic are still lacking.

Yellowfin Tuna are exploited both in the east and the west Atlantic although fishing effort is greater in the East Atlantic with a strong contribution of purse seine fisheries (ICCAT 2011). Assessment currently assumes a single stock for the Atlantic based on the continuous distribution of the species across the tropical portion of the basin, and accounting for the strong potential of Yellowfin Tunas for dispersal at all life stages. However, recent data indicate that movements of adults are restricted geographically (Schaefer et al. 2011) and studies of natural tags in Hawai indicated regional retention of juveniles and sub adults (Wells et al. 2012). Available tagging data in the Atlantic suggest relative fidelity of fish to either the eastern or western region (ICCAT 2011). Recent assessments of the Atlantic stock concluded that the stock was overfished when international thresholds are applied (ICCAT 2011). The species does not appear to be undergoing overfishing (NMFS 2014) but the status of the stock in the 2011 assessment had worsened as compared to that inferred during earlier assessments. Considering the high mobility of Yellowfin Tunas, the degree of connectivity between stocks in various regions of the Atlantic needs to be formally quantified to determine if multiple stocks need to be accounted for in assessment and management.

The Blackfin Tuna is a small tuna distributed in tropical and sub-tropical waters of the Western Atlantic Ocean (Collette and Nauen 1983). Historically, Blackfin Tunas were not sought for by recreational fishers, but they are now increasingly exploited through trolling and drift fishing along the East U.S. coast, in the Gulf of Mexico, off the Florida Keys, and in Puerto Rico. There is no commercial fishing for this species in the United States but Blackfin Tunas are

exploited commercially using pure seines and long lines in several Caribbean countries including Cuba, the Dominican Republic, the Lesser Antilles, Venezuela, and Brazil (Mathieu et al. 2013). As Yellowfin Tunas, Blackfin Tunas have a high potential for dispersal at all life stages. Tagging studies in St Vincent and the Grenadines or Bermuda revealed that a significant proportion of tagged fish were recaptured near the tagging location (Luckhurst et al. 2001, Singh-Renton and Renton 2007) sometimes over extended periods of time but other individuals tagged along the East Atlantic coast of the U.S. were recaptured far away from their tagging locations.

Current genetics research at the University of Southern Mississippi aims to clarify the structure of yellowfin and Blackfin Tuna populations in the Atlantic in order to determine if separate management of genetically distinct stocks is needed. In pilot projects, homologous microsatellites were designed and used to perform an initial survey of genetic variation among geographic populations. High density genome scans based on Single Nucleotide Polymorphisms (SNPs) are currently in development to characterize comprehensively neutral and non-neutral variations in regional stocks.

MICROSATELLITE MARKERS STUDIES

Homologous microsatellite markers were developed for each species at the beginning of this project. The characteristics of the developed markers and assay conditions are described in details in Antoni et al. (2014a,b). The loci retained for the study of spatial genetic variation in each species (14 loci for Yellowfin Tuna, 13 for Blackfin Tuna) are presented in Table 1. Most loci are highly polymorphic with on average 27.6 alleles detected per locus to date in Blackfin Tuna (range 11 - 58) and 31.9 (range 8-59) in Yellowfin Tuna.

Sampling for the initial study of population structure targeted adults of reproductive size and focused on the summer spawning season in order to describe the breeding structure of each species. Tissue sample acquisition was initiated in 2013.

Genetic Variation in Atlantic Yellowfin Tuna

Adult and sub-adult Yellowfin Tuna were collected via fishery-dependent sampling in two regions of the East Atlantic (off Senegal and Ghana) and 3 regions in the West Atlantic (U.S. East coast, Gulf of Mexico and southern Caribbean Sea offshore Venezuela). To date a total of 752 samples have been assayed at the 14 microsatellites including specimens collected during two sampling years in the northern Gulf of Mexico, Venezuela, Senegal, Ghana, and the U.S. East coast (Mid Atlantic Bight, North East Coastal, Florida East Coast and Sargasso fishing areas). Samples of juveniles from the lesser Antilles (La Martinique Island) and the Gulf of Mexico were also examined. Allele frequencies differ slightly among samples although the F_{ST} estimate from the current dataset is very low (0.002, 95% bootstrap Confidence Interval 0.001-0.003) and no clear geographic pattern was evidenced. Further analysis of the dataset is in progress and focuses on spatial and temporal autocorrelation of genotypes and the possible occurrence of cryptic demographic assemblages.

Table 1. Microsatellite markers used for the study of genetic variation in Yellowfin Tuna and Blackfin Tuna. Markers characteristics are detailed in Antoni et al. (2014a) for Blackfin Tuna microsatellites and Antoni et al. (2014b) for Yellowfin Tuna microsatellites. H_e : estimates of expected heterozygosity (average across samples); A: number of alleles detected to date.

| Blackfin Tuna | | | Yellowfin Tuna | | |
|---------------|-------|----|----------------|-------|----|
| Locus name | H_e | A | Locus name | H_e | A |
| BT4 | 0.878 | 36 | YT4 | 0.904 | 28 |
| BT11 | 0.951 | 43 | YT29 | 0.899 | 26 |
| BT18 | 0.919 | 27 | YT43 | 0.810 | 22 |
| BT20 | 0.929 | 28 | YT44 | 0.964 | 59 |
| BT22 | 0.903 | 58 | YT60 | 0.925 | 26 |
| BT27 | 0.901 | 25 | YT87 | 0.709 | 35 |
| BT29 | 0.926 | 32 | YT92 | 0.750 | 19 |
| BT31 | 0.894 | 25 | YT94 | 0.854 | 37 |
| BT68 | 0.829 | 11 | YT103 | 0.925 | 35 |
| BT81 | 0.821 | 15 | YT110 | 0.455 | 8 |
| BT83 | 0.604 | 11 | YT111 | 0.943 | 47 |
| BT88 | 0.922 | 28 | YT112 | 0.777 | 30 |
| BT95 | 0.888 | 20 | YT121 | 0.939 | 38 |
| | | | YT122 | 0.914 | 36 |

Genetic Variation in Blackfin Tuna

Samples of Blackfin Tunas were obtained from North Carolina, the Florida Keys, the northern Gulf of Mexico (offshore Louisiana), northern Brazil (Rio Grande Do Norte), Venezuela and La Martinique. Allele frequencies among the samples analyzed to date appear homogeneous (F_{ST} estimate 0.0008, 95% bootstrap Confidence Interval 0.000-0.0010) and preliminary analyses suggest occurrence of a weak pattern of isolation by distance where genetic distance increases as a function of geographic distance.

FUTURE DIRECTIONS - RESTRICTION SITE ASSOCIATED DNA SEQUENCING

The microsatellites employed in the pilot study described above are highly polymorphic and are assumed to be non-impacted by natural selection making these markers valuable tools to characterize the effects of the neutral processes of genetic drift and migration on population structure. However, microsatellites are not adapted to study the effects of divergent selection and local adaptation unless very large numbers of loci are surveyed such that some of the sampled markers are located, by chance, near a genomic region impacted by selection. The pilot studies presented above include 13 and 14 microsatellites and augmenting the numbers of microsatellites in projects involving large numbers of samples is labor intensive and impractical. Therefore, further work in progress addresses this limitation by developing high density genome scans using SNPs to characterize genome-wide variation in yellowfin and Blackfin Tuna. The double digest Restriction Site Associated DNA sequencing method after Peterson et al. (2012) is employed to assay samples. The method is expected to yield genotypes at 1,000 or

more SNPs, providing a relatively dense coverage of the genome which will allow assessing candidate regions of the genome involved in local adaptation in an outlier analysis (Whitlock and Lotterhos 2015) following which loci evolving neutrally can be separated from those impacted by selection. The analysis of neutral loci will focus on assessing occurrence of barriers to gene flow (Chen et al. 2007) and/or of cryptic structure (Pritchard et al. 2000) and patterns will be contrasted with those obtained at selected loci. Reference genomic resources are being developed as part of the project including a draft genome sequence and a linkage map for the Yellowfin Tuna. These resources will facilitate the detection of genomic regions impacted by selection and will allow accounting for physical linkage when estimating demographic parameters such as effective population size (Waples 2006, Larson et al. 2014).

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