BLUEFIN TUNA CONSERVATION RESEARCH IN THE CENTRAL-SOUTHERN MEDITERRANEAN SPAWNING AREA

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Abstract

Bluefin tuna (*Thunnus thynnus thynnus*) has been caught in the Maltese Islands since 1748. By 1995, when the Japanese bought bluefin tuna (BFT) caught by Maltese fishermen, the effort increased dramatically, however limited by the artisanal long-line gear used. Total BFT long-line fisheries landings have followed an over-all decline from a peak of 353,014kg in 1995 to 227,008kg in 2006. Close to 400 BFT specimens were sampled during six years to study variations in sizes, sex ratios, biogeographical characteristics and molecular genetics using microsatellite loci. This study's results indicate decreasing average BFT catch body size and an increasing sex bias for females. A significant genetic heterogeneity, FST value of 0.018 (P=0.015), was found among the BFT sampled in the spawning and fishing region south of the Maltese Islands.

Keywords: Conservation, Pelagic, Fisheries

Introduction

Tuna has been caught in the Maltese Islands with traps since 1748. However this fishing method was finally replaced with long-line, initially as a by-catch in the swordfish fisheries prior to fine-tuning gear for Bluefin tuna in 1995, when the Japanese bought Bluefin tuna caught by Maltese fishermen. Bluefin tuna fishing is now a seasonal activity for Maltese Fishermen undertaken between May and mid July. Each fishing expedition involves on average 4 days of effort with 40 to 50 miles of long-line, set at least three times, each extending down to 100m with bait which is mostly imported Mackerel. Boats utilized for this fishing measure between 10 to 26m. The increase in purse-seine fishing effort in the Central and Southern Mediterranean Area can jeopardize the survival of the species population and the livelihoods of Maltese long-line tuna fishermen if continued unchecked [1]. Vessels from Malta and Tunisia found themselves being joined by Italians, Spanish, French and recently increasing Libyan fishing vessels. The sudden increase in exclusive fishing zones by Tunisia and Libya further proves the increasing desire to increment the income from this fish species. In order to better understand the Bluefin tuna population structure and diversity found south of the Maltese islands, this study was started in 1998 [2,3] so as to undertake the microsatellite population genetics study presented here. This study has recently extended its sampling range West ward and East ward so as to expand on the results obtained so far.

Materials and Methods

Three hundred and eighty specimens of Bluefin tuna caught during the Maltese tuna long-line fishing season between 1998 and 2004 were measured (fork length and weight), sexed, and tissue sampled. DNA extraction from the stored tissues was carried out using the proteinase K/phenol/chloroform /isoamylalcohol method [4]. Microsatellite analyses protocols and primers used for this study were adapted from Takagi *et al.* [5]. PCR DNA amplification was carried out in an Eppendorf Mastercycler Gradient thermocycler. A 50ul reaction was used containing around 50ng DNA, 1μM of each primer, 5ul of buffer (consisting of 10 mM KCl, 10 mM (NH4)2SO4, 20 mM Tris-HCl, 2.0, mM MgSO4, 0.1% Triton X-100, pH8.8), 200 μM dNTPs, 3.5units of Taq DNA polymerase (New England Biolabs). 7.2ug of BSA was also added later. Each primer set had a flourescent label on either the forward or the reverse. The primers for the four loci considered included: tth01-TET; tth04-FAM; tth06-HEX; tth07-FAM. The temperature profile used involved an Initial denaturation for 5 min at 94°C, followed by 35 cycles of 1 min at 95°C, 1 min at 50°C, and 1 min at 74°C followed by a final extension of 10 min at 72°C. Allele frequencies were calculated as: number of the allele / total number of alleles (for that particular locus). Heterozygosity observed (Ho) and expected (He) were calculated using Arlequin ver.3: Ho is the number of heterozygotes observed as a ratio of the total number of individuals, while He is the expected number of heterozygotes if the total number of individuals were at Hardy-Weinberg equilibrium (HWE). Fixation index (FST) was used to measure Bluefin tuna population differentiation based on genetic polymorphism data, such as microsatellites. This statistic compared the genetic variability within and between populations. FST was calculated using Arlequin ver.3. This analysis was important in order to investigate the presence or absence of any significant differences between the various allele frequencies amongst Bluefin tuna from different sampling areas south of the Maltese Islands.

Results and Discussion

Bluefin tuna landings have increased through the years. However a decline in recent years is also noted though fishing efforts have increased. Between 1999 and 2004 on alternative years one may note a progressive increase of % females with the highest (70%) in 2004 showing a high bias toward females. Body size investigation indicates a clear reduction in the average size of the sampled individuals in 2003 and 2004. A close positive logarithmic relation between body size (kgs) and fork length (m) with a high R squared value of 0.86 was obtained. On investigating the genetic distance between samples caught during the six year study period (1999-2004) from three locations south of the Maltese Islands, genetic diversity between individuals sampled from regions A (SW of the Maltese Islands) and C (SE of Maltese islands) was found to be significant on a pairwise test: FST = 0.018 (P = 0.015). The molecular genetics analyses results for Bluefin tuna in this study show the number of alleles per microsatellite locus within samples varied from 5 for Ttho1 in the three locations to 13 for Tth07 in location B (situated between A and C). Allele richness per locus and sample varied from 3.894 at locus Tth01 in location C to 10.2 at locus Tth07 in location B. Average observed heterozygosities varied between 0.368, in location C at locus Tth01, and 0.925, in location C at locus Tth04. The expected heterozygosities varied between 0.536, in location C at locus Tth01, and 0.885 in location C at locus Tth07. The genotype distribution at loci Tth06 and Tth07 in location C deviated significantly from Hardy-Weinberg expectations (HWE) and thus were removed from subsequent analyses with the consequence that the FST value increased further in its significance.

Investigating the genetic distance between samples from the different locations South of the Maltese Islands during the six year study period (1999-2004) indicated an FST which varied from 0.018 (P=0.015) to 0.025 (P=0.012), when removing the two loci in location C found to be significantly deviating from the HWE obtained in this study. This clearly shows a significant difference between Bluefin tuna sampled in different locations in the Maltese Fishermen's fishing grounds thus requiring further conservation research and management considerations.

References

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