Mitochondrial DNA analysis reveals a single stocks of Frigate tuna *Auxis thazard* (Lacepède, 1800) in the northern coastal waters of Tanzania

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ABSTRACT

Frigate tuna *Auxis thazard* is an epipelagic and migratory species of family Scombridae found in the Indo Pacific Ocean. Apart from its ecological importance, the species plays an important role in terms of fishery within Indian Ocean region. The genetic structure of Frigate tuna is not documented in the Western Indian Ocean. The present study investigated the genetic diversity and structure of 35 Frigate tuna using sequence analysis of 500bp mitochondrial DNA D-loop gene from two geographically separate locations along the northern Tanzania coastal waters. The overall haplotype and nucleotide diversities were high respectively, 0.934±0.002 and 0.479±0.14. Hierarchical analysis of molecular variance (F_{ST} = 0.0035 (P = 0.3327) and pair wise differences (Φ_{ST} = 0.0014; *P*= 0.424) did not reveal a significant genetic differentiation between locations. Results were further corroborated by a none significant value of exact test of genetic differentiation (P = 0.437) and nearest neighbour statistic (S_{nn} = 0.291, P = 0.43). Thus finding of this study accepts the null hypothesis of single panmictic population of Frigate tuna in Indian Ocean. Such spatial homogeneity on genetic structure of the Frigate tuna confirms that the current management of the species as a single stock in the Indian Ocean is in agreement with our findings.

Keywords: Frigate tuna, mitochondrial DNA, D-loop, genetic structure, Tanzania

1.0 INTRODUCTION

Frigate tuna *Auxis thazard thazard* (Lacepede, 1800) is a widely distributed species and one of the smallest members of the tribe Thunnini, (the true tunas) and currently managed as a single stock throughout its range in the Indian Ocean. It is a coastal tuna species found circumglobally in tropical oceans up to depths of 50 m (Collette and Nauen 1983). The species is restricted to continental shelves and has a localized migratory habit (Maguire et al. 2006). Frigate tuna is exploited for canned products due to the excellent properties of meat, with its mild taste and low cholesterol content (Infante et al. 2004). Although larvae have high temperature tolerance between 21.6^0C and 30.5^0C, their optimum temperature is between 27^0C and 27.9^0C (Collette and Nauen, 1983). Spawning season varies in correlation with temperature and other environmental variables (Collette and Nauen, 1983). Spawning is believed to occur in several batches with fecundity of about 1.37 million eggs per year. Tanzania is fortunate to have large resources of tuna within its marine waters. However, despite the presence of tuna in Tanzania marine waters, little information on its fishery and reproductive biology (e.g. Johnson and Tamatamah, 2013) is documented. On the other hand, important information for management and conservation strategies including the species genetic structure is not yet in place in Tanzania and throughout Western Indian Ocean (WIO) region.

To date, there few studies on genetic structure reported across the Indian Ocean (Menezes et al. 2006, 2008, 2012; Kumar et al. 2012). However, no genetic stock structure information is available on frigate tuna, the third largest tuna fishery in the coastal waters of Tanzania. Recent study by Johnson et al. (*in press*) estimated exploitation rate of frigate tuna at 0.66 and optimum exploitation rate of 0.540, indicating that frigate tuna is exploited relatively at high rates in

Tanzania coastal waters. Based on the population parameters and stock estimates, the coastal tunas have been found to be exploited at or above the optimum levels. Along the coastal waters of India the exploitation rate of frigate tuna is 0.72, whereas the optimum exploitation rate is at 0.40. Thus, Pillai and Gopakumar (2003) suggest a relatively high exploitation rates in the Indian coastal waters. Since many species of pelagic fishes are under intense fishing pressure, it is imperative that their population genetic structure aiming at delineating different breeding stocks is required for proper management at both regional and international levels. Genetic markers in fisheries biology are used to determine if samples from natural populations are genetically differentiated from each other. The detection of differentiation would imply that source groups comprise different stocks and should be treated as separate management units.

There are several methods available for stock delineation, based on morphology, spawning locations, tagging, parasite loads, microchemistry and genetics. Although deployment of one or more approaches may be appropriate for resolving stock structure issues, genetics approach is sensitive and reliable. Mitochondrial DNA (mtDNA) has proven to be a useful genetic marker in population genetic studies and fisheries management due to its high mutation rate, haploid nature and maternal mode of inheritance, which makes it a sensitive indicator of genetic drift resulting from geographical subdivision (Garber et al. 2005). Sequence analyses involving the rapidly mutating mtDNA control region has been used to identify genetic stocks in a growing list of marine fish species (Garber et al. 2004; Menezes et al. 2012), including members of Scombroidei (Ely et al. 2002). This study attempts to investigate the genetic differentiation between samples of Frigate tuna from two different regions along the Tanzania coast using mtDNA control region

sequence data. Also the study was intended to identify discrete genetic units, if they exist, for management, while testing the null hypothesis of panmixia.

2.0 MATERIAL AND METHODS

2.1 DNA extraction and amplification

Muscle tissues of Frigate tuna were collected from the northern Tanzania coastal waters including Pangani and Dar es Salaam (Fig. 1). Samples were preserved in absolute ethanolat 95%. A total of 35 samples were obtained and DNA isolated using phenol chloroform methods and stored at -20^{0}C till further processing. A 500bp fragment containing the first half of mtDNA D-loop (control region) was subjected to PCR amplification using the primer set and procedure described by Menezes et al. (2006). The primer sequences were as follows: 5' CCGGACGTCGGAGGTTAAAAT 3' (forward) and 3' (forward) and 5' AGGAACCAAATGCCAGGAATA 3' (reverse). Amplification was carried out by the procedure as described by Menezes et al. (2006). The verification of successful PCR amplification was assessed by 1% agarose gel electrophoresis. DNA samples were amplified in Eppendorf Thermocycler. Amplification was carried out in 50µl reaction mixture containing 5µl of 10X buffer (100 mM Tris-HCl, pH 8·3, 15mM MgCl2, 500 mM KCl), 2µl of template DNA, 1·0µl of each primer (100 pmol), 5µl of a 2·5mM solution of each deoxyribonucleoside triphosphate (dNTP); 2·5 units of Taq DNA polymerase and milliQ water. PCR parameters consisted of 35 cycles of denaturation at 94°C for 1 minute, annealing at 55°C for 1 minute and extension at 72°C for 1 minute. Final extension was conducted at 72°C for 5 minutes. DNA extraction and PCR was conducted at Molecular Laboratory of the Mikocheni Agricultural Institute Tanzania.

2.2 DNA sequencing

PCR products were purified using PCR Cleanup Kits following the steps recommended by the manufacturer. All samples were sequenced in forward and reverse direction by the same primer as used for PCR amplification. Sequences were obtained at the DNA sequencing facility at Macrogen Genomic Laboratory South Korea using sequencing kit following manufacturer protocol.

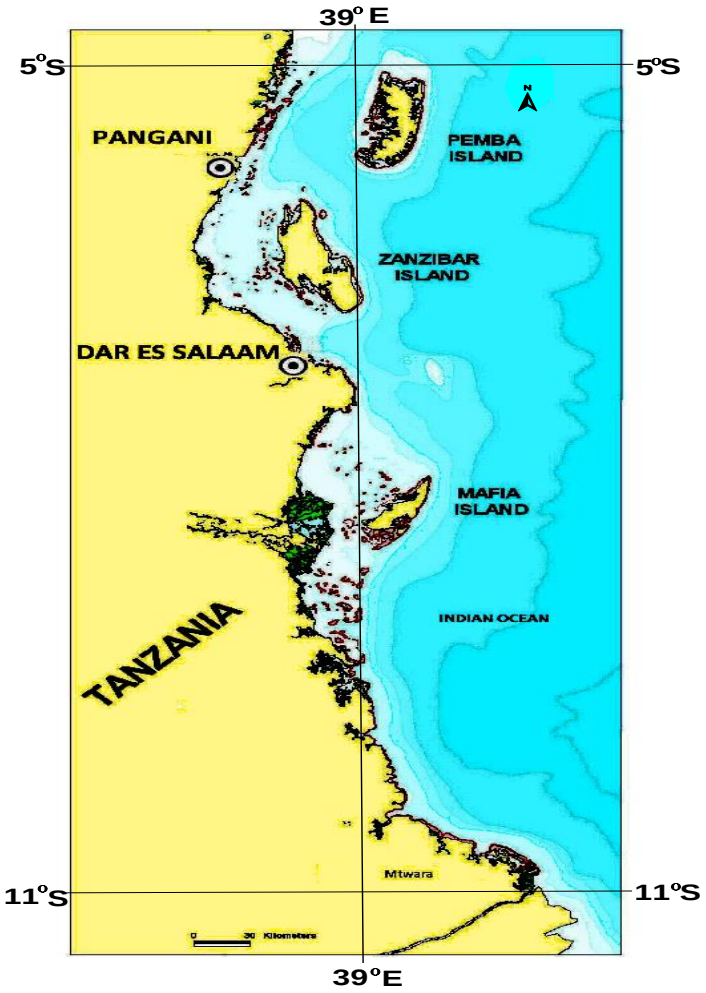

Fig 1: Map of Tanzania Coastal Waters Showing the Study Sites (Source: IMS, 2012)

2.3 Data analysis

The sequences were edited in Genious R 6.1.8 and aligned with program ClustalW (Thompson et al. 1994) in MEGA Version 6.0.6 (Tamura et al. 2013). Sequences were submitted to the GeneBank and given reference number. MEGA6.0.6 was used for calculating nucleotide composition. DnaSP 5.0.1 (Librado and Rozas 2009) was used to estimate different genetic diversity indices such as; number of polymorphic sites, haplotypes and nucleotide diversities. Genetic diversity in each sampling region was measured as haplotype diversity (H*h*), and nucleotide diversity (Pi) (Nei 1987). Analysis of molecular variance (AMOVA) and hierarchical AMOVA were used to examine the amount of genetic variability partitioned within and between populations (Excoffier et al. 1999). The hierarchical AMOVA partitions the total genetic variation between pre-hierarchical groups, yielding the measure of population indices such as F_{ST} - variation among sites, F_{SC} - variation among temporal collections within site and F_{CT} - spatial genetic differentiation between sites irrespective of time (Wright, 1969 and Nei, 1987). The nearest-neighbour statistics S_{nn} (Hudson 2000) were used to measure the extent of population differentiation by testing if the sequences with low divergence are geographically proximate. S_{nn} is a measure to find out how often closely matched sequences are from the same locality in geographical location. Significance of *S*nn was tested using 1,000 permutations.

3.0 RESULTS

3.1 Genetic variation

The genetic analyses of 35 Frigate tuna sequences at 500bp of mtDNA D-loop region produced 210bp positions after gap exclusion. A total of 72 variable sites, constituting 56 parsimony informative sites, 10 singleton variable sites and 24 haplotypes were detected. The inter-specific nucleotide frequencies were T (33.5%), C (17.9%), A (37.3%) and G (11.3%). Overall mean

haplotype diversity (*Hd*) and nucleotide diversity (Pi) were high, respectively 0.934±0.002 and 0.479±0.14 (Table 1). Also haplotype and nucleotide diversity within population was similarly high in the same order of magnitude within each sampling locality (Table 1). Of the 23 haplotypes, 11 were shared between localities while the remaining are population specific haplotypes. The most common haplotype (named #1) was well represented in all localities with a mean frequency of 33.8%.

Table 1: genetic diversity of Frigate tuna in Tanzania coastal waters

Parameters	Within populations		Between populations
	Dar es Salaam (1)	Pangani (2)	
Number of sequences	15	20	35
Number of haplotypes (h)	9	14	23
Haplotype diversity (Hd)	0.947±0.031	0.941±0.043	0.934±0.002
Number of polymorphic sites	71	69	73
Average number of nucleotide differences (k)	8.19±0.40	9.926±0.31	10.39±1.001
Nucleotide diversity, Pi(1)	0.412±0.15	0.552±0.003	0.479±0.14
Nucleotide diversity with Jukes and Cantor, Pi(1)JC	0.41±0.012	0.431±0.024	
Number of observed transitions	64	66	
Number of observed indels	0	0	
Number of observed sites with transitions	63	65	
Number of observed transversions	6	5	
Number of observed sites with transversions	7	7	
Number of substitutions	71	68	

Population genetic structure

Genetic differentiation between Frigate tuna populations was assessed using both F_{ST} and Φ_{ST} pair wise comparisons. Analysis of molecular variance (AMOVA) performed on mtDNA D-loop region indicated a none significant pair wise Φ_{ST} (0.0014; P= 0.424) genetic distances between population. This is an indication that more variations exists within than across populations. A non significant pair wise Φ_{CT} values of 0.00033 and 0.00042 (P>0.05) were revealed in Dar es

Salaam and Pangani, respectively. Hierarchical AMOVA was performed to test the significance of the partitioning of genetic variances resulting from different groupings of the populations into geographical groups. Present study reveals that variation attributed to between populations was very low at 0.25%, indicating that all of the variation (99.75%) found in samples collected from within populations. The inbreeding coefficient, F_{ST} was low to 0.0035 (P = 0.3327, P>0.05) and no significant genetic differentiation was found between populations. The spatial genetic structure between sites irrespective of time (F_{CT}) indicated a none significant positive values of 0.013 (P>0.05) in Dar es Salaam and 0.018 (P>0.05) in Pangani populations. The exact test of population differentiation (non-differentiation exact *P* values) indicated lack of differences between two sampling sites (*P* = 0.437). The genetic differentiation between populations was further tested using the nearest-neighbor (S*nn*). None significant genetic structuring (P>0.05) for the overall set (*Snn* = 0.291, P = 0.43) and within Dar es Slaam (S*nn* = 0.382, P = 0.49) and Pangani (S*nn* = 0.376, P = 0.56) populations. *Snn* provided another evidence for Frigate tuna panmixia populations in the northern Tanzania coastal waters. Hence a null hypothesis of panmictic population cannot be rejected.

DISCUSION

This study provides information on the genetic analysis of Frigate tuna in the northern Tanzania coastal waters; a portion within the Western Indian Ocean region. The analysis of the mitochondrial control region sequences revealed high level of haplotype and nucleotide diversity as well as high polymorphism. Haplotype diversity within and between populations was consistently higher and was in the same range to that reported by Kumar et al. (2012) in the Indian coastal waters. On the other hand, nucleotide diversity in our present study was higher

than those reported by Kumar et al. (2012). Generally, our findings are similar to the pattern observed by Ward et al. (1994) on other large pelagic marine fish. Likewise, haplotype diversity in our study is within the range for other tuna population at 0.60 (Chow and Ushiama 1995) and billfishes at 0.922 (Chow et al. 1997). Also overall nucleotide diversity and haplotype diversity (Table 1) were within the range reported for other tuna species by Viñas et al. (2004). Our results suggests that D-loop region is a useful genetic marker for population studies of Frigate tuna. Previous studies by Grant and Bowen (1998) classified the genetic diversity of marine fishes into four categories based on different combinations of small and large values for haplotype and nucleotide diversity of mitochondrial DNA sequences to infer different phenomenon of population history. Having higher haplotype and nucleotide diversities indicates that our species falls within the fourth category. This may be attributed to secondary contact between previously differentiated allopatric lineages or to a long evolutionary history in large stable population (Grant and Bowen, 1998). High genetic diversity in our study provides further evidence to the lack of the effect of ancient population bottleneck or founder effect on the genetic diversity. On the other hand, high genetic diversity has been reported to occur in the regions where a large population has been maintained over evolutionary time, probably due to the stability of the habitat (Hobbs et al. (2013).

The genetic variability and differentiation in Frigate tuna samples has been evaluated using mtDNA D-loop gene region in the Indian waters. The data exhibited very little divergence between the samples of eight localities analyzed, suggesting the existence of single panmictic population of Frigate tuna in Indian waters (Kumar et al. 2012). Our results are consistent with that of Kumar et al (2012). Our results are supported by none significant values of pairwise Φ_{ST} differences, AMOVA analyses, nearest-neighbour (S*nn*) and exact test of population

differentiation, which did not reveal geographically distinct stocks along Tanzania coastal waters. Our results indicate that Frigate tuna individuals are distributed randomly with respect to locality. Furthermore, lack of genetic differentiation between localities indicates the same evolutionary origin and that the population lack barriers to gene flow. In marine environment, barriers to gene flow can be created by oceanic circulation patterns, currents, and tectonic plate boundaries, behavioural control of dispersal by migration, natural selection due to ecological gradients, and historical subdivision of populations due to changes in sea level (Palumbi, 1994).

Studies conducted in Indian waters by Kumar et al. (2012) indicated significant genetic heterogeneity between samples collected from different coastal areas, signifying the existence of two genetic stocks of frigate tuna along the Indian coast. Although the results of Kumar et al. (2012) using exact test statistic is different from our results, the same study using AMOVA and pairwise comparisons among samples failed to produce any significant F_{ST} values. This is an indication that it was not possible to discern reproductive isolation between any of the samples (Boustany et al. 2008). Our study does not conform to other tuna studies whereby genetically discrete yellowfin tuna populations and two stocks of skipjack tuna have been reported using mitochondrial and nuclear DNA analyses (Dammannagoda et al. 2008, 2011) in the northwestern Indian Ocean. Also Menezes et al. (2012) using mtDNA D-loop region indicated four different stocks of skipjack tuna (*Katsuwonus pelamis*) along Indian coast.

CONCLUSION

High genetic diversity of Frigate tuna in our study provides further evidence of absence of strong directional or stabilizing selection. The mitochondrial DNA D-loop analysis indicated a non significant genetic differentiation within and between populations, thus providing a strong

evidence of single mixing population in Tanzania marine waters. It must be noted here, however, that spatial sample coverage in the present study is limited to only a very small area of this species' global distribution range. Therefore, in the absence of genetic analysis of samples representative of the entire distribution of the species, we are unable to determine whether or not this population expansion was an Indo-Pacific Ocean event or was restricted to the WIO region. However, our study provides important evidence of the genetic mixing of Frigate tuna population in Tanzania marine waters.

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