Original Research Article

Assessment of Genetic Diversity, Gene Flow and Demographic History of Frigate Tuna (*Auxisthazard)* Populations in Tanzanian Marine Waters using Mitochondrial DNA Control Region

Abstract

Aim: To investigate genetic diversity, gene flow and demographic history of frigate tuna (*Auxisthazard)* populations found in Tanzanian marine waters.

Study design: The study used a descriptive research design whereby fish samples were randomly collected from four locations and the genetic variation within and among the four populations was analyzed using mitochondrial DNA (mtDNA) control region.

Place and duration of the study: Fish samples were collected from landing sites in Tanga, Dar es Salaam, Mtwara and Zanzibar, Tanzania. The study was conducted between July 2020 and June 2021.

Methodology: A total of 100 frigate tunawere randomly sampled from small-scale fishermen at the landing sites of Dar-es-Salaam (20), Tanga (30), Mtwara (30), and Zanzibar (20). For each fish, 50g muscle tissue was obtained and put in a vial containing 95% ethanol. DNA was extracted from the muscle and a fragment of 432 bpof the mtDNA control region was amplified and sequenced. Haplotype and nucleotide diversity, gene flow and historic demographic wereestimated from 92fish samples.

Results: A total of 88 haplotypes were identified in all fish samples. The highest haplotype diversity was found in Zanzibar (1.000±0.017) and Mtwara (1.000±0.010) populations while the lowest was observed in Tangapopulation (0.992±0.012). Tangapopulation had the highest nucleotide diversity (0.078±0.018) while Dar es Salaam had the lowest (0.016±0.009). The highest genetic differentiation (F_{ST}) was found between Tanga and Dar-es-Salaam (0.178) andthe lowest was observed between Mtwara and Zanzibar (0.016). Thegenetic distancesbetween pairs of populations were small. The phylogenetic tree revealed two main clusters; the cluster of seven individuals from Tanga population and the cluster of individuals from all four populations, with

Comment [M1]: Was there not any species difference??

Comment [M2]: How many ??

no population specific sub-cluster.The number of immigrants per generation was highest between Mtwara and Zanzibar (Nm=18.310) and lowest between Tanga and Dar-es-Salaam (Nm=1.180). The neutrality test indicated negative values, suggesting a recent population expansion.

Conclusion: There is high ~~within population~~ genetic diversity within the population~~,~~ but there is no significant genetic differentiation among the four frigate tuna populations, suggesting that the four populations comprise a single panmictic population.

Keywords: Haplotype diversity, historical demography, migration rate, genetic differentiation

Abbreviations: DSFA -Deep Sea Fishing Authority, mtDNA – mitochondrial DNA, PCR-Polymerase Chain Reaction, TZS-Tanzanian Shilling, WIO-Western Indian Ocean

1. Introduction

Tanzania is bordered by Indian Ocean in the eastern side of the country and has a coastline of approximately 1,424 kilometers. This coast is useful for a number of activities, including fishing. The fishing industry contributes about 1.8% of the national Gross Domestic Product [1].It is estimated that 11.75% of the fish produced in Tanzania mainland comes from marine capture fisheries [1].The marine fishery in Tanzania is mainly artisanal, contributing about 95% of the fish produced from capture fisheries along the coast of mainland Tanzania [1]. Tunas are the third dominant group in the coastal fishery after mackerels and kingfishes. Tuna species found in Tanzanian seawater are bluefin (*Thunnusthynnus*), yellow fin (*Thunnusalbacares*), bonitos (*Sardachiliensis*), kingfishes (*Scomberomorus cavalla*), and swordfish (*Xiphias gladius*) [2].

Frigate tuna (*Auxisthazard*) is a small pelagic tuna-like member of the family Scombridae that includes tunas, mackerels, and bonitos. Frigate tuna is a highly migratory species, distributed in tropical and sub-tropical seas [3]. The species is mostly restricted to continental shelves at depths of up to 50 m [4]. Tuna have shown a high ability to reproduce over a wide range of environments. Despite their high fecundity, most tuna stocks are highly exploited. This can lead to the depletion of the species in the long run. Frigate tuna are fished for canned products due to

the excellent properties of the meat, with its mild taste and low cholesterol content [5]. In Tanzania, frigate tuna catches through artisanal fishing play an important role in the national economy and food security [2].

The major problem facing tuna is overexploitation due to overfishing [6]. Several populations of Frigate tuna are currently fully exploited or overexploited, although there is uncertainty about the results of stock assessment due to poor fishing statistics and species biology uncertainties [7]. Therefore, there is a need to establish strategies for sustainable utilization and conservation of the overexploited tuna species. Establishment of conservation strategies requires information on genetic diversity and the distinctiveness of the species. Information on the genetic distinctiveness and diversity of various frigate tuna populations is lacking in Tanzania.

The genetic diversity of frigate tuna can be assessed using microsatellite markers, single nucleotide polymorphisms (SNPs) and mitochondrial DNA. Mitochondrial DNA (mtDNA) markers are the markers of choice for the study of genetic diversity of closely related populations [8, 9]. Mitochondrial DNA sequences have proved to be a useful genetic marker in population genetic studies and fisheries management due to their high mutation rate, haploid nature, and maternal mode of inheritance, which makes it a sensitive indicator of genetic drift resulting from geographical subdivision [9]. Successful determination of genetic diversity of tuna species using mitochondrial DNA has been reported in many studies. For example, [10] assessed genetic diversity using mitochondrial DNA analysis and revealed a single stock of frigate tuna (*Auxisthazard*) in the northern coastal waters of Tanzania. The phylogenic relationship between tuna species has been analyzed using mitochondrial D-loop sequences [5], and the genetic stock structure of skipjack tuna in the northwestern Indian Ocean has been studied using mitochondrial DNA and microsatellites [11]. Therefore, mitochondrial DNA (mtDNA) markers can be used to assess the genetic diversity of frigate tuna in Tanzanian marine water.

To understand the evolutionary dynamics of a population, it is very important to quantify the level of migration. This is because migration rates affect the genetic structure of populations. Migration introduces genes of one population of a species into another population of the same species through interbreeding, thereby changing the gene pool composition of the recipient

population. The introduction of new alleles through gene flow increases variability within the population and makes possible creation of new combinations of traits. Therefore, knowledge about gene flow among different frigate tuna populations is very important for determining migration patterns and this can provide a framework for prediction and designing strategies to prevent overexploitation.

This study investigated the stock diversity of frigate tuna within Tanzanian marine waters in order to better understand the variation and connectivity of tuna species in inshore and territorial Tanzanian waters. More specifically, the study used mtDNA control region sequence data to assess the genetic diversity within and between four populations of frigate tuna in Tanzanian marine waters. The mitochondrial DNA (mtDNA) control region sequences have been shown to be a useful marker for population genetic studies in many aquatic organisms [12]. The mtDNA control region is a non-coding region and the most rapidly evolving region of mtDNA [13]. MtDNA evolves four times faster than the average nuclear gene. Hence, the mtDNA control region can be used to assess genetic variation of very closely related populations and even within species [13]. Therefore, the objective of this study was to assess the genetic diversity and structure and gene flow among four frigate tuna populations in Tanzanian marine waters. In addition, the demographic history of frigate tuna was investigated. It was expected that the study findings will enhance the conservation efforts and sustainable utilization of frigate tuna.

2. Materials and Methods

2.1 Description of the study area

The study was carried out in Tanzanian marine waters, specifically in four coastal areas; namely Tanga (located at 5° 04' 8.15 S, 39° 05' 55.50 E), Dar es Salaam (located at 5° 46 ' 33.6432 S, 39° 10' 41.9736 E), Mtwara (located at 10° 31' 0.01 S, 40° 10' 59.99 E) and Unguja Island (located at 6° 08' 26.00 S, 39° 20' 11.57 E) (Fig. 1). The landing sites visited at each site wereSahare in Tanga, Kunduchi in Dar es Salaam, Mikindani in Mtwara and Mablue in Unguja Island. The places were selected based on the presence of fishing grounds and artisanal fishermen targeting tuna and tuna like species.

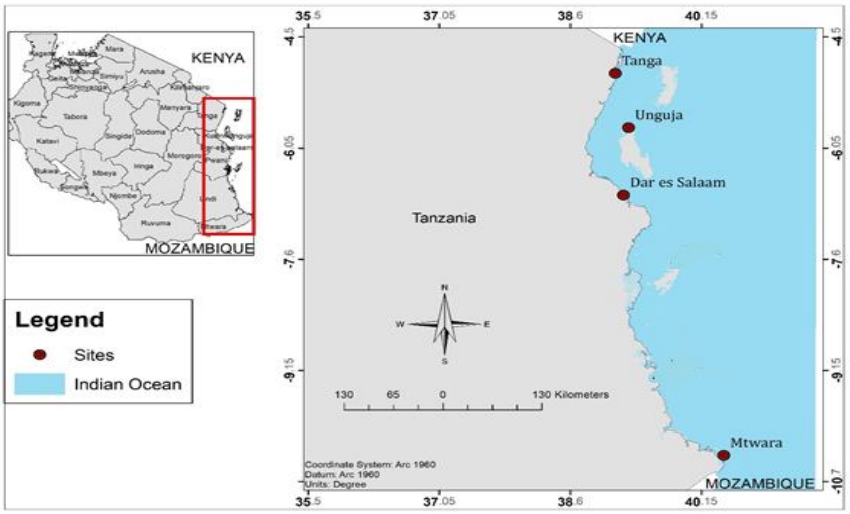

Fig.1.A map of Tanzania showing the sampling locations

2.2 Data collection

2.2.1 Fish sampling

The number of fish samples collected per site were 20 (Dar es Salaam), 30 (Tanga), 30 (Mtwara) and 20 (Zanzibar), making a total sample size of 100 fish samples from the four sites in Tanzania marine water. At each site fish samples were collected randomly from at least five different fishermen. Following collection of fish samples, a total of 50 g of muscle tissue of each fish was obtained from the area above the lateral line of the fish and below the dorsal fin and put in a cryovial tube containing 95% ethanol and labeled. The cryovial tubes were put in a cool box containing ice packs and then transported to the laboratory within 48 hours and kept in a refrigeratorat 4^{o}C. An additional sequence of *Euthynnusaffinis* (Kawakawa tuna) was downloaded from the GenBank (accession AB098092.1) and used as a reference population.

2.2.2 DNA extraction

DNA extraction from the muscle tissues was performed using a commercially available genomic DNA min extraction kit (Quick-DNA Kit) (Amersham Biosciences), according to the manufacturer's protocol. Thereafter, the presence of DNA was confirmed by gel electrophoresis on 2% agarose gel.

2.2.3 DNA quantification

To determine the DNA concentration and purity, the obtained DNA samples were quantified using a spectrophotometer (ThermoScintific, Marlborough, England, UK). The quality of DNA was determined from the concentration of DNA in the elute, by measuring the absorbance at 260 nm. The A260/A280 ratio of all DNA samples ranged from 1.703 to 2.057. The concentration of DNA was adjusted to 50 ng/µl.

2.2.4 DNA amplification

The DNA samples were sent to the Agricultural Research Council – Biotechnology Platform, South Africa (www.arc.agric;za) for polymerase chain reaction (PCR), amplification, and sequencing. A fragment of 432 bp containing the first half of the mitochondrial DNA control region (D-loop) was amplified using the primer set designed by Menezes *et al.*[14]. The sequences of the primers which were used are as follows: 5′-CCGGACGTCGGAGGTTAAAAT-3′ (forward) and 5′-AGGAACCAAATGCCAGGAATA-3′ (reverse). Amplification was carried out in a final volume of 25 µl reaction mixture that contained 2 µl of the DNA template, 0.5 µl of each of the two primers (10 mM each), 2.5 µl of the four dNTPs (10 mM dNTP), 0.2 µl of KAPA Taq Sigma-Aldrich, 2.5 µl of 10x Buffer and 16.8 µl sterilized ultrapure water (ddH_2O) in each tube. All PCR amplifications were carried out with an initial denaturation at 94 ^oC for 3 min, followed by 35 cycles, each with denaturation at 94 ^oC for 60 s, annealing at 55 ^oC for 60 s, and extension at 72 ^oC for 60 s. This was followed by a final extension at 72 ^oC for 5 min. The success of the PCR amplification was confirmed by gel electrophoresis on a 1 % (w/v) agarose gel stained with ethidium bromide. DNA was visualized under UV light. The amplified PCR fragments were purified with QIAquick® PCR purification kit (Quiagen®) by eluting the DNA in water.

2.2.5 DNA sequencing

Sequencing of the purified PCR fragments was performed using the same primers mentioned above using the Big Dye™ Terminator v. 3.0 cycle sequencing premix kit (Applied Biosystems, Foster City, CA, U.S.A.). The fragments were analyzed on an ABI PRISIM™ 3100 Genetic Analyzer (Applied Biosystems).

2.3 Data analysis

Sequences were manually edited in CLC Workbench 8.0.3 (CLC Bio-Qiagen) and in MEGA Version 6.0.6 [15] and aligned with program ClustalW [16]. Haplotypes were determined and basic diversity parameters were computed for each population using DnaSP v5 [17]. The phylogenetic relationship between individuals and populations was assessed based on the Tamura-Nei distance model using the maximum likelihood algorithm implemented in MEGA6 [15]. The analysis was done using all the generated sequences and the reference sequences of *Euthynnusaffinis* (Kawakawa tuna) downloaded from the GenBank (accession AB098092.1) with the bootstrap percentage computed after 1000 replications.

To determine population genetic diversity within and among populations, analysis of molecular variance (AMOVA) was performed using Arlequin Version 3.0 [18]. The level of gene flow among populations (Nm) based on Hudson *et al.* [19] was also calculated in DnaSP v. 4.0. NETWORK program v4.6.1.0 (copyright 2004 -2012, Fluxus Technologies Ltd.; http://www.fluxus-engineering.com) was used to draw median-joining haplotype networks to visually illustrate the population structure and relationships among various haplotypes. In addition, a neutrality test of the pairwise differences among all populations were performed to infer historical demographic and deviation of sequence variation from evolutionary neutrality. Deviations from neutrality were evaluated using Fu's Fs [20] and Tajima's *D* [21] via DnaSP v. 4.0.

3. Results

3.1 Genetic diversity

The genetic diversity was assessed based on 432 bp fragment of the mtDNA control region of 92 tuna fish tissues samples. Results on number haplotypes, haplotype diversity, nucleotide diversity and average of nucleotide differences are shown in Table 1. The total number of haplotypes from the four populations was 88, of which 27, 19, 17 and 25 haplotypes were observed in fish sampled from Mtwara, Zanzibar, Dar es Salaam and Tanga, respectively. No haplotypes were shared among the four sampling sites.

Comment [M3]: You mean all the haplotypes differed from each other.

The analysis of haplotype diversity revealed that all populations had very high haplotype diversity. The haplotype diversity of Mtwara (1.000 ± 0.010) and Zanzibar (1.000 ± 0.017) populations were the highest and the same while the lowest haplotype diversity was found in Dar es salaam (0.993 ± 0.021) and Tanga (0.992 ± 0.012) populations. The nucleotide diversity of Tanga population was the highest (Π = 0.078), followed by that of Zanzibar and Mtwara. The lowest nucleotide diversity was observed in Dar es Salaam population (Π = 0.016). Table 2 Shows that there were 119 polymorphic sites of which 23 (19.3%) were singleton variable sites and 96 (80.7%) were parsimony informative. Results on molecular diversity indices revealed 55 transitions, 10 transversions and 64 substitutions as shown in Table 3.

Table 1.Number of haplotypes, haplotype proportion, haplotype diversity and nucleotide diversity in four populations of frigate tuna (*Auxisthazard*)

Population	N	Haplotypes	Haplotype proportion (%)	K	Hd ± SD	Π ± SD

Dar es Salaam	18	17	94.4	6.719	0.993 ± 0.021	0.016 ± 0.009
Zanzibar	19	19	100	11.45	1.000 ± 0.017	0.027 ± 0.014
Mtwara	27	27	100	10.764	1.000 ± 0.010	0.025 ±0.014
Tanga	28	25	89.3	32.667	0.992± 0.012	0.078 ± 0.018
Total	**92**	**88**	**95.7**	**18.017**	**0.999 ± 0.002**	**0.043 ± 0.014**

Note: N = number of individuals, K = average of nucleotide differences, Hd = Haplotype diversity; Π: Nucleotide diversity, SD = standard deviation

Table 2.Number of conserved sites (C), number of variable sites (V), number of parsimonious informative sites (Pi) and number of singletons (S)

Population	**C**	**V**	**Pi**	**S**
Dar es Salaam	393	32	17	15
Zanzibar	370	56	30	26
Mtwara	358	66	34	32
Tanga	328	92	77	15
Total	**299**	**119**	**96**	**23**

Table 3.Number of transitions, number of transversions, number of insertions and deletions (Indel)

Parameter	**Mtwara**	**Zanzibar**	**Dar es Salaam**	**Tanga**	**Mean**	**SD**
No. of transitions	63	52	31	72	54.5	17.673
No. of transversions	3	2	0	33	9.5	15.716

No. of substitutions	66	54	31	105	64	30.952
No. of subst. sites	64	54	31	93	60.5	25.697
No. private subst. sites	5	11	3	36	13.75	15.218
No. of indel sites	0	0	0	0	0	0

3.2 Genetic differentiation and phylogenetic relationship

The analysis of molecular variance (AMOVA) showed that the percentage of variation within population constituted 90.35% while the variation among populations was only 9.64% (Table 4). The extent of genetic differentiation among the populations as indicated by F_{ST} values are shown in Table 5. The lowest F_{ST} values were found between Mtwara and Zanzibar (0.01605) and between Mtwara and Da r es Salaam (0.02113) populations. The highest F_{ST} value was found between Tanga and Dar es Salaam (0.17828), followed by that between Tanga and Zanzibar (0.14633). The genetic distance between Dar es Salaam population and Tanga population was the highest (0.0100), followed by the genetic distance between Tanga and Zanzibar (0.0087) and Tanga and Mtwara (0.0083) populations, while the genetic distance between Mtwara and Zanzibar was the lowest (0.0004). Generally, the genetic distances were low for all population pairs, except between Tanga and Dar es Salaam populations.

Table 4.Analysis of Molecular Variance

Source of variation	Sum of squares	Variance components	Percentage of variation
Among populations	85.933	0.88227	9.64575

Within populations	743.801	8.26446	90.35425
Total	829.734	9.14673	

Table 5.Estimates of average evolutionary distance (D) (below the diagonal) and level of genetic differentiation (F_{ST}) (above the diagonal) among the four frigate tuna populations

Population	DAR	ZNZ	MTWARA	TANGA
Dar es Salaam	-	0.03596	0.02113	0.17828
Zanzibar	0.00074	-	0.01605	0.14633
Mtwara	0.00082	0.0004	-	0.13865
Tanga	0.01001	0.00873	0.00827	-

Maximum likelihood (ML) tree showing the genetic relationships was reconstructed based on the 88 haplotypes obtained in this study and the reference sequences of *Euthynnusaffinis* (Kawakawa tuna) downloaded from the GenBank (accession AB098092.1). The phylogenetic tree based mtDNA D-loop sequences classified the haplotypes into two major clusters (Fig. 2). Cluster 1 which consisted of nine haplotypes was dominated by Tanga population with seven haplotypes while the other two were from Mtwara and the reference sequences of *Euthynnusaffinis* (Kawakawa tuna). The second cluster (cluster 2) included haplotypes from Dar es Salaam, Mtwara, Tanga and Zanzibar. Cluster 2 was subdivided into four sub-clusters, but there was no population specific sub-cluster.

To understand the better ~~the~~ relationships of haplotypes, the median-joining network was constructed for the identified haplotypes (Fig. 3). All haplotypes were clustered into two main clusters. In the network presented, there are no shared haplotypes. Cluster 1 had only five haplotypes from Tanga population which deviated from the other haplotypes while cluster 2 comprised haplotypes of all four populations.

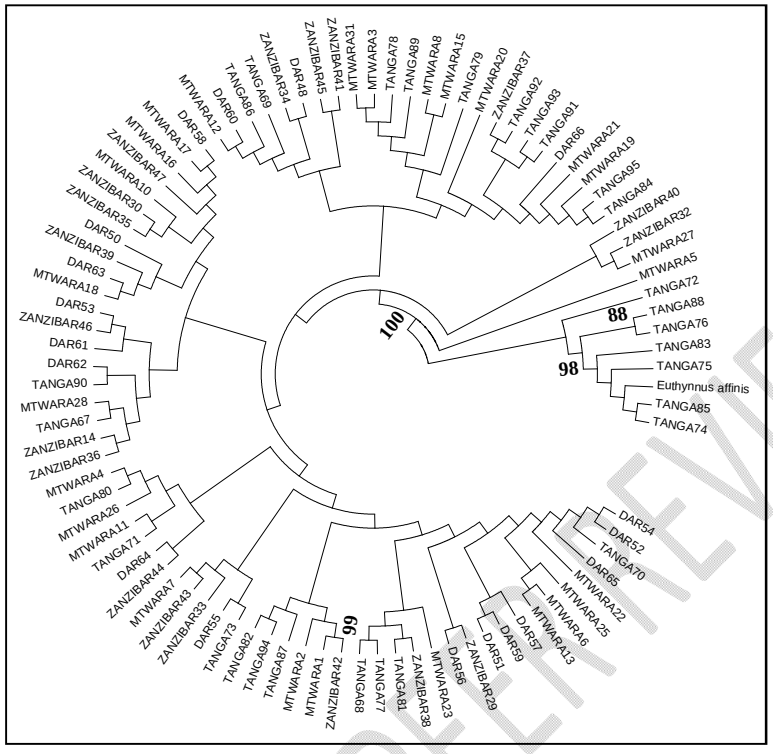

Fig.2.Maximum Likelihood (ML) phylogenetic tree of the 88 haplotypes from the four populations and the reference sequences of *Euthynnusaffinis* (Kawakawa tuna)

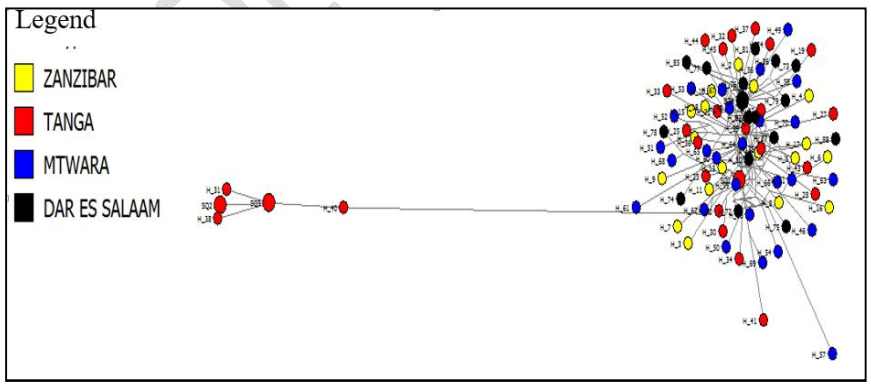

Fig.3.Median-joining network of the haplotypes from the four populations of frigate tuna

(*Auxin thazard*)

3.3 Demographic history

Population demographic history of the four populations of frigate tuna was inferred using the Tajima's D and Fu's Fs tests. The Fu's Fs and Tajima's D values (Table 6) were negative in all sampling sites and most of the p-values were statistically significant (except for Tanga Tajima's D value), which is suggestive of population growth or expansion. Significant negative values indicate an excess of rare haplotypes and rejection of the null hypothesis of neutral evolution.

Table 6.Estimates of neutrality test values in the four tuna populations

Parameter	Mtwara	Zanzibar	Dar es Salaam	Tanga
Fu's Fs	-19.767	-11.326	-10.371	-3.517
Tajima's D	-1.4986	-1.13082	-1.12015	0.90545

3.4 Gene flow among the four populations

Results for gene flow (Nm) among the four-populations are shown in Table 7. The highest number of immigrants per generation were observed between Mtwara and Zanzibar (Nm = 18.31), followed by that between Dar es Salaam and Zanzibar (Nm = 15.83) while the lowest were found between Tanga and Dares Salaam (Nm = 1.18), Tanga and Zanzibar (Nm =1.47) and Tanga and Mtwara (Nm = 1.55).

Table 7.Migration rate among four frigate tuna populations

Population	Zanzibar	Tanga	Mtwara	Dar es salaam
Zanzibar	_	_	_	_
Tanga	1.47	_	_	_
Mtwara	18.31	1.55	_	_
Dar es salaam	15.83	1.18	6.24	_

4. Discussion

4.1 Genetic diversity

The analysis of the mtDNA control region revealed relatively high number of haplotypes in the four populations, which could be attributed to large effective population size of frigate tuna. Populations having larger effective population size have more haplotypes compared to those with smaller effective population size. Also, the high number of haplotypes in the present study could be due to the high mutation rate of the mtDNA genes.

Haplotype diversity (Hd) and nucleotide diversity (Π) are important indicators of population genetic variation [22]. In this study, the mtDNA D-loop region sequence analysis showed that haplotypes diversity was very high in all populations while nucleotide diversity was relatively low. According to [13] the combination of high haplotype diversity and low nucleotide diversity is common in pelagic marine fishes. This is likely due to rapid demographic expansion of the current population from a small effective population size. This is observed if there is sufficient time for the number of haplotypes to increase through mutation but insufficient time for accumulation of large sequence differences [23]. The results of the present study are similar to the results obtained in *Teraponjarbua*by [24] and [25] who found high haplotype diversity ranging from 0.86 to 1.0 [24] and 0.99 [25]. However, the findings of the present study do not agree with [26] who reported relatively low genetic diversities ranging from 0.216 to 0.698.

4.2 Genetic differentiation and phylogenetic relationship

Results on the AMOVA and phylogenetic tree show that there is no significant population genetic structuring of*A. thazard*populationsfrom the four-sampling sites. Most of the genetic variation was found within the populations rather than between the populations. The result of the current study is similar to the findings obtained by [10] who showed high genetic variation within populations of *A. thazard*than the variation between populations.

F_{ST} is an important indicator of genetic differentiation among populations. According to [27], F_{ST} value of 0 – 0.05 is described as little differentiation, 0.05 – 0.15 as moderate differentiation, 0.15 – 0.25 as great differentiation and values greater than 0.25 as ~~very~~ thegreatest differentiation. Furthermore, Wang*et al*. [22] reported that $0 < F_{ST} < 0.05$ indicates that there is

no differentiation among the populations, $0.05 < F_{ST} < 0.15$ indicates that there is moderate differentiation; $0.15 < F_{ST} < 0.25$ indicates that there is high differentiation. In this study the F_{ST} values among the four populations revealed low to moderate level of differentiation. The low F_{ST} value observed in this study is comparable with the results reported by [10]. The extent of genetic differentiation between Zanzibar and Mtwara, Dar es Salaam and Mtwara, Dar es Salaam and Zanzibar populations were low while that between Tanga and Mtwara and between Tanga and Zanzibar populations were moderate. High level of genetic differentiation was only observed between Dar es Salaam and Tanga populations. The low level of genetic differentiation among the populations could be attributed to intermixing of different populations of frigate tuna across geographical regions due to high migration rate of the species.

Comment [M4]: Explain here the reasons for this higher level of variations than the other sites?

Assessment of genetic variation between populations based on the genetic distance indicated that the overall genetic distance between populations were low for all pairs of populations, except the genetic distances between Tanga and the rest of the populations. This means that the populations from Mtwara, Dar es Salaam and Zanzibar are closely related. The genetic distance between Tanga and Dar es Salaam, Tanga and Zanzibar and Tanga and Mtwara populations were relatively high, implying that they are more distantly related. The results of the present study are supported by Wang *et al*. [22] who showed that the genetic distance between the Heihe River *G. chilianensis* population and the Shule River G. chilianensis are lower (0.0013). Also [28] obtained the same results in bigeye tuna (*Thunnusobesus*) based on mtDNA analysis with the PCR-RFLP technique and concluded that the smaller the genetic distance values between the pair of groups, the closer the two groups are and vice versa.

Comment [M5]: What can be probable reasons of these variations whether it is due to migration, or distant location of sampling sites?

The Maximum Likelihood (ML) phylogenetic tree revealed two major clusters. The first cluster comprised of seven haplotypes from Tanga population together with the reference sequence of *Euthynnusaffinis* (Kawakawa tuna) downloaded from the GenBank (accession AB098092.1) while the second cluster had haplotypes from all four populations, with no population specific sub-cluster. In general, haplotypes specific to certain geographical sampling site did not form monophyletic groups, but appeared to be randomly distributed across the haplotype tree. These results strongly suggest that the *Auxisthazard*individuals from the four populations were panmictic with shallow genetic structure. This can be attributed to high gene flow among the

populations. Similar observation has been made by [29]who reported lack of significant geographical structure in long tail tuna (*Thunnustonggol*). It is well known that genetic differentiation among populations is the result of evolutionary processes, like migration, mutation, and drift. Thus, a highly migratory species, such as *Auxisthazard*, is expected to show limited population partitioning as a result of high migration rates among geographically separated populations. The findings in this study agree with [24] who assessed population structure of *T. jarbua*from five wild populations and conclude that there is no distinct geographical structuring among the five populations of *T. jarbua*in Malaysian waters.

Clustering pattern for the median-joining network was completely consistent with the clustering pattern of the phylogenetic tree. The median-joining network of the haplotypes reconstructed in the present study for frigate tuna (*Auxisthazard*) fish showed no genetic partitioning based on geographic location. These results are comparable to the findings of [30] who found that individuals from *A. megastoma* and *A. marmorata* populations are admixed. The high gene flow among frigate tuna (*A. thazard*) populations may be explained by its life cycle, migratory behavior and habitat use such as pelagic eggs and larvae that are spread or drift passively by ocean currents. In the network presented, there are no shared haplotypes, indicating that there is high genetic diversity as a result of high migration rate between populations. This result agrees with the results of inter-haplotypic analyses that showed that there is population expansion and high gene flow for populations in which most new haplotypes arise by recent mutation events from central wide spread haplotypes among the populations [29]. While there is no genetic structuring of *A. thazard* attributed to geographic locations based on the mtDNA control region sequences used in this study, it is interesting to note that the haplotypes seem to form two clusters, the first cluster being exclusively for few individuals from Tanga population while the second cluster having representatives of *A thazard*individuals from the four sampling locations. It is not yet clear whether this was caused by the limitation of analyzing only one locus of mtDNA used in the study and thus, further investigations are needed, including the use of many polymorphic microsatellite markers.

Comment [M6]: But migration if it is during the spawning season mixes up the gene pool limiting population diversity.

4.3 Demographic history

The results for neutrality test suggest that the population of *A. thazard* in the four sampling areas is expanding because of the strong significant negative values observed for both Fu's Fs and Tajima's D indices. Fu's Fs is more sensitive in the detection of population expansion [20]. Therefore, the results generally suggest population expansion for all the four populations of *A. thazard*. This supports the existence of panmixia population as the haplotype distributions generated describe one identical population for all the four sampling populations of *A. thazard*. The use of more markers in future studies could verify this observation.

4.4 Gene flow

The overall gene flow recorded among the four populations was high (Nm > 1), this suggests genetic connectivity among the four populations. It seems that the large geographical distances between these populations do not prevent the interbreeding of the fish from the different populations. This is due to the fact that frigate tuna has the ability to undertake long-distance migrations in ocean waters. Studies have shown that the connectivity levels between populations of marine organisms can be maintained even between long distances [31]. The results in this study disagree with [24] who reported low overall gene flow (Nm = 0.82) in *Teraponjarbua*, which suggested limited genetic connectivity among the five populations of their study. In their study they attributed the low level of gene flow to be due to the large geographical distance between the populations. Fish migration across adjacent areas follows one-dimensional stepping stone model that allows migration to adjacent populations [32]. Also, the results in the present study supports the theory that when Nm > 1 between populations the level of gene flow between the populations is higher and the genetic differentiation between them is smaller and that when Nm > 4, the gene exchange between populations is more sufficient and the genetic differentiation is smaller [33].

5. Conclusions

It is concluded that there is high haplotype diversity, but low nucleotide diversity in the *A. thazard*populations found in Tanzanian marine waters. Results on neutrality test of all populations indicate significant negative results, suggesting population expansion of *A. thazard*populations in Tanzanian marine waters. Moreover, the study revealed that there is no

significant genetic structure of A. *thazard*from the four sampling locations in Tanzanian coastal areas, suggesting that the populations are panmictic. Therefore, the frigate tuna from the four sampling sites can be regarded as a single stock unit for management purposes. However, the population from Tanga seems to be slightly differentiated from the other three populations and the gene flow between the Tanga population and the other three populations is small. Thus, the frigate tuna from Tanga coastal waters can be considered as separate population and managed differently.

9. ETHICAL APPROVAL

This research was approved by the Research Ethics Committee of Sokoine University of Agriculture and given research clearance by the Vice-Chancellor of Sokoine University of Agriculture on behalf of the Tanzania Commission for Science and Technology.

10. References

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