Research Paper

Volume : 3 | Issue : 1 | January 2014 • ISSN No 2277 - 8179

Genetic Variation in Yellowfin Tuna *Thunnus Albacares* (Bonnaterre, 1788) Along Indian Coast Using Pcr-Rflp Analysis of Mitochondrial Dna D-Loop Region

Biology

KEYWORDS : Yellowfin tuna, mtDNA, D-loop, PCR-RFLP, Mitotype, Population structure, Indian waters

Swaraj Priyaranjan Kunal	National Institute of Oceanography, Dona Paula, Goa 403004, India
Girish Kumar	National Institute of Oceanography, Dona Paula, Goa 403004, India
Maria Rosalia Menezes	National Institute of Oceanography, Dona Paula, Goa 403004, India

ABSTRACT

Yellowfin tuna Thunnus albacares is an epipelagic, oceanic species of family Scombridae, found in tropical and subtropical region of the Pacific, Atlantic and the Indian Ocean. It is a commercially important fish species. In present study, population structure of yellowfin tuna was examined using PCR-RFLP analyses of mitochondrial DNA D-loop region from eight geographically distinct locations along the Indian coast. A 500 bp segment of D-loop region was analysed for 370 yellowfin samples using six restriction enzymes (Alu I, Hae III, Hinf I, Hha I, Msp I and Rsa I), resulting in 14 composite mitotype. Analysis of molecular variance (AMOVA) showed no significant genetic differentiation among sampling localities (ΦST = 0.00185; P= 0.327). Thus finding of this study suggests single panmictic population of yellowfin tuna in Indian waters.

Introduction

Yellowfin tuna *Thunnus albacares* Bonnaterre, 1788 is globally distributed throughout tropical and subtropical waters except Mediterranean Sea (Collette and Nauen, 1983). It is a commercially important fishery worldwide and is said to be potential replacement for other endangered tuna species such as bluefin tuna (Wu et al., 2010). Though ubiquitous in presence across world Ocean, it is the Indian Ocean which is most productive areas for tuna fishing (Anganuzzi et al., 1996). In Indian Ocean, it is distributed north of latitude 30^0 S and is the most dominant species in Indian waters (Vijaykumaran and Verghese, 2010). Despite high catch and increasing commercial values of yellowfin tuna, limited studies had been done to find the stock structure of this species in Indian waters. There are various methods available for determining genetic stock structure of fish species, among them genetic approach is more effective.

Genetic analyses can provide important insights into problem of inter and intraspecific population structuring (Avise, 1994). Molecular biology techniques have become important in determining fish stock structure. There is considerable increase in the number of molecular markers available for genetic analyses of population structure for last three decades with allozyme, mitochondrial DNA (mtDNA) and nuclear DNA (nDNA) have been used for this purpose. Each marker has its own advantages and have been successfully used for population genetic analyses of several tuna species (allozyme: Ward et al., 1994b; mtDNA: Menezes et al., 2006 and nDNA: Menezes et al., 2008). The mtDNA is haploid, maternally inherited and has a rapid evolution rate in comparison to nDNA and thus provides higher level of genetic resolution than allozyme (Birky et al., 1983 and Brown et al., 1992). In particular with the advent of polymerase chain reaction (PCR), an analysis of restriction site polymorphism of mtDNA has become a widely used population genetics tool in fisheries (Ravago-Gotanco et al., 2004; Hoolihan et al., 2006; Menezes et al., 2006; Turan et al., 2009; Kumar et al., 2012b).

The mtDNA D-loop region is highly polymorphic in tunas (Alvarado Bremer et al., 1998, 1999; Chow et al., 2000). These variations have been detected using conventional PCR-RFLP analysis in several tuna species such as skipjack tuna, frigate tuna from Indian region (Menezes et al., 2006, Kumar et al., 2012b).

An understanding of population genetic variation is important for successful conservation and management of fisheries. Yellowfin tuna is one of the most studied tuna species across the globe and has been studied for the last 50 years with the earliest genetic investigation was carried out in samples from the Pacific and the Indian Ocean by Suzuki (1962). However, to the best of our knowledge, there is no report from Indian waters on genetic population structure of yellowfin tuna. The present study aims to find genetic variation in yellowfin tuna along Indian coast while testing the null hypothesis of population panmixia.

Materials and Methods

Sample collection

A total of 370 yellowfin tuna fin clip samples were collected from eight distinct geographical localities along east and west coast of India including two island samples (Fig. 1). The samples were immediately preserved in absolute alcohol and brought back to laboratory for further processing.

DNA Isolation

The fin clip samples were used for isolating high molecular weight (HMW) genomic DNA following the standard TNES-Urea-Phenol-Chloroform protocol (Asahida *et al.,* 1996). Further the DNA pellet was suspended in 50µl of Tris-EDTA buffer (pH 8.0). Qualitative and quantitative measurement of DNA was carried out using UV-VIS spectrophotometer (UV-1800, Shimadzu, Japan). Each sample was estimated to have 20-30 ng of DNA per micro litre of solution. The DNA samples were stored at 4°C prior to PCR analysis.

DNA Amplification

The D-loop region of mtDNA was amplified using the primer set designed by Menezes *et al.* (2006) (Fig. 2a). The primer sequences were as follows: 5' CCGGACGTCGGAGGTTAAAAT 3' (forward) and 5'AGGAACCAAATGCCAGGAATA 3' (reverse). DNA samples were amplified in Eppendorf Thermocycler (EP Gradient S). Amplification was carried out in 50 µl reaction mixture containing 2 µl of template DNA; 5 µl of 10X buffer (100 mM Tris-HCl, pH 8.3, 15 mM $MgCl_2$, 500 mM KCl); 1.0 µl of each primer (100 pmol); 5 µl of a 2.5 mM 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 done at 72°C for 5 minutes.

RFLP

PCR products were digested with six restriction enzymes (*Rsa* I, *Alu* I, *Hinf* I, *Hha* I, *Msp* I and *Hae* III). Restriction digestion was carried out in a 10 µl volume containing 2 µl of PCR product, 2 units of restriction enzyme, 1 µl of the appropriate buffer, and 7 µl of ultrapure water, at 37°C for overnight. Restriction fragments were resolved on a 2.5% agarose gel buffered with 1X Tris-Borate-EDTA (TBE), and stained with ethidium bromide. Bands on the gels were visualized by UV transilluminator and photographed for further analysis.

Data Analyses

The mtDNA haplotypes (mitotypes) generated by each restriction endonuclease were designated by a capital letter in order of detection. Composite mitotypes were constructed from four alphabets corresponding to the mitotypes of *Rsa* I, *Alu* I, *Hinf* I and *Hha* I, respectively. Since *Hae* III and *Msp* I did not show polymorphism, restriction pattern of these two enzymes were not used for constructing composite mitotypes. A matrix data was created, which includes presence or absence of different restriction fragment pattern with respect to each endonuclease. The presence or absence of a given fragment was indicated by 1 and 0 respectively.

The restriction site matrix was used to calculate nucleotide diversity (π) and composite mitotype diversity (h) using program ARLEQUIN version 3.11 (Excoffier *et al.,* 2005). The extent of genetic differentiation between samples was estimated using the fixation index Φ_{ST} (Wright, 1951). Estimates of expected number of migrant females between populations per generation (*Nfm*) were calculated using the formula 2 *Nfm* = ((1/ Φ_{ST})-1) (Takahata and Palumbi, 1985). The significance of Φ_{ST} was tested by 1,000 permutations for each pairwise comparison. Hierarchical analysis of molecular variance was performed to partition variance components attributable to (1) variation among groups; (2) variation among populations within groups; and (3) variation within populations, to evaluate hypothesized patterns of spatial genetic structure. The null hypothesis of population panmixia was also tested using an exact test of composite mitotype homogeneity among samples. The exact test of population differentiation of composite mitotypes tests the hypothesis that the observed distribution of frequencies is less likely than the distribution expected under panmixia. Statistical significance was estimated via 1,000 Markov Chain Monte Carlo simulations as proposed by Raymond and Rousset (1995). The population parameters θ and τ were also estimated for the yellowfin tuna samples. Tau (τ) is a relative measure of time since population expansion, but can be transformed to estimate the actual time (T) since a population expansion using formula $T = \tau/2\mu$ where μ is the mutation rate per site per generation. In the present study, the mutation rate of 3.6×10^{-8} mutations per site per year was applied for the control region sequence of yellowfin tuna as this rate has been reported for the mtDNA control region in teleosts (Donaldson and Wilson, 1999). Historical demographic/spatial expansions were investigated using Tajima's *D* test (Tajima, 1989) and Fu's *FS* test (Fu, 1997) with 1,000 permutations. ARLEQUIN was also used to estimate both Harpending's raggedness index (*Hri*; Harpending, 1994) and mismatch distributions (*SDD*), to test the goodness-of-fit of observed mismatch distributions to the theoretical distribution under a sudden expansion model (Rogers and Harpending, 1992). To examine genealogical relationships among composite mitotypes, a minimum spanning network was constructed using HapStar Version 0.5 (Teacher and Griffiths, 2011).

Results

Genetic Diversity

The primers consistently amplified a 500bp DNA fragment of mtDNA D-loop in all individual, without apparent size differences among or within individuals from different sampled populations (Fig. 2). Restriction endonuclease *Rsa* I and *Alu* I produced four mitotypes (A, B, C and D), *Hinf* I and *Hha* I produced three mitotypes (A, B and C) while *Msp* I and *Hae* III produced a single mitotype (A) giving a total of 14 composite mitotypes across the total sample size of 370 yellowfin tuna samples (Table 1). Furthermore, the polymorphic band patterns of restriction enzymes (*Rsa* I, *Alu* I, *Hinf* I, *Hha* I) are presented in Figure 3a-3h.

The composite mitotype *m*1 was most common and observed in all sampling sites followed by *m*2 (five sampling sites), *m*5 (four sampling sites), *m*3 (three sampling sites), *m*6, *m*11 and *m*12 (two sampling sites). Composite mitotype *m*4, *m*7, *m*8, *m*9, *m*10 , *m*13 and *m*14 were unique and present in VE, KO, PO, PO, PB , TU and VI respectively (Table 2). Mitotype diversity (h) and nucleotide diversity (π) were low, ranged from 0.0721 to 0.2287 and 0.1739 to 0.4875 respectively (Table 1). All the pairwise comparisons were insignificant and most of the pairwise Φ_{ST} values were negative (Table 3).

Population Genetic Structure

Analysis of molecular variance (AMOVA) performed on mtDNA RFLP data set revealed no significant genetic heterogeneity among the eight sampling sites (Φ_{ST} = 0.00185; P= 0.32747) (Table 4). The estimated value of female migrants per generation (using Φ_{ST} values) was 270 among the eight sampling sites. Hierarchical AMOVA was performed to test the significance of the partitioning of genetic variance resulting from different groupings of the populations into geographical groups. Results revealed that variation attributed to among groups composite mitotype frequency differences was very low (<1%), with almost all of the variation (99.81%) found in samples within populations (Table 4). The exact test of population differentiation (non-differentiation exact P values) showed no significant differences among eight sampling sites (P = 0.35977). A minimum spanning network (Figure 3) indicates that composite mitotypes are closely related and there is no clear pattern of composite mitotypes and geographic location among sampling sites.

Historic Demography

The ARLEQUIN analyses of mtDNA showed large differences in θ_0 (population before expansion) and θ_1 (population after expansion) within all sampling sites, suggesting rapid population expansion (Table 5). In addition, all Harpending's raggedness indices and mismatch distributions were non-significant. Tajima's D (-1.579) values were mostly negative and significant (P < 0.05) while Fu's F values were negative but insignificant. The overall tau (τ) value was estimated to be 3.000 (95% confidence interval), which reflects the time of founding of yellowfin tuna in Indian waters of 83,333 years ago.

Discussion

The genetic variability and differentiation in yellowfin tuna samples has been evaluated using PCR-RFLP analysis of mtDNA D-loop region. The data exhibited very little divergence among the samples of eight localities analyzed, suggesting the existence of single panmictic population of yellowfin tuna in Indian waters. Hence, the null hypothesis of population panmixia cannot be rejected. The outcome is well supported by non-significant values of pairwise Φ_{ST} (Table 3) and AMOVA analyses, which did not revealed geographically meaningful group along Indian coast (Table 4). The null hypothesis is further corroborated by exact test of population differentiation which gave non-significant P value, indicating that the mitotypes were distributed randomly with respect to locality.

It has been observed that marine species have low genetic subpopulation differentiation than the fresh water ones. For instance, PCR-RFLP analysis studies have shown no genetic differentiation among Pacific bigeye tuna samples (Alvarado-Bremer et al., 1998; Chow et al., 2000; Durand et al., 2005). Similarly population genetic involving mtDNA revealed low level of genetic differentiation in Pacific yellowfin tuna samples (Scoles and Graves, 1993; Ward et al., 1994, 1997; Appleyard et al., 2001).

In principle, for marine organisms the degree of gene flow is determined by the dispersal abilities of organisms and the presence/absence of geologic or oceanographic barriers. The evolution of genetically distinct populations (stock) is attributed to the disjunct geographical distribution. However, the apparent lack of barriers to dispersal in the marine environment effectively reduces heterogeneity among populations, often making it difficult to differentiate discrete populations (Palumbi, 1992; Shulman and Birmingham 1995; Menezes *et al*., 2008). Also, low level of intraspecific gene flow and reduced population structure had been reported in marine fisheries compared to freshwater fishes, likely due to fewer barriers to dispersal in the marine environment (Ward et al., 1994). Oceanic pelagic fishes such as yellowfin tuna in present study conform to this pattern.

In particular, genetic differentiation is low among tuna populations within and between the Pacific and the Indian Ocean (Alvarado Bremer et al., 1998; Grew and Hampton, 1998; Chow et

Volume : 3 | Issue : 1 | January 2014 • ISSN No 2277 - 8179

al., 2000; Appleyard et al., 2002; Durand et al., 2005; Chiang et al., 2008; Wu et al., 2010) because of the occurrence of continuous, circumtropical pelagic environment and a wide range of suitable spawning grounds.

There were close relatedness among composite mitotypes and insignificant differences in their frequencies among the sampled populations. This homogeneity indicates gene flow between the sampled populations in Indian waters, since even small rates of migration between populations should eventually homogenize any neutral genetic variation (Crow, 1986; Schultz and Cowen, 1994; Menezes et al., 2008 and Kumar et al., 2012).

A recent population bottleneck or founder event by single or few mtDNA lineages may cause low genetic diversity at both composite mitotypes and nucleotide level (Grant and Bowen, 1998). There were large difference between θ_0 (population before expansion) and θ_1 (population after expansion) within all the sampling localities in present study, as expected during rapid population expansion.

Rates of gene flow as low as a few fish per generation are sufficient to prevent genetic differentiation by genetic drift (Wright, 1931; Hauser and Ward, 1998). Insignificant genetic differentiation and high rates of gene flow (*Nfm* =270) observed in present study supports single genetic stock yellowfin tuna in Indian waters. Similarly, Wu et al (2010) observed low and insignificant Φ_{ST} values, indicating low genetic differentiation and suggested the presence of possible gene flow between the Western Pacific and the Western Indian Ocean. A recent study carried out in Indian waters also revealed low level of Φ_{ST} value and high rates of gene flow in little tuna, concluding single stock around Indian waters (Kumar et al., 2012a).

The tau (τ) values for mtDNA data set were same for all sampling sites (τ = 3.000), which further corresponds that the estimated time for population expansion of yellowfin tuna in Indian waters is 83,333 years ago(95% confidence interval). Thus, recent founding and insufficient time to attain the migration-drift equilibrium among populations of yellowfin tuna could be the possible reason for lack of genetic structure observed in present study.

The resolution of stock structure is important for effective and sustainable management of fisheries. However failure to reject null hypothesis does not necessarily means lack of stock structure, but only that it was not observed. This may be owing to the marker selected or the presence of sufficient gene flow to prevent accumulation of significant genetic divergence or insufficient time to attain the significant genetic differences (Graves and McDowell, 2003). Ward (2000) observed that by increasing sample size or using different class of marker with different population dynamics may reveal heterogeneity and restrictions on gene flow among regions once classed as homogeneous. Interestingly, a recent study involving mtDNA and nuclear DNA markers showed fine scale genetic heterogeneity in yellowfin tuna samples around Srilanka (Dammangodda et al., 2008). This finding warrants the need to carryout studies in Indian waters taking into consideration of both mtDNA as well as nuclear DNA.

Acknowledgement
The authors are grateful to S. R. Shetye, Director, National Institute of Oceanography (NIO) for his support. Financial support by a grant-in-aid project "Genetic characterization of tunas using DNA markers" from the Department of Science and Technology (DST), New Delhi to M.R.M is gratefully acknowledged. SPK and GK are grateful to DST for their fellowship support. This paper forms a part of the Ph.D. research work of SPK.

Figure

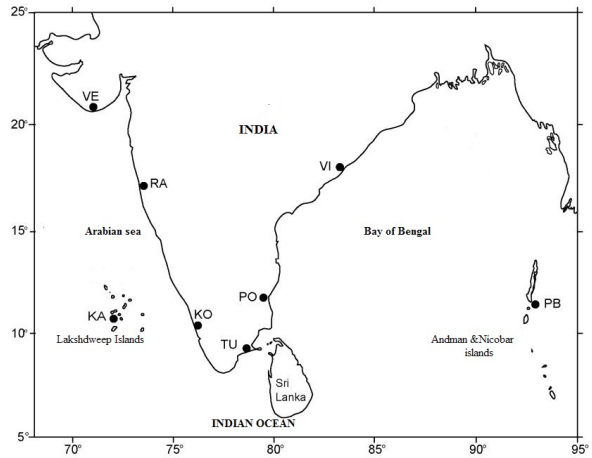

Figure 1. Map showing location of sampling sites along Indian coast VE, Veraval; RA, Ratnagiri; KO, Kochi; KA, Kavaratti; PB, Port-Blair; TU, Tuticorin; PO, Pondicherry; VI, Vizag.

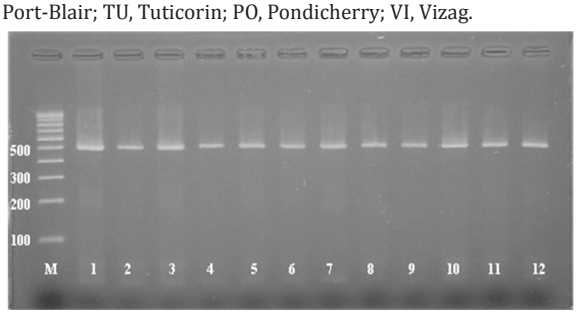

Figure 2. 500bp PCR amplified product of yellowfin tuna. Where M is the 100bp DNA marker and 1-12 is the amplified DNA product.

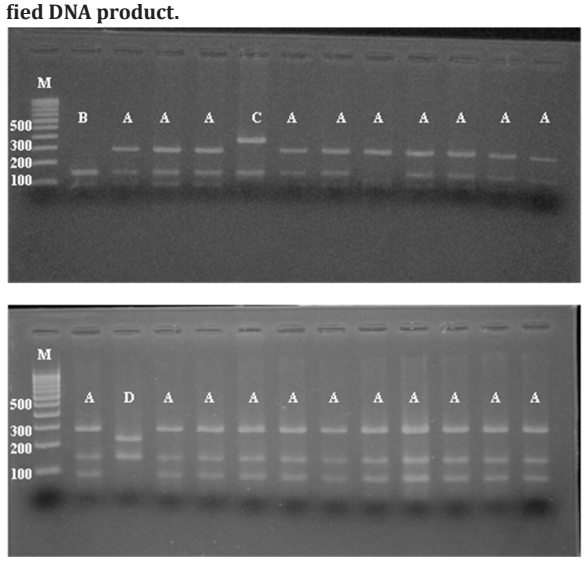

Figure 3 a, b. The restriction digestion of mtDNA (D-loop) of yellowfin tuna with enzyme *Rsa* I. Where M is the 100bp DNA marker and A, B, C and D are different restriction patterns.

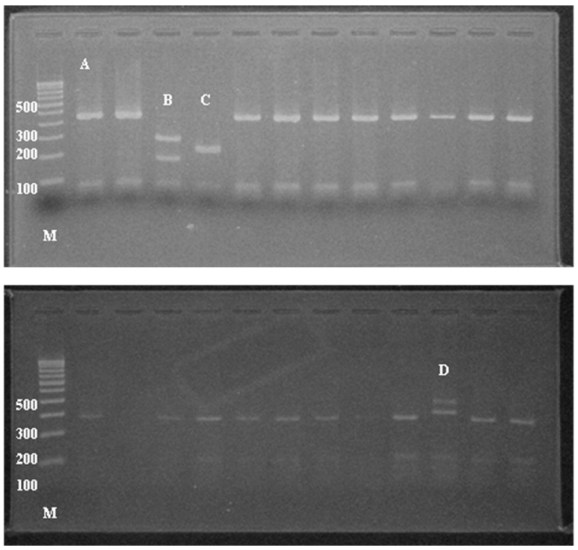

Figure 3 d, e. The restriction digestion of mtDNA (D-loop) of yellowfin tuna with enzyme *Alu* I. Where M is the 100bp DNA marker and A, B, C and D are different restriction patterns.

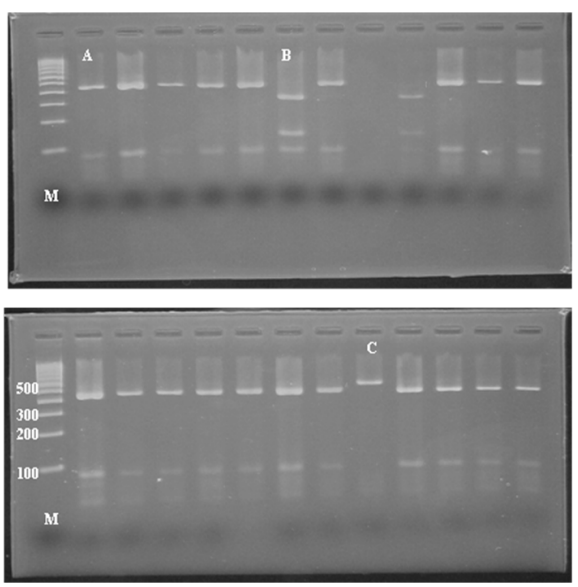

Figure 3 f g. The restriction digestion of mtDNA (D-loop) of yellowfin tuna with enzyme *Hinf* I. Where M is the 100bp DNA marker and A, B and C are different restriction patterns.

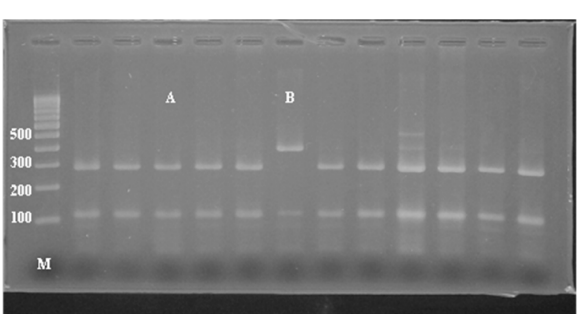

Figure 3 h. The restriction digestion of mtDNA (D-loop) of yellowfin tuna with enzyme *Hha*I. Where M is the 100bp DNA marker and A, B are different restriction patterns.

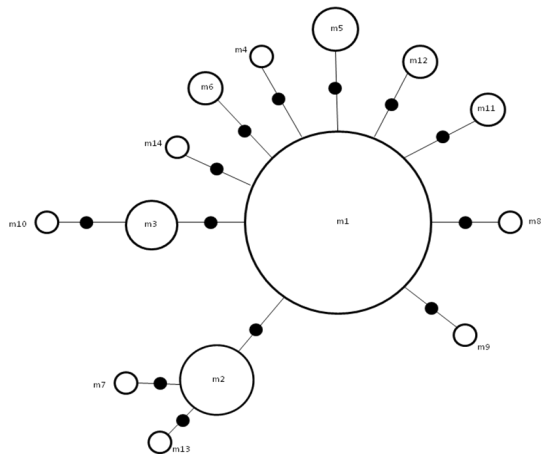

Figure 4. Minimum spanning network showing relationships among 14 mitochondrial DNA D- loop region composite mitotype. Each circle represents a unique composite mitotype in the sample. The size of each circle represents relative frequency of each composite mitotypes.

Table 1. Estimates of genetic diversity within populations based on RFLP: number of samples (*n*); number of mitotypes (*nh*); mitotype diversity (*h*) and nucleotide diversity (π).

Population	Location	Date of collection	n	nh	h	π
Veraval (VE)	20.54°N 70.22°E	October, 2007	58	5	0.2263	0.4670
Ratnagiri (RA)	16.98°N 73.3°E	January, 2008	12	2	0.1667	0.3333
Kochi (KO)	9.58°N 76.16°E	February, 2008	50	5	0.1551	0.3902
Pondicherry (PO)	11.56°N 79.50°E	July, 2008	60	6	0.2203	0.4587
Port-Blair (PB)	11.40°N 92.46°E	July, 2008	55	3	0.0721	0.2154
Tuticorin (TU)	8.49°N 78.08°E	September, 2009	55	5	0.2061	0.4875
Vizag (VI)	17.42°N 83.15°E	February, 2009	57	5	0.2287	0.4699
Agatti (AG)	10.50°N 72.12°E	November, 2008	23	2	0.0870	0.1739
Total			370	14	0.1704	0.3745

Table 2. Relative frequencies of 14 mitotypes from RFLP data. Letters reflects individual composite mitotypes for restriction enzymes *Rsa*I, *Alu*I, *Hinf*I, and *Hha*I (left to right)

S.No	Mitotype	VE	RA	KO	PO	PB	TU	VI	KA
1	AAAA	0.879	0.917	0.92	0.883	0.964	0.891	0.877	0.957
2	BAAA	0.0172	0.0833	0.02	0	0	0.0545	0.0702	0
3	AAAB	0.0345	0	0	0.0333	0.0182	0	0	0
4	AAAC	0.0172	0	0	0	0	0	0	0
5	CAAA	0.0517	0	0.02	0.0333	0	0	0.0175	0
6	AABA	0	0	0.02	0.0167	0	0	0	0
7	BABA	0	0	0.02	0	0	0	0	0
8	AACA	0	0	0	0.0167	0	0	0	0
9	DAAA	0	0	0	0.0167	0	0	0	0
10	CAAB	0	0	0	0	0.0182		0	0
11	ACAA	0	0	0	0	0	0.0182	0.0175	0
12	ABAA	0	0	0	0	0	0.0182	0	0.0435
13	BBAA	0	0	0	0	0	0.0182	0	0
14	ADAA	0	0	0	0	0	0	0.0175	0

Volume : 3 | Issue : 1 | January 2014 • ISSN No 2277 - 8179

Table 3. Pairwise Φ_{ST} (below diagonal) and p (above diagonal) values among populations of yellowfin

Sample	VE	RA	KO	PO	PB	TU	VI	KA
VE		0.71171	0.31532	0.82883	0.61261	0.11712	0.24324	0.18919
RA	-0.02529		0.99099	0.65766	0.31532	0.99099	0.99099	0.50450
KO	0.00215	-0.03714		0.70270	0.16216	0.22523	0.39640	0.21622
PO	-0.00931	-0.02002	-0.00611		0.81081	0.03604	0.12613	0.39640
PB	-0.00457	0.00932	0.01157	-0.00813		0.01802	0.03604	0.31532
TU	0.01265	-0.04193	0.00235	0.01558	0.02625		0.82883	0.51351
VI	0.00464	-0.04733	-0.00323	0.00996	0.02291	-0.01270		0.32432
KA	0.01020	0.01421	0.00714	0.00023	0.00399	-0.00291	0.00952	

Table 4. Results of analysis of molecular variance (AMOVA) testing genetic structure of yellowfin tuna based on mitochondrial D-loop region sequence data.

Structure tested (sampling sites)	Variance	Percentage of Variation	Φ Statistic	p-values
One groups (VE, RA, KO, PO, PB, TU, VI, KA)				
Among populations	0.00037	0.19	Φ_{ST} =0.00185	P= 0.32747
Within populations	0.19970	99.81		
Three groups (VE, RA, KO, PO, TU, VI); (KA) and (PB)				
Among groups	0.00030	0.15	Φ_{CT} = 0.00147	P= 0.40567
Among populations within groups	0.00025	0.12	Φ_{SC} = 0.00125	P= 0.51906
Within populations	0.19970	99.73	Φ_{ST} = 0.00272	P= 0.33431

Table 5. Demographic parameters of yellowfin tuna based on mitochondrial D-loop region RFLP: τ, θ_0, θ_1, Tajima's *D* test and Fu's *Fs* values, Harpending's Raggedness index (*Hri*), and sum of squared differences (*SDD*)

Population	τ	θ_0	θ_1	**Tajima's *D***	**Fu's *Fs***	Hri	SSD
VE	3.000	0.00176	99999	-1.581*	-2.066	0.6947	0.0610
RA	3.000	0.00000	99999	-1.451	0.431	0.7500	0.0370
KO	3.000	0.00000	99999	-1.576*	-2.711*	0.7435	0.0239*
PO	3.000	0.00000	99999	-1.933**	-3.366**	0.6973	0.0576*
PB	3.000	0.00000	0.44990	-1.674*	-1.190	0.8662	0.0049
TU	3.000	0.00176	99999	-1.316	-1.990	0.6898	0.0430
VI	3.000	0.00000	99999	-1.584*	-2.063	0.6939	0.0625*
KA	3.000	0.00000	0.09927	-1.514*	-0.153	0.8487	0.0107*
Mean	3.000	0.00044	99999	-1.579 *	-1.638	0.7480	0.0376

***, ** Significant at $P < 0.05$ and $P < 0.01$ respectively.**

REFERENCE

1. J.R Alvarado Bremer, B. Stequert, N.W. Robertson and B. Ely 1998. Genetic evidence for inter-oceanic subdivision of bigeye tuna (Thunnus obesus) populations. Marine Biology, 132:547–557. doi: 10.1007/s002270050420; | 2. A. A. Anganuzzi, K. Stobberup and N.J. Webb, (1996) Proceedings of the Sixth Expert Consultation on Indian Ocean Tunas, Colombo, Sri lanka, 25-29 September 1995, 373. | 3. S.A. Appleyard, , Ward, R.D. and P.M. Grewe 2002. Genetic stock structure of bigeye tuna in the Indian Ocean using mitochondrial DNA and microsatellites. Journal of Fish Biology, 60:767-770. doi: 10.1111/j.1095-8649.2002.tb01701.x; | 4. T. Asahida, , Kobayashi, T., Saitoh, K. and Nakayama, I. (1996) Tissue preservation and total DNA extraction from fish stored at ambient temperature using buffers containing high concentration of urea. Fisheries Sciences, 62:727-730. | 5. J.C. Avise, 1994. Molecular markers, natural history, and evolution. Chapman & Hall, New York. | 6. C.W Birky, Maruyama, T. and Fuerst, P. 1983. An approach to population and evolutionary genetic theory for genes in mitochondria and chloroplasts and some results. Genetics, 103:513-527. | 7. J.R Brown, Beckenbach, A.T. and Smith, M.J. 1992. Mitochondrial DNA length variation and heteroplasmy in populations of white sturgeon (Acipenser transmontanus). Genetics, 132:221-228. | 8. S. Chow, Okamoto, H., Miyabe, N., Hiramatsu, K. and Barut, N. 2000. Genetic divergence between Atlantic and Indo-Pacific stocks of bigeye tuna (Thunnus obesus) and admixture around South Africa. Molecular Ecology, 9:221-227. doi: 10.1046/j.1365-294x.2000.00851.x; | 9. B.B. Collette, and Nauen, C.E. 1983. FAO species catalogue, Scombrids of the world. An annotated and illustrated catalogue of tunas, mackerels, bonitos, and related species known to date. FAO Fish Synopsis, 125:1-137. | 10. S.T. Dammannagoda,., Hurwood, D.A. and Peter, B.M. 2008. Evidence for fine geographical scale heterogeneity in gene frequencies in yellowfin tuna (Thunnus albacares) from the north Indian Ocean around Sri Lanka. Fisheries Research, 90:147-157. | 11. K.A. Donaldson and Wilson, R.R. 1999. Amphi-Panamaic geminates of snook (Percoidei: Centropomidae) provide a calibration of the divergence rates in the mitochondrial DNA control region of fishes. Molecular Phylogenetics and Evolution, 13:208-213. doi: 10.1006/mpev.1999.0625; | 12. J.D. Durand, Collet A, Chow, S., Guinand, B. and Borsa, P. 2005. Nuclear and mitochondrial DNA markers indicate unidirectional gene flow of Indo-Pacific to Atlantic bigeye tuna (Thunnus obesus) populations, and their admixture off southern Africa. Marine Biology, 147:313-322. doi: 10.1007/s00227-005-1564-2; | 13. B. Ely, , Jordi, V., Jaime, R., Bremer, A., Donna, B., Luciano, L., Kelly, C., Labrie, A.V. and Thelen, E. 2005. Consequences of the historical demography on the global population structure of two highly migratory cosmopolitan marine fishes: the yellowfin tuna (Thunnus albacares) and the skipjack tuna (Katsuwonus pelamis). BMC Evolutionary Biology, 5: 19. doi: 10.1186/1471-2148-5-19; | 14. L. Excoffier, Laval, G. and Schneider, S. 2005. Arlequin version 3.11: an integrated software package for population genetics data analysis. Evolutionary Bioinformatics, 1:47-50. | 15. Fu, Y.X. 1997. Statistical tests of neutrality of mutations against population growth, hitchhiking and background selection. Genetics, 147:915-925. | 16. W.S. Grant and Bowen, B.W. 1998. Shallow population histories in deep evolutionary lineages of marine fishes. insights from sardines and anchovies and lessons for conservation. Journal of Heredity, 89:415-426. doi: 10.1093/jhered/89.5.415; | 17. Graves, J.E. and McDowell, J.R. 2003. Stock structure of the world's istiophorid billfishes: a genetic perspective. Marine and Freshwater Research, 54:287-298. | 18. P.M. Grewe and Hampton, J. 1998. An assessment of bigeye (Thunnus obesus) population structure in the Pacific Ocean based on mitochondrial DNA and DNA microsatellite analysis. SOEST (School of Ocean & Earth Science Technology) Publ 98–05, JIMAR (Joint Institute for Marine & Atmospheric Research) Contribution 98–320. University of Hawaii at Manoa, HI. | 19. R.C. Harpending, 1994. Signature of ancient population growth in a low resolution mitochondrial DNA mismatch distribution. Human Biology, 66:591-600. | 20. J.P. Hoolihan, , Anandn, P. and Herwerden, L. 2006. Mitochondrial DNA analyses of narrow-barred Spanish mackerel (Scomberomorus commerson) suggest a single genetic stock in ROPME sea area (Arabian Gulf, Gulf of Oman and Arabian Sea). ICES Journal of Marine Sciences, 63:1066-1074. | 21. G. Kumar, Kunal, S.P., Menezes, M.R. and Meena, R.M. 2012a. Single genetic stock of kawakawa Euthynnus affinis (Cantor, 1849) along the Indian coast inferred from sequence analyses of mitochondrial DNA D-loop region. Conservation Genetics, 13:1119-1131. | 22. G. Kumar, Kunal, S.P. and Menezes, M.R. 2012b. Low genetic variation suggests single stock of kawakawa Euthynnus affinis (Cantor, 1849) along the Indian coast. Turkish Journal of Fisheries and Aquatic Sciences, 12:371-380. | 23. Menezes, M.R., Kumar, G. and Kunal, S.P. 2012. Population genetic structure of skipjack tuna Katsuwonus pelamis from the Indian coast using sequence analysis of the mitochondrial DNA D-loop region. Journal of Fish Biology, 80:2198-2212. | 24. M.R. Menezes, Ikeda, M. and Taniguchi, N. 2006. Genetic variation in skipjack tuna Katsuwonus pelamis (L.) using PCR-RFLP analysis of mitochondrial DNA D-loop region. Journal of Fish Biology, 68 (a):156-161. | 25. M. R. Menezes, Noguchi, D., Nakajima, M. and Taniguchi, N. 2008. Microsatellite development and survey of genetic variation in skipjack tuna Katsuwonus pelamis (L.). Journal of Fish Biology, 73:463-473. doi: 10.1111/j.1095-8649.2008.01912.x; | 26. S.R. Palumbi 1992. Marine speciation on a small planet. Trends in Ecology and Evolution, 7:114-118. doi: 10.1016/0169-5347(92)90144-Z; | 27. M. Raymond and Rousset, F. 1995. An exact test for population differentiation. Evolution, 49: 1280-1283. | 28. R.G. Ravago-Gotanco and Juinio-Men˜ ez, M.A. 2004. Population genetic structure of the milkfish, Chanos chanos, based on PCR-RFLP analysis of the mitochondrial control region. Marine Biology, 145:789–801. | 29. Rogers, A.R. and Harpending, H. 1992. Population growth makes waves in the distribution of pairwise genetic differences. Molecular Biology and Evolution, 9:552-569. | 30. D.R. Scoles and Graves, J.E. 1993. Population genetic structure of yellowfin tuna, Thunnus albacares, from the Pacific Ocean. Fish Bulletin US, 91:690–698. | 31. Shulman, M.J. and Bermingham, E. 1995. Early life histories, ocean currents, and the population genetics of Carribean reef fishes. Evolution, 49:897-910. | 32. A. Suzuki 1962. On the blood types of yellowfin and bigeye tuna. American Naturalist, 96:239-246. | 33. F. Tajima 1989. Statistical method for testing the neutral mutation hypothesis by DNA polymorphism. Genetics, 10:512-526. | 34. N. Takahata and Palumbi SR (1985) Extranuclear differentiation and gene flow in the finite island model. Genetics, 109:441-457. | 35. A.G.F. Teacher and Griffiths, D.J. 2011. HapStar: automated haplotype network layout and visualization. Molecular Ecology Resources, 11:151-153. doi: 10.1111/j.1755-0998.2010.02890.x; | 36. Turan, C., Gurlek, M., Yaglioglu, D. and Ozturk, B. 2009. Genetic differentiation of Mediterranean horse mackerel (Trachurus mediterraneus) populations as revealed by mtDNA PCR-RFLP analysis. Journal of Applied Ichthyology, 25:142–147. doi: 10.1111/j.1439-0426.2009.01223.x; | 37. K. Vijayakumaran, and Varghese, S.P. 2010. Update on the status of tuna fisheries in India. IOTC-2010-SC-Inf12 | 38. R. D. Ward, Elliott N.G., Grewe, P.M., and Smolenski, A.J. 1994. Allozyme and mitochondrial DNA variation in yellowfin tuna (Thunnus albacares) from the Pacific Ocean. Marine Biology, 118:531–539. | 39. R.D. Ward, 2000. Genetics in fisheries management. Hydrobiologia, 420:191-201. doi: 10.1023/A:1003928327503; | 40. R.D. Ward, Eliot, N.G., Inne, B.H., Smolenski, A.J., and Grewe, P.M. 1997. Global population structure of yellowfin tuna Thunnus albacares inferred from allozyme and mitochondrial DNA variation. Fisheries Bulletin, 95:566-575. | 41. R.D. Ward, Woodmark, M. and Skibinski, D.O.F. 1994. A comparison of genetic diversity levels in marine, freshwater and anadromous fishes. Journal of Fish Biology, 44:213-232 doi: 10.1111/j.1095-8649.1994.tb01200.x; | 42. B.S. Wright, 1951. The genetical structure of populations. Ann Eugen 15:323-354 doi: 10.1111/j.1469-1809.1949.tb02451.x; |