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Evidence for fine geographical scale heterogeneity in gene frequencies in yellowfin tuna (*Thunnus albacares*) from the north Indian Ocean around Sri Lanka

Sudath T. Dammannagoda *, David A. Hurwood, Peter B. Mather

School of Natural Resource Sciences, Queensland University of Technology, GPO Box 2434, Brisbane, Queensland 4001, Australia

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Abstract

Yellowfin tuna are currently considered by the member nations of the Indian Ocean Tuna Commission to constitute a single stock in the Indian Ocean due to a lack of knowledge about yellowfin tuna population structure in this region. Previous studies of Indian Ocean yellowfin tuna based on morphology and fisheries data have hinted at the presence of multiple stocks in the region, and further, that stocks may mix in the north western Indian Ocean around Sri Lanka. To better understand the genetic stock structure of yellowfin tuna in the north western Indian Ocean, we examined genetic variation in 285 yellowfin individuals collected over a period of 4 years from six fishing grounds around Sri Lanka and a single fishing ground in the Maldive Islands. We screened variation in both the mitochondrial ATP*ase* 6 and 8 region (498 bp) and three microsatellite loci. Significant genetic differentiation was detected among sites for mitochondrial DNA ($\Phi_{ST} = 0.1285$, $P < 0.001$) and at two microsatellite loci ($F_{ST} = 0.0164$, $P < 0.001$ and $F_{ST} = 0.0064$, $P < 0.001$), while spatial analysis of molecular variance of mtDNA data identified three genetically heterogenous groups namely; western, south eastern and all remaining sites. These results suggest the possibility that genetically discrete yellowfin tuna populations may be present in the north western Indian Ocean.

Keywords: ATP*ase*; Indian Ocean; Microsatellites; Population structure; *Thunnus albacares*; Yellowfin tuna

1. Introduction

Yellowfin tuna (YFT) constitute the second largest tuna fishery worldwide (FIGIS, 2006). Among the principal market tuna fisheries, YFT were responsible for a global catch of 1.5 million metric tonnes (mt) in 2006 (FIGIS, 2006), which represents 32% of all tuna fished commercially that year. In the Indian Ocean, YFT also represent the second largest tuna catch comprising 25% of the total catch there (IOTC, 2006). As a result of expansion of an industrial fishery equipped with purse seine nets, the Indian Ocean YFT catch has increased more than seven fold from 66,200 mt in 1982 to 506,900 mt by 2004 (IOTC, 2006). The Indian Ocean Tuna Commission expressed alarm in a recent report at the increasing rate of the YFT catch in the region which it considered well above the maximum sustainable yield (IOTC, 2006). Moreover, unlike industrial YFT fisheries in the Atlantic and Pacific Oceans, the Indian Ocean tuna fishery provides a very important resource for developing coastal nations and constitutes the major food protein source for very large populations in the region. Depletion of Indian Ocean YFT stocks could compromise food security in a number of developing nations in the Indian Ocean. In Sri Lanka, the YFT fishery comprises 32% of the tuna fishery and provides the major animal protein source for an island population of 20 million. Currently Sri Lanka produces approximately 30,000 mt of YFT from its EEZ (IOTC, 2007), and a collapse of YFT stocks in the region may compromise food security there, and also an important export industry.

Currently, YFT in the Indian Ocean are considered to constitute a single stock (IOTC, 2006) but this recognition is based on only limited knowledge and scientific assessment of YFT population structure. Some recent analyses of fisheries data from the Indian Ocean have suggested however, the presence of more than a single stock in the region (IOTC, 2006). The majority of YFT stock assessment studies to date, have been undertaken in the Pacific and Atlantic Oceans, while Indian Ocean stocks have yet

* Corresponding author. Tel.: +61 7 31385051; fax: +61 7 31381535.
E-mail address: s.dammannagoda@qut.edu.au (S.T. Dammannagoda).

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to be studied in any detail. Early genetic studies that attempted to delineate stocks in the Pacific and Atlantic Oceans using allozymes and mitochondrial DNA (mtDNA) RFLP markers, did not recognise any inter- or intra-oceanic population structuring (e.g. Sharp, 1978; Ward et al., 1994; Scoles and Graves, 1993). Recently, genetic studies of Indian Ocean YFT attempted to detect population differentiation between collections of 40 individuals taken from sites in the far eastern and far western Indian Oceans using mtDNA (Chow et al., 2000) and nDNA (Nishida et al., 2001) markers but reported no evidence of significant differentiation. This may have resulted however, from a lack of sensitivity in the molecular markers employed and the lack of statistical power provided by the relatively small sample sizes examined.

A global genetic study of YFT in the Atlantic, Indian and Pacific Oceans that screened both allozyme and mtDNA RFLP markers by Ward et al. (1997) argued for existence of at least four discrete stocks in the three major oceans identified as; Atlantic, Indian, west-central Pacific and east Pacific. Use of genetic markers with high resolution (e.g. mtDNA sequencing, nuclear DNA microsatellites) and larger sample sizes, has meant that some genetic studies on tuna species were able to detect significant population differentiation even within ocean basins. A study of YFT from eight sites in the western Pacific using microsatellite markers identified very low, but significant differentiation (Appleyard et al., 2001). While a very recent study of YFT in the Atlantic and Pacific Oceans employed RFLP markers to show low genetic differentiation between the Atlantic and Pacific populations while no genetic differentiation was evident within oceans (Ely et al., 2005).

In the Indian Ocean, most YFT stock delineation studies that have been conducted to date, have been based on morphometry/fish length data or weight frequency data only and have produced inconsistent results. Kurogane and Hiyama (1958) suggested that three stocks were present, identified as western and two eastern, while Morita and Koto (1970) suggested two stocks were present that they referred to as 'eastern' and 'western' based on morphometric data. Nishida (1992) used industrial long line fishery data from across the Indian Ocean to suggest that eastern and western stocks were limited by 40–90°E and 70–130°E, respectively. In addition, he recognised two minor stocks 'far eastern' and 'far western'. Interestingly, this report also suggested that the major eastern and western stocks in the Indian Ocean may mix around Sri Lanka.

Reliable and informative data on YFT population structure will be essential to allow development of better management strategies for the species in the Indian Ocean and will help to conserve wild stocks in the future. While non-genetic methods can only infer different fish breeding units, a population genetics approach can directly test the hypothesis that genetically different breeding units may exist (e.g. Ward, 2000). Lack of detailed population genetic studies on Indian Ocean YFT stocks has constrained development of scientific management strategies for the species, and so the IOTC currently manages Indian Ocean YFT as a single stock.

Although YFT are generally considered to be highly migratory fish, recent tagging studies have shown that they can show limited dispersal and often remain close to their natal waters (e.g. Yesaki and Waheed, 1992; Schaefer and Fuller, 2006). If this observation were broadly true, it could result in fine geographical scale genetic heterogeneity. Studies that have reported evidence for fine geographical scale population structure in some otherwise pelagic marine fish have increased recently (e.g. Atlantic cod: Ruzzante et al., 1998; Knutsen et al., 2003, and Atlantic bluefin tuna: Carlsson et al., 2004).

Given this background, here we examined the extent of genetic differentiation among YFT populations collected between 2001 and 2004 in waters of the north western Indian Ocean around Sri Lanka and the Maldive Islands using mtDNA markers and three microsatellite loci. The objective was to test for genetic differentiation among major fishing grounds around Sri Lanka to evaluate whether YFT catches in this region could represent heterogeneous stocks.

2. Materials and methods

2.1. Sampling

YFT samples were collected from commercial fishing operations from six sites around Sri Lanka and a single site in the Maldive Islands (Fig. 1). Sampling sites were selected to represent major fishing grounds in Sri Lankan waters, and a single collection was included from the Maldive Islands as an outgroup to compare the levels of genetic diversity of YFT populations from two geographically remote regions. Samples were collected between 2001 and 2004, with muscle tissue collected for approximately 50 individuals per site (Table 1). Individuals varied in length from 55 cm to 70 cm at all sites except for the KK and KR sites where individuals were generally larger (mean length of 138 cm and 90 cm, respectively). Muscle tissue samples were removed from fish and stored in 95% ethanol for later genetic analyses.

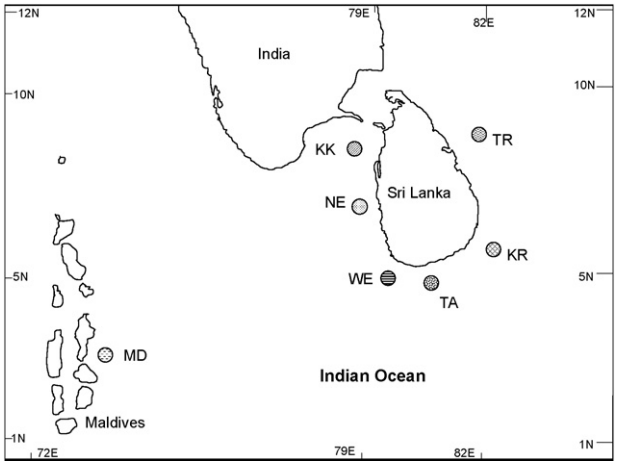

Fig. 1. Sampling sites of YFT in the north western Indian Ocean.

Table 1
Details of samples collected for analysis

Population	Location	Date	Sample size (*n*)	Average length (cm)
Kandakuliya (KK)	79°12′, 8°20′	April 2002	51	138
Negombo (NE)	79°18′, 6°057′	June 2001	6	67
		August, October 2003	27	
Weligama (WE)	80°18′, 5°034′	March 2001	3	55
		September 2002	15	
		November 2003	19	
Tangalle (TA)	81°14′, 5°042′	April 2002	14	60
		November 2003	17	
Kirinda (KR)	82°23′, 6°017′	June 2001	52	90
Trincomalee (TR)	81°51′, 8°058′	September 2004	39	70
Maldives (MD)	73°09′, 40°20′	November 2003	42	60
Total collection			285	

2.2. DNA extraction, PCR and screening for variation

Total genomic DNA was extracted from each sample using either phenol–chloroform (Sambrook et al., 1989) or a modified salt extraction method (Miller et al., 1988). For mtDNA the whole ATP synthase subunits 6 and 8 region (ATP*ase* 6, 8) was targeted using the ATP 8.2 L (5′AAA GCR TYR GCC TTT TAA GC 3′) and COIII.2H (5′ GTT AGT GGT CAK GGG CTT GGR TC 3′) (http://striweb.si.edu/bermingham/research/primers/index.html) primers resulting in a ∼950 bp fragment. Two internal primers were then designed for this study yielding a 540 bp product (forward primer: 5′ CCT AGT GCT AAT GGT GCG ATA AA 3′; reverse primer: 5′ TTC CTC CAA AAG TTA TAG CCC AC 3′) that was used for further analysis. mtDNA PCR reaction consisted of 2.5 μl Roche 10X buffer, 0.5 μl 25 mM Fisher $MgCl_2$, 1 μl Roche deoxy nucleotide tri phosphate (dNTP), 1 μl 10 mM primer, 0.2 μl Roche Taq DNA polymerase, 1 μl DNA template (∼200 ng/μl) to a final volume of 25 μl with 16 μl ddH_2O. mtDNA PCR conditions were, 5 min at 94 °C for initial denaturation, then 30 cycles of 40 s at 94 °C, 40 s at 52 °C, 40 s at 72 °C, with the final extension step 8 min at 72 °C. mtDNA variation was assayed using Temperature Gradient Gel Electrophoresis with outgroup heteroduplex analysis (TGGE/OGHA) as described in Lessa and Applebaum (1993) and Campbell et al. (1995). Each unique haplotype identified using TGGE/OGHA was sequenced. Initially, haplotypes were sequenced in both directions. As forward and reverse sequences were perfectly complementary, haplotypes were then sequenced in the forward direction only. All sequencing was performed at the Australian Genome Research Facility (AGRF) using a 3730xl sequencing platform. PCR products of unique haplotypes were purified by ethanol precipitation, and sequenced using the forward primer and the BigDye terminator Sequencing Ready Reaction v3.1 kit (Applied Biosystems, CA, USA).

YFT genomic libraries were developed specifically for this study using a magnetic bead method as described in Glenn and Schable (2002). Tetranucleotide microsatellite markers were targeted, isolated and trialed to produce three polymorphic loci (i.e. UTD402, UTD494 and UTD499; Electronic Appendix) that were optimised to screen for population variation. Microsatellite PCR reaction mix consisted of ∼50 ng/μl DNA 1 μl, 1.25 μl of 10X PCR buffer (Roche), 0.25 μl of 25 mM $MgCl_2$, 0.5 μl of 10 mM dNTP (Roche), 0.5 μl of each 10 mM forward and reverse primers, 0.1 μl of Taq (Roche) and ddH_2O to a final volume of 10 μl. PCR conditions were; 5 min at 94 °C for initial denaturation, then 30 cycles of 30 s at 95 °C, 30 s at relevant annealing temperature (UTD402 at 51 °C, UTD494 at 49 °C, UTD499 at 52 °C), 30 s at 72 °C and a final extension step at 72 °C for 8 min. Microsatellite polymorphisms were analysed on a Gelscan 2000 System (Corbett Research) in 5% acrylamide gels and run according to the manufacturer's instructions. A 50–350 bp size standard (Tamra 350) was run at both ends of each gel and in two additional lanes to determine allele size. In addition to the size standard, an allele reference standard was generated for which YFT allele sizes were known and this standard was run in two additional lanes to ensure consistent allele scoring across all gel runs. Microsatellite allele sizes were scored using One D-scan 2.05 (Scanalytics, Inc., 1998).

2.3. Statistical analysis

2.3.1. mtDNA variation

mtDNA haplotype sequences were edited and aligned in BioEdit version 7.0.1 (Hall, 1999) and the sequence data set was then tested for deviation from neutral expectations using both Tajima's *D* (Tajima, 1989) and Fu's F_S (Fu, 1997) tests implemented in Arlequin version 2.00 (Schneider et al., 2005). Significance for both of these tests was determined using a coalescent simulation process in DnaSP 4.10 (Rozas et al., 2003) with 1000 replicates. *P* values of multiple neutrality tests were adjusted using the Bonferroni correction (Rice, 1989).

A mtDNA parsimony cladogram of haplotypes was constructed (at 95% level connectivity) using TCS version 1.18

(Clement et al., 2000). Haplotype networks reconstruct the genealogical history of haplotypic variation and illustrate the evolutionary relationship among unique haplotypes. Under coalescent principles, internal haplotypes in a network are assumed to be ancestral, while tip haplotypes are considered younger, more recently derived types (Templeton et al., 1987; Templeton and Sing, 1993; Crandall, 1996). A mtDNA parsimony cladogram can provide information on the demographic and geographical history of a population including past population expansions, and bottlenecks. Frequency and site information were incorporated into the YFT network here to illustrate the distribution of haplotypes among locations.

Genetic variation was examined using several standard diversity indices including; the number of polymorphic sites (S), haplotype diversity (H_d), and molecular diversity indices including nucleotide diversity (π) (Nei, 1987), the average number of pair wise nucleotide differences (k) (Tajima, 1983), and expected heterozygosity based on number of segregating sites (θ_s) (Watterson, 1975) implemented in Arlequin (Schneider et al., 2005) for the total YFT sample collection and for individual temporal and/or geographic samples.

Population genetic analyses were performed using Arlequin version 2.00 (Schneider et al., 2005) and DnaSP 4.10 (Rozas et al., 2003). Differentiation among spatially and temporally differentiated sites was estimated using the fixation index (Φ_{ST}) (Excoffier et al., 1992), that includes information on mitochondrial haplotype frequency (Weir and Cockerham, 1984), and genetic distances among unique haplotypes. For all Φ_{ST} analysis, the Tamura and Nei (1993) distance method was used as it accounts for mutational rate heterogeneity among nucleotides within the gene fragment.

An analysis of molecular variance (AMOVA) was used to examine the amount of genetic variability partitioned within and among YFT populations (Excoffier et al., 1992). Hierarchical AMOVA was used to investigate the effects of temporal sampling. Hierarchical AMOVA partitions total genetic differentiation (Φ_{ST}) among pre-defined groups, yielding two measures of genetic differentiation; Φ_{SC} describing variation among sample populations within groups, and at a higher level of the hierarchy Φ_{CT} describing differentiation among groups (Excoffier et al., 1992). In this study, hierarchical groups were organised in two ways, year wise groups and site wise groups. For year wise groups, sites were grouped based on year of sampling (i.e. four groups—2001, 2002, 2003, and 2004). Genetic variation was therefore partitioned among year (Φ_{CT}), among sites within years (Φ_{SC}), and within sites. Using this hierarchical grouping, we tested if any inferred structure among sampling sites remained stable over time. For site wise groups, samples at each site taken in different years were grouped together (i.e. seven groups—KK, NE, WE, TA, KR, TR, and MD). Genetic variation was partitioned among sites, irrespective of sample year (Φ_{CT}), and within sites among years (Φ_{SC}). Using this hierarchical grouping, we investigated the stability of individual sample sites over time. The significances of variance components for each hierarchical comparison were tested using 1000 permutations.

Population structure was also examined by determining genetic differentiation estimates between all pairs of sites (pair wise Φ_{ST} analysis). Significance of pair wise site comparisons was tested using the permutation process as above. In all instances with multiple tests, P values were adjusted using the Bonferroni correction (Rice, 1989).

Spatial structure was investigated further using spatial analysis of molecular variance (SAMOVA) (Dupanloup et al., 2002), which identifies groups of sample sites that are most similar and geographically meaningful. SAMOVA uses the statistics derived from an AMOVA, and incorporates geographical information on sampling sites (i.e. geographic distances among sites) with a simulated annealing approach to maximise the Φ_{CT} among groups of populations as well as identifying possible genetic barriers between them, without pre-defining populations (Dupanloup et al., 2002). Thus, SAMOVA defines groups of samples that are geographically homogeneous and also maximally differentiated from each other (Dupanloup et al., 2002).

To measure the extent of population differentiation by testing if sequences with low divergence are geographically proximate, the nearest-neighbour statistic S_{nn} (Hudson, 2000) was estimated in DnaSP 4.10 (Rozas et al., 2003). S_{nn} is a measure of how often closely matched sequences are from the same locality in geographical space. Significance of S_{nn} was tested using 10,000 permutations.

2.3.2. nDNA variation

Microsatellite data were checked for presence of null alleles, large allele dropout or errors in scoring due to stutter bands using Micro-checker software version 2.2.3 (Oosterhout et al., 2004).

Each locus was tested for deviation from Hardy–Weinberg equilibrium (HWE) in Arlequin version 2.00 (Schneider et al., 2005) with significance of deviations in observed heterozygosity from expected heterozygosity tested using an Exact test (Guo and Thompson, 1992). The possibility of linkage disequilibrium among loci was investigated using the method of Slatkin and Excoffier (1996) in Arlequin with 1000 permutations ($\alpha = 0.05$). P values were adjusted using the Bonferroni correction (Rice, 1989).

Measures of genetic variation included number of alleles and expected and observed heterozygosities, calculated in Arlequin. An analysis of molecular variance (AMOVA) was used to examine the amount of genetic variability partitioned within and among populations (Excoffier et al., 1992) for the entire microsatellite data set and for each locus.

2.3.3. Statistical power

Further, we tested whether our microsatellite data set had sufficient statistical power to detect genetic heterogeneity at various true levels of divergence using the POWSIM software (Ryman and Palm, 2006) as described by Ryman et al. (2006). This programme uses sample sizes, number of loci, and allele frequencies, and simulates genetic sampling from multiple populations that have drifted apart to a predefined expected degree of divergence defined as F_{ST}. Samples from these populations are used to test the hypothesis of genetic homogeneity and to estimate the α error at each locus separately, using both

Table 2
Statistical tests of neutrality

Population	Tajima's D	Fu's F_S
Total collection	−1.914 (0.013)	−15.804 (0.000)
KK	−1.065 (0.149)	−1.685 (0.182)
NE	−0.406 (0.363)	0.026 (0.477)
WE	−1.325 (0.092)	−1.225 (0.208)
TA	0.180 (−0.419)	0.636 (0.430)
KR	−1.138 (0.131)	−2.835 (0.065)
TR	−1.619 (0.044)	−1.329 (0.181)
MD	−1.688 (0.036)	−4.011 (0.002)

Estimates and associated P values. Significance level after Bonferroni correction; $\alpha = 0.05/7 = 0.007$.

Fisher's Exact test and traditional chi-square approaches, respectively.

3. Results

3.1. mtDNA variation

3.1.1. Tests for neutrality

Tests for neutrality for each sample population are presented in Table 2. When sampling sites were considered separately, there was no strong indication that samples deviated from neutral expectations with both Fu's F_S and Tajima's D in most cases being nonsignificant. However, F_S for the Maldives sample (MD) resulted in a significantly large negative value reflecting either the presence of multiple slightly deleterious alleles (haplotypes) or more likely, a recent demographic fluctuation resulting in mutation/drift non-equilibrium (Tajima, 1989) had occurred. The fact that F_S for the entire sample collection showed a significant large negative value ($F_S = -15.804$, $P < 0.001$) however, does not necessarily indicate a recent population expansion, but more likely results from the effect of sampling a structured population.

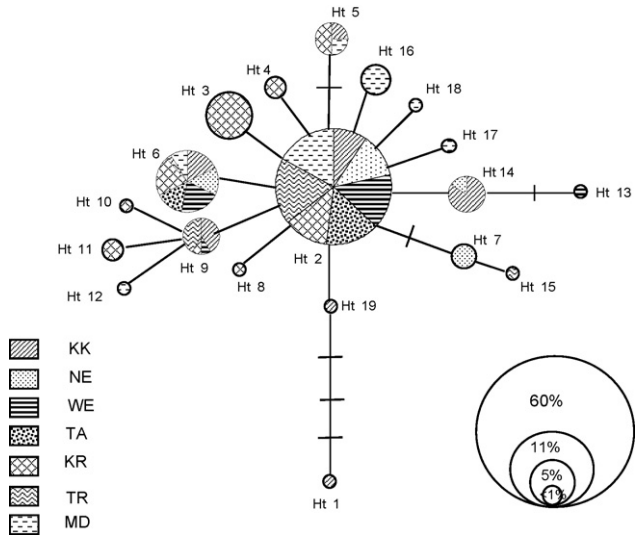

Fig. 2. Parsimony cladogram of YFT haplotypes showing the evolutionary relationship among haplotypes. Each circle represents a unique haplotype in the sample, and the size of each circle represents the relative frequency of each haplotype. Hatchings and their percentage in each circle represent the presence of each haplotype at different sites and their relative abundance. Cross bars between circles represent the haplotypes that were not found in the sample.

3.1.2. Genetic variation

Genetic analyses were conducted on 285 YFT individuals, from six fishing grounds around Sri Lanka and a single site from the Maldive Islands (Fig. 1; Table 1). mtDNA haplotype sequence analysis produced an alignment of 498 bp that included a partial fragment of the ATP*ase* 6 and 8 gene regions.

Table 3
Haplotype frequency distribution among sampling sites of YFT

Haplotype	Site							Total	Haplotype frequency (%)
	KK	NE	WE	TA	KR	TR	MD		
Ht1	1	0	0	0	0	0	0	1	0.35
Ht2	16	21	26	26	21	32	29	171	60.00
Ht3	0	0	0	0	17	0	0	17	5.96
Ht4	0	0	0	0	2	0	0	2	0.70
Ht5	1	0	0	0	2	0	1	4	1.40
Ht6	6	5	7	5	7	1	3	34	11.93
Ht7	0	3	0	0	0	0	0	3	1.05
Ht8	0	0	0	0	1	0	0	1	0.35
Ht9	5	0	1	0	1	5	0	12	4.21
Ht10	0	0	0	0	1	0	0	1	0.35
Ht11	0	0	2	0	0	0	0	2	0.70
Ht12	0	0	0	0	0	0	1	1	0.35
Ht13	0	0	1	0	0	0	0	1	0.35
Ht14	21	4	0	0	0	0	0	25	8.77
Ht15	0	0	0	0	0	1	0	1	0.35
Ht16	0	0	0	0	0	0	6	6	2.11
Ht17	0	0	0	0	0	0	1	1	0.35
Ht18	0	0	0	0	0	0	1	1	0.35
Ht19	1	0	0	0	0	0	0	1	0.35
No. of samples	51	33	37	31	52	39	42	285	

Table 4
Measures of variation for YFT samples

Population	h	S	H_d	k	(π)	(θ_s)
Total collection	19	20	0.936	0.839	0.002	3.211
Kandakuliya (KK)	7	8	0.722	1.094	0.002	1.778
Negombo (NE)	3	2	0.400	0.804	0.002	0.960
	4	4	0.595	0.838	0.002	1.028
Weligama (WE)	1	–	–	–	–	–
	3	4	0.514	0.819	0.002	1.230
	4	3	0.521	0.766	0.002	0.858
Tangalle (TA)	2	1	0.462	0.463	0.001	0.322
	2	1	0.117	0.118	0.000	0.296
Kirinda (KR)	8	7	0.721	0.989	0.002	1.770
Trincomalee (TR)	4	5	0.317	0.434	0.001	1.183
Maldives (MD)	7	7	0.507	0.624	0.001	1.627

Number of haplotypes (h), number of polymorphic sites (S), gene diversity (H_d), mean pair wise nucleotide difference (k), nucleotide diversity (π), expected heterozygosity per site based on number of segregating sites (θ_s).

A total of 21 nucleotide sites were variable (segregating sites) that defined 19 unique haplotypes (Table 3; Fig. 2 and Electronic Appendix). Analysis of variable sites showed that all mutations were synonymous (i.e. they did not alter the amino acid sequence).

Overall haplotype diversity (H_d) was high (0.9366) for the mtDNA region when compared with other tuna studies (e.g. Martinez et al., 2005; Carlsson et al., 2004, 2007; Ely et al., 2005), and individual geographic population haplotype diversity was also high. Nine haplotypes were singletons, and the most abundant (Ht2) and second most abundant haplotype (Ht6) were found at all sites sampled (Table 3; Fig. 2). Overall nucleotide diversity, and average number of pair-wise nucleotide differences were 0.002 and, 0.839, respectively. Measures of genetic variation for each site are presented in Table 4.

3.1.3. Phylogenetic relationships

The parsimony cladogram (Fig. 2) showed that all haplotypes were closely related to the most ancestral haplotype (Ht2) (i.e. under coalescent theory, the haplotype that was most common, widespread and central to the network). While another two internal haplotypes were consistent with coalescent theory (Ht9 and Ht14), two other haplotypes (Ht5 and Ht6) representing tips in the cladogram were also relatively widespread (Fig. 2). Pair wise percentage divergence (uncorrected) among haplotypes in the parsimony cladogram ranged from 0% to 2.0%. While most haplotypes were usually one, and sometimes two base pairs different from their nearest relative, Ht1 was relatively divergent from the rest of the cladogram. This haplotype was represented by a single individual from site KK.

3.1.4. Population differentiation

Pattern of YFT haplotype diversity among sites (Table 3; Fig. 2) showed that Ht2 was at highest frequency at all sites except for site KK where Ht14 was most frequent (41.17%). Ht14 was found only at site KK and the adjacent NE site. The south-eastern site KR also showed another haplotype (Ht3) at relatively high frequency (32.69%) and this haplotype was present only at this site.

Results of hierarchical AMOVA analysis are summarised in Table 5. Among all sites across years, highly significant genetic differentiation was evident at least among two sites (global $\Phi_{ST} = 0.1285$, $P < 0.001$). Hierarchical AMOVA of groups (i.e. sample collections at different sites in 2001) constituted a single group as were samples in years 2002, 2003, and 2004. This implies that the overall genetic composition of YFT populations around Sri Lanka remained stable over the sampling period. Significant spatial genetic differentiation was evident however, among sites within years ($\Phi_{SC} = 0.0791$, $P < 0.001$). As no temporal variation was evident within sites among years, temporal collections per site across years (for NE, WE and TA) were pooled for further analyses.

Structure of sampled YFT was supported by the nearest-neighbour statistic S_{nn} (Hudson, 2000) estimated in DnaSP, which was significant ($S_{nn} = 0.287$, $P < 0.001$). Pair wise Φ_{ST} analyses were conducted for the entire mtDNA data set using the Tamura and Nei (1993) distance method. Overall they show that most genetic differentiation among sites was limited to that between KK and most other sites and between KR and all other

Table 5
Genetic structuring of YFT populations based on AMOVA on mitochondrial ATP*ase* region sequence data

Structure tested	Observed partition		Φ Statistics
	Variance	%Total	
(1) Total collection (2001, 2002, 2003, 2004)			
Among sites (global Φ_{ST})	0.06256	12.85	$\Phi_{ST} = 0.1285^{***}$
Within sites	0.42415	87.15	
(2) Among years			
Among years (temporal)	0.02307	4.72	$\Phi_{CT} = 0.0472$
Among sites within years (spatial)	0.03686	7.54	$\Phi_{SC} = 0.0791^{***}$
Within sites	0.42917	87.75	$\Phi_{ST} = 0.1225$
(3) Among sites			
Among sites (spatial)	0.05072	10.43	$\Phi_{CT} = 0.1043^{*}$
Among years within sites (temporal)	0.00622	1.28	$\Phi_{SC} = 0.1428$
Within sites	0.42917	88.29	$\Phi_{ST} = 0.1171$

$^{*}P < 0.05$, $^{***}P < 0.001$.

Table 6
mtDNA pair wise Φ_{ST} among sampling sites of YFT for entire collection, after Bonferroni correction

	KK	WE	TA	KR	TR	MD	NE
KK	0.000						
WE	0.129***	0.000					
TA	0.160***	−0.07	0.000				
KR	0.199***	0.100***	0.108**	0.000			
TR	0.174***	0.029	0.061	0.117***	0.000		
MD	0.179***	0.045	0.033	0.119***	0.044	0.000	
NE	0.084	0.028	0.019	0.113***	0.062	0.055	0.000

Initial $\alpha = 0.05/21 = 0.002$. $^{**}P < 0.01$, $^{***}P < 0.001$.

sites (Table 6) accounting for 8/10 significant values after Bonferroni correction for multiple tests (Table 6). This result was supported by SAMOVA analysis (Table 7). The best SAMOVA grouping (i.e. the highest F_{CT} value) indicated three YFT groups. These were; KK, KR and all remaining sites as a single group (NE, WE, TA, TR, MD) ($F_{CT} = 0.1458$, $P = 0.048$).

3.1.5. nDNA variation

3.1.5.1. Genetic variability, Hardy–Weinberg and linkage disequilibrium estimates. No significant problems with null alleles, large allele dropouts or other genotyping errors were detected ($P > 0.05$) for the YFT microsatellite data set. Estimates of genetic diversity for the three microsatellite loci screened are summarised in Table 8 and Electronic Appendix. Sample sizes varied among loci due to a small number of individuals that could not be scored at some loci due to amplification problems. The number of individuals amplified for the three loci for all sites however, were generally high ($n = 26$–54) except at the WE site ($n = 8$) for locus UTD499.

Number of microsatellite alleles per locus ranged from 6 (at locus UTD402 at NE) to 22 (at locus UTD494 at NE). After Bonferroni correction, tests for Hardy–Weinberg equilibrium revealed significant heterozygote deficiencies at locus UTD402 and locus UTD494 at the NE site and at locus UTD402 in the KR site ($P < 0.001$). Linkage disequilibrium was detected only for UTD494 and UTD499 at NE in 2003.

3.1.5.2. Population structure. While AMOVA analysis of nDNA data showed no significant genetic differentiation across all loci (global $F_{ST} = -0.0633$, $P = 0.462$) (Table 9), analysis of individual loci showed structure at two (i.e. UTD494 and UTD499) out of three loci.

3.1.5.3. Statistical power. Table 10 shows the statistical power of the microsatellite data set to detect various levels of true population differentiation (F_{ST}) between populations based on sample sizes, number of loci, and average allele frequencies. Our microsatellite data set will detect a true F_{ST} of as low as 0.0025 with a probability of more than 80%, and the same data set can detect true differentiation of 0.01 with a probability of 100%. The α error (corresponding to the probability of obtaining false significance when the true $F_{ST} = 0$) was close to 5% in all cases.

4. Discussion

To date, no detailed studies of YFT genetic stock structure have been conducted in the Indian Ocean at any significant scale. Lack of genetic information on population structure in the Indian Ocean has led regional management authorities to continue to treat YFT populations across the Indian Ocean as a single management unit based on analyses from other ocean basins and a general perception that YFT possess high dispersal potential. Some earlier and recent studies of tuna morphology and fisheries data have suggested that YFT population structure in the Indian Ocean may however, be more complex, and suggested the presence of more than a single stock (Kurogane and Hiyama, 1958; Morita and Koto, 1970; Nishida, 1992; IOTC, 2006). Results of the present study support this view and suggest that YFT population structure in the Indian Ocean may be more complex than current management suggests. Data here indicate that some fine-scale population structure may be present, at least among specific sites around Sri Lanka. All approaches used here for mtDNA analyses investigating population structure provided strong indication that two sampled sites (KK and KR) were significantly different from the remaining population pool. This finding was not supported however, by combined microsatellite AMOVA analysis, that indicated that sampled sites were effectively panmictic for nuclear genes even though two of the three nDNA loci showed evidence for some structure. While one con-

Table 7
Population structure based on mtDNA differentiation of YFT (in SAMOVA)

No. of groups	Structure	Variation among groups	Variation (%)	F_{CT}	P
2	(KK) (NE, WE, TA, KR, TR, MD)	0.0609	13.15	0.1315	0.147
3	**(KK) (KR) (NE, WE, TA, TR, MD)**	**0.0654**	**14.59**	**0.1458**	**0.048**
4	(KK) (KR) (TR) (NE, WE, TA, MD)	0.0532	12.24	0.1223	0.0762
5	(KK) (KR) (TR) (MD) (NE, WE, TA)	0.052	12.14	0.1214	0.0342
6	(KK) (KR) (TR) (MD) (NE) (WE, TA)	0.0562	13.2	0.132	0.043

The row in bold type indicates the details of geographically meaningful groups with maximum genetic differentiation.

Table 8
Estimates of genetic diversity for three microsatellite loci among YFT

Sample	Locus			Average across loci
	UTD402	UTD494	UTD499	
KK				
n	54	54	53	54
a	16	20	17	17.667
H_e	0.708	0.927	0.918	0.851
H_o	0.722	0.981	0.925	0.876
HW (*P*)	0.383	0.580	0.433	
NE				
n	26	45	47	39
a	6	22	17	15.000
H_e	0.666	0.916	0.881	0.821
H_o	0.280	0.773	0.870	0.641
HW (*P*)	**0.000**	**0.000**	0.711	
WE				
n	33	44	8	28
a	10	20	8	12.667
H_e	0.875	0.934	0.879	0.896
H_o	1.000	1.000	1.000	1.000
HW (*P*)	0.932	1.000	1.000	
TA				
n	42	40	41	41
a	10	18	19	15.667
H_e	0.542	0.930	0.912	0.794
H_o	0.571	0.875	0.927	0.791
HW (*P*)	0.579	0.053	0.192	
KR				
n	44	49	24	39
a	9	20	14	14.333
H_e	0.749	0.941	0.924	0.871
H_o	0.667	1.000	0.900	0.856
HW (*P*)	**0.000**	0.152	0.219	
TR				
n	35	45	25	35
a	7	19	14	13.333
H_e	0.599	0.915	0.897	0.804
H_o	0.536	0.857	0.960	0.784
HW (*P*)	0.024	0.549	0.793	
MD				
n	42	42	43	42
a	10	20	14	14.667
H_e	0.585	0.938	0.911	0.811
H_o	0.548	0.929	0.977	0.818
HW (*P*)	0.785	0.716	0.700	

Number of samples (*n*), number of alleles (*a*), expected heterozygosity (H_e), observed heterozygosity (H_o), Probability values of concordance with Hardy–Weinberg expectations (HW) (*P*). Values in bold type are significant probability estimates after Bonferroni correction for multiple tests (initial $\alpha = 0.05/21 = 0.0023$).

sistently non-significant locus may constitute an outlier, power analysis suggests that a combination of the three loci and the respective numbers of alleles present provided sufficient power to detect structure if it was present.

A variety of explanations are available for this apparent anomaly between mtDNA and nuclear markers. For example, potential for differences in male vs female biased dispersal can explain intra-specific differences in patterns of mtDNA and nDNA variation (e.g. Prugnolle and de Meeus, 2002; FitzSimmons et al., 1997; Lyrholm et al., 1999; Pardini et al., 2001). If female YFT do not disperse as extensively as males, this may produce structure in the mtDNA variation while it is absent for nDNA. While evidence for female 'philopatry' is virtually absent for YFT, some studies have commented on male dominance in YFT breeding populations in the Indian Ocean (IOTC, 2006). Significant genetic differentiation was observed however, for mtDNA data for bigeye tuna but not for nDNA data in the Atlantic Ocean, and this pattern was explained by male mediated gene flow (Durand et al., 2005).

Alternatively, lower estimates of genetic differentiation in nuclear microsatellite markers compared with a mtDNA marker is expected, as the effective population size for the nuclear DNA genome is four times higher than for the mtDNA genome (Birky et al., 1989). Hence, the mtDNA genome is much more sensitive to genetic drift effects and therefore low but significant genetic differentiation (if present), is more likely to be detected with mtDNA markers. Drift effects in mtDNA can also be intensified if the population had undergone a sudden population expansion in the recent past, for example following a population bottleneck. When a population has experienced a recent sudden expansion, recombination increases in the nDNA and hence can dilute most of the genetic drift effects resulting in low or no genetic differentiation in nDNA. In fact, the star-like phylogeny observed in the YFT mtDNA data suggests that populations have probably experienced a recent sudden population expansion. Ward et al. (1997) also reported that global YFT populations had expanded recently, as did Ely et al. (2005).

Previous studies of YFT genetic population structure in the Indian Ocean however, did not find evidence for population structuring (Ward et al., 1997; Chow et al., 2000; Nishida et al., 2001). The discrepancy between these earlier results and our study may reflect the relatively low sample sizes used in previous studies, and the comparatively low resolving power of genetic markers and statistical analyses used there. In contrast, our study examined 285 YFT from six regions around Sri Lanka and one collection from the Maldive Islands using highly sensitive markers (mtDNA sequencing data and nDNA microsatellite data), in addition to applying powerful statistical techniques capable of detecting fine geographical scale genetic heterogeneity, if present.

It is clear from the data set here, that the genetic structure observed among our sites, results largely from genetic heterogeneity at two sites, the KK and KR populations. Individuals sampled at these sites were very large sized fish caught from long line fishing operations. This contrasted with collections from most other sites, where sampled individuals were generally smaller (Table 1). Thus, individuals at the KK and KR sites may have dispersed from elsewhere, perhaps from more distant locations in the western and eastern Indian Oceans, respectively, towards Sri Lanka. According to IOTC (2006), YFT spawning grounds in the Indian Ocean exist in the equatorial region (0–10°S), with the main spawning grounds identified in the western Indian Ocean (west of 75°E), while secondary spawning grounds are reported in the Mozambique Channel, near Sri Lanka, and off the western Australian coast in the eastern Indian

Table 9
Genetic structuring of YFT populations based on microsatellite data

Structure tested	Observed partition		F statistics (F_{ST})	P
	Variance	%Total		
AMOVA for all three loci				
Total collection (2001, 2002, 2003, 2004)				
Among populations	−0.0538	−6.34	−0.0633	0.462
Within populations	0.9043	106.34		
Locus by locus AMOVA for total collection				
UTD402				
Among populations	0.0011	0.372	0.00373	0.185
Within populations	0.3051	99.627		
UTD494				
Among populations	0.0029	0.6355	0.00635	0.0009
Within populations	0.4659	99.3645		
UTD499				
Among populations	0.0075	1.635	0.01635	0.0000
Within populations	0.4545	98.3649		

Ocean. A feeding ground that is well recognised has also been reported in the Arabian Sea (IOTC, 2006), which is northwest of Sri Lanka and relatively close to the KK site. Given that there is potential for three heterogeneous groups in the study area (based on discrete spawning locations), there is a possibility that KK individuals may have originated from the western Indian Ocean spawning grounds and had dispersed towards feeding grounds adjacent to the KK site. In contrast, KR individuals may have originated in the eastern Indian Ocean spawning grounds, while individuals in the remaining sampled sites may have come from locally spawned fish. Prevailing monsoon ocean currents around Sri Lanka are likely to move fish towards Sri Lanka from the eastern and western Indian Oceans, during the two monsoon seasons, respectively, as evidenced by tagging studies (Yesaki and Waheed, 1992). Another factor that may influence the observed pattern was differences in age and size classes at spawning. Atlantic bluefin tuna from different areas of the Atlantic Ocean spawn at different ages and size classes and this variation has been argued to be under genetic control (Nemerson et al., 2000). We observed a similar pattern here for YFT, with body length of spawning adults at the KK and KR sites, much larger than YFT from remaining sites. This observation may indicate that KK and KR individuals come from genetically different spawning stocks. Additional support for this model of YFT population structure around Sri Lanka comes from the suggestion by Nishida (1992) based on longline fishery data that Sri Lanka is an area of overlapping boundary between eastern and western Indian Ocean YFT stocks.

Thus, data presented here in combination with indirect evidence of heterogeneity in YFT populations sampled in the Indian Ocean from other sources, suggest that populations in this region may not be panmictic. This finding warrants further genetic analysis of YFT populations in the Indian Ocean that focus on comparisons of larvae or post larvae (young of the year) sampled from putative geographically discrete spawning grounds in the region. This study would clarify whether spawning aggregations are discrete (e.g. Carlsson et al., 2004, 2007), and hence help to resolve Indian Ocean YFT stock structure. If further studies verify the existence of multiple genetically independent stocks in the north western Indian Ocean, this finding should be paramount when decisions are made concerning future management and conservation options for Indian Ocean stocks that are already over exploited.

Table 10
Statistical power for detecting various true levels of population differentiation (F_{ST}) by means of Fisher's exact test when using the present microsatellite loci, allele frequencies, and sample sizes

True F_{ST}	Power
0.001	0.251
0.0025	0.802
0.005	0.986
0.01	1.000
0.02	1.000
0.025	1.000
0.05	1.000

The power is expressed as the proportion of simulations that provide statistical significance at the 0.05 level.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.fishres.2007.10.006.

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