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# Stock Structure of Indo-Pacific King Mackerel (*Scomberomorus guttatus*) in the Persian Gulf Using Microsatellite Loci

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**Abstract:** In order to assess the genetic differentiation within and between wild populations of Indo-Pacific king mackerel (*Scomberomorus guttatus*) five microsatellite markers were used (J43Sc, L42Sc, D61Sc, H96Sc, C83Sc). Population structure and genetic divergence were investigated by 200 individuals from, Lengeh, Dayyer, Boushehr and Abadan in the northern coasts of Persian Gulf. All the markers produced polymorphic PCR products, which amplified of the four populations. Genetic differentiation, as measured by the fixation index, $F_{st}$, was determined to estimate stock structure. Results identified one genetic stock with sufficient gene flow between all the four regions to prevent regional genetic differentiation from occurring. Almost 82.21% of the genetic variation was observed within individuals and 2.05% among populations. Results revealed that adopting a single-stock model and regional shared management could probably be appropriate for sustainable long-term use of this important resource.

**Key words:** *Scomberomorus guttatus* · Microsatellite markers · Persian Gulf · Stock structure

## INTRODUTION

The Indo-Pacific King mackerel, *Scomberomorus guttatus*, locally recognized as Ghobad (Iran), is one of the most ecologically and commercially important migratory fish species exploited in Persian Gulf. This species is a pelagic predator found throughout the Indo-West Pacific: Persian Gulf, India and Sri Lanka to southeast Asia, north to Hong Kong and Wakasa Bay, Sea of Japan. This species inhabit in the coastal waters at depths between 15-200 m and sometimes entering turbid estuarine waters. The diet mainly consists of small schooling fishes like sardines and anchovies, though squids and crustaceans are also consumed. Large adults may be solitary, whereas juveniles and young fish occur in small schools. They are found in small schools, which undertake long-shore migrations. In waters off Iran in the northern Persian Gulf, *S. guttatus* has a short spawning season, with fishing peaks in the months between late December and January [1].

The threat from the increasing overfishing and the potential of recruitment failure associated with the intensive harvest of immature fish has been of particular concern for this species in this region [2]. Ever-increasing pressures on fisheries resources intensify the need to identify stock structure in exploited populations. Understanding fish stock structure is an important component of successful and sustainable long-term management [3]. Genetic methods have the advantage over other stock identification methods in that their results are not affected by environmental parameters, as is usually the case for phenotypic characters [4].

Molecular methods are the most important tools for defining stock structure [5] and for evaluating levels and patterns of genetic diversity in fishes [6]. Microsatellite DNA loci is one of these tools, which contain tandem repeated motifs of 1-6 base pairs and are found throughout the genome of all prokaryote and eukaryote genomes investigated so far [7]. Among the various currently available DNA markers that can be used to examine genetic diversity at the molecular level, the most informative and polymorphic are microsatellite DNA markers [8]. Because of the very high levels of genetic variation which are often detected at individual microsatellite loci, the large number of loci that can be screened, their relative ease of analysis and use of microsatellites loci in taxonomically related species (e.g. those of a single genus), microsatellite is currently widely used to assess genetic structure [7].

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Fig 1: Map of the Persian Gulf indicating the four sites where *S. guttatus* were sampled

Therefore, the main objective of this study was employ five microsatellite markers to determine whether this species forms a single stock in the Persian Gulf, or it is genetically subdivided into distinctly separated populations and we expect that our results will be useful for fisheries managers.

## MATERIALS AND METHODS

Fish samples were collected from four sites along the northern coast of the Persian Gulf over a 3-month period (March-May 2009); Lengeh, Dayyer, Boushehr and Abadan (Fig. 1). These fish were caught by artisanal fisherman. A small piece of caudal fine (20 mg) was removed and transported to the laboratory in absolute ethanol and stored at -20°C until analyzed. Sample size was 50 individuals per population. Primers sequences specific for five microsatellite loci described and used in a previous study carried out on *Scomberomorus commerson* by van Herwerden *et al*. (2006) were applied in this study [9] (Table 1).

DNA was extracted from 200 individual using a standard phenol/chloroform extraction procedure, then visualized by gel electrophoresis (0.8% agarose) and quantified by spectrophotometric assay. Some individuals failed to amplify with all markers and were rejected from the study. Polymerase Chain Reaction (PCR) amplification was performed in 20 µl reaction volume containing 100 ng of template DNA, 10 pmol of each primer, 400 µM each of the dNTPs, 1 U of *Taq* DNA polymerase (Cinnagen, Iran), 1.5 mM $MgCl_2$ and 1× PCR buffer. PCR temperature profile consisted of pre-denaturation at 94°C for 3 min followed by 30 cycles of denaturation at 94°C for 30 s, annealing at primer-specific temperature for 30 s (Table 1), extension at 72°C for 30 s and final extension at 72°C for 3 min. PCR products were separated by electrophoresis on an 8% denaturing polyacrylamide gel and visualized by silver staining.

ONE-Dscan (1-D Gel Analysis) software version 2.03 was used to determine the alleles detected by electrophoresis.

Table 1: Microsatellite markers from *S. commerson*, annealing temperature, $T_a$ and size of amplified fragment

| Locus | Repeat motif | Primer 5′>3′ | $T_a$ (°C) | Size (bp) |
|---|---|---|---|---|
| C83Sc | $(TG)_5TC(TG)_{11}$ | F: ACGCAGCAATGCACCGTGG | | |
| | | R: AAGAATCAACACAAACAGCACC | 58°C | 170-190 |
| H96Sc | $(CA)_{13}GA(CA)_2GA(CA)_4$ | F: AAAGAATGGAAATTCAGATCAC | | |
| | | R: TAAAATGACATCATCCCATGG | 56°C | 180-206 |
| J43Sc | $(TG)_5TT(TG)_7AG(TG)_4$ | F: TGATCTAATCAATGGGAGAGG | | |
| | | R: TGCTCACATGTGCAAGCAAT | 57°C | 150-184 |
| L42Sc | $(TG)_4CC(TG)_{15}$ | F: ATGGCAACGGCGAGATTAAGG | | |
| | | R: TCCAGAACAGCAGCAGTTTCC | 59°C | 278-380 |
| D61Sc | $(CA)_{11}AA(CA)_3$ | F: CTATCAGCAATTAAGTGTACTAC | | |
| | | R: TGTGAGAGGTTCAACAATG | 58°C | 260-358 |

The recorded microsatellite genotypes were examined to evaluate genotyping errors, estimate a large allele dropout and null allele frequency for each locus using MICROCHEKER 2.2.3 [10]. Pairwise linkage disequilibrium was tested with ARLEQUIN software version 3.5.1.2 [11] and Genepop software version 4.0 [12]. ARLEQUIN software was also used in order to calculate observed (Ho) and expected (He) heterozygosities.

Using Genepop software allele number, allele frequencies and statistical significance of Hardy-Weinberg equilibrium (HWE) were analyzed. The probability of each locus pair across all populations to be in HWE was estimated by Markove chain method. Markov chain parameters for all tests were 1000 dememorization, 100 batches and 1000 iterations per batch steps. Fisher's method was used to assess genotypic disequilibrium by determining exact *P*-values for each locus pair across all populations. Sequential Bonferroni corrections were performed to reduce the group-wide type I error rate (as per Holm, 1979 in Rice, 1989). Wright's index $F_{st}$ and analysis of molecular variance (AMOVA) were carried out using ARLEQUIN software. *N*m values among the four populations were computed using GenAlex software version 6 [13].

## RESULTS

Evaluation of genotyping errors by MICROCHEKER revealed no evidence for large allele dropout and null allele frequency at all loci. The five microsatellite loci used to assess genetic diversity in four geographic populations were polymorphic and 58 alleles identified in 200 individuals. The number of alleles per locus ranged from 10 (H96Sc) to 20 (D61Sc). The number of alleles per locus per population ranged from 10 (H96Sc in Abadan population) to 20 (D61Sc in Dayyer population) alleles within individual populations. Observed heterozygosities ranged from 0.875 to 1.000 (Table 2). All of the five loci showed significant deviation from HWE in all populations (Table 2). None of the five loci were found to be in LD ($p$>0.05).

Table 2: Genetic diversity and HWE in four populations *s.guttatus* in northern coasts of Persian Gulf

| Locus | Lengeh | Dayyer | Boushehr | Abadan |
|---|---|---|---|---|
| C83Sc | | | | |
| *N* | 48 | 49 | 47 | 47 |
| *A* | 14 | 13 | 14 | 15 |
| He | 0.702 | 0.732 | 0.653 | 0.690 |
| Ho | 1.000 | 0.975 | 0.975 | 1.000 |
| $F_{is}$ (*P*-value) | -0.425 (0.000)* | -0.333 (0.000)* | -0.492 (0.000)* | -0.449 (0.000)* |
| H96Sc | | | | |
| *N* | 49 | 50 | 49 | 48 |
| *A* | 14 | 12 | 16 | 10 |
| He | 0.710 | 0.689 | 0.722 | 0.568 |
| Ho | 1.000 | 1.000 | 0.875 | 1.000 |
| $F_{is}$ (*P*-value) | -0.408 (0.000)* | -0.452 (0.000)* | -0.133 (0.000)* | -0.761 (0.000)* |
| J43Sc | | | | |
| *N* | 49 | 48 | 50 | 48 |
| *A* | 16 | 15 | 14 | 18 |
| He | 0.744 | 0.710 | 0.749 | 0.688 |
| Ho | 1.000 | 1.000 | 1.000 | 1.000 |
| $F_{is}$ (*P*-value) | -0.343 (0.000)* | -0.408 (0.000)* | -0.334 (0.000)* | -0.455 (0.000)* |
| L42Sc | | | | |
| *N* | 50 | 49 | 47 | 49 |
| *A* | 18 | 18 | 16 | 12 |
| He | 0.739 | 0.748 | 0.728 | 0.500 |
| Ho | 1.000 | 1.000 | 1.000 | 1.000 |
| $F_{is}$ (*P*-value) | -0.354 (0.000)* | -0.338 (0.000)* | -0.375 (0.000)* | -1.000 (0.000)* |
| D61Sc | | | | |
| *N* | 50 | 48 | 50 | 49 |
| *A* | 18 | 20 | 16 | 12 |
| He | 0.799 | 0.803 | 0.758 | 0.805 |
| Ho | 1.000 | 1.000 | 1.000 | 1.000 |
| $F_{is}$ (*P*-value) | -0.251 (0.000)* | -0.246 (0.000)* | -0.320 (0.000)* | -0.242 (0.000)* |

*N*: number of individual fish sampled; *A*: number of alleles; He: expected heterozygosity; Ho: observed heterozygosity; $F_{is}$: correlation of alleles within individuals; *P*: *P*-value of Chi-Square tests for Hardy-Weinberg equilibrium. Asterisked $F_{is}$ *P*-values indicate genotype frequencies not in Hardy–Weinberg equilibrium following sequential Bonferroni correction.

Table 3: Population pairwise $F_{st}$ values for five loci are shown below the diagonal for four samples of *S. guttatus*

| | Lengeh | Dayyer | Boushehr | Abadan |
|---|---|---|---|---|
| Lengeh | - | 0.975 | 0.940 | 0.0832 |
| Dayyer | 0.004 | - | 0.910 | 0.859 |
| Boushehr | 0.011 | 0.016 | - | 0.897 |
| Abadan | 0.038 | 0.033 | 0.025 | - |

*N*m are shown above the diagonal and $F_{st}$ values are below.

Table 4: AMOVA analyses for samples of *S. guttatus* into four populations

| Source of variation | d.f. | Sum of squares | Percentage of variation |
|---|---|---|---|
| Among populations | 3 | 24.383 | 2.05 |
| Among individuals within populations | 196 | 503.290 | 15.74 |
| Within individuals | 200 | 393.000 | 82.21 |
| Total | 399 | 920.673 | 100 |

Level of partitioning was either among populations among individuals within populations and within individuals; d.f., degrees of freedom are shown

Low $F_{st}$ values were observed among all population pairs, ranging from 0.004 to 0.038 (Table 3). Pairwise *P*-values between any of two populations indicated no statistically significant in the genetic differentiation among populations into the northern coasts of Persian Gulf. AMOVA analysis showed only 2.05% of the total genetic variation among populations. The genetic variation among individuals within populations was 15.74% and almost all the genetic variation detected within individuals (82.21%) (Table 4).

## DISCUTION

In the present study, the first analysis of *S. guttatus* populations in the Persian Gulf was carried out based on five neutral microsatellite loci among the four sites. Results obtained from microsatellite data revealed a genetic connectivity between the four sampled sites across the Persian Gulf.

In general, marine species have low levels of genetic differentiation for several reasons: (1) the overall absence of clear barriers to distribution in the marine environment effectually reduces heterogeneity between populations; (2) a small number of migrants per generation are adequate to remove genetic differentiation; (3) marine species usually have high fecundities and dispersal abilities [14], these are particularly true of highly migratory vagile species with planktonic larvae such as members of the genus Scomberomorus [1].

Microsatellite DNA markers used in this study showed higher levels of genetic diversity. Observed heterozygosities ranged from 0.875 to 1.000. It indicated that the microsatellite technique is more powerful for polymorphic analysis than other techniques and thus it is valuable in population genetics studies [15, 16].

The *F*-statistics showed that $F_{is}$ estimates of five loci were negative. $F_{is}$ values indicating that there is heterozygote excess relative to HWE (Table 2). Heterozygote excess in populations is not as common as the heterozygote deficiency and therefore has not been fully theoretically explored. Over dominant selection favoring heterozygotes, associative over dominance [17] and negative assortative mating are generally used to explain heterozygote excess in natural populations [18, 19 and 20]. All the loci displayed significant deviation from HWE in all populations. Deviation from Hardy-Weinberg proportions indicates either selection, population mixing or nonrandom mating and its detection is one of the first steps in the study of population structure [21]. Thanks to the distance among locations, specific characteristics of Persian Gulf, as kind of a homogenous body of water [22] and described lifestyle of Indo-Pacific king mackerel, migration, mutation, natural selection and gene flow between different populations are probably the other descriptions for the mentioned deviation between the four sites [23].

$F_{st}$ analysis showed pairwise $F_{st}$ in the four sites was low (0.004-0.038) and no statistically significant values of *P*-value suggesting no genetic differentiation among this species in Persian Gulf and the four stocks could be considered as an admixed single stock. The *N*m values showed that there must be sufficient gene flow between all Persian Gulf sites sampled. The lack of this species' genetic differentiation is related to the adult and larval pelagic life history and wide-ranged alongshore migration.

In conclusion, this study extends our knowledge of the genetic diversity of *S. guttatus*, particularly; provide useful information on the genetic variation and differentiation in the Iranian populations. The data generated from this research it was accepted that Indo-Pacific king mackerel of Persian Gulf is composed of one

population and suggesting that regional shared management could probably be appropriate for sustainable long-term use of this important resource. Therefore, we expect that our results will be useful for fisheries managers.

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