Genetic Differentiation of Narrow-Barred Spanish Mackerel (Scomberomorus commerson) Stocks Using Microsatellite Markers in Persian Gulf

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Abstract: Scomberomorus commerson is not considered as an endangered species in Persian Gulf, but recent studies indicated the decline of this population and genetic management strategies are needed. In order to assess the genetic differentiation within and between wild populations of Spanish mackerel, five neutral microsatellite markers were used. Population structure and genetic divergence were investigated by 50 individuals at each site from Lengeh, Dayyer, Boushehr and Abadan in the northern coasts of Persian Gulf. All the markers produced polymorphic PCR products which amplified to the four populations. Genetic differentiation, as measured by $F_{st}$, was determined to estimate stock structure. Results identified one genetic stock with sufficient gene flow between all the four sites to prevent genetic differentiation from occurring. Only 2.98% of the genetic variation was observed 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 in Persian Gulf.

Key words: Genetic Differentiation · Microsatellite Marker · Persian Gulf · Scomberomorus Commerson · Stock Structure

INTRODUCTION

The narrow-barred Spanish mackerel, Scomberomorus commerson (Lacepède, 1800), locally recognized as Sheer (Iran) and Kingfish (English), is one of the most ecologically and commercially important migratory fish species exploited in Persian Gulf. This species is an epipelagic predator found throughout the Indo-West Pacific, including contiguous distribution within Persian Gulf [1] and also from Hong Kong and Japan to Australia [2]. Kingfish occurs from the edge of the continental shelf to shallow coastal water where it is found along drop-offs, gently sloping reefs and lagoon waters from depths of 10-70 m [3]. The diet mainly consists of small fishes like anchovies, clupeids and carangids, though squids and shrimps are also consumed [4]. 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, but importantly permanent resident populations have also been reported [5]. In the waters of Iran in the northern Persian Gulf, S. commerson has a short spawning season, with peaks in reproductive output between May and June [6]. The feeding migration coincides with reduced water temperatures and an increase in the abundance of small pelagic species [7].

Many fish population in the Persian Gulf have been heavily exploited and effort may be above optimum levels for some species [8]. 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 [9].

There is a lack of information on movements, migrations and spawning activities to describe the stock structure of kingfish in the Persian Gulf. 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 [10]. Stock structure is conventionally determined by differences in phenotypic parameters of fish sampled from different locations. Genetic methods have the advantage over other stock identification methods as their results are not affected by environmental parameters, compared with phenotypic characters [11]. Molecular genetic techniques offer the ability to identify and delineate fish stock structure where it may not be apparent from phenotypic or behavioral characteristics [12].

Genetic methods are the most important tools for defining stock structure and evaluating levels and patterns of genetic diversity in fishes [13]. 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. 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. Because of the very high levels of genetic variation that are often detected at individual microsatellite loci, the large number of loci that can be screened and their relative ease of analysis, microsatellites are currently widely used to assess genetic structure [14]. Microsatellites have been extensively used to describe stock structure and evaluate genetic diversity of marine fish species, such as *Scomberomorus cavalla* [15], *Lethrinus miniatus* [16] and *Trachurus trachurus* [11] and *Scomberomorus guttatus* [17].

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 fin (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 by van Herwerden et al. [13] were used in this study (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 the 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

![Fig 1: Map of the Persian Gulf indicating the four sites where *S. commerson* were sampled.](image)

**Table 1: Microsatellite markers from *S. commerson*.**

<table>
<thead>
<tr>
<th>Locus</th>
<th>Repeat motif</th>
<th>Primer 5’&gt;3’</th>
<th>T&lt;sub&gt;a&lt;/sub&gt; (°C)</th>
<th>Size (bp)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C85Sc</td>
<td>(TG)₃(TG)₆</td>
<td>F: ACGCAACACATGCACCCGG &lt;br&gt;R: AGGAATCCAACACAAACGACACC</td>
<td>58°C</td>
<td>168-198</td>
</tr>
<tr>
<td>H96Sc</td>
<td>(CA)₅Ga(CA)₆Ga(CA)₄</td>
<td>F: AAGAATGGAATTCAGATCAC &lt;br&gt;R: TAAAATGACATCATCCATCCTGG</td>
<td>56°C</td>
<td>178-216</td>
</tr>
<tr>
<td>J43Sc</td>
<td>(TG)₃TT(TG)₆AG(TG)₄</td>
<td>F: TGATCTAATCAATGGGAGAGG &lt;br&gt;R: TGGTACATCTGTGCCAAGCAAT</td>
<td>57°C</td>
<td>148-188</td>
</tr>
<tr>
<td>L42Sc</td>
<td>(TG)₃CC(TG)₆</td>
<td>F: ATGCGAACCGCGAGATTAAGG &lt;br&gt;R: TCCAGAACACGAGCAAGCTTCCCC</td>
<td>59°C</td>
<td>276-388</td>
</tr>
<tr>
<td>D61Sc</td>
<td>(CA)₅AA(CA)₅</td>
<td>F: CTATCAGCAATTAAGTACTAC &lt;br&gt;R: TGGAGAGGTCTCAACATG</td>
<td>58°C</td>
<td>260-346</td>
</tr>
</tbody>
</table>

*T<sub>a</sub>* annealing temperature and size of amplified fragment.
polymerase (Cinnagen, Iran), 1.5 mM MgCl₂, 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 3 min 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.

The recorded microsatellite genotypes were examined to evaluate genotyping errors, estimate a 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 66 alleles identified in 200 individuals. The number of alleles per locus ranged from 14 (H96Sc) to 21 (D61Sc), with an average of 13.2. The number of alleles per locus per population ranged from 7 (H96Sc in Abadan population) to 21 (D61Sc in Lengeh population) within individual population. The average expected and observed heterozygosities were 0.910 and 0.986, respectively. Expected heterozygosities ranged from 0.803 (H96Sc) to 0.947 (L42Sc) (Table 2).

**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 66 alleles identified in 200 individuals. The number of alleles per locus ranged from 14 (H96Sc) to 21 (D61Sc), with an average of 13.2. The number of alleles per locus per population ranged from 7 (H96Sc in Abadan population) to 21 (D61Sc in Lengeh population) within individual population. The average expected and observed heterozygosities were 0.910 and 0.986, respectively. Expected heterozygosities ranged from 0.803 (H96Sc) to 0.947 (L42Sc) (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 ($p>0.05$).

Low $F_{is}$ values were observed among all population pairs, ranging from 0.007 to 0.047. Pairwise $P$-values between any two populations indicated no statistically significant in the genetic differentiation among populations into the northern coasts of Persian Gulf (Table 3).

AMOVA analysis showed only 2.98% of the total genetic variation among populations. The genetic variation among individuals within populations was 12.99% and almost all the genetic variation detected within individuals (84.03%) (Table 4).
DISCUSSION AND CONCLUSION

There have been a number of studies on the genetic structure of *S. commerson* based on different molecular markers and focusing in different areas of its distribution, such as allozymes [21] and microsatellite markers [22] in the Arabian Sea and mitochondrial DNA [23] in the ROPME sea area. van Herwerden et al. [13] based on microsatellites marker indicated that there were two genetic stocks in the populations of *S. commerson*, one of them was restricted to one locality (Dhofar) in the Arabian Sea, the other widespread with sufficient gene flow between Bandar Abbas in Persian Gulf, Muscat and Musandam in Gulf of Oman, Al-Wusta in Arabian Sea and Yeman in Gulf of Aden. However, an adaptation to a single-stock model in the ROPME sea area was suggested by Hoolihan et al. [23].

In the present study, the first analysis of *S. commerson* populations in the northern coasts of 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 [24], these are particularly true of highly migratory vagile species with planktonic larvae such as members of the genus *Scomberomorus* [25, 15].

Microsatellite DNA markers used in this study showed higher levels of genetic diversity. It indicated that the microsatellite technique is more powerful for polymorphic analysis than other techniques and thus it is valuable in population genetics studies [26].

*F*<sub>S</sub> values indicating that there is heterozygote excess relative to HWE. Heterozygote excess in populations is not as common as the heterozygote deficiency and therefore has not been fully theoretically explored. Overdominant selection favoring heterozygotes [27], associative overdominance [28] and negative assortative mating are generally used to explain heterozygote excess in natural populations [29, 30].

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 [31]. The distance among locations, specific characteristics of Persian Gulf, as kind of a homogenous body of water and described lifestyle of kingfish, migration, mutation, natural selection and gene flow between different populations are probably the other descriptions for the mentioned deviation between the four sites [32].

It is concluded that there was no genetic differentiation among Spanish mackerel in Persian Gulf and the four stocks could be considered as an admixed single stock. The presence of the widespread stock 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.

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REFERENCES


