Population genetic structure of the white sardine, *Sardinella albella*, in the Persian Gulf and Sea of Oman by analysis of mitochondrial control region

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Abstract

Several studies on the white sardine: *Sardinella albella*, have focused on the identification of stock composition and behavior. In this study population genetic structure and historical demography of *S. albella* along the cost of the Persian Gulf and Sea of Oman were investigated with a 500-bp segment of mt-DNA control region. In total 40 samples were collected from 3 locations: Jask in Sea of Oman, Qeshm in Strait of Hormuz and Lengeh in the Persian Gulf during 2012-2013. 33 haplotype were obtained none of which were presented in all sampling sites. Analysis of molecular variance (AMOVA) indicated low genetic differentiation among regions (F_{ST} =0.024, *p*<0.05). The average pair wise differences between regional population were small but significant (0.0158-0.165). Molecular variance explained by differences among three regions was significantly different from zero but the F_{ST} did not show clear phylogeographic isolation. This observation can support the conclusion that *S. albella* has a widespread dispersal potential.

Keywords: Sardinella albella, Clupeidae, Sardine Fish, Persian Gulf and Sea of Oman

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Introduction

The genetic of fish structure populations is important not only because of fundamental interest in biotic evolution (Tudela et al., 1999) but also for the management of fisheries (Roldan et al., 2000). So the biological and ecological knowledge about their populations can be relevant in devising suitable management strategies.

Sardines have a very important ecological role in marine ecosystems because they are first consumers in marine food chain and they are the main food of valuable species as tuna. So a decrease in their population will decline fishing of these species (Whithead, 1988). There are 10 genera of Clupeidae in the south of Iran. Sardinella albella is one of the most abundant species inhabiting the tropical and subtropical coastal regions in Indo -pacific from east of Africa, Red Sea, Persian Gulf, Sea of Oman and Indian ocean up to Indonesia and new Gionea (Whithead, 1985) (Fig. 1). In Iran Sardinella albella lives in estuaries along the north coast of the Persian Gulf and Sea of Oman. Adults make schools that can be maintained as a dark span during the day but at night schools scatter and migrate to deep areas to feed. In the breeding season, adults migrate to coastal areas from mid April to the end of June. Their eggs and larva are pelagic (Van Zailing et al., 1993). The life history of Sardinella albella and their passive and active dispersal mechanism have a direct influence on population structure (Liu et al., 2009). Because of the importance of the

identification of stock structure in stock assessment and harvest management (Whithead, 1985), in this study we investigated population the of Sardinella labella in 3 sites located along the north coast of the Persian Sea of Oman. Gulf and The mitochondrial control region serves as the origin of replication for the mitochondrial genome and is usually more variable than other coding genes (Avis, 1994; Parker et al., 1998). So it is an ideal marker for assessing genetic structure of recently diverged or closely related species or populations (Avis, 1994; Rand, 2000). We used partial sequence of mitochondrial control region (D-Loop) to test the hypothesis that indicates: there are no significant genetic differences between sampling zones.

Materials and methods

Sampling

The white sardines used in the present study were collected between autumn 2012 and summer 2013 from commercial landing in Sea of Oman (Jask), strait of Hormoz (Qeshm) and the Persian Gulf (Lengeh). Geographic locations and sample sites are given in Fig. 2.

The dorsal fin of each individual was obtained and preserved in 98% ethanol for genomic DNA extraction.



Figure 1: The map of global distribution of Sardinella albella (Whithead, 1985).



Figure 2: Sampling sites in the Persian Gulf and Sea of Oman.

DNA extraction, amplification, and sequencing

Genomic DNA was isolated using the phenol- chloroform standard method. The primers reported by Somante *et al.* 2000 were used to amplify a fragment of the control region (D-Loop) of mtDNA, (Fig. 3).

Polymerase chain reaction (PCR) was performed in a total volume of 25 µL reactions containing 1×PCR buffer, 200 mM dNTPs, 1.5 mM MgCl₂, 0.3 mM of each primer, 0.5 units Taq DNA polymerase (Fermentaze) and 1 µL of DNA. PCR cycling consists of an initial 94° C for denaturation at 5 min, followed by 30 cycles of 45 sec at 95° C, 45 sec at 52° C, and 2 min at 72° C, with a final elongation of 5 min at 72°C. After visualization on a 1% agarose gel, the appropriate PCR products were sent to the micro gen lab for sequencing. The primers used for sequencing were the same as those for PCR amplifications.

Data analyses

The sequences for all individuals were aligned and edited by BioEdit software (Hall, 1999) and Mega5 (Tamura et al., 2011) which uses Clustal w algorithm. Molecular diversity indices such as number of haplotypes, polymorphic sites, transition, transversions, and indels were obtained by using the program ARLEQUIN 3.5 (Excoffier et al., 2011). Population genetic structure was analyzed by using the analysis of molecular variation (AMOVA), which estimates the proportion of genetic variation within and among

populations. Genetic differentiation between populations was assessed by comparing the average number of pair wise differences between population, within populations (PiX and PiY) and corrected average the pair wise difference (PiXY-(PiX+PiY)/2) using the program ARLEQUIN 3.5 (Excoffier et al., 2011). The significance of differentiation between pairs of populations was tested by exact test (Raymond and Rousset, 1995) using 10,000 Markov chain steps. Molecular pairwise F_{ST} was estimated to evaluate genetic differentiation between pairs of data set. The demographic history was investigated by examine the mismatch distributions (Rogers and Harpending, 1992; Ray et al., 2003; Excoffier, 2004). The parameters of demographic expansion τ , θ_{0} , θ_{1} were estimated. τ index is the time since population expressed unites of expansion as time mutational (Rogers and Harpending, 1992). θ_0 and θ_1 are the mutation parameters before and after population growth. The overall validity of the estimated demographic model is tested by the distribution of a tested statistic (SSD) (the sum of squared differences) between the observed and estimated mismatched distribution. To test whether the D-loop data confirm to neutrality's expectations, the Tajima's D (Tajima, 1989) and Fu's Fs (Fu, 1997) statistics were used. The significance of neutrality was tested with a coalescent simulation program (1000 simulations) as implemented in ARLEQUIN 3.5 (Excoffier et al., 2011).

Results

Genetic diversity

A 500 bp fragment of the control region (D_Loop) of 40 specimens was sequenced. 15 polymorphic sites defined 33 haplotypes among specimens (GenBank accession no. are from KP714734 tile KP714763). This high genetic variability is evident by the large haplotype diversity. No haplotype was presented in all zones (Table 1). Average values of pair wise differences among populations were between 3.25 and 5.01 and corrected average of pair wise difference ranged from 0.051 to 0.1313 (Table 2). These results demonstrated that nucleotide diversity is low and haplotype diversity is high in Lengeh and both nucleotide diversity and haplotype diversity are high in Jask (Table 1). However in Qeshm the haplotype diversity is high and the nucleotide diversity is low.

Geographical structure of genetic variation

The genetic difference was detected in the average number of the pairwise differences (Table 2). The average number of pairwise differences and corrected number of pairwise differences showed minor but significant differentiation between localities (Table 2). The most difference is between Qeshm and Lengeh. AMOVA test ($k_2 p$, p < 0.05) of genetic diversity showed low variation between populations and high variation within them and fixation index of genetic diversities (F_{ST}) between 3 localities was low too (Table 3). Pairwise F_{ST} between Lengeh and Qeshm is low but is more than that between Jask and Qeshm (Table 4).

Patterns of historical demography

None of the mismatched analyses deviated significantly (p>0.05) from expectations under the sudden expansion model, and hence analysis of demographic patterns was suitable (Table 5). The mismatch distribution for both Lengeh and Qeshm was unimodal, and closely matched the expected distribution under the sudden expansion modal. But the mismatch distributions for Jask were multimodal and much smoother, suggesting that this population has not always remained stationary (Fig.4). All Tajima's D and Fu's F_s values were negative (p < 0.05) suggesting the effects of populations growth (Table 5).







Stocks	Ν	nh	H± SD	π±SD	Mean number of
					pair wise differences
Jask	13	10	0.9615 ± 0.0496	0.01287±0.0073	6.052 ± 3.082
Qeshm	12	11	0.9848 ± 0.0403	0.00808 ± 0.004	3.789 ± 2.05
Lengeh	15	11	0.8952 ± 0.0704	0.00541 ± 0.0034	2.536 ± 1.44

Table 1: Sample size (n), number of haplotype (nh), haplotype diversity (H), nucleotide diversity (π) and standard deviation (SD) for each group.

Table2: Pair wise population differences for mitochondrial control region sequences of *Sardinella albella* (*p*<0.05). Above diagonal: average number of pair wise differences between populations (PiXY). Diagonal elements: average number of pair wise differences within population (PiX). Below diagonal: corrected average number of pairwise difference (PiXY-(PiX+PiY)/2).

	Lengeh	Qeshm	Jask	
Lengeh	2.535	3.294	4.347	
Qeshm	0.1313	3.789	5.010	
Jask	0.0513	0.0874	6.055	

Table3: Hierarchical analysis of molecular variance (AMOVA) of mtDNA control region haplotypes of *Sardinella albella* ($k_2 p, p < 0.05$).

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Source of	df	Sums of squares	Variance	Percentage of				
variation			component	variation				
Among populations	2	5.345	0.04876 va	2.35				
Within populations	37	74.93	2.02513 vb	97.65				
Total	39	80.275	2.07389					
Fixation index		F _{ST} : 0.024						

 Table 4: F_{ST} estimates between geographical populations of Sardinella albella

based on mitochondrial control	region sequences haplotypes (<i>p</i> <0.05).	

	Lengeh	Qeshm	Jask
Lengeh	0.000		
Qeshm	0.044	0.000	
Jask	0.01656	0.0158	0.000

Table 5:Results of Tajima's D, Fu's Fs, mismatch distribution test (SSD), corresponding *p*-value and the mismatch distribution parameter estimates based on *the Sardinella albella* mt DNA control region in three populations (p<0.05).

Samples	Tajima's D		Fu's Fs							
	D	р	Fs	р	SSD	р	T	θ	θ_1	
Lengeh	-1.282	0.076	-5.514	0.000	0.0215	0.09	2.5	0.000	9999.0	
Qeshm	-1.282	0.097	-7.736	0.000	0.0111	0.56	3.2	0.000	9999.0	
Jask	-1.197	0.114	-4.898	0.006	0.0106	0.55	3.7	0.0138	34.6.9	



Mismatch distribution (demographic expansion) jask





Figure 4: Mismatch distributions of the *Sardinella albella* mtDNA control region in populations of Lengeh, Qeshm and Jask.

Discussion

The genetic structure of marine organisms can be attracted by many poorly understood variables such as physical oceanographic factors including currents, tides, and violent storms. The biological factors such as local recruitment, larval transport potential, or reproductive strategy can influence the populations too (Palumby, 1994). Some barriers such as large distances, massive river outflows or unsuitable habitats can separate the distribution of some marine fish populations (Liu et al., 2009). Some studies have provided evidence of long - distance dispersal linking populations at the scale of ocean basins (Bowen et al., 2001) and the others indicate that clear divisions have been found over relatively short geographic distances (Guarnieo *et al.*, 2002; Hoffman *et al.*, 2005; Bernal – Ramirez *et al.*, 2003; Carlin *et al.*, 2003; Rodriguez, 2011). The meaningful divergence of genetic signals can be maintained for widely distributed fishes whose populations might have been subjected to the effects of such complex variables (Ward *et al*, 1994; Aripin and Showers, 2000; Atarhouch *et al.*, 2006).

Historical demography and genetic diversity

Populations of fishes often show high haplotypes but medium to low nucleotide diversities (Vaughan et al., 1992; Grant and Bowen, 1998 Dudgeon et al., 2000 ; Fauvelot et al., 2003 ; Chen et al., 2004 ;). In 1997 Bowen and Grant investigated the phylogeography of the sardines (*Sardinops* spp.) as a widely distributed species in temperate upwelling zones and assessed biogeography models for them. Bowen and Avise (1990) studied the influence of zoogeographic factors and life-history patterns of Atlantic menhaden, *Brevoortia tyrannus*. In 2004, Bay *et al.* investigated genetic structure of *Chlorurus sordidus* and found high genetic diversities in it. The high genetic diversities of these species were ascribed to their long stable evolutionary history (Grant and Bowen, 1998).

In this study the mismatch distribution (Fig.4) for both Lengeh and Qeshm were clearly unimodal and none of the mismatch distributions (Table 5) deviated significantly (p>0.05) from expectations under sudden expansion model (Rogers and Harpending, 1992) or there is a high rate of migration between near demes (Ray et al., 2003; Excoffier,2004). But the mismatch distribution of Jask population is bimodal and suggested that the population is in equilibrium (Rogers and Harpending, 1992). Although Fu's value was negative and significantly deviated from a neutral rate, the Tajima's D was negative but not significant (*p*>0.05). Population and mutation expansion rate heterogeneity have opposite effects on Tajima's D statistics (Aris-Brosou and Excoffier, 1996). The negative amount of Tajima's D and Fu's Fs is due to genetic expansion of population in the 3 localities. The high amount of $\theta 1$ can confirm expansion of genetic diversity. The result of increase in genetic diversity is stability of the population. These results demonstrated that our data fit well with the model of spatial expansions of population.

High haplotype diversity and low nucleotide diversity (Table 1) was found in the hyper variable portion at the 3' end of the mitochondrial control region among the three localities. According to Grant and Bowen's (Grant and Bowen, 1998) populations in 3 localities have all experienced rapid population expansion after a period of low effective population size. This criterion enhances the retention of new mutation (Avise et al., 1984). The results were consistent with mismatch distribution analysis and the neutrality test (Table 5). However the high haplotype diversity and low nucleotide diversity in Qeshm may be result in migration of some groups for feeding in the sampling site (Grant and Bowen, 1998).

Population genetic structure

Pelagic marine fishes generally show low levels of genetic diversity among geographic regions because of high dispersal potential during planktonic eggs, larval or adult history stages as a migratory habit and absence of physical barriers between ocean basins or adjacent continental margins (Palumbi, 1994; Grant and Bowen, 1998; Hewitt, 2000). In 2006 Atarhouch et al. investigated populations of Moroccan sardines (Sardinia pilchardus) and find that the genetic diversity can be the result of adaptation of fish to the environment and stability of populations, number of effective reproductive females and dimension of population. Mangulas et al. (2006) investigated the population structure in Atlantic and Mediterranean anchovy (Engraulis encrasicolus) by studying mitochondrial DNA. The results revealed а mosaic pattern of phylogeographical structures for this species and also diversity among populations became low and that within become high. populations **Species** characters environmental and conditions influence gene flow between localities and cause differences in phenotypic and genotypic characters. Rodriguez et al. (2011) investigated population structure of Pacific sardine (Sardinops sagax) and found that high ability of migration of adults and the California current system. which disperses eggs and larva, may be due to weak phylogeographic structures in this species. The adults of Sardinella albella have horizontal migrations and prefer to vertically migrate to costal margins for breeding and their larvae and juveniles are regarded as pelagic. So the high genetic diversity in Sardinella labella populations shows stability because of the creation of large schools. In contrast to the high genetic diversity populations, low within genetic diversity was detected among them (Table 3). These criteria show gene flow between localities. Oceanic and sea currents can effect dispersal of fishes. Among populations, differences might be the result of passive dispersal of eggs and larva and active dispersal of adults or existence of gene flow

between populations (Bowen and Grant, 1997; Grant and Bowen, 1998; Hewitte, 2000). There is in current water along north coastal areas of the Persian Gulf from the Sea of Oman (Hunter, 1983; Pous et al., 2004) that can be the cause of passive transports of eggs & larva from the Sea of Oman to the Persian Gulf and the occurrence of gene flow between them. **Better** environmental conditions in the Sea of Oman, and the monsoons of the Indian Ocean, resulted in differences in morphological characters between animals of the Sea of Oman and the Persian Gulf 1990: (Emara, Alabdessalam, 1995; Cole, 1998; Feron, 1999; Aripin, 2000; Salarpouri et al., 2003; FAO, 2010; Salarpouri et al., 2014;). In our study morphological showed meaningful characters differences between sampling sites (Rahimi et al., 2013) but investigation of genetic populations of S. albella revealed no meaningful genetic differentiation between populations and Tajima's D and Fu's Fs show genetic expansion in 3 localities. High amounts of θ_1 also demonstrate expansion of genetic diversity, so these results demonstrated that S. abella populations may be having stability and widespread dispersal in the Persian Gulf and the Sea of Oman. However the high haplotype diversity and low nucleotide diversity in Qeshm may be the result of migrations of some groups for feeding in the sampling site (Grant and Bowen, 1998). It seems that observed differences in morphological characters are only in the phenotype level and the

populations of Sardinella albella in the Persian Gulf and Oman Sea have no genetical differences. On the other hand observed genetic low variation (Fst=0.02) did not have enough ability to separate the populations in the sample sites. So our hypotheses: there are no significant genetic differences between sampling zones, can be accepted. But more investigation with other gene targets must be done to approve this conclusion. These results can be useful to manage the source of this fish in the Persian Gulf and the Sea of Oman.

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