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Application of AFLP molecular marker for genetic analysis of black pomfret *Parastromateus niger* from the Persian Gulf

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Abstract

Black pomfret *Parastromateus niger* is a commercially important fishery resource in the Persian Gulf but harvesting its stocks lacks genetic identification of populations. AFLP technique was applied to analyze genetic diversity and population structure of 32 fish from coastal waters of Bandar Abbas, Bushehr and Abadan with 7 *Eco*RI/*Mse*I primer pair combinations. In total, 381 bands were produced of which, 46 were polymorphic (12.07%). Percentage of polymorphic bands was higher in Bushehr samples (91.30%) than in Abadan (84.78%) and Bandar Abbas (73.91%) samples. The highest level of heterozygosity based on Nei's coefficient and Shannon's index was observed in Bushehr fish (0.38±0.16 and 0.54±0.21). Observed and effective alleles ranged from 1.73±0.44 and 1.53±0.40 in Bandar Abbas samples to 1.91±0.28 and 1.70±0.34 in Bushehr samples. The average F_{st} was 0.19 indicating high genetic differentiation among the three locations. Gene flow with mean of 1.93 was the lowest level between Bandar Abbas and Abadan (1.24). Nei's genetic identity revealed the least genetic similarity between the samples of Bandar Abbas and Abadan (0.77). AMOVA analysis demonstrated 81% of the genetic variation within populations and 19% among populations. The UPGMA dendrogram clustered all 32 individuals into 3 groups. In some cases individuals from the same region were grouped together but in most cases, gene exchange was observed to be common among the groups. Analyses provided evidence for genetic differentiation among the three locations, indicating separate populations of black pomfret in the northern Persian Gulf.

Keywords: Black pomfret, *Parastromateus niger*, AFLP molecular markers, Population structure, Persian Gulf

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Introduction

Studies on population genetics have attracted much attention because genetic diversity and variance are particularly important for the sustainability of species. Population genetic analyses can provide indirect measures of connectivity among populations (Bossart and Prowell, 1998; Waples, 1998; Hellberg *et al*., 2002; Thorrold *et al*., 2002). Moreover, to manage a fishery resource effectively, it is important to investigate the genetic diversity and population structure because each stock must be managed separately to optimize yield. Failure to identify and manage individual population units can lead to local over-fishing and ultimately to severe over-fishing and decline of populations (Zhang *et al*., 2006).

Molecular markers are effective tools for analyzing genetic variation and population structure (Englbrecht *et al*., 2000; Whitehead *et al*., 2003). There are several kinds of molecular markers among which, amplified fragment length polymorphism (AFLP) is a method developed for genomic DNA fingerprinting (Vos *et al*., 1995). AFLP uses both the techniques of restriction endonuclease digestion and polymerase chain reaction (PCR) amplification of restriction fragments, and thus as a multi-locus fingerprinting technique possesses the powers and overcomes the weaknesses of the RFLP and RAPD methods (Vos *et al*., 1995; Liu *et al*., 2009). Simultaneous screening of a large number of polymorphic loci, high reproducibility due to high stringency of PCR, and relatively high cost effectiveness are the main advantages of the AFLP method (Liu and Cordes, 2004).

AFLP has been applied to study genetic variation and population structure of many fishes, such as rainbow trout (Young *et al*., 1998), ayu *Plecoglossus altivelis* (Seki *et al*., 1999), common carp (Wang *et al*., 2000), channel catfish *Ictalurus punctatus* (Liu *et al*., 1998; Mickett *et al*., 2003), striped mullet *Mugil cephalus* (Liu *et al*., 2009) and so on.

The Carangidae are diverse marine fishes that include ecologically and economically important species such as the black pomfrets, Jacks, scads, trevallies, pampano, amberjacks, and queenfish (Reed *et al*., 2002). The black pomfret, *Parastromateus niger*, a member of the family carangidae, is a benthopalagic species distributed along the continental shelf throughout the Persian Gulf and western Pacific Oceans, ranging from the coast of South Africa east through Indonesia to Queensland, Australia and the southern coast of Japan and China (Witzell, 1978; Pati, 1983; Smith-Vaniz, 1999). Black pomfret makes a daily vertical migration from muddy bottoms (15–40 m in depth), where it spends its days, to the surface at night, presumably to feed on zooplankton and shows schooling behavior (Smith-Vaniz, 1999).

The black pomfret contributes to the commercial fisheries of the countries bordering the Persian Gulf including Iran (Bishop, 2003) and is ecologically and economically important in this region. The fishing of this species has been increasing during the recent years in Iran according to the statistics of the Iranian Fisheries Organization (www.shilat.com) and so, there is a concern about overfishing of its stocks.

Genetic variation is particularly important for the sustainability of species and so, much attention has been paid to research on population genetics. Loss of heterozygosity could have a deleterious effect on population fitness, and the population size must be kept at a certain level (Reed and Frankham, 2002). Furthermore, important information could be attained by investigations of genetic diversity and population structure to program resource recovery. Molecular techniques also offer the ability to identify and monitor fish stock structure (Tudela *et al*., 1999). Although understanding genetic structure is important for the management and conservation of exploited species, presently, little information is available on the genetic structure of *P. niger* in the Persian Gulf. In the only published study, Abdali *et al*. (2007) designed a research to introduce molecular markers for the identification and differentiation of four species of Carangidae in the Persian Gulf and Oman Sea using PCR-RFLP. AFLP technique has become one of the major methods for studies of genetic diversity, particularly for species like black pomfret, whose genomic sequence which is required to identify markers is not available (Bensch and Akesson, 2005; Meudt and Clarke, 2007). In the present study, black pomfret from three geographic locations in coastal waters of the Persian Gulf were analyzed using AFLP to characterize population genetics and stock structure.

Materials and methods

Fish sampling

A total of 32 adult black pomfret were collected in May 2012 directly from the sea at 3 coastal locations of the Northern Persian Gulf (Fig. 1). The sampling locations were coastal waters of Bandar Abbas (BA), Bushehr (Bu) and Abadan (Ab). Geographic locations and sample sizes are presented in Table 3. Muscle samples from each individual were cut, and then, preserved in 95% ethanol for DNA extraction.

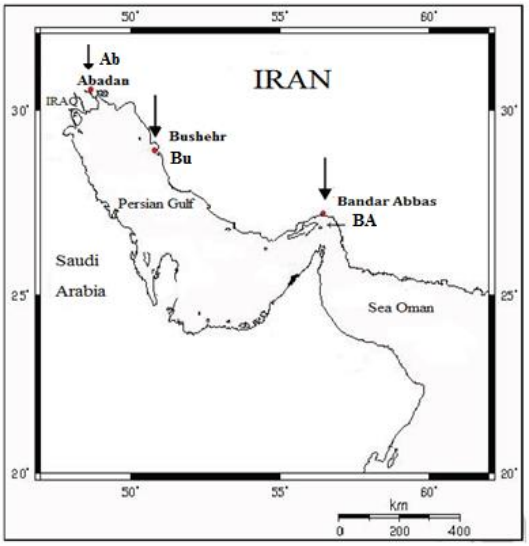

Figure 1: Locations for sample collection of black pomfret in the Persian Gulf.

AFLP analysis

Total genomic DNA was extracted from muscle tissue using the standard phenol–chloroform method (Sambrook *et al*., 2001). DNA concentration was measured with a UV spectrophotometer. The quality of extracted DNA was assessed by 1.0% agarose gel electrophoresis. Procedures of AFLP analysis were essentially based on Vos *et al*. (1995) with some modifications. Seven primer combinations (E-ACA/M-CCG, E-ACC/M-CCG, E-ACC/M-CCC, E-ACA/M-CAA, E-ACA/M-CCC, E-AAC/M-CAA, and E-ACC/M-CCG) were chosen for AFLP analysis. Sequences of AFLP adapters and primers are listed in Table 1. About 300 ng of genomic DNA was digested in a 20 ml volume with 5 U *Eco*RI (MBI Fermentas), 5 U *Mse*I (MBI Fermentas), and 2 µl 10X digestion buffer R at 37°C for 2:30 h and at 67°C for 2:30h, and then double-stranded adapters were ligated to the restriction fragments at 20°C for 10 h after adding 2 U T4 DNA ligase (MBI Fermentas), 0.5µl T4 DNA ligase buffer, 0.5µl *Eco*RI adapter (2µmol/l) and 0.5µl *Mse*I adapter (20µmol/l) with a final volume of 25 µl. Pre-amplification PCR reaction was conducted in a 50µl volume containing 5µl restriction–ligation mixture, 5µl 10× PCR buffer, 4.0µl 2.5mM dNTP, 3µl 25mM $MgCl_2$, 1.5µl *Eco*RI C+ primer (50 ng/µl), 1.5µl *Mse*I A+ primer (50 ng/µl), 1 U *Tag* polymerase (Cinnagen). The pre-amplification PCR program was set at 94°C for 2 min followed by 20 cycles consisting of 30 s at 94°C, 30 s at 56°C and 1 min at 72°C, followed by 5 min of final extension at 72°C. Selective amplification reactions were similar to pre-amplification reactions, substituting 30 ng of each selective primer (*Eco*R+3 and *Mse*+3 primer), and 0.5 ml of the pre-amplification product. Selective amplification reactions were conducted using the following profile: an initial denaturation step of 2 min at 94°C; 12 cycles with 30 s at 94°C, 30 s at 65°C decreasing with 0.7°C per cycle, 2 min at 72°C; 23 cycles with 30 s at 94°C, 30 s at 56°C, 2 min at 72°C; followed by 10 min at 72°C. PCR products were run on 6.0% denaturing polyacrylamide gel electrophoresis (PAGE) for 2.5h at 50°C on the Sequi-Gen GT Sequencing Cell (Bio-Rad, USA), and finally detected using the silver staining technique modified from Merril *et al*. (1979).

Table 1: Oligonucleotide adapters and primers used in AFLP analysis.

Primer		Sequence (5´-3´)
Adapters	*Eco*RI-1	CTCGTAGACTGCGTACC
	*Eco*RI-2	CATCTGACGCATGGTAA
	*Mse*I-1	GACGATGAGTCCTGAG
	*Mse*I-2	TACTCAGGACTCAT
Primer of pre-amplification	*Eco*RI + 1	GACTGCGTACCAATTCA
	*Mse*I + 1	GATGAGTCCTGAGTAAC
Primer for selective amplification	E-ACA	GACTGCGTACCAATTCACA
	E-ACC	GACTGCGTACCAATTCACC
	E-AAC	GACTGCGTACCAATTCAAC
	M-CCG	GATGAGTCCTGAGTAACCG
	M-CCC	GATGAGTCCTGAGTAACCC
	M-CAA	GATGAGTCCTGAGTAACAA

Data analysis

Clear and unambiguous bands in length ranging from 100 to 600 bp were considered as usable. AFLP bands were scored as "1" if present or "0" if absent excluding the smeared and weak ones by visual inspection, and transformed into 0/1 binary character matrix. For the three locations, the percentage of polymorphic loci (*P*), observed number of alleles (*Na*), effective number of alleles (*Ne*), genetic diversity (*H*), Shannon's information index (*I*), Nei's genetic diversity and genetic distance were calculated by POPGEN1.32 (Yeh and Boyle,1997). The gene flows were estimated using the equation: N_m ¼ (1-Fst)/4F_{st} (Wright, 1949). F_{st} values were analyzed using GenAlEx 6.5 (Excoffier *et al*., 2005). For determining genetic relationships among fish from the three samples, similarity coefficients were estimated and the phenogram was constructed based on un-weighted pair-group method (UPGMA; Sokal and Michener, 1958). The bootstrap test was performed with 1000 replications in NTSYS (version 2.02; Rohlf, 2005). Molecular variances within and among the three samples were estimated by analysis of molecular variance (AMOVA) using software GenAlEx 6.5 (Peakall and Smouse, 2012).

Results

AFLP polymorphism and genetic variation for the three locations

A total of 381 loci were identified, with a size range of 100–600 bp, using 7 AFLP primer combinations from 32 individuals among the three populations, of which, 46 loci (12.07%) were polymorphic (Table 2). The average number of bands scored per primer pair was 54.42 (range 42-73). The number of polymorphic loci amplified by each primer combination over all samples was 4-12, with an

average of 6.51 polymorphic loci per primer combination (Table 2).

The percentage of polymorphic loci, observed and the effective number of alleles, Nei's genetic diversity and Shannon's informative index for each sample are summarized in Table 3. The percentage of polymorphic loci varied within samples from 73.91% to 91.30%. The values of N_a were from 1.73±0.44 to 1.91±0.28 and N_e from 1.53±0.40 to 1.70±0.34. Nei's genetic diversity and Shannon's informative index ranged from 0.29±0.20 to 0.38±0.16 and 0.43±0.28 to 0.54±0.21, respectively.

The average F_{st} was 0.19 (p<0.001), indicating significant genetic differentiation among samples. Pair-wise F_{st} value among samples ranged from 0.15 to 0.22 and samples were significantly different (p<0.001; Table 4). Additionally, pair-wise F_{st} analysis indicated the greatest genetic difference existed between samples of Bandar Abbas and Abadan; whereas, the difference between Abadan and Bushehr was the smallest. Moreover, gene flow (N_m) ranged from 1.24 (between BA and Ab) to 2.80 (between Bu and Ab).

Population structure

Nei's genetic distance analysis among the three locations was 0.16 –0.26 (Table 5). The greatest genetic distance was between samples of Ab and BA, and the smallest difference was between samples of Bu and Ab. Nei's genetic identity also revealed the greatest genetic similarity between the samples from Bu and Ab locations (0.85) and the least genetic similarity between the samples from BA and Ab locations (0.77).

The UPGMA dendrogram clustered all 32 individuals into 3 groups. In some cases individuals from the same region were grouped together but in most cases, gene exchange was observed to be common among the groups. The 2-level hierarchical AMOVA analysis indicated that 81% of the genetic variation contained within samples and 19% occurred among samples. There were significant differences (p<0.001) among samples using the significance test with 1000 permutations (Table 6).

Table 2: Number of loci generated by primer combinations.

Primer	N_1 [a]	N_2 [b]	P [c] (%)
combinations			
E-ACA/M-CCG	59	4	%06.77
E-ACC/M-CCG	45	5	%11.11
E-ACC/M-CCC	42	6	%14.28
E-ACA/CAA	73	12	%16.43
E-ACA/M-CCC	54	5	%09.25
E-AAC/M-CAA	62	9	%14.51
E-ACC/M-CCG	46	5	%10.86
Average	54.42	6.51	-
Total	381	46	%12.07

(a)N_1: number of loci, (b) N_2: number of polymorphic loci, (c) P: percentage of polymorphic loci.

Table 3: Parameters of genetic diversity for samples of black pomfret.

Sample	Geographic location	Sample size	P [a]	Na [b]	Ne [c]	H [d]	I [e]
BA	53°11´ E 27°17´ N	10	73.91	1.73(0.44)	1.53(0.40)	0.29(0.20)	0.43(0.28)
Bu	50°83´E 28°92´N	11	91.30	1.91(0.28)	1.70(0.34)	0.38(0.16)	0.54(0.21)
Ab	48°18´E 30°20´N	11	84.78	1.84(0.36)	1.62(0.37)	0.34(0.18)	0.50(0.24)

(a) *P*: percentage of polymorphic loci, (b) *Na*: observed number of alleles, (c) *Ne*: effective number of alleles, (d) *H*: Nei's (1978) gene diversity, (e) *I*: Shannon's information index, f: Numbers in parentheses are standard deviation.
BA: Bandar Abbas samples, Bu: Bushehr samples, Ab: Abadan samples

Table 4: Pairwise F_{st} (below diagonal) and gene flow (N_m) (above diagonal) between the three natural populations of black pomfret based on AFLP data.

Sample	BA	Bu	Ab
BA	****	1.75	1.24
Bu	0.20	****	2.80
Ab	0.22	0.15	****

BA: Bandar Abbas samples, Bu: Bushehr samples, Ab: Abadan samples

Table 5: Nei's genetic identity (above diagonal) and genetic distance (below diagonal).

Sample	BA	Bu	Ab
BA	***	0.78	0.77
Bu	0.25	***	0.85
Ab	0.26	0.16	***

BA: Bandar Abbas samples, Bu: Bushehr samples, Ab: Abadan samples

Table 6: Hierarchical AMOVA results for the three populations of black pomfret.

Source of variance	d.f.	Sum of squares	Variation component	Percentage of variation	*p*
Among samples	2	58.3602	1.95	19%	<0.01
Within samples	29	241.32	8.32	81%	<0.01
Total	31	299.688	10.27	Fst = 0.19	

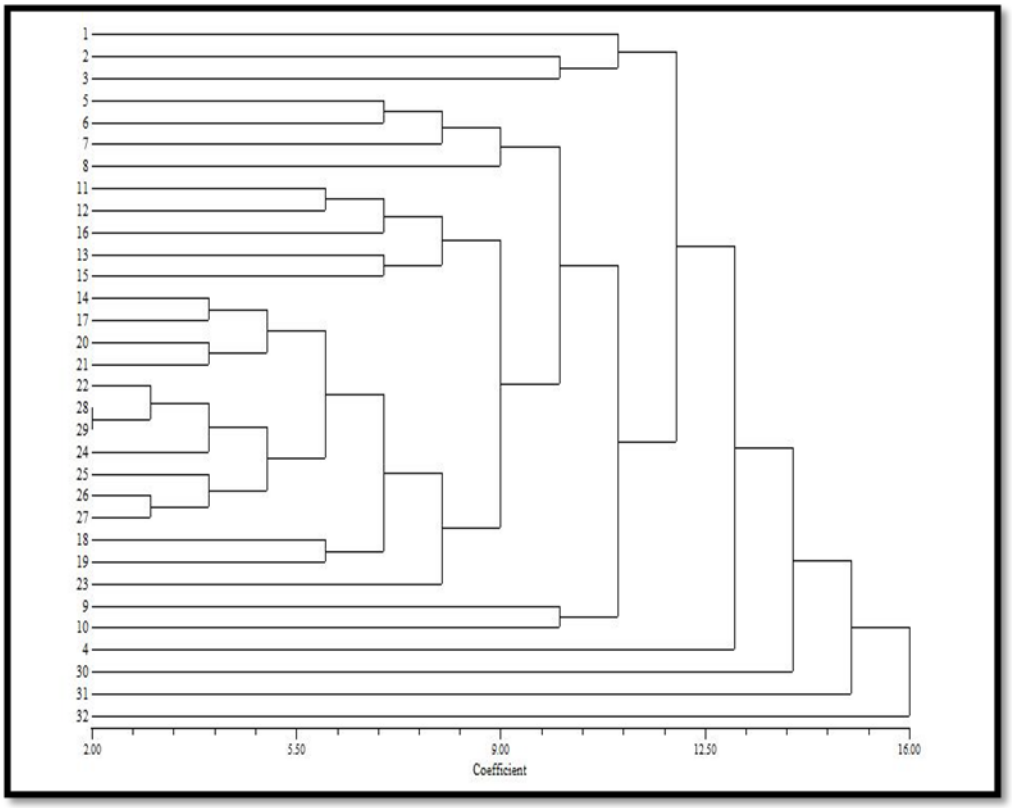

Figure 2: UPGMA dendrogram of 32 individuals of black pomfret based on the Dice similarity coefficients (1-10: Bandar Abbas samples, 11-21: Bushehr samples, 22-32: Abadan samples).

Discussion

The conservation of genetic variation is important for the long-term interest of any species (Falk and Holsinger, 1991). In this study, the seven AFLP primer combinations showed a high ratio of polymorphic loci. According to the polymorphic bands in the samples, the highest and lowest diversity were calculated in the populations of Bushehr (91.30) and Bandar Abbas (73.91), respectively. The level of Nei's heterozygosity was also higher in Bushehr samples (0.38±0.16) than in those from other locations. According to the theory of population genetics, greater populations tend to keep genetic diversity at higher levels (Jones and Wang 2012; Wang, 2013). Therefore, there seems to be greater populations of black pomfret present in Bushehr than in Abadan and Bandar Abbas regions.

Hunting and overuse of food resources and environmental pollution

are among the main threats to global biodiversity and genetic variation (Novacek and Cleland, 2001; Dudgeon *et al*., 2006; Lewis, 2006). The difference in the genetic diversity of populations could be attributed to various parameters including to environmental pollutions. Research on the effect of environmental pollution on fish genetic diversity has been focused in some fish ecotoxicological studies. For example, Maes *et al*. (2005) showed a reduced genetic variability in European eel *Anguilla anguilla* (L.), collected from the sites that were polluted with heavy metals. The impact of pollutants or toxicants, such as heavy metals, pesticides or industrial waste, on the genetic diversity and structure of natural populations is a reduced genetic variability in polluted populations (Ma *et al*., 2000, Belfiore and Anderson, 2001; Ciftci and Okumus, 2002). It seems that the intense environmental pollutions caused by different anthropogenic activities like the release of industrial wastewaters and oil extraction processes are one of the possible reasons for decreased diversity in Bandar Abbas samples compared to those from other locations.

Observing higher levels of genetic variation in the Bushehr population could be also attributed to the geographic differences between sampling locations and the physical-chemical conditions of the study area. Differences in salinity (Nielsen *et al*., 2004) and temperature (Banks *et al*., 2007), and other geographical and physical-chemical factors can shape population structure of marine organisms and contribute to the levels of genetic diversity. In coastal waters of Bushehr, the entrance of fresh water from Mond, Helle and Dalaki Rivers into the sea and mixing of fresh and salt waters in this region, which is one of the important fishing sites, causes some environmental changes including the decrease in salinity and the increase in food richness in the area. These parameters would consequently result in more biodiversity in this region. Therefore, the level of genetic diversity may be affected by these changes, too. The same results were reported by Shokoohmand *et al*. (2011), who stated that genetic diversity of Jinga shrimp *Metapenaeus affinis* revealed by microsatellites collected from the mouth of Arvand and Bahmanshir Rivers (Lifeh- Busaf estuaries) where the water flows into the Persian Gulf is higher than that collected from the Bahrekan region in the sea. Also, Bechari *et al*. (2012) showed that the level of genetic diversity in mudskipper fish *Periophthalmus waltoni* revealed by RAPD markers was higher in Hendijan region in Khuzestan province where the mixing of fresh water of Zohreh River with salt water of the Persian Gulf occurs. This environmental condition results in the increase of biodiversity and genetic variation in this region.

The structure of natural populations can be viewed from two standpoints: the demographic structure, which is affected by processes such as birth, death and dispersal; and the genetic structure, which is influenced by processes such as mutation, drift, selection and gene flow (Slatkin, 1994). Two factors from these different standpoints that are tightly linked are dispersal (and migration) and gene flow, which refer, respectively, to the movement of individuals and gametes among populations. Bushehr is geographically located between the regions of Bandar Abbas and Abadan and so, the black pomfret population in Bushehr has the opportunity to breed with the two other populations and hence, this may be one of the more reasons for higher level of observed genetic variability.

According to the statistics of the Iranian Fisheries Organization, the amount of black pomfret fishing reaches 1716 tons in Hormozgan Province. Indeed, most of the catch of black pomfret occurs in the coastal waters of Hormozgan and this rate has always been higher in this region than in Bushehr and Abadan. According to the theory of population genetics the level of genetic variation in populations is positively correlated with the population size so, the decrease in the stocks of one species can led to the decrease in genetic diversity. The same situation exists in Bandar Abbas region and the decrease in black pomfret stocks due to overfishing and having less opportunity for breeding compared to other locations are other possible reasons for the decreased diversity in this region.

In this study, The level of genetic diversity of black pomfret (H=0.29±0.20 to 0.38±0.16 and I= 0.43±0.28 to 0.54±0.21) was greater than those of many other marine fishes, i.e., silver pomfret *Pampus argenteus* (Zhao *et al*., 2011), small yellow croaker *Larimichthys polyactis* (Han *et al*., 2009), striped mullet *M. cephalus* (Liu *et al*., 2009), Yellow Drum *Nibea albiflora* (Han *et al*., 2006), Olive flounder *Paralichthys olivaceus* (Zhang *et al*., 2004) and Red seabream *Pagrus major* (Wang *et al*., 2001). This level of diversity was also higher than that in *Pomadasys kaakan* in the Persian Gulf in the sampling locations similar to that in the present study (Salari Ali Abadi *et al*., 2012) and in striped mullet also found in the Persian Gulf (Faqih Ahmadani *et al*., 2011). Large population size is believed to be responsible for the great levels of genetic diversity in many marine fishes (Avise, 2002).

The mean F_{st} value among populations was 0.19 (0.15 to 0.22). According to Wright (1978), F_{st} values below 0.05 indicate low genetic differentiation, values from 0.05 to 0.15 moderate differentiation, values from 0.15 to 0.25 high differentiation and values above 0.25 very high differentiations. Therefore, according to

the results of this study, there is a high genetic differentiation among the black pomfret populations. Zhao *et al*. (2011) found the F_{st} value of 0.11 among six populations of silver pomfret *P. argenteus* from the Yellow and East China Seas, which indicates a moderate genetic differentiation. Results of the pair-wise F_{st} analysis indicated that there was a high genetic differentiation (0.22) between the samples of Bandar Abbas in the east and the samples of Abadan in the west of the Persian Gulf. This level of differentiation indicates a strong population structure. Population genetic structuring in widely distributed marine species has also been previously reported (Chapman, 1999; Abubert and Lightner, 2000; Rhodes *et al*., 2003). High genetic differentiation of Black pomfret populations in the Persian Gulf is consistent with the biology of this species and in fact, confirms a low amount of migration between populations in the Persian Gulf. Black pomfret has low swimming speed and shows schooling behavior. In schooling behavior, the fish tend to breed with each other in the population (Carpenter *et al*., 1997). In stocks of fish that live together and have schooling behavior, the produced population homogeneity results in a high differentiation with other populations in the long term. Results of this study provide evidence for separate black pomfret stocks in the northern Persian Gulf, and indeed, the F_{st} values among the three locations were all significantly different (p<0.001), suggesting that there could be at least three black pomfret stocks in the Persian Gulf.

Gene flow (N_m) is one of the main components of population structure, as it governs to what extent each local population of a species is a separate evolutionary unit. If there is a lot of gene flow between local populations, then that set of populations evolve together, while if there is low gene flow, each population evolves practically alone (Slatkin, 1994). About the level of gene flow, although this value (mean 1.93) is not high in the present study, it indicates that there is a moderate amount of migration between populations. It seems that having pelagic eggs and larvae (Smith-Vaniz, 1984) is one of the main reasons for N_m>1 in this study for black pomfret. However, low level of gene flow also confirms high genetic differentiation among black pomfret populations in the Persian Gulf.

Based on the AMOVA analysis, the genetic variation was found to be mostly within populations (81%) and not so much among populations (19%). The AMOVA analysis also supported a significant differentiation among black pomfret populations. In silver pomfret *P. argenteus* from the Yellow and East China Seas, AMOVA analysis showed 89.3% of the genetic variation within samples and 10.7% among samples indicating significant differentiation among populations suggesting at least

six populations are present in the two seas (Zhao *et al*., 2011).

However, most marine fish show low levels of genetic differentiation among geographic regions. This is likely due to the dispersal during the life history of planktonic eggs and larvae, or juvenile or adult migrations between ocean basins or adjacent continental margins (Hewitt, 2000; Rhodes *et al*., 2003). With developing cities and industries along the coast lines and discharging the contaminants into the seas, it's probable that some spawning areas and nursery and feeding sites are destructed. Therefore, different populations shape in some separated habitats in a short geographically distant area (Qian *et al*., 2011). It seems that this also happened in the Persian Gulf and therefore, more within population differentiation is probably induced.

Genetic similarity was 0.80 (0.77-0.85) between the black pomfret populations of different sampling sites in this study. Nei's genetic distance analysis revealed that the differences between the three populations were within the limits of populations from the same species (Thorpe, 1982; Thorpe and Sole-Cava, 1994), indicating that they did indeed belong to the same species. According to the results, maximum genetic distance and minimum genetic similarity was observed between the samples of Bandar Abbas and Abadan. This observation is in accordance with the long geographical distance between these two regions. It is known that genetic differentiation generally increases with increasing geographical isolation. The animals which are geographically closer to each other have more genetic similarity and with the increase in geographical distance, genetic distance also increases (Zhao *et al*., 2007; Zawadzki *et al*., 2008; Zhao *et al*., 2011).

Our results provide evidence for separate black pomfret stocks in the northern Persian Gulf, and indeed, the F_{st} values and AMOVA analysis among the three samples were all significantly different, suggesting at least three populations are present in this sea. Moritz (1994) proposed the concept of management unit (MU), for populations with high genetic differentiation. Using this concept, the results probably indicate black pomfret in the northern Persian Gulf present at least three fisheries management units.

Since the results of this survey are the first molecular approval of the existence of different genetic populations of black pomfret in the Persian Gulf, the data could be used for the conservation of gene pool and for fishery management in this valuable species. Therefore, more supervision on genetic management programs for sustainable fishery and also more genetic and biological research would definitely assist in black pomfret populations' conservation.

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