Assessing genetic diversity of populations of bartail flathead (*Platycephalus indicus* Linnaeus, 1758) in the Northern part of the Persian Gulf using AFLP markers

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Abstract

Genetic diversity of six populations of bartail flathead (*Platycephalus indicus* Linnaeus, 1758) was investigated using amplified fragment length polymorphism (AFLP). A total of 118 reproducible bands amplified with ten AFLP primer combinations were obtained from 42 fishes that were collected from six different locations in the northern part of the Persian Gulf. The percentage of polymorphic bands was 57.06%. Nei's genetic diversity was 0.1808±0.2074 - 0.2164±0.1903, and Shannon's index was 0.2675±0.2949 - 0.3280±0.2727. The results of AMOVA analysis indicated that 66% and 34% of the genetic variation occurred within and among the populations and gene flow was 0.6454. The estimated level of population differentiation as measured by average *Fst* value across all loci was 0.327. Analyses revealed significant genetic differentiation with low gene flow among the six locations, indicating at least six separate populations of bartail flathead in the northern part of the Persian Gulf.

Keywords: Genetic diversity, Bartail flathead, *Platycephalus indicus*, AFLP, Persian Gulf.

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Introduction

Genetic diversity is of great importance to the sustainability of populations (Hamrick *et al*., 1991). The level of genetic diversity may reveal information about historical population sizes and structure (Sivasundar and Hey, 2003). For management of a species, knowledge of intraspecific genetic variations may help to assess extinction risks such as inbreeding and evolutionary potential in a changing world (Hedrick, 2001).

Molecular markers are useful for population genetic studies to assess influences of various factors on genetic diversity and population structure (Englbrecht *et al*., 2000; Whitehead *et al*., 2003). Among many types of molecular markers, amplified fragment length polymorphism (AFLP) has been demonstrated as a useful tool for population genetic studies of fish (Liu *et al*., 2003), particularly for those species whose genome information is lacking. AFLP is highly efficient for the determination of genetic variations as a multi-locus molecular marker technique (Vos *et al*., 1995). Because it is easy, fast, inexpensive and robust, AFLP has been successfully used to study genetic diversity and relationships in yellow back sea bream (Xia and Jiang, 2006), Lutjanus sp. (Zhang *et al*., 2004), tilapia (Agresti *et al*., 2000), flounder (Liu *et al*., 2005), rainbow trout (Young *et al*., 1998), catfish (Liu *et al*., 1998, 1999; Mickett *et al*., 2003; Simmons *et al*., 2006), striped mullet (Liu *et al*., 2009), spottedtail goby (Song *et al*., 2010) and silver pomfret (Zhao *et al*., 2011).

The Persian Gulf is located in one of the hottest areas in Asia. It is a semi-enclosed sea that is located between the latitudes of 25°-32° N and longitudes of 48°-56°E. The water consists of a shallow continental shelf with an average depth of 35m, which increases from the Arvand estuary and reaches a maximum of 100 meters in the Hormuz strait (IFRO Experts Group, 2006). Due to the very hot climate and high evaporation rate, the water salinity can be increased up to 40 grams per liter, Apart being one of the busiest oil transship areas of the world, Persian Gulf also provides fishing grounds for the fisheries industry of its surrounding countries. The family platycephalidae is one of the fish families living in the Persian Gulf. The most commonly known platycephalids are flatheads, which are mailnly distributed in the tropical and d temperate marine waters. They are characterized by an elongated body, depressed head and large mouth with the lower jaw longer than the upper one. These benthic fish are frequently found on muddy or sandy bottoms of the continental shelf at depths down to 300m, and frequently shallower than 100m (Knapp, 1999; Froese and Pauly, 2007). *P. indicus* is a large platycephalid that inhabits temperate and tropical coastal waters of the Indo-West *Pacific* (Jordan and Richardson, 1908). *P. indicus* is a benthic fish found on sandy or muddy

bottoms in very shallow areas of estuaries and near shores up to the depth of 25m. This species is the dominant species of Platycephalidae family, in northern part of the Persian Gulf (Iran) and is captured mainly by bottom trawl and gillnet (Parsamanesh *et al*., 2000). *P. indicus* species is one of the most expensive fishes in province (South of Iran). Because it is one of the most favoured edible marine fish in the southern coastal areas of Iran (specially Khuzestan and Bushehr) as well as the other countries along the Persian Gulf , this fish is a target species for capture (Hashemi and Taghavi, 2013).

Despite its economic and ecological importance, genetic diversity for this species has not been studied before. Therefore, in order to prevent further decline of the genetic resources, an understanding of its genetic structure and diversity, and geographical distribution is essential. In this study, we investigated the genetic diversity and structure within and among six populations of bartail flathead, using AFLP technique to assess the level of genetic diversity among these populations. This study provides baseline information on genetic background of this species, and might be beneficial to population conservation and fisheries management of this species.

Materials and methods

Fish samples

P. indicus (n = 42) collected from 6 locations were used in this study (Fig. 1, Table 1). The samples were mainly collected off the coastal areas of the northern Persian Gulf during September 2012 and transfered to the genetic labratory of Persian Gulf Research and Studies Center in Bushehr. All individuals were identified based on the morphological characteristics, and a piece of caudal fin was taken from each individual and preserved in 95% ethanol for DNA extraction.

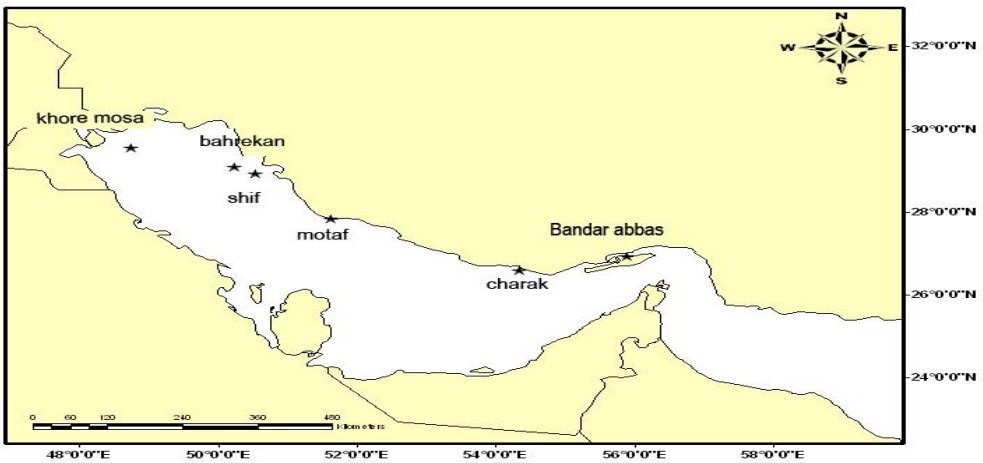

Figure 1: Locations of six *Platycephalus indicus* sample sites in the Persian Gulf.

Table 1: Sampling information of *Platycephalus indicus* including sampling sites, sample size and several genetic diversity indices.

Pop ID	Sampling site	Sample size	Observed number of alleles	Effective number of alleles	Number of polymorphic loci	Proportion of polymorphic loci	Nei's gene diversity	Shannon's information index
1	charak	7	1.610±0.489	1.339±0.358	72	61.02%	0.202±0.193	0.307±0.275
2	Bandar Abbas	7	1.533±0.501	1.374±0.409	63	53.39%	0.209±0.215	0.306±0.305
3	Shif	7	1.610±0.489	1.354±0.380	72	61.02%	0.206±0.199	0.311±0.282
4	Motaf	7	1.644±0.480	1.359±0.348	76	64.41%	0.216±0.190	0.328±0.272
5	Bahrekan	7	1.533±0.501	1.314±0.365	63	53.39%	0.185±0.197	0.279±0.284
6	Khore Mosa	7	1.491±0.502	1.318±0.393	58	49.15%	0.180±0.207	0.267±0.294

Genomic DNA extraction and AFLP method

Genomic DNA was isolated from muscle tissue by proteinase K digestion followed by a standard phenol–chloroform method (Li *et al*., 2002). Procedures of AFLP were essentially based on Vos *et al*. (1995) and Wang *et al*. (2000). About 100 ng genomic DNA was digested with 1 unit of *EcoR* I and *Mse* I (NEB) at 37 °C for 6 h. Double-stranded adapters were ligated to the restriction fragments at 20 °C overnight after adding 1 mL 10 ligation buffer, 5 pmol *EcoR* I adapter (*EcoR* I-1/*EcoR* I-2; Table 2), 50 pmol *Mse* I adapter (*Mse*I-1/*Mse*I-2; Table 2), 0.3 unit of T4 DNA ligase (Promega) with a final volume of 10 mL. Preamplification PCR reaction was conducted using an Eppendorf Thermocycler (Mastercycler 5334) with a pair of primers containing a single selective nucleotide. Amplification was performed at an annealing temperature of 53 °C for 30 s. The 20 mL PCR product mixture was diluted 10-fold with distilled water and used as templates for the subsequent selective PCR amplification. The selective amplifications were carried out in 20 mL PCR reaction volume containing 1 mL productions of preamplifications, 1X PCR reaction buffer, 150 mMol each dNTP, 30 ng of each selective primer, and 0.5 unit of Taq DNA polymerase on a gradient thermal cycler (Mastercycler 5334) with a touchdown cycling profile of 9 cycles of 30 s at 94 °C, 30 s at 65 °C (1 °C at each cycle), and 30 s at 72 °C followed by the cycling profile of 28 cycles of 30 s at 94 °C, 30 s at 56 °C, and 1 min at 72 °C. The final step was a prolonged extension of 7 min at 72 °C. PCR products were run on 6.0% denaturing polyacrylamide gel electrophoresis (PAGE) for 2.5 h 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). Sequences of AFLP adapters and primers are listed in Table 2. Ten primer combinations (E-ATC/M-CTG, E-AAG/M-CCC, E-ACC/M-CAG, E-ACT/M-CAT, E-AGC/M-CCT, E-AGC/M-CGT, E-ATC/M-CGT, E-AAG/M-CAG, E-ACT/M-CCT and E-ACT/M-CTG) were chosen for AFLP analysis (Table 2).

Table 2: Adaptors and primer combinations sequences used in the study.

Primer		Sequence
Adaptors	EcoRI-Adaptor	5′ - GACGATGAGTCCTGAG- 3′ 5′ - TACTCAGGACTCAT- 3′
	MseI-Adaptor	5′ - CTCGTAGACTGCGTACC- 3′ 5′ - AATTGGTACGCAGTCTAC- 3′
Pre-amplification primer	EcoRI	5′-GACTGCGTACCAATTC- 3′
	MseI	5′-GATGAGTCCTGAGTAA- 3′
Selective amplification primer	E-ATC/M-CTG	5′-GACTGCGTACCAATTCATC- 3′ 5′-GATGAGTCCTGAGTAACTG-3′
	E-AAG/M-CCC	5′-GACTGCGTACCAATTCAAG-3′ 5′-GATGAGTCCTGAGTAACCC-3′
	E-ACC/M-CAG	5′-GACTGCGTACCAATTCACC- 3′ 5′-GATGAGTCCTGAGTAACAG-3′
	E-ACT/M-CAT	5′-GACTGCGTACCAATTCACT- 3′ 5′-GATGAGTCCTGAGTAACAT-3′
	E-AGC/M-CCT	5′-GACTGCGTACCAATTCAGC- 3′ 5′-GATGAGTCCTGAGTAACCT- 3′
	E-AGC/M-CGT	5′-GACTGCGTACCAATTCAGC- 3′ 5′-GATGAGTCCTGAGTAACGT-3′
	E-ATC/M-CGT	5′-GACTGCGTACCAATTCATC- 3′ 5′-GATGAGTCCTGAGTAACGT-3′
	E-AAG/M-CAG	5′-GACTGCGTACCAATTCAAG-3′ 5′-GATGAGTCCTGAGTAACAG-3′
	E-ACT/M-CCT	5′-GACTGCGTACCAATTCACT- 3′ 5′-GATGAGTCCTGAGTAACCT- 3′
	E-ACT/M-CTG	5′-GACTGCGTACCAATTCACT- 3′ 5′-GATGAGTCCTGAGTAACTG-3′

Data analysis

For the six samples, 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 POPGENE32 (Yeh and Boyle, 1997). *Fst* values were analyzed using ARLEQUIN 3.1 (Excoffier *et al*., 2005). Genetic relationships among fish from the six samples were estimated by constructing an NJ tree based on Nei's genetic distance (Nei, 1978) in TFPGA 1.3. Molecular variances within and among the six samples were estimated by analysis of molecular variance (AMOVA) using software GenALEx6.5 (Peakall and Smouse, 2005)

Results

AFLP polymorphism and genetic variation for the six samples

A total of 681 bands were identified using10 AFLP primer combinations from 42 individuals among the six populations, of which, 118 bands were polymorphic, and the percentage of polymorphic bands was 17.32% (Table 3). The number of polymorphic loci amplified per primer combination varied from 8 to 20, with an average of 11.8 (Table 3). The percentage of polymorphic loci within populations ranged from 49.15% to 64.41%, with an average of 57.06%. The values of observed number of alleles were from 1.491±0.502 to 1.644±0.480 and the effective number of alleles from 1.314±0.365 to 1.374±0.409. Motaf population showed the greatest percent of polymorphic loci 64.41%), the highest Nei's gene diversity (0.216±0.190) and Shannon's information index (0.328±0.272), while Khore Mosa population showed the lowest proportion of polymorphic loci (49.15%), the lowest Nei's gene diversity (0.180±0.207) and the Shannon's information index (0.267±0.294) (Table 1). The average *Fst* was 0.327 (p<0.001), indicated significant genetic differentiation among the six populations. The pairwise *Fst* value ranged from 0.107 to 0.494, the largest being between Bandar Abbas and Khore Mosa populations, and the lowest between Shif and Motaf populations (Table 4).

Table 3: Number of bands generated by ten primer combinations.

primer	Number of loci	Number of polymorphic loci	Proportion of polymorphic loci
E-ATC/M-CTG	74	8	10.81 %
E-AAG/M-CCC	73	13	17.80 %
E-ACC/M-CAG	62	17	27.41%
E-ACT/M-CAT	77	13	16.88 %
E-AGC/M-CCT	53	7	13.20 %
E-AGC/M-CGT	80	15	18.75 %
E-ATC/M-CGT	28	6	21.42 %
E-AAG/M-CAG	93	11	11.82 %
E-ACT/M-CCT	74	20	27.02 %
E-ACT/M-CTG	67	8	11.94 %
total	681	118	17.32%

Table 4: Nei's genetic distance (below diagonal) and pair-wise Fst (above diagonal) between populations. The symbol "*" indicates that the populations are significantly genetically different.

Pop	1	2	3	4	5	6
1		0.371*	0.286*	0.282*	0.340*	0.314*
2	0.307		0.425*	0.392*	0.489*	0.494*
3	0.178	0.401		0.107	0.175*	0.367*
4	0.221	0.400	0.082		0.194*	0.374*
5	0.189	0.459	0.195	0.249		0.308*
6	0.224	0.492	0.099	0.147	0.146	

Population structure

Nei's genetic distance analysis among the six samples was 0.082–0.492 (Table 4). The greatest genetic distance was between samples from Bandar Abbas and Khore Mosa, and the smallest difference was between samples from Shif and Motaf. An UPGMA dendrogram for the six populations was constructed based on Nei's genetic distance and two clusters occurred (Fig. 2). Samples of Shif and Motaf clustered together to form cluster I. Cluster II was shaped from the samples of Charak, Bandar Abbas, Bahrekan and Khore Mosa.

The results of AMOVA analysis indicated that 66% of the genetic variation existed within populations and 34% among populations. There were significant differences (p<0.001) among samples using the significance test with 1000 permutations (Table 5).

Table 5: Results of analysis of molecular variance

Source	df	SS	MS	Est. Var.	%
Among Populations	5	387.714	77.543	8.690	34%
Within Populations	36	601.571	16.710	16.710	66%
Total	41	989.286		25.401	100%

df: Degrees of freedoms, SS: Sum of squares, Est. Var.: Variance components, %: Percentage of variation

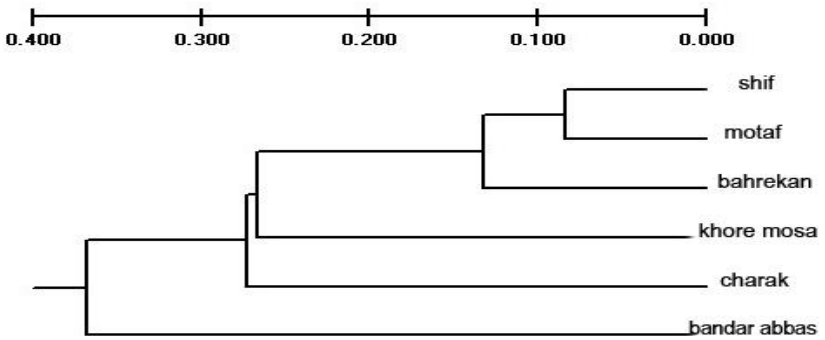

Figure 2: UPGMA dendrogram based on Nei's (1978) genetic distance among the six populations.

Discussion

AFLP is a multilocus PCR-based DNA fingerprinting technique (Liu, 2007). AFLP's most important point is its capacity for inspecting an entire genome for polymorphism and its reproducibility (Blears *et al.*, 1998). As compared to other markers such as RFLP and RAPD, AFLP provides a much greater level of polymorphism and covers large genomes well. However, AFLPs has been rarely applied in animal ecological and evolutionary studies (Sonstebo *et al.*, 2007).

The conservation of genetic diversity is important for the long-term interest of any species (Falk and Holsinger, 1991).The level of polymorphism and genetic diversity was greater than those of many other marine fishes, i.e., *Nibea albiflora* (Han *et al.*, 2006), *Pagrus*

major (Wang *et al*., 2001), *Paralichthys olivaceus* (Zhang *et al*., 2004), *Larimichthys polyactis* (Han *et al*., 2009) and *Synechogobius ommaturus* (Song *et al*., 2010). Large population size is believed to be responsible for the great levels of genetic diversity in many marine fishes (Avise, 1998). However, most marine fish show low levels of genetic differentiation among geographic regions. This is likely due to the dispersal during life history of planktonic eggs and larvae, or to juvenile or adult migrations between ocean basins or adjacent continental margins (Palumbi, 1994; Hewitt, 2000). This situation does not apply to the genetic diversity of bartail flathead found among geographic areas.

Population genetic structuring in widely distributed marine species has been reported (Chapman, 1999; Abubert and Lightner, 2000; Rhodes *et al*., 2003) and our results provide evidence for separate bartail flathead stocks in Persian gulf, because, the *Fst* values among the six samples were all significantly different (p<0.001), suggesting at least six populations are present in this gulf. The AMOVA analysis also supported a significant differentiation among populations. Mickett *et al*. (2003) suggested that an *Fst* value of 0.4456 indicates a high genetic differentiation of channel catfish (*Ictalurus punctatus*) populations and that 0.1763 indicates a moderate genetic differentiation. An *Fst* value of 0.047 corresponded to a moderate genetic differentiation of Asian arowana (*Scleropages formosus*) populations (Yue *et al*., 2004). We found that the *Fst* value among populations was 0.327, which indicates a high genetic differentiation.

Our results detected that the gene flow between populations from Khore Mosa to Bandar Abbas was restricted (gene flow average=0.6454) and that genetic divergence is significant (Table 4). The Motaf population, connecting the eastern and western populations, has limited gene exchange with either eastern or western populations, which will increase the level of heterozygosity. In addition, the relatively stable environment of the Motaf population might also be advantageous to the accumulation of genetic variation. The low values of heterozygosity and polymorphism found in bartail flathead from Khore Mosa can be explained either by genetic drift, a founder effect, or by strong directional selection as a consequence of the smaller number and geographic isolation of that population.

Since the movements of adults are restricted to a small area, the effective immigration between populations are likely caused by larval movement. Until recently, studies have supported the view that the swimming ability of larvae was weak and relatively ineffectual (Blaxter, 1986). Larval exchanges among populations induced by marine currents are therefore expected to be the most important factor facilitating gene flow. The AMOVA analysis revealed that 34% of

the genetic variation was between populations (Table 5), indicating the low gene flow between the six bartail flathead populations, which is consistent with the result based on pairwise genetic distance (*Fst*) analysis.

In conclusion, molecular markers are effective methods to delineate genetic diversity and structure of the population, and can provide effective conservation and management strategies for the species. The results that the limited gene flow existed among different populations of *P. indicus* point out the need to take into account separate fishery stocks along the Persian Gulf coast. In addition, the ability of larval dispersal and environmental factors like currents are expected to be the effective factors in shaping the present structure of this species. So, when appropriate management strategies are to be emplemented, larval dispersal period and the environmental factors should also be taken into consideration.

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