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PRIMARY RESEARCH PAPER

Fine-scale population genetic structure of Endangered Caspian Sea trout, *Salmo caspius*: implications for conservation

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Abstract Many populations of Caspian Sea trout (*Salmo caspius*)—a nationally endangered species in Iran—have been extirpated or depleted due to anthropogenic impacts. The Lar National Park hosts large populations of Caspian Sea trout, which have not been subject to fisheries management programs before, but the population/s also face different human-related threats that may endanger their sustainability. A total of 357 Caspian Sea trout collected from different streams in Lar National Park were genotyped at 7978 filtered SNP using Genotyping-By-Sequencing to document population genetic structure and the contribution of each population/habitat to lake-run trout fisheries. Our results revealed a fine-scale population genetic structure, which is probably a product of factors including natural and artificial barriers to gene flow, geographic distance, and behavioral differences between resident and lake-run trout. Mixed-Stock Analyses revealed a high contribution from four panmictic populations of the national park to lake-run fish and almost no contribution from streams located in upper reaches or from streams with hydrochemical or physical barriers. Our results highlighted the necessity for a more serious conservation plan for both the populations contributing greatly to lake

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fisheries and the highly diverged upstream populations due to their uniqueness.

Keywords Genotyping-by-sequencing · Hydrochemical barriers · Lake-run fish stock · Mixed-stock analysis · Population genetics structure · Resident Caspian Sea trout

Introduction

Species in marginal habitats are vulnerable due to ecological and environmental extremes to which they are exposed (Antunes et al., 2006). These extreme conditions, along with human activities and climatic changes, threaten the sustainability of such populations (Jonnson & Jonnson, 2011). This is the case for Caspian Sea trout *Salmo caspius* Kessler, 1877 populations in the Southern Caspian Sea basin and the nearby inland basins which face intensive anthropogenic activities such as habitat destruction, fishing, and ecological extremes like the effects of climatic changes (Sedighkia et al., 2018).

Clarification of population genetic structure is one of the most important steps in management and conservation of all species. Due to imprinting (the phenomenon by which young migratory animals learn and memorize the properties of the environment where they were born and use these cues to migrate back to their place of origin) and spawning migrations to natal habitats, anadromous salmonids usually show moderate to pronounced population genetic structure (Jonnson & Jonnson, 2011). The few previous studies pertaining to population genetic structure in Iranian Caspian Sea trout *S. caspius* documented a pattern of pronounced genetic differentiation among the Iranian populations from different lakes and closely located streams (Vera et al., 2011; Hashemzadeh Segherloo et al., 2012). *Salmo caspius*, native to the southern Caspian Sea basin (Kottelat and Freyhof, 2007), is considered endangered at the national level, and during the last four decades it has been supported via restocking activities by the Iranian Fisheries Organization (Kiabi et al., 1999; Abdoli, 2000). This species is threatened by different factors, including spawning ground destruction, dam construction, pollution, poaching, and introduction of non-native species, namely Rainbow trout *Oncorhynchus mykiss* (Walbaum, 1792) (Kiabi et al., 1999; Abdoli, 2000; Niksirat & Abdoli, 2009). Many populations of Caspian Sea trout have been extirpated (Kiabi et al., 1999), and the number of breeders returning to spawning grounds has decreased sharply in many others (Niksirat & Abdoli, 2009). The pronounced inter-drainage genetic differentiation along with considerable reduction of Caspian Sea trout returning to rivers (Niksirat & Abdoli, 2009; Vera et al., 2011; Hashemzadeh Segherloo et al., 2012) can be interpreted as the effect of disconnection among resident populations, which is partly due to the loss of natural inter-drainage connection caused by loss of migratory forms (Kottelat & Freyhof, 2007; Almodóvar et al., 2012). Each isolated population of *S. caspius* can be considered as a genetic resource important in management and conservation of the species over its natural distribution. Such resources can be used to augment or restock populations and habitats, which are genetically and ecologically close to the donor population (Hallerman, 2003). In addition, knowledge of fine-scale population structure and the importance of different populations for the sustainability of the species can be beneficial in management and conservation (Carlsson et al., 1999; Carlsson & Nilsson, 2000; Swatdipong et al. 2013; Mäkinen et al. 2015), since such data can be utilized in management measures with the aim of restocking or augmentation.

In the Caspian Sea basin, both the migratory and resident forms of the Caspian Sea trout exist (Abdoli, 2000). In the southern Caspian Sea basin, Lar National Park is one of the unique and un-managed habitats of both forms of the Caspian Sea trout (no trout artificial breeding program has been implemented in the park) that is protected by the Iranian Department of Environment (Esteve et al., 2017). The lake-run trout migrate to Lar Lake (a reservoir created after construction of the Lar Dam) and return back to streams for spawning. The lake-run Caspian Sea trout likely represents the migratory forms existing before their migration path to the Caspian Sea was blocked by the Lar Dam and hence they migrate to the Lar Dam reservoir. As salmonid fishes exhibit imprinting behavior (Jonnson & Jonnson, 2011), it is possible that Caspian Sea trout in different streams of the Lar River drainage can develop population genetic structure. Until a few years ago (2013), the Caspian Sea trout in the Lar National Park were exposed to recreational fishing (only in the lake) and

unintentional introduction of exotic bait fishes probably by anglers. During the years that fishing was permitted, a decrease in trout landings was observed. Hence, the Iranian Department of Environment stopped fishing activities in the national park for recovery of the lake-run trout stock. The presence of nomad human communities during summer that settle very close to the streams may threaten survival of the Caspian Sea trout via physical damages to the stream habitats and wastewater released to the streams. Additionally, their livestock may cause soil erosion and, hence, increased sedimentation and spawning ground degradation that can cause population loss. Clarification of population genetic structure and estimation of the contribution from each stream population to lake-run trout can be helpful in identifying differentiated subpopulations and important habitats to be protected by management measures (Carlsson et al., 1999; Carlsson & Nilsson, 2000; Mäkinen et al., 2015).

In this study, we assessed the Caspian Sea trout distributed in different streams in the Lar National Park (southern Caspian Basin, Iran) using the Genotyping-By-Sequencing (GBS) approach to discover possible population genetic structure in different habitats. Next Generation Sequencing (NGS) approaches analyzing hundreds to thousands of loci provide a good representation of the complete genome in both model and non-model species and can clarify even very fine-scale population structures (Catchen et al., 2013; Benestan et al., 2015; Andrews et al., 2016; Jones & Good, 2016) and provide a powerful means of performing Genetic Stock Identification (Anderson et al., 2008; Mäkinen et al., 2015). More specifically, our main goals were to (a) determine subpopulations and their related habitats, and (b) determine contribution of each population to lake-run trout stock as a measure of the importance of different habitats to the sustainability of lake-run fish, to be considered in future management measures.

Materials and methods

Study area

Lar National Park with a surface area of 30,000 ha hosts the Lar River and its tributaries (Sedighkia et al., 2018). The National Park (Fig. 1) is located in the Alborz Mountains, around 55 km from Tehran City, Iran. The Lar River originates at elevation of over 4300 m above sea level, and after receiving the Khoshkehrood, Gelsardab, Siahpalas, Elarm, Absefid, Emam Bahtak, and Dalichay streams, drains into the Haraz River. The Lar River, with a length of 55 km and an average slope of 3%, flows from north-west to south-east before draining to the Haraz River that flows toward the Caspian Sea in the north (I.R.G.O.A.F., 2005).

Sampling

A total of 165 specimens were collected from different streams in the Lar River drainage using a gasoline HACH electro-shocker (1 KW, 100–200 V) in August 2017 (Fig. 1, Table 1). In addition to these, 192 fin clips that we previously collected from the Lar Lake and different streams during 2009–2010 were also included in this study (Table 1). Electrofishing was performed over more than 100-m length intervals at each locality to reduce the probability of repeatedly sampling family (Hansen et al., 1997). Captured fish were sedated with clove powder and the pectoral fin was clipped, coded, and stored in 95% ethanol for subsequent molecular analyses. Collected fish were left in water for recovery. Sampling was performed in accordance to permit no. 96/8592 (2017-05-29) issued by the Iranian Department of Environment, and all procedures and ethical protocols conducted while working with the sampled fish were confirmed by the research council of the Environmental Sciences Research Institute (ESRI) (Shahid Beheshti University, Tehran, Iran). About 24 h after sampling, alcohol was refreshed and the preserved specimens were kept at -20 ^{o} C.

DNA extraction

DNA was extracted using the salt extraction method of Aljanabi and Martinez (1997) with an additional treatment with RNAase (Hashemzadeh Segherloo et al., 2018). The quality of extracted DNA samples was checked via electrophoresis on a 1% agarose gel. Degraded DNA samples were excluded from further treatments. Concentrations of DNA samples were determined using a NanoDrop2000 spectrophotometer (www.thermofisher.com).

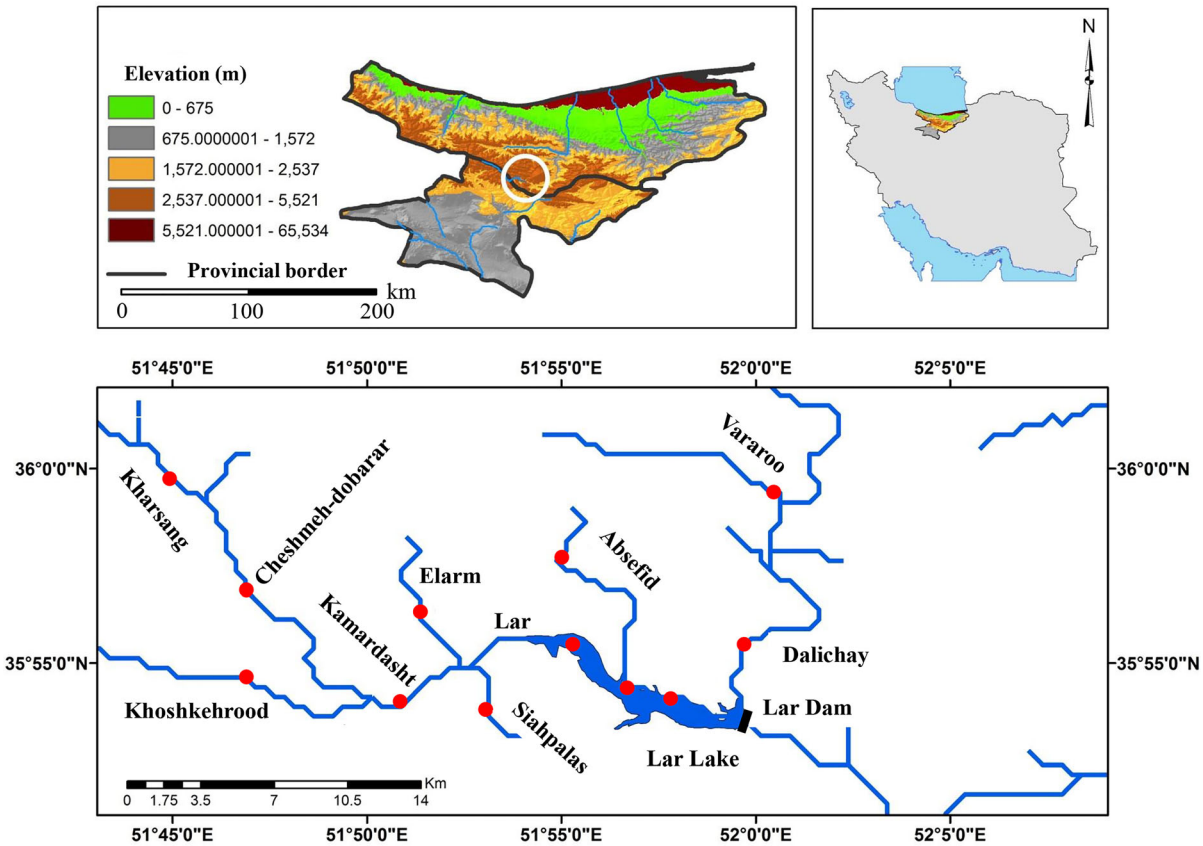

Fig. 1 Location of Lar Lake (top panels) and distribution of sampling sites (bottom panel)

Table 1 Sampling details

Locality	N^a	Collection date	Population density in 100 m^2 (N/m^2)	Coordinates (UTM)	
				Longitude	Latitude
Elarm	30	2017	221	579,160.98	3,975,320.02
Dalichay	15	2017	96	589,540.63	3,975,474.86
Vararoo	30	2017	233	590,455.90	3,980,361.46
Absefid	30	2017	96	583,952.26	3,978,947.35
Kamardasht	30	2017	204	575,731.21	3,973,165.67
Siahpalas	30	2017	345	579,796.30	3,974,412.46
Lake Lar	134	2009–2010	NA	–	–
Dalichay	15	2009	NA	589,658.42	3,975,771.87
Kharsang	13	2009	NA	565,174.81	3,985,912.61
Khoshkehrood	12	2009	NA	574,089.17	3,972,804.77
Cheshmeh-dobarar	18	2009	NA	569,438.24	3,981,431.28

[a]Sample size

Library preparation and sequencing

The exact concentration of DNA was determined using AccuClear™ Ultrahigh-sensitive dsDNA quantification kit (https://biotium.com) using a Spark™ 10 M multimode microplate reader (www.tecan.com) and concentrations were normalized around 10 ng/µl. The libraries for Genotyping-By-Sequencing were prepared following Mascher et al. (2013). DNA samples with concentrations around 10 ng/µl were digested using the *Pst I* and *Msp I* restriction enzymes. The digested DNA samples were then barcoded using individual-specific oligonucleotide sequences and ligated to adaptors for amplification. Each set of 96 individuals was multiplexed and amplified in a single tube. Ninety-six individuals were included on each sequencing chip (a total of eight chips: two chips for each 96 individuals); additional details can be found in Bernatchez et al. (2016). Individuals of the same population were dispersed in a random order on different chips to avoid effects of common chip or sequencing reactions. Sequencing was performed using Ion Torrent technology at the IBIS genomic analysis platform (Laval University, Quebec City, Canada).

Bioinformatics

Data processing

The raw sequence reads were trimmed with Cutadapt (Martin, 2011) in order to remove the adapter sequences, and sequence quality was assessed using FastQC (Andrews, 2010). The sequences were extracted and trimmed (trimmed length: 80 bp) using process_radtags in STACKS V.1.48 (Catchen et al., 2013). Trimmed sequence reads were aligned to the Atlantic salmon *Salmo salar* Linnaeus, 1758 reference genome (PRJNA72713) (Lien et al., 2016) with bwa (0.7.17−r1188, options: $-k = 19$, $-c = 500$, $-O = 0.0$, $-E = 2.2$, $-T = 0$) and samtools (1.9, options: $-S$, $-b$, $-q = 1$, $-F = 4$, $-F = 256$, $-F = 2048$). Then, pstacks was performed to extract the stacks aligned to the reference genome (options: $-m = 1$, –model_type = snp, –alpha = 0.05) and to identify single-nucleotide polymorphisms (SNPs) at each locus. The loci were grouped together across individuals and cataloged using cstacks, with a maximum between loci mismatch parameter of 1, and then loci from each individual were matched against the catalog to identify the alleles at each locus using sstacks. Then, the state of loci was written as VCF (variant call format) output using the populations program (options: $-r = 0.5$, $-p = 1$, $-m = 4$). VCF output from the populations program was further filtered using the script 05_filter_vcf.py from stacks workflow (https://github.com/enormandeau/stacks_workflow) with the following parameter values: $-m = 4$, $-p = 60$, $-a = 0.01$, $-A = 0.05$, and $-H = 0.6$.

Analyses of admixture and introgression

A Bayesian clustering analysis was first performed on the SNP data using ADMIXTURE V.1.23 (Alexander et al., 2009). The admixture analysis was run for 2000 bootstraps and the groups were set from 1 to 10 (K). K was selected according to the cross-validation error (CV), *i.e.,* the K values corresponding to the fewest cross-validation errors were selected. To visualize the distribution of each admixture cluster, different genomic clusters (Q values) identified among all specimens were then plotted on a geographic map using DIVA-GIS 7.5.0 (http://www.diva-gis.org/). In addition to the admixture analysis, the SNP data were analyzed using principal components analysis (PCA) using *Adegenet* R package (Jombart & Collins, 2015). To reduce the effect of missing data on PCA analysis, the missing data were imputed using the *random forest* R package (Breiman, 2006) based on the sampling localities of the specimens. To formally assess the inter-population admixture and gene flow, all possible combinations of three-population (f_3; X: B, C) and four-population (f_4; A, B:C, D) (Keinan et al., 2007; Patterson et al., 2012) tests implemented in TreeMix (Pickrell & Pritchard, 2012) were assessed. Furthermore, in order to find the best-fitting model of gene flow among the analyzed populations, we reconstructed the maximum likelihood (ML) population tree and then modeled inter-branch gene-flow events using TreeMix. To account for linkage disequilibrium, we grouped each 500 consecutive SNPs along the genome together. We then reconstructed ML trees with different numbers of migration events and calculated the variance explained by different models with different numbers of migration events.

Genetic differentiation

To quantify the extent of genetic differentiation among populations, pairwise F_{ST} estimates (Weir & Cockerham, 1984) were calculated using the R *STAMPP* package (Pembleton et al., 2013). To assess the extent of correlation between population differentiation and geographic distances, a Mantel test was performed between genetic distance and geographic distance matrices using R. To produce the matrix of geographic distances, the water-line (the linear distances of sampling localities to the mouth of the stream plus the distance between mouths of each pair of streams) distances between each pair of sampling localities were calculated in kilometres using Google Earth.

Genetic stock identification

Genetic stock identification (GSI), also referred to as mixed-stock assessment (MAS), is an approach whereby contributions of different populations to a mixed-stock fishery are determined for sustainable management of fisheries stocks (Anderson et al., 2008; Swatdipong et al., 2013). Individuals caught from a common fishing ground are assigned to their population of origin based on highest probability of relatedness of their genetic markers to different known baseline populations (individual assignment) or the genotypes in a mixed fisheries sample are attributed to genotype frequencies in the known source populations (Swatdipong et al., 2013). To estimate the contributions of different populations in habitats/streams to lake-run trout, a trained population assignment test was performed using the *assignPOP* ver. 1.1.4 R package (Chen et al., 2018). In order to perform the assignment test, we treated specimens collected from the lake as of unknown origin and treated samples collected from each locality as source reference data (*i.e.,* source populations) for trained clustering analysis. Before performing the assignment test, the accuracy of assignments was cross-validated using Monte-Carlo and *K*-fold cross-validation approaches. In Monte-Carlo cross-validation, the mean assignment accuracy is calculated via resampling random sets of training individuals and in *K*-fold cross-validation approach, the probability of membership for all individuals is measured by using one group as test group and $K - 1$ groups as training groups, so this approach provides the possibility for each individual to be tested once (Chen et al., 2018). In cases where there was no significant population differentiation between population pairs, the undifferentiated populations were pooled and considered as a single unit. We used 10%, 25%, 50%, and all loci as training loci to assess the effect of using different proportions of training loci. Low-variance loci, i.e., the loci with the major allele existing in over 95% of individuals, were excluded from the analysis. For baseline assign.MC analysis, the options were F_{ST} locus sample method: F_{ST}, principle component retaining criteria: Kaiser–Guttman, and machine-learning model: svm. The options used for unknown population assignment test to baseline (assign.X) were as follows: PC retaining criteria: Kaiser–Guttman and machine-learning model: tree (Chen et al., 2018). In addition to using a full set of known individuals from each locality, to avoid assignment bias resulting from different baseline sample sizes and to compare the results produced by different sample sizes, we used equal numbers of fish (24 individuals for each population) for baseline data for assignment of unknown individuals to baseline. When using equal sample sizes of 24 individuals from each stream, the Kharsang population was excluded from assignment analysis due to its lower sample size (ten individuals). In addition, we used a maximum likelihood algorithm implemented in ONCOR (http://www.montana.edu) to assign lake-run trout to different baseline populations. This method calculates the probability of origination of each lake-run individual's genotype from one of the baseline populations (Mäkinen et al., 2015). To calculate confidence intervals for assignments, we used 5000 bootstrap replicates.

Stock assessment

During sampling at each locality, the total length of fish was recorded to the nearest 1 mm using a measuring board. To estimate population density, the surface area of the sampled section of each stream was calculated and the upstream and downstream of the sampling sites were blocked with two seine nets. In addition, the physical and chemical properties of water at each sampling site were recorded using a HACH Sension + EC5 Portable Conductivity/TDS meter (www.hach.com). Further, we had recorded the same

environmental data during 2009 in different seasons (Supporting data I).

Results

Genomic data

In order to ensure sufficient sequence coverage per individual, only the 263 trout with more than 2000,000 sequences were kept for further analyses. Among these, after running STACKS, only individuals that had less than 20% of missing data after a first filtration step were kept for further analyses. Data from these samples were re-run through the *populations* program and filtered with the 05_filter_vcf.py script using the same parameters. The output VCF file from populations contained 61,825 SNPs (43,035 loci) before filtering and 9255 SNPs located within 7978 loci shared among all individuals after filtering. We kept only the SNPs with the highest MAF (Minor Allele Frequency) at each locus.

Clustering analyses

On the basis of having fewest cross-validation errors, the most appropriate numbers of admixture clusters fitting the data for analyzed specimens were 2 (CV = 0.1478), 3 (CV = 0.1511), 4 (CV = 0.1524), and 5 (CV = 0.1571) (Fig. 2). For K = 2-5, the Vararoo and to various degrees the Absefid populations were associated with their own admixture cluster. For all K values Vararoo had its own admixture cluster (the Vararoo cluster). At K = 4–5 in the Absefid Stream individuals were admixed among the Vararoo, Lar (see below), and a different prevailing admixture cluster (named the Absefid cluster hereafter). The most frequent cluster included fish from Lake Lar and Elarm, Kamardasht, Khoskehrood, Cheshmeh-dobarar, and Siahpalas streams, named Lar cluster hereafter (Figs. 2 and 3). Specimens analyzed from Kharsang Stream showed to be admixed between Vararoo and Lar clusters at K = 2, but were composed of pure individuals for a third admixture cluster (named Kharsang cluster hereafter), pure individuals for the Lar cluster, and individuals admixed between the Lar and Kharsang clusters at K = 3–5. Spatially, at K = 3, the eastern tributary to Lake Lar had one dominant cluster (Vararoo cluster; shown in green), the upper western tributary had one cluster (Kharsang cluster; blue), while the Lar Lake stock, most fish in the western tributary and some in the eastern tributary belonged to a third cluster (Lar cluster; shown in red). Although, K = 2 was the fittest number of Admixture clusters, based on the clustering patterns revealed in Principal Component Analysis (see below) and physical properties of the national park and the connectivity of habitats, K = 3–4 probably reflects the Caspian Sea trout populations more realistically.

In Principal Component Analysis, Caspian Sea trout from Vararoo Stream were well discriminated from Caspian Sea trout of most other streams (Lar Lake and Elarm, Kamardasht, Khoskehrood, Cheshmeh-dobarar, and Siahpalas Streams) along the first PC axis (19.59% of total variation; Fig. 4). Caspian Sea trout collected from Dalichay and Absefid streams were intermediate between Vararoo and Lar clusters (see above) along the first PC axis. The second PC axis (1.21% of total variation) did not discriminate Caspian Sea trout from Vararoo and Dalichay from the Lar PC cluster. Caspian Sea trout from the Absefid Stream and a few individuals from the Kharsang Stream were isolated from one another and from other groups along the second PC axis. In addition, a few individuals from the Kharsang Stream were isolated from other populations along the third PC axis (1.01% of total variation) but none of the populations were discriminated along the fourth PC axis (0.76% of total variation).

Population differentiation

Pairwise population differentiation (F_{ST}) values were generally pronounced and highly significant between Vararoo Caspian Sea trout and each of the other populations analyzed (0.296–0.497; Table 2). The other populations that showed significantly high pairwise F_{ST} values to other populations were the Dalichay (0.060–0.299) and Absefid (0.097–0.296) stream populations, respectively. Although significant, the F_{ST} between Kharsang and Cheshmeh-dobarar (0.031), Siahpalas (0.039), Elarm (0.042), Khoskehrood (0.025), and Kamardasht (0.042) populations were less pronounced compared to the values calculated between Vararoo (0.06–0.497), Dalichay (0.06–0.296), and Absefid (0.97–0.296) and the noted populations. The Mantel test showed a significant

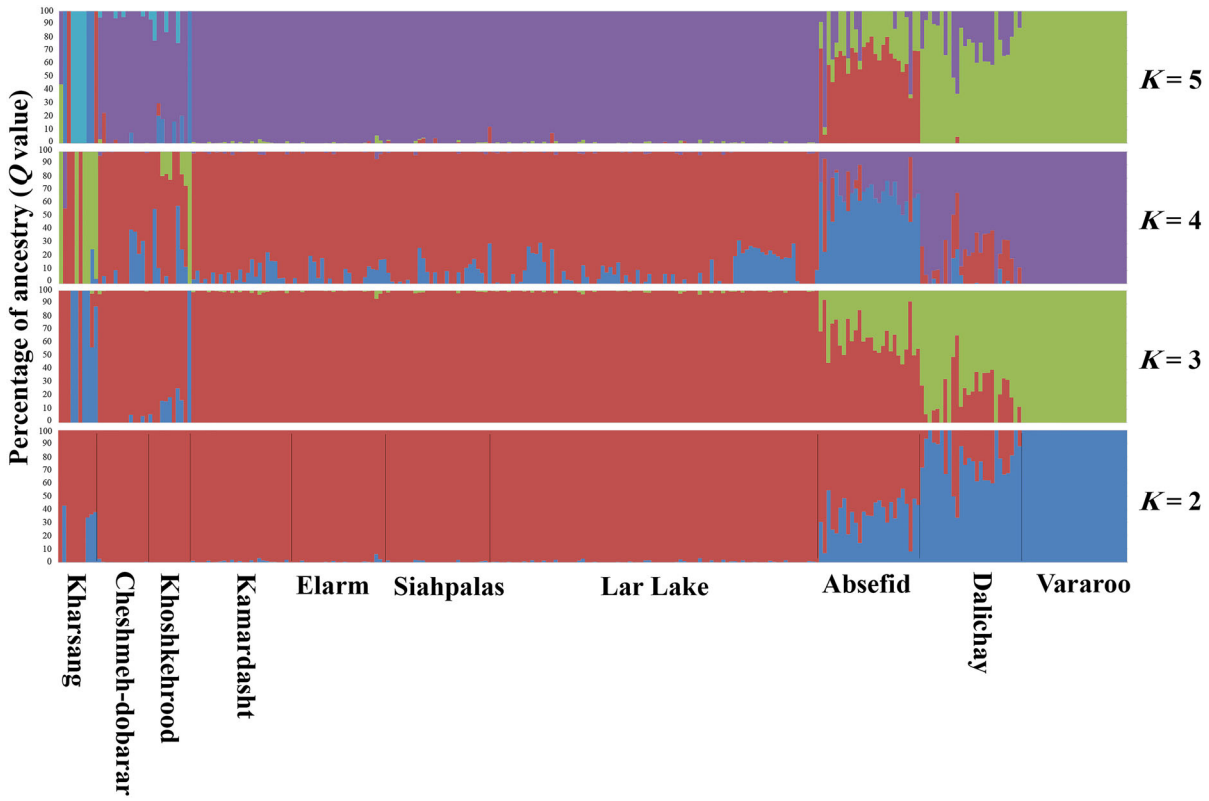

Fig. 2 Admixture graphs for different K values (2–5). The black lines depicted on the graph for K = 2 delineate populations

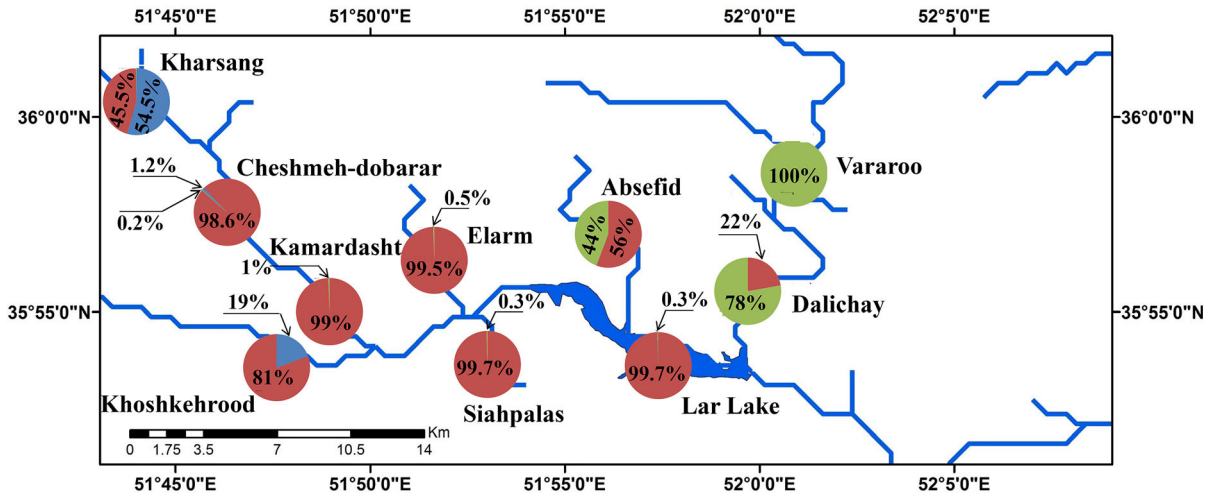

Fig. 3 Spatial distribution of different admixtures for k = 3, in studied areas. Admixture clusters are shown using different colors. Admixture clusters are red: Lar cluster (the most frequent), green: Vararoo cluster, and blue: Kharsang cluster. The names are selected in accordance to the localities where the admixture clusters were observed with high frequency. The percentage of each contributing cluster on pie charts is the average value calculated for each locality

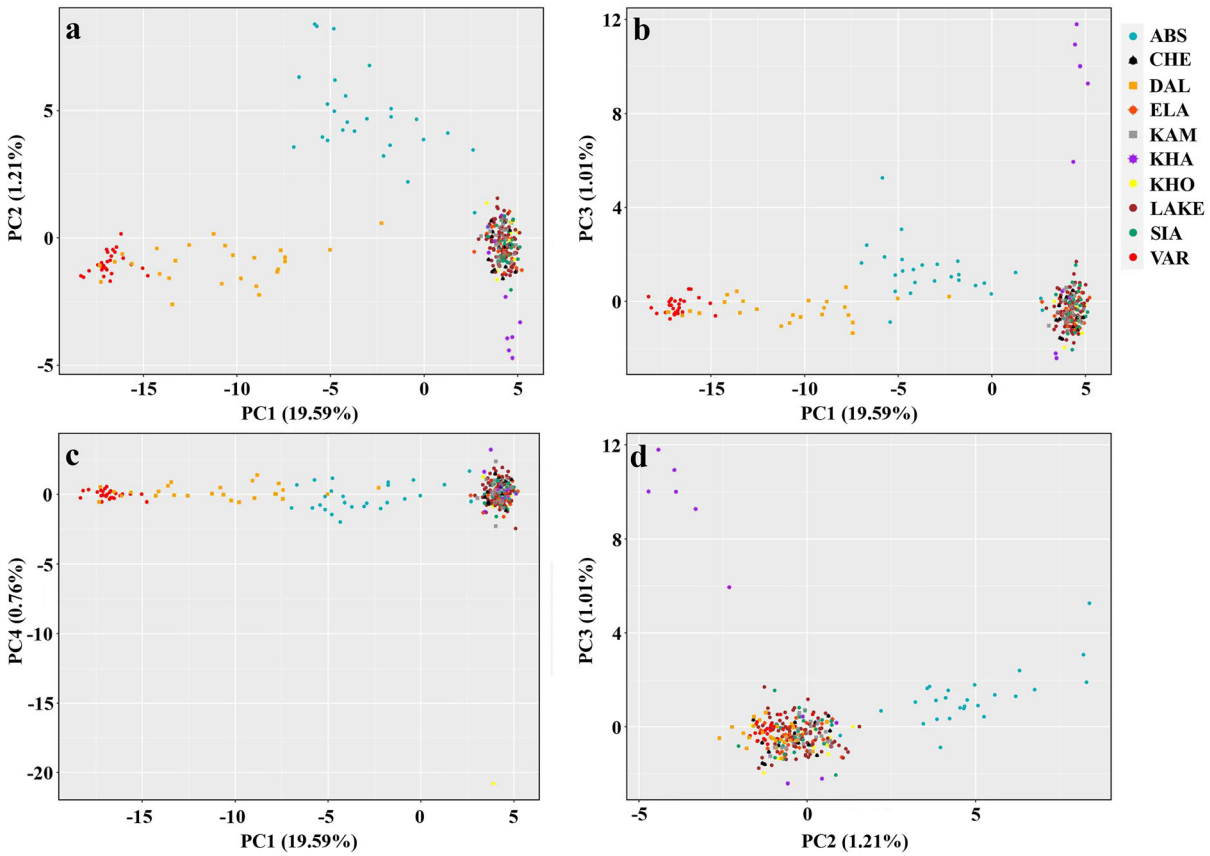

Fig. 4 Distribution of individuals from different sampling localities along the first, second, third, and fourth PC axes. *ABS* Absefid, *CHE* Cheshmeh-dobarar, *DAL* Dalichay, *ELA* Elarm, *KAM* Kamardasht, *KHA* Kharsang, *KHO* Khoskehrood, *LAKE* Lar Lake, *SIA* Siahpalas, *VAR* Vararoo

correlation between geographic and genetic distance matrices (R^2= *0.646*; $P < 0.001$), suggesting isolation-by-distance.

Three- and four-population tests

Results of the four-population test (f_4) suggested the occurrence of important and highly significant gene flow between the Vararoo, Dalichay, and Absefid populations ($|Z$ score$| \gg 2$). Among other populations, Caspian Sea trout from Cheshmeh-dobarar Stream also showed low levels of gene flow from Vararoo Stream, but the Z score was much smaller compared to that calculated for gene flow between Vararoo and Dalichay and Absefid streams. No gene flow was detected between Vararoo and other populations (Supporting data II). Significant gene flow also was detected among all other populations. Results from the three_population test (f_3) showed that Dalichay and Absefid populations were admixed populations (Z score $\ll 0$; Supporting data III). In all cases, one of the ancestral populations for Absefid and Dalichay was the Vararoo population. TreeMix analysis suggested three migration events (Fig. 5), including one from Dalichay to Absefid (red arrow), one from Kharsang and Cheshmeh-dobarar to Dalichay, and one from Cheshmeh-dobarar to Khoskehrood. This model with three migration events explained 99.959% of the variance of the relatedness between populations.

Genetic stock identification

In assignment cross-validation pre-tests performed only on the baseline data, increasing the number of individuals from 10 to 15 and to 20 increased assignment accuracy to 95% or more. Increasing the proportion of loci used in assignment test showed that

Table 2 Pairwise fixation index (F_{ST}) (lower diagonal) and Nei (1972) standard genetic distance (upper diagonal) calculated for each pair of populations

	Absefid	Cheshmeh-dobarar	Dalichay	Elarm	Kamardasht	Kharsang	Khoskehrood	Siahpalas	Vararoo
Absefid	–	0.010	0.011	0.009	0.008	0.014	0.011	0.009	0.020
Cheshmeh-dobarar	0.101*	–	0.025	0.003	0.003	0.008	0.005	0.003	0.040
Dalichay	0.141*	0.299*	–	0.024	0.024	0.030	0.027	0.024	0.003
Elarm	0.101*	0.007	0.295*	–	0.002	0.007	0.004	0.002	0.040
Kamardasht	0.097*	0.004	0.293*	0.000	–	0.007	0.004	0.002	0.039
Kharsang	0.120*	0.031*	0.289*	0.042*	0.042*	–	0.009	0.007	0.045
Khoskehrood	0.097*	0.005	0.296*	0.004	0.006	0.025*	–	0.004	0.043
Siahpalas	0.099*	0.007	0.291*	0.000	0.000	0.039*	0.006	–	0.039
Vararoo	0.296*	0.497*	0.060*	0.475*	0.470*	0.466*	0.497*	0.464*	–

*Significant F_{ST} values ($P < 0.01$)

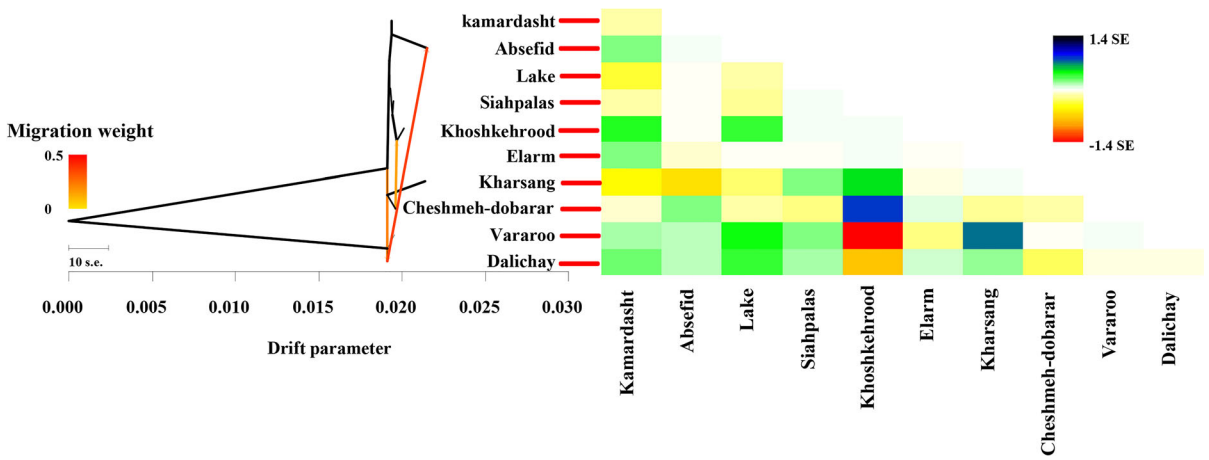

Fig. 5 *Left* Maximum Likelihood tree with three migration events reconstructed using TreeMix for 7978 loci in windows of 500-base-pair blocks. The drift parameter refers to the amount of genetic drift occurred between the populations. The color legend on the left side shows migration weight. *Right* residual fit from the maximum likelihood tree. The colors denote the magnitude of standard error (SE): the darker the color, the higher the SE

the assignment accuracy with 25% and 50% of the loci resulted in higher accuracy compared to 10% of the loci. Population assignment results showed that the majority of lake-run fish (100% in maximum likelihood assignment method, and 98.75% in machine-learning method of assignpop) were assigned to Caspian Sea trout from the pooled sample (Siahpalas, Kamardasht, Cheshmeh-dobarar, Elarm and Khoskehrood streams; Table 3). No lake-run fish was assigned to population from Vararoo, Kharsang, Absefid, and Dalichay streams (0 or 1% depending on baseline sample size). There was no significant difference between the assignments using equal baseline sample sizes or the full set of baseline samples.

Stock assessment

Trout population density in Siahpalas Stream was greater than in other streams (345 per 100 m^2; total length ± standard error (TL) = 146.68 ± 5.78 mm). The density of Caspian Sea trout varied among the other sampling sites in the following decreasing order

Table 3 Results for assignment of unknown individuals caught from the Lake Lar to different possible source populations in streams in the Lar National Park

Locality	Assignpop (all)	Assignment percentage Assignpop (24)	ONCOR	95% CI
Absefid	0.00	1.25	0.00	0.000, 0.000
Dalichay	1.25	0.00	0.00	0.000, 0.000
Kharsang	0.00	–	0.00	–
Pooled sample set[a]	98.75	98.75	100.00	1.000, 1.000
Vararoo	0.00	0.00	0.00	0.000, 0.000

Assignments for both Maximum Likelihood (ONCOR) and assignpop were performed using equal (24) and full (all) baseline sample sizes

[a]Samples with no differentiation including samples from the Lake and Kamardasht, Elarm, Cheshmeh-dobarar, Siahpalas, and Khoshkehrood streams

in Vararoo (233; TL = 154.08 ± 6.34 mm), Elarm (221; TL = 152.26 ± 6.21 mm), Kamardasht (204; TL = 115 ± 10.8 mm), Dalichay (96; TL = 135.6 ± 13.8 mm), and Absefid (96; TL = 193.1 ± 16.3 mm).

Discussion

Population genetic structure

Among the Caspian Sea trout populations analyzed, a population genetic structure was clarified that is concordant with the significant within river/stream population structure reported in brown trout Linnaeus, 1758 and other salmonids (Carlsson et al., 1999; Hébert et al., 2000; Spidle et al., 2001; Taugbøl, 2008; Dionne et al., 2009; Kitanishi et al., 2009; Ozerov et al., 2010; Wellband et al., 2012; Kelson et al., 2015; Mäkinen et al., 2015). In particular, our results showed that the Vararoo population, which inhabits elevation of 2970 m above sea level and higher, was highly divergent compared to other populations. No admixture clusters existing in other streams of the Lar River drainage were observed in the Vararoo population, but the Vararoo cluster contributed to fish from Dalichay (downstream of Vararoo) and Absefid (the most proximate stream to Vararoo after Dalichay) streams. This supports the occurrence of one-way gene flow from Vararoo to downstream and nearby streams. These findings imply that the divergence of the Vararoo Caspian Sea trout population is likely due to physical obstacles impassable to upstream migration, especially due to about 500 m elevation difference within 8.5 km separating Dalichay and Vararoo and the several steep reaches of the river that in combination with high flow rate can act as barriers to upstream movement of fish. In addition, this can also be related to the behavior of the resident form of trout that shows very limited movement in streams (Pettersson et al., 2001; Olsson & Greenberg, 2004; Vøllestad et al., 2012). Due to the admixture from Vararoo, the Dalichay population showed more genomic similarity to that population than to any others. It may also be due to limited gene flow from the lake and other streams, since there is an obstacle in the mouth of Dalichay that limits the upstream migration of lake-run fish into this stream.

At $K = 4$–5 the Caspian Sea trout in the Absefid Stream were admixed among the Vararoo, the Lar, and the dominant Absefid clusters. In contrast to the context for the Vararoo and Dalichay populations, in the Absefid Stream there is no visible physical obstacle for fish migration, such that the development of the Absefid cluster is most likely related to other environmental factors. Namely, in downstream reaches of the Absefid Stream sulfurous springs drain into the stream, and along the impacted section of the stream, the frequency of fish dramatically decreases (personal observations of the authors). Sedighkia et al. (2018) documented a high-temperature gradient between the upstream and downstream sections of the Absefid Stream. They indicated that in downstream sections of the Absefid Stream, the substrate is covered with calcareous sediments that, in suspension, contribute to increased water temperature and a

decrease in dissolved oxygen to critical limits. Because of this, these authors concluded that Caspian Sea trout habitat in Absefid Stream was becoming fragmented. This hydro-chemical change may act as a barrier that limits fish movement in either direction between the lake and upstream sections of the Absefid Stream.

Previous studies dealing with population structure in brown trout showed that fish inhabiting the upper reaches of rivers were resident (Swatdipong et al., 2013; Mäkinen et al., 2015). It has also been shown elsewhere that resident brown trout populations show very limited movement, which may promote significant population structure, as we observed here (Olsson & Greenberg, 2004; Vøllestad et al., 2012). Hence, we propose that the Vararoo Caspian Sea trout are probably resident and no fish from Vararoo and Dalichay enter the lake. Assuming that the Vararoo fish are resident Caspian Sea trout, we hypothesize that the Vararoo Caspian Sea trout had migrated to Absefid Stream before the creation of the artificial Lar Lake a few decades ago, when the two streams were on continuous river ecosystem. After creation of the lake, migration of resident Caspian Sea trout from Vararoo to Absefid Stream via the lake ecosystem was likely hampered due to the fact that resident Caspian Sea trout avoid entering the lake ecosystem.

In the lake and streams including Siahpalas, Elarm, Kamardasht, and Cheshmeh-dobarar, no or very weak population genetic structure was detected, which may be related to their proximity to one another and also the absence of physical–chemical obstacles to gene flow (Bohlin et al., 2001). Moreover, the environmental conditions (hydro-chemical properties) are similar among these streams (Supporting data I). Hence, the weak population genetic structure can also be a result of weak spatial differences. In addition, more pronounced exchange is expected between geographically proximate populations compared to the populations that are geographically more distant (Jonnson & Jonnson, 2011), which was confirmed by significant Mantel test in our results.

The different admixture cluster observed in the Kharsang and Khoshkehrood streams that coexist with the Lar cluster probably shows that similar to the eastern streams of the national park, resident populations also exist in these localities. In addition, the Kharsang cluster can be related to the factors hampering dispersal such as physical barriers. In downstream and upstream sections relative to the mouth of Cheshmeh-dobarar Stream, the main stream regularly dries up at two sections, which might be a driving factor in development of the Kharsang cluster. The dried section between the Kharsang and Cheshmeh-dobarar streams is stable, but the dry section downstream of Cheshmeh-dobarar is not and contains water mostly during spring. The coexistence of the Kharsang and the Lar clusters in the Kharsang and Khoshkehrood might result from colonization of the upper reaches of the Lar River drainage by the Lar cluster when the dry sections may become flooded, temporarily.

Genetic stock identification

Our results showed that Siahpalas, Elarm, Kamardasht, Cheshmeh-dobarar, and Khoshkehrood Streams comprise a single panmictic unit that largely contributed to the lake-run fish, whereas almost no contribution from other populations was detected. This may be related to different ecological factors including close proximity of these localities to the lake, less energetic cost of migration from lake to these streams, higher frequency of spawning grounds, and the absence of physical or ecological barriers to migration, like non-suitable habitat patches or waterfalls. These streams provide suitable spawning habitats for Caspian Sea trout (Sedighkia et al., 2018). This may explain the high trout density we observed in those streams. Moreover, it is plausible that the energetic cost of feeding migration from Siahpalas, Kamardasht, Elarm, Cheshmeh-dobarar, and Khoshkehrood streams to the lake may be lower for juvenile trout in these systems due to their proximity to the lake. When population density in an ecologically preferred habitat increases, there is expected to be a higher intensity of competition for food and space (Jonnson & Jonnson, 2011); to minimize the energy used for competition, fish may select different habitats (Bohlin et al., 2001; Tabatabaei et al., 2015). Hence, we propose that due to high population density in theses streams and therefore a high level of competition for food and space, more fish from these streams migrate to the lake to decrease competition.

Our results showed that trout from Dalichay, Vararoo, Absefid, and Kharsang streams had no or minimal contribution to lake-run trout. Mäkinen et al. (2015) and Swatdipong et al. (2013) assessed the

contribution of brown trout of different rivers in lake systems in northern Finland, and observed that there were limited contributions from upper reaches of rivers to lake-run brown trout stock. This is concordant with our results, since Vararoo, and Kharsang streams are located in upper reaches of the Lar drainage. In accordance with other studies on resident brown trout movement behavior (Olsson & Greenberg, 2004; Vøllestad et al., 2012), Mäkinen et al. (2015) and Swatdipong et al. (2013) using microsatellite loci concluded that the insignificant contribution from the upper reaches might be related to the fact that the fish in upper reaches are resident and do not migrate to the lake. Our data also seem concordant with their results, since the barriers that exist in Dalichay and Vararoo would be expected to block only upstream migration but not dispersal from upstream to downstream.

Conclusion

Overall our results revealed the existence of fine-scale population structure of some Caspian Sea trout populations inhabiting different streams in the Lar River drainage. Lack of significant population structure among other streams may reflect the differential impacts of different habitat landscape either hampering or enhancing the potential for gene flow. Our data highlighted that resident populations inhabiting the upper reaches of tributaries have no or insignificant contributions to the lake-run trout population, and the contributions from lower reaches of streams are high. Among the populations that we assessed, the Vararoo population is currently not protected by the national park system. Yet, our results revealed that the Vararoo population is a highly genetically divergent entity of Lar trout, such that our results provide rational support for expanding the coverage of the national park to ensure the conservation and management of this apparently unique lineage of Caspian Sea trout. In addition, stream habitats including Siahpalas, Elarm, and Kamardasht had the highest contribution to lake-run trout, and these habitats are adjacent to settlement sites of nomads, the grazing activities of their livestock may cause habitat degradation. Hence, we propose that protection of these habitats must be considered seriously in Lar trout conservation and management measures.

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Data availability VCF files and other Data are available on request.

Compliance with ethical standards

Conflict of Interest The authors declare that they have no conflict of interest.

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