



Genetic Structure Profile of Rainbow Trout (*Oncorhynchus mykiss*) Farmed Strains in Greece

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Abstract

The rainbow trout, *Oncorhynchus mykiss*, comprises a non-indigenous species of the European freshwater ecosystem. Due to its remarkable advantages of fast-growing and facultative adaptability in various habitats under different conditions, it has become the most dominant commercially reared species of freshwater aquaculture in Greece. Despite its economic importance, there is a dearth of population genetic studies regarding the origin of any posterior introduction by the private aquaculture sector. In this study, a polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) assay was used, in order to assess variation in five mitochondrial DNA protein-encoding regions and the control region, covering more than 5,500 bp of the 16,600 bp total mitochondrial genome size. The samples derived from 10 rainbow trout farms throughout Greece representative of the freshwater aquaculture sector. The conducted population structure analysis revealed two major clusters among the farmed-rainbow trout populations. Moreover, the overall genetic diversity was substantially attributed to the diversity within populations rather than among them. In any case, both factors obtained from AMOVA analysis were statistically significant. The obtained haplotype network reinforced the existence of two central haplotype clusters among the farmed-rainbow trout populations in the Greek freshwater aquaculture sector.

Keywords: *Oncorhynchus mykiss*, freshwater aquaculture, genetic structure, founder populations.

Introduction

The rainbow trout, *Oncorhynchus mykiss*, has a worldwide distribution, fact that is strongly related to its remarkable fast-growing and facultative adaptability in various habitats, under different conditions. The commercial aquaculture took advantage of these attributes and in 2012 its global production reached a total of 732,432 tons, rendering it among the most dominant reared commercial species of freshwater aquaculture with an estimated economic value of 2.6 billion Euros (FAO, 2013).

The native range of the rainbow trout includes south-western Alaska to the Sierra Madre Occidental in Mexico and tributaries of the Pacific Ocean in Asia (Behnke, 2002). As far as its naturalized range is concerned, it is currently distributed throughout the eastern coast of North America where self-sustaining populations have proliferated (Behnke, 2002). It comprises a non-indigenous species of the Greek freshwater ecosystem (Economidis *et al.*, 2000). Since its deliberate introduction as eggs from Switzerland in the early 1950s, focusing primarily on the enhancement of the domestic freshwater fish

production and the economic growth of the sector, it inhabits in aquaculture facilities. These are established mainly in rivers, streams and lakes throughout Greece (Economidis *et al.*, 2000). Since the 1950s, the origin of any posterior introduction by the private aquaculture sector has not been documented. Particularly, there are 94 producers in total, of which only 6 major companies produce more than 100 tons, 14 medium ones produce from 50 to 100 tons and 74 small establishments produce less than 50 tons (Framian, 2009; Anonymous, 2009). In 2006, the total production of rainbow trout in Greece accounted for 3,643 tons, contributing to a value of 10 million Euros to Gross National Income (GNI) (Framian, 2009).

Despite the fact that rainbow trout comprises the major freshwater reared species in Greece, literature review revealed a dearth of population genetic studies. Likewise, only two population genetic studies of reared *O. mykiss* have been conducted in Europe, the first referred to northern and eastern Europe (Finland, Denmark, Sweden, Norway, Estonia and Poland) (Gross *et al.*, 2007) and the second regarding to Norwegian fjords (Glover, 2008). Particularly,

according to Gross *et al.* (2007) the majority of European strains presented similar levels of variation with Shasta strain derived from California, USA, apart from two Polish strains. In contrast, Glover's (2008) study revealed a considerable genetic variation within and among rainbow trout strains farmed in Norway, a fact that enabled management authorities to identify the farm of origin for escapees.

On the contrary, in other continents and mainly in northern America several population genetic studies have been conducted for natural stocks (McCusker *et al.*, 2000; Narum *et al.*, 2004; Deiner *et al.*, 2007; Williams *et al.*, 2007; Heath *et al.*, 2008) and reared rainbow trout (Sajedi *et al.*, 2003; Silverstein *et al.*, 2004; Johnson *et al.*, 2007; Camarena-Rosales *et al.*, 2008; Zhao *et al.*, 2008). Briefly, concerning the natural rainbow trout populations, McCusker *et al.* (2000) assessed mtDNA variation of *O. mykiss* in northern America by means of RFLPs, revealing two phylogenetically distinct mitochondrial lineages (resident and anadromous). Likewise, Narum *et al.* (2004) using six microsatellites detected a genetic structure between the two major drainages, Walla Walla River and Touchet River, regarding the anadromous and resident trout forms. Heath's *et al.* (2008) study, by means of three types of genetic markers (microsatellites, major histocompatibility complex - MHC, RFLPs), revealed that the migratory and resident forms of *O. mykiss* were genetically differentiated in two different groups. On the other hand, concerning reared rainbow trout, Silverstein's *et al.* (2004) study results, by means of nine microsatellites, implied that three examined domesticated strains (Clear Springs, Troutlodge and University of Washington) were differentiated. Moreover, Johnson *et al.* (2007), using twelve microsatellites studied genetic structure among annual broodstocks, revealing substructure for many generations. Finally, Camarena-Rosales *et al.* (2008) studied the variation and composition of mtDNA haplotypes of four hatcheries from northwestern Mexico, focusing on the mtDNA region between Cytb and D-loop by means of RFLPs. The obtained results showed that trout hatcheries presented more unique haplotypes in comparison with the native populations.

Despite the fact that worldwide aquaculture is the fastest growing animal food-producing sector, the used aquaculture broodstocks are yet to be genetically adequately characterized. Population genetic research using mtDNA markers has contributed substantially to aquaculture management (Billington, 2003) and to the elucidation of genetic relationship among aquaculture strains. Among other advantages (Benzie *et al.*, 2002), their use became quite popular among aquaculture geneticists due to the fact that they successfully revealed significant genetic differences among reared populations (Sajedi *et al.*, 2003; Camarena-Rosales *et al.*, 2008).

The aim of the present study is (1) to evaluate

the specific genetic variation of the mtDNA using PCR-RFLP within and among populations of *O. mykiss* derived from different freshwater aquacultures throughout Greece; (2) to document the current mtDNA composite haplotypes of each aquaculture unit/farm. Both these aims are putting the underpinning stone for future development of selection breeding programs, which would in the long term increase the domestic production, in the framework of the implementation of a sustainable ecosystem conservation management plan.

Materials and Methods

According to the sampling procedure, a total of 564 *Oncorhynchus mykiss* specimens were collected. The samples derived from 10 rainbow trout farms sited in six rivers, two streams and one spring throughout Greece, representative of the freshwater aquaculture sector. Fish were stunned and transported to Aquatic Animal Genetics Lab in UTh according to the Council Regulation (EC) No 1/2005 that sets the framework for the protection and welfare of farmed fish in Hellas. Concerning the infrastructure facilities of each aquaculture, these consisted of raceways adjacent to rivers, streams and springs. The majority of aquaculture enterprises use broodstocks which are mostly derived from the local State Aquaculture Institutes with an inner replenishment every three years, but there are also reported occasional imports of fry fish from abroad. In Figure 1 details concerning the sampling procedure are presented and the coordinates of the sampling sites are indicated.

Total DNA was extracted from muscle tissue, using phenol-chloroform protocol of Taggart *et al.* (1992). Taking into account the study design, all samples were included in the composite RFLP analysis of five mitochondrial DNA protein-encoding regions (including the whole control region and two tRNA genes), covering approximately more than 5,500 bp of the 16,600 bp total mitochondrial genome size. Specifically, a segment consisting of the entire Control Region-Cytb gene (2,096 bp) was amplified using the newly designed forward CR₁-(5'-CAACTACAAGAACCTAAT-3') and reverse CR₂-(5'-TAACAGGAACATAAGGAT-3') primers, taking into account the GenBank *O. mykiss* sequences. Moreover, the amplification of the entire NADH dehydrogenase subunits 5 and 6 (ND5/6) segment (2,500 bp) was applied, using the primers ND_L and ND_G (Sajedi *et al.*, 2003). Likewise, a fragment of 948 bp, which comprises a part of ATPase-VI and COIII, was amplified using the newly designed forward AT₁-(5'-GCAGGYATTACTACTCTA-3') and reverse AT₂-(5'-AGGTAAAGCTGAAGTGTA-3') primers based on *O. mykiss* sequences from GenBank.

In all cases, DNA amplification reaction mixtures consisted of approximately 100 ng template DNA, 10 µl 5× PCR Buffer, 2.5 mM MgCl₂, 200 µM

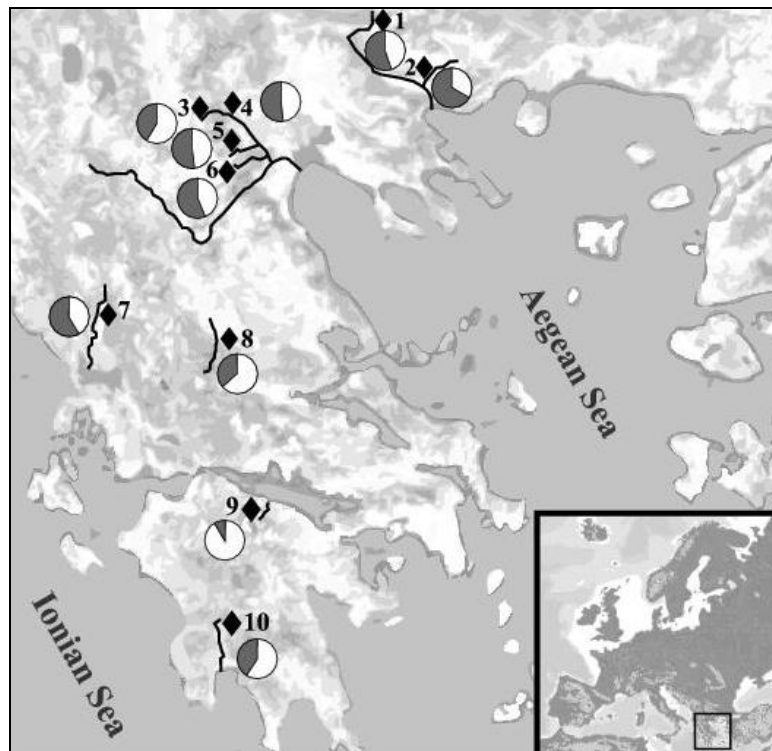


Figure 1. Map of Greece showing sampling sites of farmed-rainbow trout. Each freshwater aquaculture population is coded with rhombus [1. Agistro (N = 59), 41°22'50.39''N 23°26'18.15''E; 2. Agios Ioannis (N = 57), 41°5'57.30''N 23°35'15.15''E; 3. Edesseos 1 (N = 59), 40°48'30.94''N 22°0'57.25''E; 4. Edesseos 2 (N = 57), 40°48'25.00''N 22°1'19.12''E; 5. Arapitsa (N = 55), 40°36'47.46''N 22°2'49.15''E; 6. Tripotamos (N = 55) 40°28'26.68''N 22°10'37.37''E; 7. Louros (N = 54), 39°25'39.82''N 20°50'43.41''E; 8. Megdovas (N = 57), 39°13'1.53''N 21°44'43.74''E; 9. Krathes (N = 54), 38°1'4.07''N 22°15'5.36''E; 10. Bourmia (N = 57), 37°19'54.77''N 21°55'53.43''E.] Pies denote the presence of two putative clusters K (white, grey color) in each population revealed by STRUCTURE analysis.

of each dNTP, 30 pmol of each primer, 1.2 units of Taq Polymerase (Kapa Biosystems, USA) and sterilized water was added to a final volume of 50 μ l. The optimized amplification conditions performed in the MJ Research PTC-200 Gradient Thermal Cycler (BIO-RAD, USA) were an initial denaturation at 95°C for 5 min, a strand denaturation at 95°C for 1 min, annealing at 56.2°C (for CR₁ and CR₂) or 60°C (for ND_L and ND_G) or 62°C (for AT₁ and AT₂) for 1 min, a primer extension step at 72°C for 1.30 min, repeated for 34 cycles and a final extension step at 72°C for 10 min. The length of PCR products was verified by electrophoresis on 1.5 % agarose gel containing 0.5 μ g ml⁻¹ ethidium bromide.

Initially, a preliminary screening of a representative sample of PCR products with 10 restriction endonucleases (*AluI*, *AvaII*, *EcoRI*, *HaeIII*, *HinfI*, *HpaII*, *MspI*, *NciI*, *RsaI*, *TaqI*) was conducted. The representative sample consisted of 5 individuals per population, focusing on the detection of at least two restriction patterns for each enzyme. Digestions were performed by incubation in 10 μ l reaction volumes according to manufacture instructions (New England Biolabs Inc., USA). Afterwards, the separation of the PCR products digested fragments was accomplished by electrophoresis on agarose gel 2 % stained with ethidium bromide and immersed in 1 ×

TAE (Tris base-acetic acid-EDTA) buffer. The restriction patterns on stained gel were visualized under UV light and photographs were taken using the MiniBIS Pro gel documentation system (BioSystematica, United Kingdom).

Data Analysis

Initially, the obtained distinct restriction patterns were coded with capital letters, generating composite haplotypes (genotypes) consisting of 10 capital letters, one for each restriction enzyme (Table 1). Moreover, genotypic richness, R (G/N ratio), per population was estimated, where G denotes the number of different observed composite genotypes and N denotes the number of the tested individuals. ARLEQUIN 3.5 software (Excoffier and Lischer, 2010) was used for the estimation of fixation index F_{ST} (Wright, 1951), as a measure of genetic differentiation, in parallel with haplotype diversity h (Nei 1987), nucleotide diversity π (Nei and Tajima, 1981) and Tajima's D index (Tajima, 1989). Tajima's D index was estimated in order to identify deviations from the neutral theory of mutation drift equilibrium. ARLEQUIN 3.5 software (Excoffier and Lischer, 2010) was also used for the estimation of statistical significance of pairwise differentiation, taking into account the composite

Table 1. Twenty-four composite genotypes (haplotypes-denoted with capital letters) based on RFLP digests of six restriction endonucleases (*AluI*, *AvaII*, *HaeIII*, *HinfI*, *RsaI*, *Taq^oI*) in ND5/6, one restriction enzyme (*HaeIII*) in ATPase VI-COIII and three restriction enzymes (*AvaII*, *HinfI*, *Taq^oI*) in Control Region - Cytb fragments of mtDNA; relative frequencies of haplotypes per population, sample size (*N*), genotypic richness (*R*), haplotype diversity (*h*) and nucleotide diversity (π), including standard deviation (*SD*) and Tajima's *D* index (ns = non-significant)

Haplotype	Composite genotype	Agistro	Ag. Ioannis	Edesseos 1	Edesseos 2	Araptisa	Tripotamos	Louros	Megdovas	Krathes	Bournia
1	BBBBBAABBB				0.04	0.04					
2	BBBBABABAB					0.04			0.04		
3	BBBBAAACAB					0.04					
4	BBBBAAABAB							0.04			
5	BABBBAAABAB							0.04		0.12	
6	BABABABBBB			0.03							
7	BABABAABBB		0.07	0.45	0.26	0.32	0.04	0.09	0.07	0.17	0.15
8	BABABAABAB	0.45	0.22	0.15	0.07	0.08	0.40	0.25	0.44	0.46	0.19
9	BABABAABAA	0.03									
10	BABABABAB				0.04						
11	BABAAAABAB		0.04		0.04						
12	BABAAAAAAB					0.04		0.04	0.07	0.17	0.25
13	ABBBBAABBB				0.07						
14	ABBBABABAB	0.32	0.19	0.25	0.26	0.24	0.04	0.04	0.19		0.15
15	ABBBAAACAB		0.11	0.03		0.08					
16	ABBBAAACAA	0.03									
17	ABBBAAABAB	0.07	0.30	0.03	0.10	0.04	0.52	0.5	0.04		0.07
18	ABBAABABAB		0.07			0.04					
19	ABBAAAABAB					0.04					
20	ABABAABBAB	0.07									
21	ABABAABBAA	0.03		0.03	0.04				0.11	0.08	0.15
22	ABABAAABAA			0.03							0.04
23	AABABAABAB				0.04				0.04		
24	AABAABABAB				0.04						
<i>N</i>		59	57	59	57	55	55	54	57	54	57
<i>R=G/N</i>		0.119	0.123	0.136	0.193	0.200	0.073	0.130	0.140	0.093	0.123
<i>h</i>		0.71±0.06	0.83±0.04	0.74±0.06	0.87±0.04	0.85±0.05	0.59±0.06	0.7±0.08	0.77±0.07	0.74±0.07	0.86±0.03
π		0.32±0.17	0.26±0.14	0.31±0.17	0.31±0.17	0.31±0.17	0.21±0.12	0.22±0.12	0.32±0.17	0.18±0.10	0.36±0.19
Tajima's <i>D</i>		1.40 ^{ns}	1.76 ^{ns}	0.96 ^{ns}	1.27 ^{ns}	2.34 ^{ns}	1.49 ^{ns}	0.97 ^{ns}	1.04 ^{ns}	-0.73 ^{ns}	1.64 ^{ns}

haplotype frequencies (Raymond and Rousset, 1995). Analysis of molecular variance (AMOVA) (Excoffier and Lischer, 2010) was applied on restriction sites using the ARLEQUIN 3.5 software (Excoffier and Lischer, 2010). Fixation index and variance components were permuted 10,000 times using a Bonferroni correction (Rice, 1989).

Additionally, the Bayesian cluster analysis was implemented using STRUCTURE 2.3 software (Pritchard *et al.*, 2000), enabling the classification of individuals of each population into *K* clusters according to their membership coefficients. The parameters burn-in length and simulation length were finally set at 500,000 and 1,000,000 repetitions respectively after the conduction of a preliminary test, where three independent repeats were run for each value of *K* ($1 \leq K \leq 14$). The adequate value for *K* was chosen, taking into account the HARVESTER online software, which provided the likelihood value for each *K* value (Earl and Von Holdt, 2012).

The visualization of the genetic relationships among the farmed-rainbow trout populations was applied through the conduction of Principal Coordinates Analysis (PCoA) (Jombart *et al.*, 2009) with Nei's standard genetic pairwise distances by

means of GenAlex 6.5 (Peakall and Smouse, 2012). Pairwise geographic distances among freshwater farmed-rainbow trout aquacultures were calculated as Euclidean distances in kilometers (km). A Mantel's Test (Mantel, 1967; Mantel and Valand, 1970) (1,000 permutations) was applied to test the relationship between matrices of geographical distance and Nei's genetic distance using GenAlex 6.5 (Peakall and Smouse, 2012). Phylogenetic relationships among the generated composite haplotypes were examined by calculating an unrooted haplotype network by means of NETWORK 4.6.1.2 (Fluxus Technology Ltd; www.fluxus-engineering.com). The Median Joining (MJ) and Maximum Parsimony (MP) algorithms were used in order to calculate the adequate haplotype network (Bandelt *et al.*, 1999).

Results

According to the obtained restriction patterns, six enzymes (*AluI*, *AvaII*, *HaeIII*, *HinfI*, *RsaI*, *Taq^oI*) presented polymorphism in ND5/6 region; three enzymes (*AvaII*, *HinfI*, *Taq^oI*) in CR - Cytb region and one enzyme (*HaeIII*) in ATPase-VI-COIII region, respectively. Four restriction endonucleases (*AluI*,

HaeIII, *RsaI*, *Taq^oI*) recognize tetranucleotide sequences and two enzymes (*AvaII* and *HinfI*) recognize pentanucleotide sequences. The composite haplotypes consisted of 10 restriction endonucleases, generating a total of 23 restriction sites. The restriction sites analysis showed 24 different haplotypes among the screened individuals and the relative haplotype frequencies of the 10 populations are presented in Table 1. The relative haplotype frequencies ranged from 0.03 to 0.52 among the different populations of freshwater aquacultures. Taking into account the relative haplotype frequencies, it seemed that the different composite genotypes within populations ranged between 4 and 11. Additionally, it is worth noting that only the composite genotype 8 (4.17%) was presented in all rainbow trout aquacultures. In contrast, among the 24 different composite genotypes, three were detected solely in populations of Agistro and Edesseos-2 rivers; two haplotypes in Arapitsa population and one haplotype in Edesseos-1 and Louros populations, respectively. Furthermore, the highest average haplotype diversity ($h = 0.87 \pm 0.04$) was presented in Edesseos-2 population and the highest nucleotide diversity ($\pi = 0.36 \pm 0.19$) was presented in the population of Bournia spring. As far as the genotypic richness R is concerned, the lowest value $R = 0.16$ was presented in the population of Tripotamos river and the highest value $R = 0.44$ was presented in the population of Arapitsa river. Tajima's D index was non-significant for all populations (Table 1).

Regarding the pairwise F_{ST} values, it was revealed that the highest value 0.3496 was presented

between Agios Ioannis stream and Krathes river populations ($P < 0.001$; Table 2), with a significant ($P < 0.001$) average F_{ST} value 0.056 (Table 3). The global exact test of sample differentiation based on haplotype frequencies was statistically significant ($P < 0.0001$). Taking into account the differentiation tests among all pairs of samples, it seems that the majority of population pairs present haplotype frequency distribution that differs with highly significant probability (Table 2). According to the AMOVA results obtained, only 5.65% of the overall genetic diversity was attributed to be among populations and the substantial 94.35% of variation exists within populations (Table 3).

The use of software STRUCTURE 2.3 unraveled two putative clusters ($K = 2$; Figure 2) among the 10 populations considering the membership coefficients which sum to 1 for each individual. The vast majority of populations appeared to have individuals in both clusters. Nevertheless, the percentages of the two clusters in the examined populations varied considerably (Figure 1). Similarly to the results of STRUCTURE 2.3 software, the use of NETWORK 4.6.1.2 indicated that all the examined farmed-rainbow trout populations derived from two founder nodes (Figure 3).

According to PCoA, the genetic differences among the 10 populations were visualized and each population assigned a location in a low-dimensional space (Figure 4). The obtained results showed that the first axis revealed a higher percentage of variation 77.64 %, in comparison with the low percentage of variation of the second and third axes 13.82 % and

Table 2. Pairwise differentiation exact tests of farmed-rainbow trout is presented below diagonal, and population pairwise (F_{ST}) values (after Bonferroni correction) are presented above diagonal

	AGS	AIO	IED	2ED	ARA	TRI	LOU	MEG	KRA	BOU
AGS	-	0.0102	0.0235	-0.0116	-0.0018	0.0125	0.0164	-0.0090	0.2026***	0.0125
AIO	0.0063**	-	0.1008*	0.0117	0.0139	0.0130	0.0045	0.0799	0.3496***	0.0688*
IED	0.0001***	0.0019**	-	0.0006	0.0017	0.0643	0.0695	-0.0085	0.1076*	0.0285
2ED	0.0004***	0.0335*	0.4279	-	-0.0246	0.0251	0.0249	0.0150	0.2316***	0.0359
ARA	0.0003***	0.0341*	0.8031	0.6936	-	0.0306	0.0240	0.0141	0.2143***	0.0269
TRI	0.0001***	0.0674	0.0001***	0.0001***	0.0001***	-	-0.0381	0.0370	0.2641***	0.0445
LOU	0.0001***	0.1512	0.0001***	0.0010**	0.0001**	0.7687	-	0.0443	0.2742***	0.0375
MEG	0.2641	0.0077**	0.0029**	0.0124**	0.0077**	0.0004***	0.0006***	-	0.0860**	-0.0084
KRA	0.0001***	0.0001***	0.0001***	0.0001***	0.0003*	0.0001***	0.0005***	0.0935	-	0.1252*
BOU	0.0009***	0.002**	0.0083**	0.0211*	0.0257*	0.0001***	0.001**	0.2283	0.0475*	-

*** $P < 0.001$, ** $P \leq 0.01$, * $P < 0.05$. Abbreviations - AGS: Agistro, AIO: Ag. Ioannis, IED: Edesseos1, 2ED: Edesseos 2, ARA: Arapitsa, TRI: Tripotamos, LOU: Louros, MEG: Megdovas, KRA: Krathes, BOU: Bournia.

Table 3. Analysis of molecular variance (AMOVA) among and within populations. F_{ST} index represented the sum of variation among populations and variation within populations divided by total variation

	df	Sum of squares	Variance components	Percentage of variation	Fixation Index
Among populations	9	75.453	0.19456 Va	5.65	$F_{ST} = 0.056^{***}$
Within populations	554	825.422	3.24969 Vb	94.35	
Total	563	900.875	3.44426		

(*** $P \leq 0.001$)

8.54 %, respectively. The Eigenvalues were 0.608 for the first axis, 0.108 for the second axis and 0.067 for the third axis, expressing the level of variation on each axis. Taking into account the bi-dimensional scatter-plot of PCoA, the farmed-rainbow trout populations were clearly separated into four groups along coordinates 1 and 2. The discriminated groups were Arapitsa-Megdovas-Bournia / Edesseos1-Louros-Krathes / Agistro-Tripotamos-Edesseos2 / Agios Ioannis. Nevertheless, Mantel's Test resulted in a non-significant correlation between genetic and geographical distances ($R^2 = 0.1891$, $P > 0.05$).

Discussion

In the present study, more than 5,500 bp of the 16,600 bp total mitochondrial genome size were

assessed using PCR-RFLP, in order to evaluate the specific genetic variation within and among populations of rainbow trout derived from different farms throughout Greece. Particularly, apart from the ND5/6 and the Control Region-Cytb entire regions, the 948 bp fragment of ATPase VI-COIII protein-encoding regions of *O. mykiss* had never been assessed by means of restriction endonucleases prior to this study. However, the study of these protein-encoding regions in various aquatic animals (Katsares et al., 2003) and relative species, such as *S. trutta* (Giuffra et al., 1994; Apostolidis et al., 2008) confirmed the existence of polymorphism.

The conducted population structure analysis revealed the presence of two central haplotype clusters among the farmed-rainbow trout populations in the Greek freshwater aquaculture sector (Figure 1;

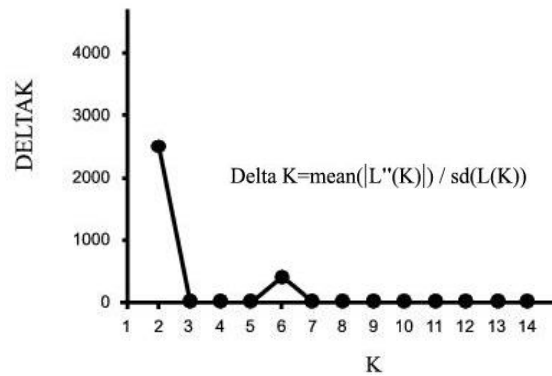


Figure 2. Selection of the adequate number of clusters (K) taking into consideration the highest peak, which represents the most likely number of clusters ($K = 2$) according to the Pritchard Bayes Formula.

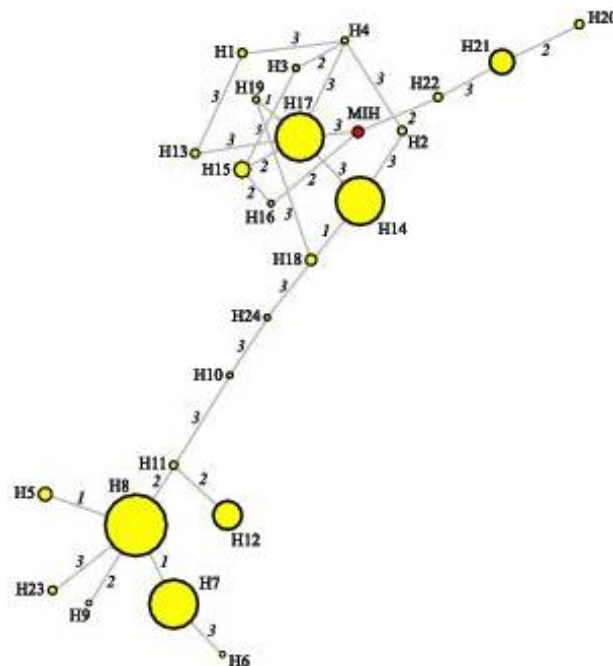


Figure 3. Median-joining network indicating the phylogenetic relationships among mtDNA haplotypes of the 10 farmed-rainbow trout populations. Each haplotype (H1-H24) is represented by a yellow circle, while the missing intermediate haplotype (MIH) is represented by a red circle. The mutational step between haplotypes is indicated by italicized numerals.

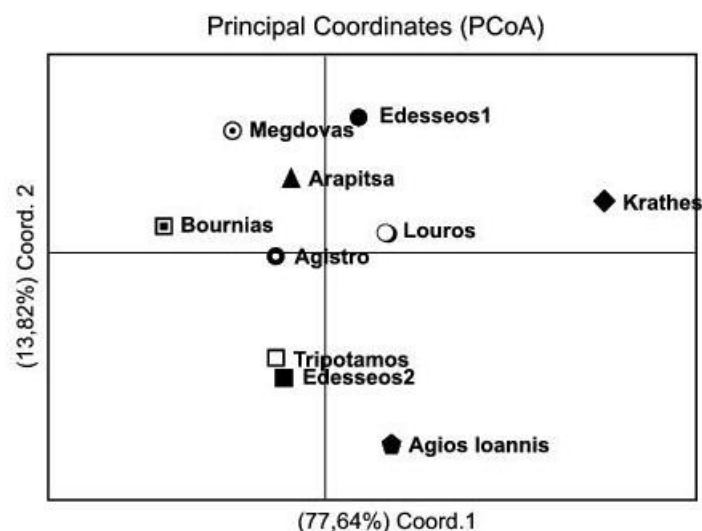


Figure 4. Principal Coordinates Analysis (PCoA) of farmed-rainbow trout populations. The amount of variation explained by each axis is 77.64 % for Coordinate 1 and 13.82% for Coordinate 2.

Figure 3). The coexistence of haplotypes from both clusters in all populations might denote the common broodstock gene pool. Only the population of Krathes River appeared to have an extremely high percentage of individuals belonging to the 1st cluster, in contrast to the others which have an admixture of both clusters revealed from the structure analysis. Moreover, the extent of polymorphism was, as expected, lower compared to the diversity recorded for natural populations. Such levels of genetic variability in reared populations comparatively to the natural populations is a common phenomenon, taking into account a considerable number of conducted population genetic studies regarding reared and natural populations of other aquatic species, such as common carp (*Cyprinus carpio*) (Zhou *et al.*, 2004; Kohlmann *et al.*, 2005) and Atlantic salmon (*Salmo salar*) (Norris *et al.*, 1999).

Apart from Tripotamos population, the vast majority of the examined reared populations presented substantial high haplotype diversity and a relatively low nucleotide diversity, implying that nearly all haplotypes were related to each other. This may be typical of diversity from former source populations. This fact might reinforce the potential common broodstock gene pool of the studied strains. Specifically, in the case of Tripotamos population, the haplotype diversity was quite low relative to nucleotide diversity, denoting the existence of few haplotypes which presented high divergence among them. This may be characteristic of divergent haplotypes from multiple source regions. In the present study, the majority of Tajima's *D* (Tajima, 1989) values were non-significantly positive, implying that there is a deficit of low-frequency mutations compared to our expectations under neutral theory. On the contrary, only Krathes River showed a non-significantly negative Tajima's *D* value denoting an excess of low-frequency mutations compared to our expectations under neutral theory.

The results of AMOVA analysis indicated that only 5.65% of the total genetic diversity accounted for the detected variance among populations. However, a substantial variance of 94.35% was detected within populations. Moreover, the level of differentiation among the farmed *O. mykiss* populations ranged from -0.0381 to a substantial high value of 0.3496 with an average F_{ST} value of 0.056. In other European studies, such as the study among 12 European farmed rainbow trout strains (Gross *et al.*, 2007) and the study of nine rainbow trout farms in Norway (Glover, 2008), the reported pairwise F_{ST} values ranged from 0.04 to 0.36 with an average F_{ST} value equal to 0.14 and from 0.001 to 0.127 with an average F_{ST} value equal to 0.053, respectively. It seems that the obtained values from the present study were within the range of the observed values in other European countries. Furthermore, in Western Australia the introduced farmed-rainbow trout presented a similar range of F_{ST} values with European countries (Gross *et al.*, 2007), ranging from 0.016 to 0.404 with an average F_{ST} value equal to 0.19 (Ward *et al.*, 2003). On the contrary, both in the USA and in northern Iran the range of F_{ST} values reported for the reared rainbow trout was low, ranging from 0 to 0.048 with an average F_{ST} value 0.028 in the USA (Silverstein *et al.*, 2004; Johnson *et al.*, 2007) and from 0.062 to 0.080 with an average F_{ST} value 0.072 in northern Iran, respectively (Yousefian *et al.*, 2012).

Although genetic differentiation among populations was statistically significant (after Bonferroni correction) for most of the pairwise comparisons, either for haplotype frequencies distributions (exact tests) or pairwise F_{ST} values, the overall genetic diversity was substantially attributed to the diversity within populations rather than among them. Thus, taking into account the reported average F_{ST} values of farmed populations in Norway and Iran, in comparison with the obtained F_{ST} values of Greek farmed-rainbow trout populations, it can be inferred

that farmed-rainbow trout populations in Greece possess similar levels of polymorphism. However, it seems that the domesticated populations of Western Australia and those in northern and eastern European countries presented a substantially higher divergence in comparison with Greek farmed-rainbow trout populations.

According to PCoA results, the first two coordinates explained 81.2% of the variation revealed by the distance matrix. The bi-dimensional scatterplot does not reveal a discrimination of populations according to their geographical distance. In accordance with the latter, the Mantel's Test results indicated a non-significant relationship between geographic distance and Nei's genetic distance matrices.

Conclusions

The current study comprises the first attempt to assess the genetic variation among and within farmed rainbow trout populations throughout Greece. The obtained percentage of overall genetic variation that was observed, was assumed to be within populations rather than among populations. The percentage of genetic variation among populations to the overall reported variation reflected the admixture of the two founder populations (strains), pinpointing a common gene pool broodstock for all freshwater aquacultures throughout Greece. This fact was reinforced by the obtained haplotype network, which clearly revealed the existence of two founder nodes throughout Greek farms. Finally, this effort could act as the foundation for further experiments in the molecular genetics characterization of rainbow trout (i.e. Heterozygosity Fitness Correlation-HFC), contributing to the adoption of sophisticated rearing programs by the freshwater aquaculture companies.

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