POPULATION GENETIC STRUCTURE OF GREATER AMBERJACK (*SERIOLA DUMERILI)* IN THE MEDITERRANEAN SEA AND EASTERN ATLANTIC OCEAN

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Introduction

Greater amberjack (*Seriola dumerili*) is an epipelagic species with a distribution throughout the world and especially in the Mediterranean Sea. It is a promising species for the Mediterranean aquaculture and it is expected to lead to the growth and diversification of the sector, due to its high growth performance and market value (see in Šegvić-Bubić *et al*. 2016). The study of the genetic variability in the species aims at providing the necessary information to better characterize brood stocks and achieve significant gains when breeding programs will follow. The objective of the present was to identify any stock genetic differences using microsatellites and mitochondrial markers in populations sampled from the eastern Atlantic Ocean and the Mediterranean Sea.

Materials and Methods

Nine populations were sampled and a total of 254 wild-origin fish were collected; these fish constitute actually the brood stocks in private companies and public institutions. There were 3 populations from Greece (Chalkidiki, Astakos, Crete), 1 from Italy (Lambedusa), 3 from Spain (Gran Canaria, Tenerife, North Tenerife), 1 from Turkey (Mersin), and one from Cyprus.

DNA was extracted from fin clips and its quality and quantity were determined using a NanoDrop 1000 spectrophotometer v3.7 (Thermo Fisher Scientific). For microsatellites, samples were genotyped in a single multiplex reaction with 10 loci (Sdu29, 31, 32, 34, 36, 37, 39, 40, 41 and 46) (Renshaw *et al*. 2006, 2007), following the QIAGEN multiplex PCR plus kit manufacturer's guidelines. After the amplification, PCR products were loaded on ABI 3730-XL Genetic Analyzer and fluorigrams were evaluated (genotyped) using STRand 2.4.110 software.

All samples were also analyzed by sequencing part the mitochondrial D-Loop region. First, a PCR reaction was prepared to amplify the D-Loop sequence following the PCR conditions as described in Šegvić-Bubić *et al.* (2016), then the PCR products were evaluated on 1.5% agarose gel and visualized with UV transilluminator to check the presence of a single sharp DNA band (expected size around 400 bp), and last the product was purified and used to prepare a sequence reaction run on the ABI 3730-XL Genetic Analyzer. Sequencing results were analyzed with the MEGA 6.06 software.

We estimated a series of population genetics parameters (heterezygosities, Fst, Fis, etc.) using the GenALEx 6.503. Additionally, we run a Bayesian Analysis using Structure 2.3.4 software. The number of different D-loop haplotypes were determined using the DAMBE 6.4.51 and the network of unique haplotypes was constructed using Network 5.0.0.1.

Results and Discussion

Microsatellite analyses were based on all ten loci; the mean number of alleles is bigger in the populations from Cyprus and Astakos, followed by Lambedusa and Tenerife whereas the lowest is detected in Gran Canaria and Crete (Table I). However, data from the Crete should be treated with caution because we had only 4 samples. For expected and observed heterezygosity, the Atlantic samples seem to have the highest values, whereas the Lambedusa population was the only one showing signs of inbreeding. Bayesian analysis differentiated the Atlantic from the Mediterranean populations, but there was little differentiation between the Mediterranean populations.

Table I: The mean values of number of alleles (Na), observed heterezygosities (Ho), expected heterezygosities (He), Fixation index (Fis), and (*) significant at 5%.

SAMPLE	Na	Ho	He	Fis
GRAN CANARIA	4.400	0.74	0.612	-0.19
NORTH TENERIFE	6.900	0.762	0.71	-0.033
TENERIFE	8.000	0.727	0.742	0.044
LAMBEDOUZA	8.300	0.617	0.68	0.104*
ASTAKOS	9.700	0.683	0.704	0.038
CRETE	4.100	0.7	0.609	-0.006
CHALKIDIKI	6.200	0.674	0.682	0.04
TURKEY	6.800	0.641	0.674	0.079
CYPRUS	9.300	0.676	0.704	0.049

Our mtDNA data set was finally based on 250 haplotypes. The MtDNA analysis of the D-Loop sequence lead to 35 different haplotypes. The construction of network between the different haplotypes revealed two different phylogroups. The first one consisted of 27 haplotypes found in the majority of samples (212 fish) whereas the second one of 8 haplotypes found in 38 specimens. Only the Gran Canaria sample showed only phylogroup A haplotypes, and in all other 8 populations the percentage of phylogroup B ranged from 7.7% in N. Tenerife to 33% in Chalkidiki (average 18%).

Current results confirm those reported by Šegvić-Bubić *et al.* (2016) in greater amberjack. Nuclear data (microsatellite markers) corroborated differentiation between the Atlantic and Mediterranean basins, whereas mitochondrial DNA shows two different lineages or phylogroups (A and B), present in almost every sample. This last evidence is indicative of deep divergence in the species, usually attributed to secondary contact between groups of ancient populations that followed separate evolutionary processes in allopatry and finally got together after the removal of an ecological or a geographical barrier. For the aquaculture industry, the interest is to study breeding performance of fish belonging to each one of the two phylogroups and additionally examine the interbreeding between them.

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