XV EUROPEAN CONGRESS OF ICHTHYOLOGY

Life+ TROUT Project (LIFE12 NAT/IT/0000940) for the recovery and conservation of Mediterranean trout (*Salmo trutta complex*) in the central Apennines (Italy).

Caputo Barucchi V[1], Carosi A[2]*, Giovannotti M[1], La Porta G[2], Splendiani A[1], Lorenzoni M[2]

[1]DiSVA, Università Politecnica delle Marche, via Brecce Bianche, 60131 Ancona, Italy;
[2] Dept. of Chemistry, Biology and Biotechnologies University of Perugia, via Elce di Sotto, 06100 Perugia, Italy.

INTRODUCTION

The Mediterranean brown trout (*Salmo trutta complex*) is one of the freshwater fish species complex at greater risk of extinction in the Mediterranean area. The introduction of alien invasive species and their interaction with the native fauna from an ecological and genetic point of view represents one of the main threats to this species. Currently, the genetic identity of the Mediterranean trout is strongly affected by the occurrence of introgressive hybridization with the Atlantic trout, resulting in loss of genetic variability; the hybridization was favored by stocking programs carried out in the last century. The aim of the project, started in November 2013, was to make safe the last populations of Mediterranean trout in seven river basins of central Apennine, where native populations has been threatened by stocking with Atlantic trout. The information collected are the indispensable premise for the adoption of the necessary strategies for conservation of the Apennine trout.

THE APENNINE TROUT

The trout is a wide-ranging species complex native to Eurasia and North Africa: the northernmost populations are in Iceland, Northern Scandinavia and Russia, while the southern limit is represented by the mountain streams of Northern Morocco; the species has also been introduced in several other locations around the world (North and South - America, Australia, etc). The Apennine trout (Fig. 1) has been considered a vulnerable species in Europe, critically endangered in Italy and it is included in the Red List category Near Threatened due to water abstraction, overfishing and stocking of non-native trouts (resulting in hybridization and competition). It is suspected that in the last 10 - 12 years the population has declined by almost 30%, and it will continue to do so as water abstraction will most likely increase.

STUDY AREA

The project focuses on 14 sites included in the Natura 2000 EU wide network of nature protection areas, established under the 1992 Habitat Directive. The project involved also the territory of the National Park of Monti Sibillini. The study area comprises 7 watersheds (Fig. 2): Metauro, Cesano, Esino, Potenza, Chienti, Nera and Tenna; a total of 25 watercourses were investigated with 32 sampling stations.

MATERIAL AND METHODS

A census of the fish fauna by elettrofishing was carried out at each sampling stations. All captured fishes were identified and counted. For all specimens, total length and weight was measured. Genetic analysis was performed by using mitochondrial (D-loop fragment of 310 bp) and nuclear markers (locus *LDH-C1* * and 12 microsatellites). Bayesian clustering implemented in Structure 2.3.2.1 was used for estimating the number of groups (k) represented by all sampled individuals (Pritchard et al., 2000; Falush et al., 2003)).

ACTIONS

The project's main actions concern the genetic and demographic characterization of trout populations in 32 sites led us to select: i) four sites for the collections of adults, needed for the production of genetically pure juveniles trout, and ii) six sites to carry out the alien trout eradication. Another significant activity of the project concern the hydrological characterization of the water courses surveyed by the Minimum flow analysis (MFA), adopting the MesoHABSIM method (Parasiewicz, 2007), in order to increase the chances of long term survival of Apennine trout populations in the sites involved in the above restocking actions, and more generally, in the study area.

RESULTS

Main expected results are the following:
✓Identification of natural populations of Apennine trout in the study area and their protection through specific changes in existing regulation.
✓Establishment of genetically pure wild populations with fish produced in a provincial hatchery specifically adapted to manage wild native broodstock.
✓Widening Apennine trout habitat availability by removal of nonnative trout in areas to be restocked.
✓Restocking of streams selected in previous actions with pure juvenile trout.
✓Assessment of the summer river flows in order to increase the chances of survival of Apenninian trout populations in compliance with the regulations on minimum flow and water quality.
✓Involvement of local sport fishing community to increase their awareness of the problem and ease their acceptance of more restricting fishery and fish management rules.
At present the genetic and demographic characterization of the trout populations is completed. Table 1 and Figures 3-4 showed the results of abundance analysis of the fish assemblages divided by basins. The standing crop's mean values can be considered in the standard range, according to the small size and the limited productivity that generally characterized the watercourses analyzed, as most of the Apennine rivers. The differences of the mean values among basins were highly statistically significant to the ANOVA analysis (density: F= 5.24, p=0.001; standing crop: F=12.42, p=0.001).
The results of genetic analysis separated clearly native and aliene genomes (Fig. 5a) and showed the presence of three distinct genetic stocks of native Apennine trout:, respectively in the Nera, Tenna and Chienti /Potenza/Metauro/Esino river basins (Fig 5b). Populations characterized by less introgression with the alien genome are the source of wild individuals to produce pure juvenile trouts in captivity, currently underway at the provincial plant of Cantiano. Four moderately introgressed populations are subject to supportive breeding activities. Six stream rivers where the trouts showed mainly alien genetic characteristics were selected for the eradication activities.

REFERENCES
Falush D., Stephens M., Pritchard J.K. (2003). Inference of population structure using multilocus genotype data: Linked loci and correlated allele frequencies. *Genetics*. 164: 1567-1587
Lorenzoni M., Ghetti L., Carosi A., Dolciami R. (2010) La fauna ittica e i corsi d'acqua dell'Umbria. Sintesi delle Carte Ittiche Regionali dal 1986 al 2009. Petruzzi Editore, Perugia.
Lorenzoni M., Esposito L. (2011). La Carta Ittica delle Marche. Regione Marche, Assessorato Caccia e Pesca, Ancona.
Parasiewicz P. (2007). Arena the mesoHABSIM model revisited. *River Res. Appl.* 23: 893-903.
Pritchard J.K., Stephens M., Donnelly,P. (2000). Inference of population structure using multilocus genotype data. *Genetics*. 155: 945-959.

Fig. 1 Apennine trout

Protected areas
Sampling site
Fig. 2 Study area and location of sampling sites

Basin	Density (ind/m²)			Standing crop (g/m²)		
	Number of observations	Average	Standard deviation	Number of observations	Average	Standard deviation
Metauro	20	0.08	0.07	20	3.34	3.49
Esino	4	0.74	0.47	4	16.51	8.19
Tenna	8	0.14	0.10	8	5.73	3.98
Tevere	10	0.42	0.43	10	12.99	7.99
Cesano	8	0.26	0.22	8	7.32	4.86
Chienti	8	0.29	0.38	8	4.02	2.71
Potenza	6	0.45	0.16	6	26.11	14.30

Tab. 1 Density and standing crop: summary statistics by basins

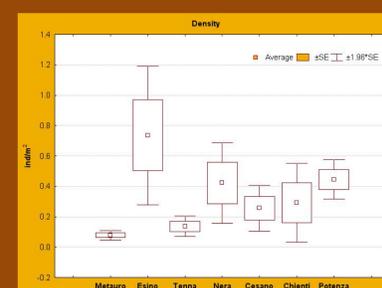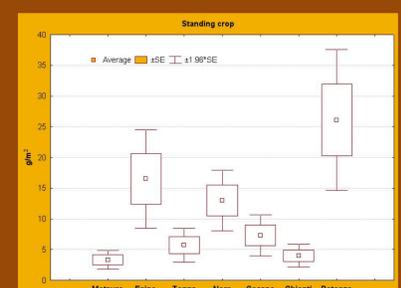Fig. 3 Comparison of density mean values among basins

Fig. 4 Comparison of standing crop mean values among basins

a)
Alien genome
Native genome

b)
Tenna basin
Nera basin
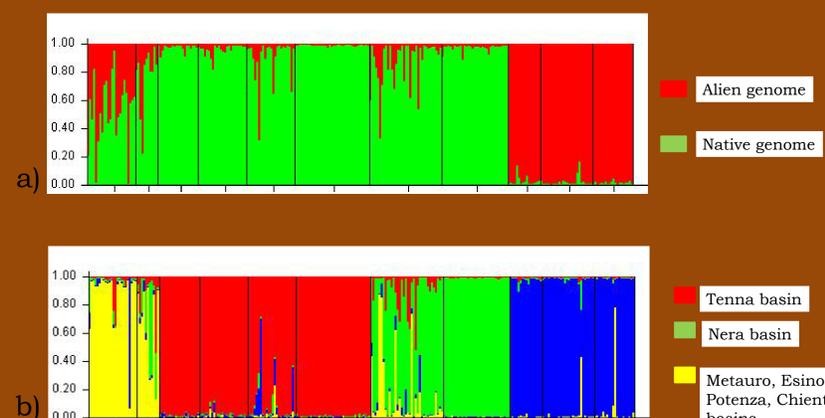Metauro, Esino, Potenza, Chienti basins

Fig. 5 Structure analysis results. a) At the first hierarchy level (k = 2), river samples are best explained by the presence of two major genetic group (alien and native genomes); b) at a lower hierarchy level (k = 4), additional genetic sub-structuring is evident at basin level.