Life NAT.SAL.MO

PROTECT THE NATIVE TROUT

Life

LIFE17 NAT/IT/000547

NATURA 2000

LAYMAN'S REPORT

Recovery of *S. macrostigma*

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Recovery of S. *macrostigma*; application of innovative techniques and participatory governance tools in rivers of Molise

Action E1.2

PROJECT DETAILS

Duration: **01/07/2018 – 31/03/2023**
Total project budget: **€ 2.631.434**
Project Area: **Italy (Molise), Romania (North-Est)**
Section: **Natura**

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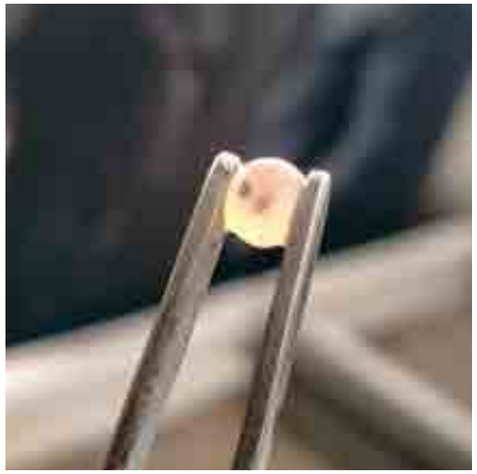
Female stripping

ABSTRACT: The Project in 1 Minute

The Mediterranean trout is an endangered species at national and European level, mainly due to the hybridization with other introduced introgressive strains and the degradation of natural habitats. To ensure sustainable management of this fish resource, LIFE Nat.Sal.Mo. has successfully introduced innovative techniques ranging from the application of methodologies and biotechnologies never used before for the Mediterranean trout, to habitat requalification through river reconnections.

Eyed eggs in incubator

A major focus has been given to expanding the range of the native trout, also by eradicating allochthonous fish species, but most of all supporting artificial reproduction and through the creation of the first Mediterranean Trout Sperm Cryobank, the only one of its kind in Europe. Eggs, collected from females by stripping, are fertilized using frozen semen and incubated in suitable facilities. Then, in due time, they are planted in their home river sites through the construction of artificial nests (**cocooning technique**).



Eyed egg

Gamete collection

Many other actions have been put into practice, from habitat requalification (removing any waste from spawning sites) to reconnecting river tracts that impeded on the trout ascent during spawning migrations.
The main innovations introduced by Nat.Sal.Mo can be considered as follows:

- Creation of the **first Mediterranean Trout Sperm Cryobank**, the only one of its kind in Europe, hosted in the Department of Agricultural, Environmental and Food Sciences, University of Molise
- Use of the **cocooning** technique to plant eyed eggs produced through artificial reproduction
- Real and heartfelt involvement of local communities including through the signing of the **“River Contracts”**.

1. INTRODUCTION

Trout, Volturno river

The main objective of the Nat.Sal.Mo Project (Recovery of ***S. macrostigma***: Application of innovative techniques and participatory governance tools in rivers of Molise - LIFE17 NAT/IT/000547), funded by the European Union, is to ensure the recovery and conservation of **native Mediterranean trout** and its habitat in the Biferno and Volturno river basins (Molise region – Southern Italy).

This species is endemic of the Mediterranean area and is identified in Annex II of the Habitats Directive and classified as vulnerable in Europe and endangered in Italy.
In the project area, populations of this species show great intraspecific variability in morphology, behaviour and evolutionary history due to genetic differences that have allowed various populations to adapt to different environmental conditions. In the project area there are **two main distinct populations**: an Adriatic Mediterranean trout population inhabits the Biferno river, that presents a diagnostic mtDNA marker shared with the marble trout (***S. marmoratus***), while a Tyrrhenian population lives in the Volturno river basin with specimen characterized by four evidently dark marks on their sides to constitute a "striped" phenotype. The Mediterranean trout is an endangered species at national and European level, mainly due to the hybridization with other introduced introgressive strains and the degradation of natural habitats.

The main **threats** for this species inside the project area are the same threats as those recorded in other parts of its original range:

Threat 1: Genetic introgression, due to the introduction of zootechnical trout strains of Atlantic North-European origin caused by activities related to sport fishing

Threat 2: Loss of spawning habitat: Multiple barriers located in the tributaries of the main rivers impede on the ascent of the breeders modifying their life cycle. The spawning grounds are altered by the presence of waste on the riverbed. The Biferno river springs in Bojano, for example, are highly frequented by a large number of wild breeders during the spawning period, but the site is severely altered by waste material of different origin (tires, electrical appliances, etc…).

Threat 3: Poorly regulated fishing: It is necessary to analyse the demographic parameters and migration patterns of the autochthonous trout populations to draw up management plans and regulations in order to promote sustainable fishing and, then recover and preserve the Mediterranean trout in the future.
LIFE Nat.Sal.Mo, as a European project for the safeguarding of the Mediterranean trout in the Volturno and Biferno rivers of Molise, has achieved (and partially exceeded) its objectives by using innovative techniques. The project was led by the **University of Molise** - Department of Agriculture, Environment and Food which coordinated a technical-scientific partnership made up of MTRG (Mediterranean Trout Research Group), **Legambiente**, **StudioGiuliano** srl, local administrations such as the **Molise Region**, **Oratino Municipality** and **Rocchetta a Volturno Municipality**, and the **Romanian Universities of Iasi and Sibiu**.

2. OBJECTIVES

The main objective of LIFE Nat.Sal.Mo has been to ensure the recovery and conservation of native Mediterranean trout populations in the Biferno and Volturno river basins (Molise region – Southern Italy). This species is endemic of the Mediterranean area and is identified in Annex II of the Habitats Directive.
Other specific objectives were:

- **to recover the genetic integrity of native populations of *S.macrostigma***, threatened by introgressive hybridization with introduced strains brown trout (***Salmo trutta***) of North-European origin in both Adriatic (Biferno river) and Tyrrhenian (Volturno river) drainages of Molise;

- **to protect and restore the functionality of habitats**, such as the river connectivity and the quality of spawning grounds, promoting the ecological connection between the SCIs inside the targeted area;

- **to encourage the natural reproduction** of less or non introgressed wild native breeders;

- **to expand the range of the species** inside the project area;

Project meeting in Romania

Sources of the Volturno river

- **to optimize the semen cryopreservation protocols** for the target species in order to ensure the maximum genetic variability during the artificial supportive breeding practices; moreover, artificial nests will be created made of natural material collected from the riverbed to plant eyed eggs;

- **to update the current fishing regulations** considering the biological, ecological and ethological characteristics of native salmonids populations inhabiting the two main Molise basins;

- **to generate a positive socio-economic return** and increase environmental awareness;

- **to foster the adoption of participatory governance tools such as River Contracts**, to ensure the sustainability of the conservation measures applied not only during the project but also after the end of the same, as well as to guarantee the preservation and transferability of good practices in similar contexts both in the national and in other EU Member States territories;

- **to forge a close cooperation among partners** to transfer the innovative methodologies applied and developed in Molise waters during the project to similar contexts, both in Italy and in other EU Member States, and generate at least two replicability assessments in two different states, with at least one different species. Thus creating, in Molise, a participatory model of natural resource recovery and management that can be adopted by administrations and bodies elsewhere in Europe.

- **to assess the replicability of the methodologies** developed within the LIFE Nat.Sal.Mo. project in the Romanian territory (identified as a breeding site for other species of community interest) with at least one different species.

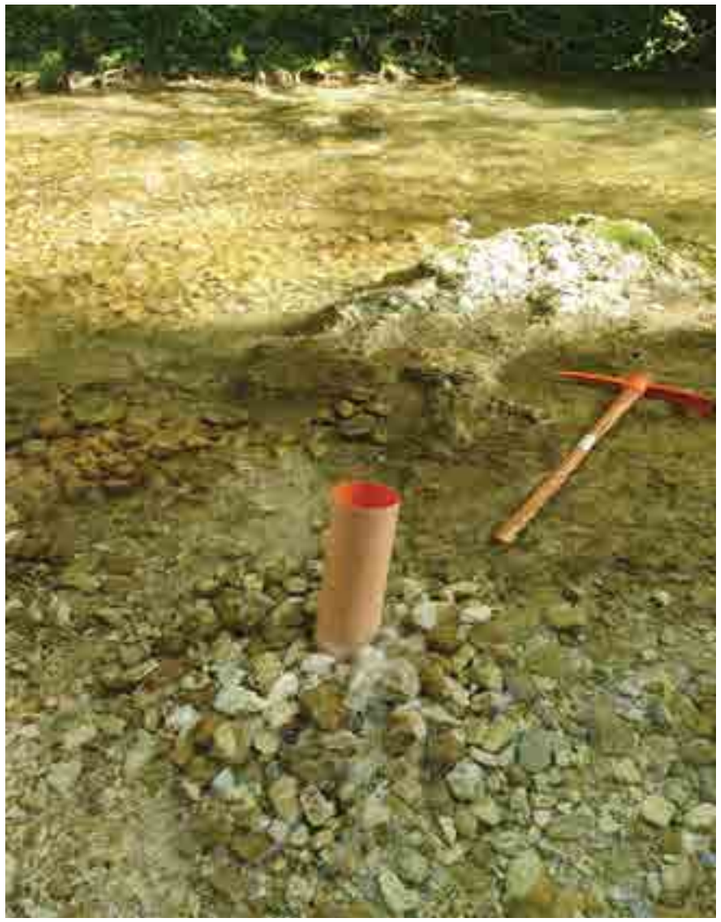
3. ACTIONS

To significantly reduce the genetic introgression in native trout populations in the project area, two main strategies were undertaken:

1) allowing access to the main natural spawning grounds only to non introgressed wild breeders;

2) using a part of these breeders for artificial reproduction with frozen semen and fertilization schemes in order to increase the genetic variability of the offspring.

The selective access of native trout to the main spawning grounds has been possible by intercepting the breeders' upstream migration with fixed traps and then selecting wild breeders by means of genetic and morphological analysis. Hybrid and non-native individuals have been isolated from the natural river networks or downstream of insurmountable barriers. The transport of fish has followed the requirements provided by inherent law (Decree Law 148/2008, D.L. 03/08/2011).
The native Mediterranean trout habitat was reconstructed by recovering the natural spawning sites (removing any waste and upgrading the habitat) and increasing river connectivity (creating fish passes).



Nesting

In order to promote the dispersion of native trout inside the project area, an innovative technique involving the planting of eyed eggs close to hatching in semi-artificial nests constructed directly within the riverbed was used for artificial reproduction and restocking. Two facilities for egg incubation and temporary maintenance of wild breeders have been installed at Rocchetta a Volturno (Volturno river) and Oratino (Biferno basin).
Having tested and identified an efficient freezing protocol has allowed the creation of the first native trout sperm cryobank in Europe. Artificial fecundation of eggs using frozen semen in combination to fertilization schemes has thus supported natural reproduction, ensuring a wide genetic variability of native populations.
Planting eyed eggs in semi-artificial nests reduces the phenomena of domestication to artificial feeding.

Semi- artificial nest

Eggs in the nest

Local associations of fishermen have actively participated as to the issue of recreational fishing regulations to support the biological and reproductive characteristics of thenative trout populations.
The engagement of all local stakeholders has allowed generating a positive social and economic impact that culminated in the signature of a "River Contract" for both river basins involved to ensure the sustainability over time of the results achieved during the project. Local schools (primary and secondary) were involved through the implementation of a specific Environmental Education program. Semen cryopreservation and semi-artificial nesting techniques have been transferred to the Romanian territory, and replicability actions have been produced in two different EU Member States.

4. RESULTS

The River Contracts

The project succeeded in involving local communities not only throughout its duration, but also in its continuation over time, in particular by entering into “River Contracts”, i.e., voluntary agreements for the observation of good practices and compliance with rules to protect the species and habitats, with stakeholders - administrations, public bodies, the productive sector, professional associations (fishermen, environmental associations and citizens) and local authorities; a tool, therefore, for participatory and shared management of the river resource. The signature of 2 river contracts (the first in the Molise Region), one for the Biferno basin and one for the Alto Volturno, took place in Campobasso involving, until March 2023, a few dozen entities that have subscribed the document. This means that, in the territory, more than 40 municipalities, companies and stakeholders believed in this tool for innovative and sustainable governance. The conservation model developed in the Molise region is ready to be exported to the rest of the Italian territory and other countries in the European Union.

Signature of the river contracts

The Sperm Cryobank

In the laboratories of UniMol’s Department of Agriculture, Environment and Food, the first Mediterranean Trout Sperm Cryobank in Europe was created, thanks to the identification of a cryopreservation protocol. Sperm cryopreservation has several advantages for biodiversity conservation: this technique, in fact, through the use of semen samples from already genotyped breeders always available, allows ensuring the widest genetic variability to the offspring. In this way, thanks to the stock of frozen and ready-to-use semen, it is possible to avoid keeping male individuals in breeding for a long time, releasing them immediately after collection in their natural habitat, thus preserving them from domestication to safeguard their wildness.

Sperm Cryobank

Habitat requalification

Thanks to the intervention of Regione Molise, Mediterranean trout spawning sites have been upgraded by removing litter in the riverbed and constructing fish passes through which the potamodromous fish fauna can reach the spawning beds upstream of artificial barriers

Artificial reproduction and the construction of semi-artificial nests (cocooning)

Artificial reproduction involves the “stripping” of breeders, who are given a gentle abdominal massage that emulates the fish natural contractions during gamete emission.

The eggs thus obtained are fertilized through the addition of cryopreserved semen and are incubated in special facilities at the Municipality of Oratino and the Municipality of Rocchetta a Volturno until they reach the “eyed egg” stage. During the LIFE Nat.Sal.Mo. project, eyed eggs close to hatching have been planted in their home streams through the construction of semi-artificial nests, made of gravel and river stones, that provide shelter in the early stages of development for the new hatchlings. Thus, the entire biological cycle is concluded in the natural environment, ensuring the wildness of the offspring. Likewise, it is also possible to have the biological cycle concluded at ichthyogenic facilities, to generate what will become the future spawning grounds that will enable production for the entire Molise region.

Cocooning

Environmental Education

LIFE Nat.Sal.Mo has also involved the youngest: educational and active participation activities have been addressed to primary and secondary school classes in the region (8 comprehensive schools and one multi-sports centre) for a total of 642 pupils involved, depending on the season, both in field meetings and inside the school facility. Pupils were offered an educational path to discover the animal and plant biodiversity of the project areas and insights were shared on the inclusion of human activities in natural settings. At the final project meeting, prizes were awarded to the winners of the contest *Vivi il tuo territorio: la vita nei fiumi molisani* (Live Your Territory: Life in the Molise Rivers), with the participation of a large number of classes. Then, to celebrate the success of the project and to highlight the importance of an endemic fish species of the recovered Mediterranean area, an initiative was also dedicated to urban art. In fact, on the perimeter wall of the Giovanni Paolo II Primary School of the Istituto Comprensivo Statale Leopoldo Montini, former Student House, an artistic mural dedicated to the Mediterranean trout was created. The artwork was commissioned from Smake, an artist from Molise, born in 1988, member of the Malatesta Association, which has been organizing the Draw the Line Festival in Campobasso since early 2011, i.e. an urban regeneration project through street art.

Environmental Education

LIFE NAT.SAL.MO IN FIGURES

2 River Contracts implemented

40 Entities and associations who have signed the River Contracts (to date)

200 Number of fishermen reached by information and awareness activities

Over **5,000** square meters of river habitat upgraded

2 river reconnections carried out

2 Incubators created

161 native males from which semen was collected

2,091 doses of semen stored and currently hosted in the cryobank

88 native females used for egg production

More than **300,000** eggs fertilized in the incubators of Rocchetta and Oratino

Over **200,000** eggs planted using the cocooning technique

0.05 final degree of introgression compared to the initial **0.22**

From **+70%** to **94%** increase of Mediterranean trout to date in the areas covered by the project

3 foreign universities involved in transferability actions

Approximately **500,000** people reached by media relations activities

1,500 social followers and more than 5,000 unique visitors on the website (about **30,000** clicks per year)

108 participants in open forums

642 pupils who participated in educational activities in schools

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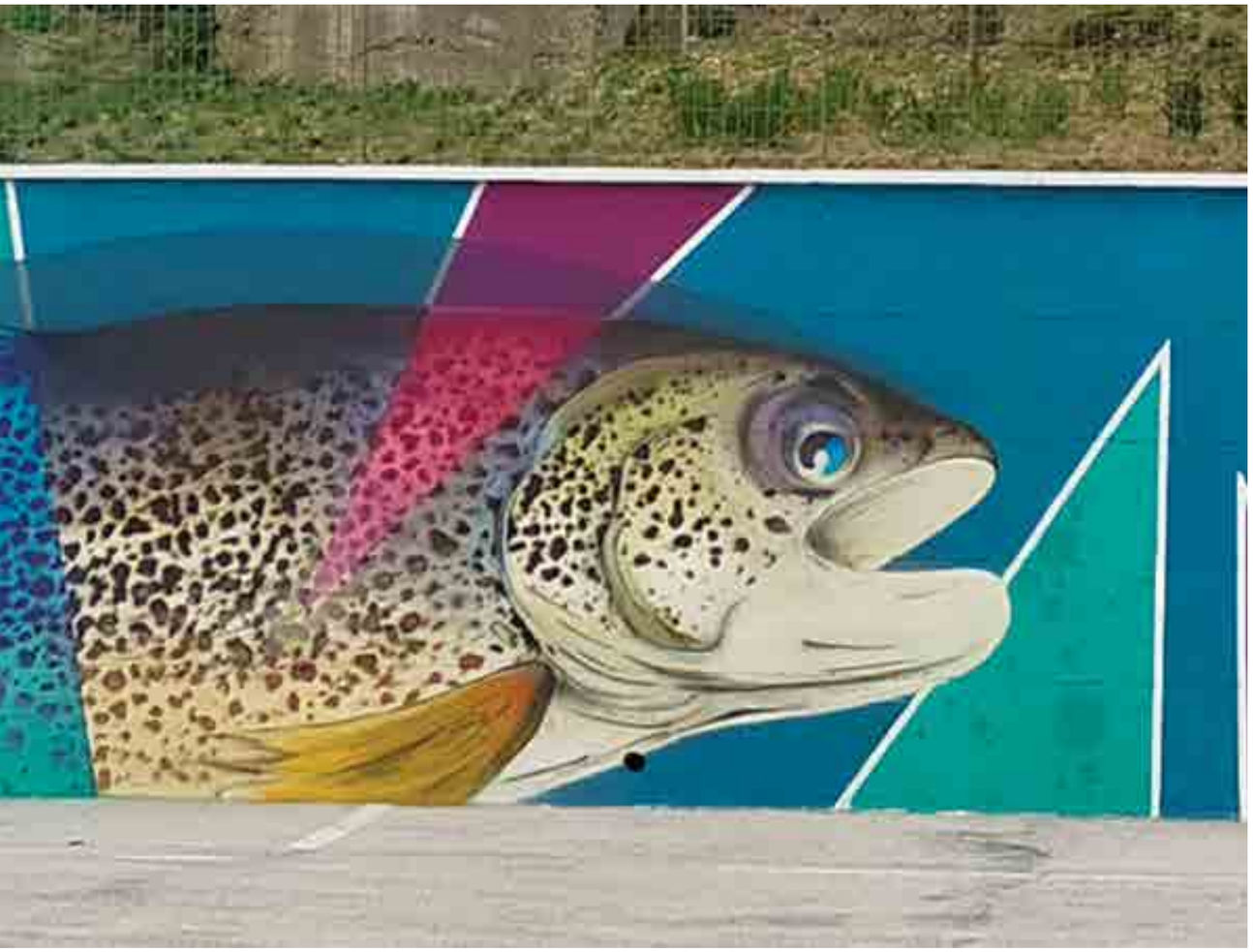

Project Murales – artist Smake

GLOSSARY

Cocooning: innovative technique for planting eyed eggs close to hatching in semi-artificial nests built directly in the riverbed.

Cryobank: a place where frozen genetic material is stored in haploid (oocytes, sperm) or diploid (embryos) form. Semen doses from genotyped Mediterranean trout donors are cryopreserved in the Nat.Sal.Mo semen cryobank.

Ecological connectivity: interconnected system of habitats whose biodiversity has to be safeguarded

Eyed: definition of an oocyte when its fertilization is successful.

Hybridization: Process whereby two animals of different species reproduce resulting in offspring with genetic (and, often, morphological) characteristics intermediate to the parental species. Such offspring may be sterile or fertile.

Introgression: through a series of crosses, the permanent incorporation of genes from one population into the genome of another population capable of reproduction.

River connectivity: in the river, the succession of ecosystems that gradually merge into one another seamlessly. Maintaining or restoring river connectivity is essential for some migratory river species such as precisely the Mediterranean trout.

Potamodromous: a potamodromous species lives and moves exclusively in fresh water.

Project Meeting, Oratino

coordinatore beneficiario

Università degli Studi del Molise

beneficiari associati

COMUNE DI ORATINO

COMUNE DI ROCCHETTA A VOLTURNO

LEGAMBIENTE

Mediterranean Trout Research Group

REGIONE MOLISE

STG

ULBS