LIFE Nature and Biodiversity

TECHNICAL APPLICATION FORMS

Part A – administrative information

LIFE 2018

FOR ADMINISTRATION USE ONLY

LIFE18 NAT/IT/000931

LIFE Nature and Biodiversity project application

Language of the proposal:

English (en)

Project title:

Salmo ceTtii REcovery Actions in Mediterranean Streams

Project acronym:

LIFE STREAMS

The project will be implemented in the following Member State(s) and Region(s) or other countries:

France	Corse
	Provence-Alpes-Côte d' Azur
Italy	Abruzzi
	Basilicata
	Calabria
	Campania
	Emilia-Romagna
	Lazio
	Liguria
	Molise
	Piemonte
	Sardegna
	Sicilia
	Toscana
	Umbria

Expected start date: 01/09/2019 **Expected end date:** 30/11/2023

LIST OF BENEFICIARIES

Name of the **coordinating** beneficiary: PARCO NAZIONALE DELLA MAJELLA

Name of the associated beneficiary:	AGENZIA FORESTALE REGIONALE PER LO SVILUPPO DEL TERRITORIO E DELL' AMBIENTE DELLA SARDEGNA
Name of the associated beneficiary:	Istituto Superiore per la Protezione e la Ricerca Ambientale
Name of the associated beneficiary:	LEGAMBIENTE ONLUS
Name of the associated beneficiary:	Noesis snc
Name of the associated beneficiary:	Ente Parco di Montemarcello-Magra-Vara
Name of the associated beneficiary:	Parco Nazionale delle Foreste Casentinesi, Monte Falterona e Campigna
Name of the associated beneficiary:	PARCO NAZIONALE DEI MONTI SIBILLINI
Name of the associated beneficiary:	Ente Parco Nazionale del Pollino
Name of the associated beneficiary:	Università degli Studi di Perugia - Dipartimento di Chimica Biologia e Biotecnologie

LIST OF CO-FINANCERS

SUMMARY DESCRIPTION OF THE PROJECT (Max. 3 pages; to be completed in English)

Project title:

Salmo ceTtii REcovery Actions in Mediterranean Streams

Species/habitats/biodiversity

Scientific name: *Salmo macrostigma* (An II 92/43/EEC) or *Salmo cettii,* according with Art 17 of Italian National summary.

Population Size: in most of the Italian range the species is considered extinct and replaced by populations of the allochthonous Atlantic *Salmo trutta* or its hybrids (Splendiani et al., 2016, Biological Invasions, 18: 2029-2044). Most of the populations of *S. cettii* are fragmented and isolated in small mountain streams, so it is likely that the whole species in Italy is limited to a few thousand individuals.

Conservation status: “unfavorable bad” with a declining trend (Art 17 Italian National Summary)

Conservation threats

1- the lack of a range-wide strategy to stop introgression phenomena with the Atlantic genome of domestic origin. Data shows also that in the 7.04% of populations the level of introgression was mild (i.e., presence of alien “genes” < 10%) and in the 30.99% was high (10-50%). In the rest of the samples (59.16%) the rates of introgression ranged from severe to completely introgressed. *S. cettii* populations with only autochthonous genetic variants are: western Alps 20%, Apennines 2.82%, Sardinia 50% and Sicily 33.33%.

2- habitat alterations, related to several factors, as decreasing of water resources due to climate changes, increasing of pollutants in freshwater, and river fragmentation. The presence of weirs (physical barrier) limits the upward movement of adults and causes a reduction in the abundance of populations, exposing them to risks of local extinction. To give an idea of the importance of the problem, in the Umbria region the presence of a weir was recorded every 5 km of river within the potential range of the Mediterranean trout.

Even the water abstraction prevents trout movements upstream (hydrological barriers). The reduction in flow rates causes a decrease in the population abundances and an alteration of the age structure, due to the lack of adult specimens. The flow rates decrease also reduces the dilution of pollutants. Habitat alterations also reduce the trout's ability to adapt to climate changes. Due to rising temperatures the trout will tend to move progressively upstream and this range shift will be impossible without the river continuity.

The effects of the river habitats alterations on the Mediterranean trout can be measured in terms of number of specimens, abundance and age structure of populations. The baseline for the average population abundance in the study area is a standing crop values less than 10 g/m^2 of (Life TROTA = 8,40 g/m^2). The age structure evaluation using the Proportional Stock Density Index (PSD) indicates that trout populations are far from the optimal range (35 – 65) for a lack of adult specimens (Life TROTA, PSD = 21.64; Pedicillo et al., 2010, KMAE, 399: 1-15 = 20.28).

3- illegal stocking. The extent of illegal stocking has so far escaped all control. Anyway, it is possible that the recent worsening of the introgressive hybridization rates in the wild Mediterranean trout populations is mainly due to this illegal practice, since in National Parks all other ways of stocking of alien trout are banned. Currently the problem is widely unknown because of the lack of categorization of the specific crime type.

Project objectives:

The main objective of this project is the recovery and the conservation of Mediterranean trout (*Salmo cettii* or *Salmo macrostigma* in Directive 92/43/EEC, annex II), the endemic salmonid of the mediterranean area and the only native trout of the central/southern Italy, protected by the “Habitat Directive”.

Mediterranean trout is classified as vulnerable species in Europe and, according the Art 17 its conservation status is considered “unfavourable bad” in Italy.

In particular, the present proposal, capitalizing the experience of LIFE TROTA (LIFE12/NAT/IT/000940), aims at designing and applying to the whole native range of the species a comprehensive strategy to improve the conservation status of *Salmo cettii* through a set of combined actions aimed at eliminating the sources of introgression and ameliorating the introgression rates, improving the quality of freshwater habitats and combating the phenomenon of illegal stocking

This general objective could be specified in four specific objectives:

-Develop and pilot test a set of supplemental conservation strategies aimed at restoring the native S. cettii genetic biodiversity through the elimination of sources of introgression, selective fishing on alien trout and supportive breeding of the native populations,

-Design and apply in the protected areas of *S.cettii* native range an integrated system of monitoring and improvement of the quality of freshwater habitat based on the application of the minimum vital flows and the decreasing of river fragmentation in according with the Water Framework Directive (2000/60 CE)

-Design and apply Guidelines for the conservation and management of *S. cettii* which will represent the main reference framework for the species conservation actions in its whole native range

-Develop a strategy for combating illegal stocking phenomenon based on stakeholders awareness and engagement, improvement of territorial control system, improvement of the regulatory and legislative framework

Actions and means involved:

PREPARATORY

A1 START UP (M 1-3) PNM

WHAT: to set up the legal and technical bases for project implementation

HOW

Administrative procedures

Setting up of Managerial bodies

A2 OPERATIVE PROTOCOL (M 1-8) ISPRA

WHAT: to produce an agreed operative protocol for ecological and genetic characterization of the identified rivers

HOW

- Protocol designing: detailing methods and approaches for rivers characterization

- Parks’ Technicians training

A3 POPULATION AND HABITAT CHARACTERIZATION (M 8-13) UNIPG

WHAT: to collect data for the genetic and demographic description of populations and habitat characterization

HOW

- detection of “source” and “sink” populations

- detection of optimal sites for the installation of mobile hatcheries

- detection of river segments for selective fishing activities

- localization of optimal rivers segment for alien trout eradication

- detection of sites for fish passage installation

CONCRETE CONSERVATION

C1 REPRODUCTION OF WILD PURE SPAWNERS (M 13-51) PMAGRA

WHAT: To produce the pure wild trout to be used for restocking and restoration

HOW

- Hatchery installation for artificial reproduction along the rivers

- Genetic selection: wild spawners selection for artificial reproduction

- Artificial reproduction in 2 seasons

C2 SUPPLEMENTAL CONSERVATION STRATEGIES (M 13-51) PNM

WHAT: To improve population condition in rivers with average introgression (ca 50%) through supplemental strategies

HOW

- Direct translocation of pure wild trout from “source sites”

- Selective fishing engaging anglers duly selected and trained

- Stocking with native fry of local origin (i.e., from the same river basin) to support native population with native fry produced in the hatcheries

- Selective fishing and stocking with native trout combining the previous two strategies

C3 ALIEN SPECIES REMOVAL (M 13-51) PNFC

WHAT: To restore pure native population in river with high introgression (<80%) through removal alien trout and restocking

HOW

- Selective fishing with trained anglers

- Eradication through electrofishing

- Restocking after the eradication

C4 FRESHWATER HABITAT IMPROVEMENT (M 13-51) UNIPG

WHAT: To preserve the *S. cettii* freshwater habitat by the reduction of river fragmentation

HOW

- Removal of physical barriers to obtain a maximum defragmentation length and to facilitate the movements of the highest number of native species also through fish passes

- Removal hydraulic barriers to guarantee the minimum vital flow

C5 NATIONAL GUIDELINES FOR *Salmo cettii* CONSERVATION (M 13-51) ISPRA

WHAT: To design National guideline for the management of *S.cettii* in compliance with the principles of biological conservation integrating "habitat" “alien species” and "water framework" directives

HOW

- Designing of guidelines according to the results obtained in the pilot areas

- Guidelines application in the protected areas directly and indirectly engaged through the updating of SCIs conservation measures

C6 ACTION AGAINST ILLEGAL STOCKING (M 5-51) FORESTAS

WHAT: To implement a combined set of actions aimed at fighting the phenomena of illegal stocking with alien trout

HOW

- Territorial control enforcement through the installation camera trap and training of voluntary guards

- Awareness raising through events and labs on the risk related with illegal stocking

- Regulation updating to prevent the phenomenon and to create an effective system of detection and classification of the complaints

D. MONITORING (M 5-51)

WHAT: The actions aim to

1) Assess the results of the conservation actions for a continuous improvement of the adopted approach

2) Value the results obtained in the 6 pilot areas to transfer the methodology in the 14 transfer areas

3) Gather data on the effectiveness of the different strategies to design National Guidelines applicable in the *S. cettii* native range

HOW

D1 Monitoring of project results to evaluate the impact in terms of population and habitat status (13-51) ISPRA

D2Monitoring on socio economic impact to value the impact on stakeholders' behaviours and local economy (5-51) UNIPG

D3Monitoring on ecosystem functions restoration to value the impact on the quality of habitat and biodiversity (5-51) UNIPG

E. AWARENESS AND DISSEMINATION (M 1-51) LEG

WHAT: To ensure project replicability and transferability and maximizing its impacts

HOW

E1 Communication Awareness and dissemination actions are foreseen to:

1) guarantee the wide visibility of project activities and results

2) engage local stakeholders

3) exchange of know-how with complementary initiatives

E2 Replicability and transferability: during the last 2 project years 11 protected areas will be objective of transfer interventions aimed at applying the *S. cettii* conservation strategy

F. PROJECT MANAGEMENT (M 1-51)

WHAT: Set up an effective technical and financial managerial system

HOW

- Project strategic coordination: PNM

- Operative and financial coordination: Noesis

Expected results (outputs and quantified achievements):

CONCRETE RESULTS AND IMPACTS IN TERMS OF ELIMINATION OF SOURCES OF INTROGRESSION AND AMELIORATION OF THE INTROGRESSION RATES:

- Individuation and protection of at least 11 pure wild populations.

- Creation of at least 11 new pure wild populations.

- Increase of autochthonous genotype of 5% respect to the *ex ante* baseline.

- Decrease of alien genotypes respect of 5% respect to the *ex ante* baseline.

- Eradication of at least 11 alien populations.

Production of at least 66.000 native fry.[ML1]

-Translocation of at least 3.600 wild native trout.[ML2]

CONCRETE RESULTS AND IMPACTS IN TERMS OF REDUCTION OF FLUVIAL FRAGMENTATION AND IMPROVEMENT OF THE QUALITY OF FRESHWATER HABITAT

- Guarantee the MVF in 51 sites.

- Decrease of river fragmentation by removing 5[ML3] barriers.

- Union of previously isolated populations by removing 5 barriers and increasing their number to twice.

- Increase of average abundances of wild populations up to a minimum of 10 g/m^2.

- Increase of average quality of age structures (PSD) of wild populations up to a minimum of 35.

- Decrease probability of extinction of local populations by increasing colonization rates.

- Decrease probability of extinction of local populations by decreasing emigration (drift) rates.

CONCRETE RESULTS AND IMPACTS IN TERMS OF CREATION OF A COMMON REFERENCE FRAMEWORK FOR THE *SALMO CETTII* CONSERVATION ACTIONS

- Adoption of common guidelines for the *S. cettii* populations management and conservation in 17 protected areas covering a relevant part of the *S. cettii* native range.

- Adoption of common guidelines for the *S. cettii* habitat management in 17 protected areas covering a relevant part of the *S. cettii* native range.

CONCRETE RESULTS AND IMPACTS IN TERMS OF STRATEGY FOR COMBATING ILLEGAL STOCKING

- Increase of the number of notification in the targeted area updating the regulation in the 6 pilot

areas.

- Increase the awareness of the problem in citizenship involving at least 3.000 people in awareness events.

- Creation of 6 groups of voluntary guards engaging at least 100 local volunteers.

Sustainability of the Project Results:

STREAMS is designed to guarantee the full sustainability of the project results foreseeing a three-steps architecture:

The pilot application of a combined set of conservation strategy in 6 pilot protected area selected according with 3 specific criteria a) advanced knowledge of trout population an habitat status to guarantee the technical readiness of the interventions b) coverage of the whole native range (from Calabria to Alps) c) wide heterogeneity of conservation, environmental, climatic, legal and socio economic characteristics.

the creation of a comprehensive and unique reference framework for all the protected area managing authority at national level with National Guidelines on the S. cettii conservation strategy, designed by ISPRA according with the compared analysis of the results obtained in the six pilots and applied in all the 17 engaged protected areas.

the start-up of the process of application of the Guidelines on the whole S cettii native range through a peer- to-peer approach where once the interventions have been tested and validated in the selected areas, will support the technicians of the other 11 identified areas, in order to transfer attune and concretely apply the S. cettii conservation strategy according to with the national Guidelines produced by ISPRA

According with this scheme, the project will guarantee:

Activities' continuation in the pilot areas: All the concrete activities foreseen in the project, artificial reproduction (C1) supplemental conservation strategy (C2) alien species removal and restocking (C3) freshwater habitat improvement (C4) and actions against Legal stocking (C6), will be kept active beyond project end, becoming part of the SCIs Management plan and conservation measure (C5). The resources to guarantee the continuation will be identified in the Pilot area managing authorities own budget furthermore specific interventions to further exploit in other sites/river stretches the conservation actions will be identified activating specific funding lines foreseen in the Regional Structural Funds Operative Programmes (mainly Rural Development Programme) and in the Ministry of Environment specific Initiatives.

Transferability and replicability: During the last two years of the project the 11 transfer sites will start the characterization of the population and habitat, select the fittest conservation strategy, concretely plan the needed conservation actions, update the management plan and identify specific funding for implementing the identified actions in the above-mentioned funding lines. The project transferability also foresees the direct engagement of France regions and protected areas for them the replication of the project will be managed through a process of adaptation of the Guidelines to the specific national legal and regulatory framework and conservation approach

Is your project significantly climate-related? Yes [X] No [ ]

The biological implications of climate changes, include the moving upstream for many species and the reduction of physical habitat caused by the decrease water flows: presumably climate changes will worsen the environmental restrictions, mainly for the species living on the edge of their range, with fragmented populations and limited shelters, as the case of Mediterranean trout (Lorenzoni et al., 2014: Biologia Ambientale, 28: 2, 67-73). The project includes the removal of some barriers that prevent the movements of the fish populations. The barriers can be of two types: i) physical barriers, such as dams, weirs or other, and ii) hydraulic barriers, which essentially consist of river stretches where, due to excessive water withdrawal for human purposes, remains an insufficient amount of water to ensure aquatic life. In the long term removal of barriers, action envisaged by the project, will increase the chances of survival of the fish fauna, raising its resilience under climate changes conditions. Climate changes will lead to the worsening of the particular environmental conditions (in terms of hydrological instability, particularly long drought periods, variability of ecological conditions) that characterize the watercourses of the Mediterranean region, an area already particularly vulnerable to the reduced availability of water. For these areas, climate models predict inter annual temperature

variations more pronounced in the summer season and more frequent events of particularly high temperatures (Almodovar *et al*., 2012: https://doi.org/10.1111/j.1365-2486.2011.02608.x): in this context rivers and aquatic communities can only benefit from the reduction of water withdrawals for human purposes.

Moreover, the Mediterranean can take advantage by its greater ability to adapt to extreme events then the non native trout (Caputo et al., 2010: DOI: 10.1080/11250000802589576). Recent research has shown that in this species females mature their eggs asynchronously, so that the reproductive period is able to extends for over six months (Gortázar et al., 2007: Ecology of Freshwater Fish 16: 515–527). The presence of reproducing individuals for a long period increases the chances of reproductive success for populations inhabiting rivers with variable environmental conditions: the longer spawning period facilitates the capacity of the population to avoid negative consequences of unpredictable phenomena.

The proposal addresses the following project topic(s):

- Projects aimed at improving the conservation status of habitat types or species of Community Interest, provided, their status is not 'favourable/secure and not declining' or 'unknown' according to the most recent overall assessments that Member States have provided at the relevant geographic level according to Article 17 of the Habitats Directive or to the most recent assessments according to Article 12 Birds Directive and EU-level bird assessments.

Reasons why the proposal falls under the selected project topic(s):

The present proposal clearly falls under the project topics n.2: Projects aimed at improving the conservation status of habitat types in Natura 2000 sites or species (including bird species) of Community Interest, provided, their status is not “favourable/secure and not declining” or “unknown” according to the most recent overall assessments that Member States have provided at the relevant geographic level according to Article 17 of the Habitats Directive or to the most recent assessments according to Article 12 Birds Directive and EU-level bird assessments. The coherence with the project topics n.2 is clear when we take into consideration that the present project is aimed at improving the conservation status of S. cettii that has been classified as: "unfavorable bad" in all the three Italian ecoregions (Alpine, Continental e Mediterranean) of the III Italian National Report of Article 17. The project aims to reverse the current trend that sees the species suffer over time a progressive reduction in the range, a decrease of abundance and an increase in the level of genetic introgression with a multi-level approach that, starting from the most important Italian National Parks, intends to extend to the whole national territory.

What are the Project Partnership details for the Project:

In order to guarantee the high value of the demonstrative actions and the maximum level of project replicability and transferability STREAMS Consortium is composed by two levels of partners:

PROJECT BENEFICIARIES:

PROTECTED AREA MANAGING AUTHORITIES:

Majella N.P - PNM

Pollino N.P- PNP

Foreste Casentinesi N.P - PNFC

Monti Sibillini N.P - PNMS

MAGRA R.P – PMAGRA

Agenzia Forestale Regionale della Sardegna – FORESTAS

PROFILE

- in charge of *S. cettii* conservation strategy in protected areas

- advanced knowledge of trout population a habitat status ensuring the technical readiness of the actions

- coverage of the whole native range

- wide heterogeneity of conservation, environmental, climatic, legal and socio-economic characteristics

ROLE: Pilot testing and transferring the *S. cettii* conservation strategy

SCIENTIFIC PARTNERS:

ISPRA

PROFILE: Scientific support to Italian Ministry of environment. Wide experience in fish species genetic analysis and habitat ecological characterization

ROLE: designing of intervention protocol, coordination of habitat and species characterization and conservation actions' scientific coordination, designing of the Guidelines and supporting for implementation in protected areas

Università di Perugia (UNIPG)

PROFILE: wide experience in fish species demographic analysis and habitat ecological characterization (UNIPG)

ROLE: designing of intervention protocol, coordination of habitat and species characterization and conservation actions' scientific coordination

COMMUNICATION:

LEGAMBIENTE ONLUS – LEG

PROFILE: larger environmental NGO in Italy deeply engaged in conservation policies species and habitat

ROLE: Coordination of awareness, communication and dissemination strategy

MANAGEMENT:

NOESIS

PROFILE: Company specialized in LIFE project management, partner of other LIFE approved project

ROLE: Supporting the PNM in all the activities related with technical operative management (monthly monitoring, partnership coordination, quality control, administrative financial coordination) in order to guarantee the project smooth implementation

EXTERNAL PARTNERS:

Abruzzo, Lazio e Molise NP

Sirente Velino RP

Alpi Marittime RP

Monviso RP

Tosco Emiliano NP

Parks and Biodiversity of Romagna MA

Umbria Region

Gran Sasso e Monti della Laga NP

Cilento NP

Aspromonte NP

Corse - Federation de la peche

PROFILE: Protected areas managing authorities in charge of S. cettii conservation strategy

ROLE: Activation a peer to peer transfer process foreseeing: population and habitat characterization, conservation strategy selection and planning, funding line identification. For the engaged French transfer areas it is envisaged a specific action aimed at adapting the project approach to the French legal and regulatory framework

The project will be co-financed by beneficiaries' own contributions

Expected constraints and risks related to the project implementation and how they will be dealt with

R=Risk P=Probability M=Mitigation

R: Presence of sink population
P: unlikely
M: the pilots' selection is based on previous analysis confirming the presence of pure populations

R: Health problems in hatcheries
P: likely
M: Prevision of veterinary protocol based on previous experience and steady control of veterinary personnel for the early detection of health problems

R: engagement of local anglers in selective fishing
P: likely
M: the actions is preceded by awareness and consensus building actions aimed at explaining the actions and guarantee their engagement

R: Effectiveness of the eradication actions
P: unlikely
M: LIFE+TROTA demonstrates that a two-years eradication through electrofishing guarantee a reduction of alien species >95%. STREAMS, to further improve the results reducing the effort, foresees the combination with selective fishing

R: Illegal stocking difficult detection
P: likely
M: STREAMS foresee a combined set of actions combining awareness, locals engagement, control and regulation updating

R: Engagement of transfer areas
P: unlikely
M: All the listed pilot already confirmed their interest and will sign a specific commitment letter for the full application

R: Adoption of guidelines
P: unlikely
M: The engaged managing authorities already confirmed their commitment in updating SCIs conservation measures

Risk LIFE project Management
P: likely

M: A SME specialized in LIFE management will support the whole consortium in handling the managerial and financial procedures

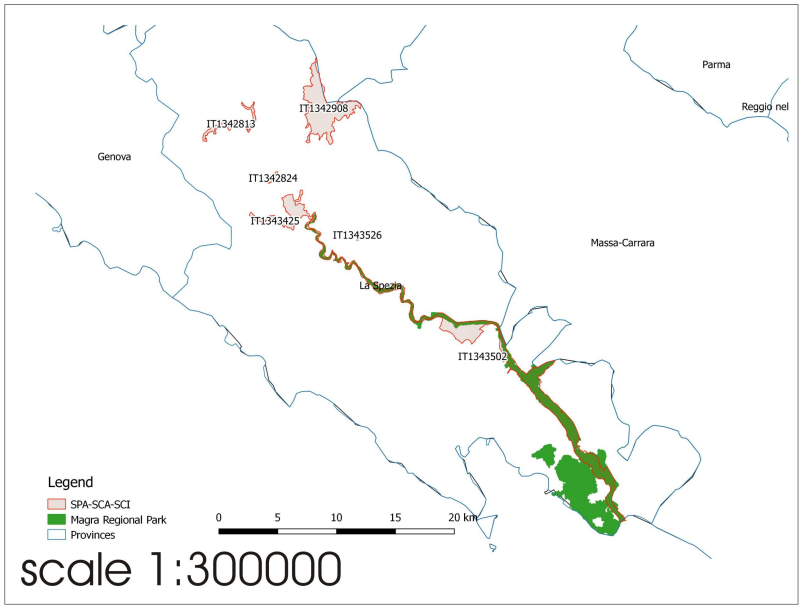
MAP OF THE COUNTRY LOCATION OF THE PROJECT AREA

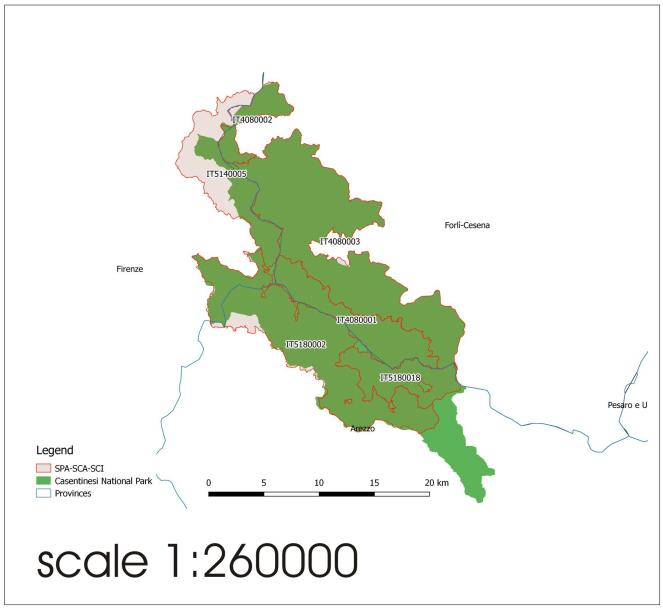
SCALE 1:70000000

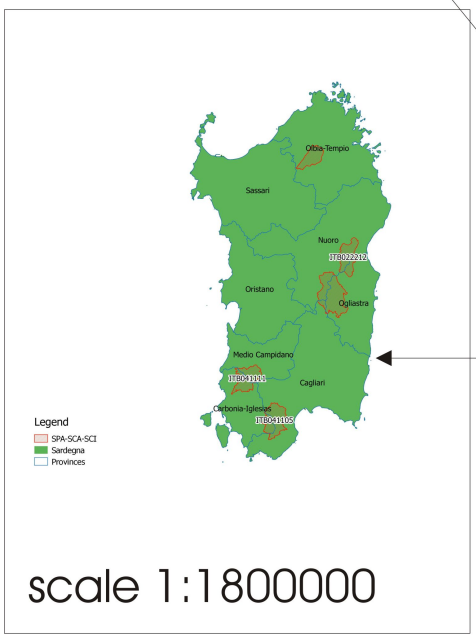
Country location of the project areas: Italy

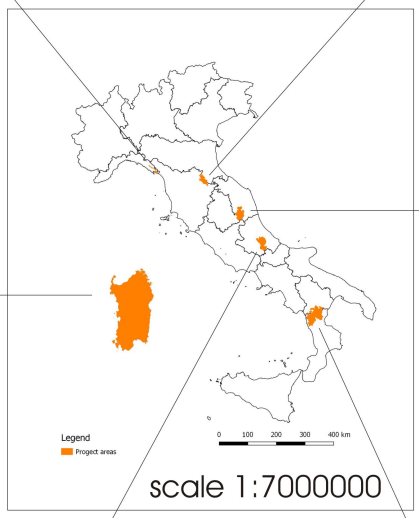
MAP OF THE REGION LOCATION OF THE PROJECT AREA

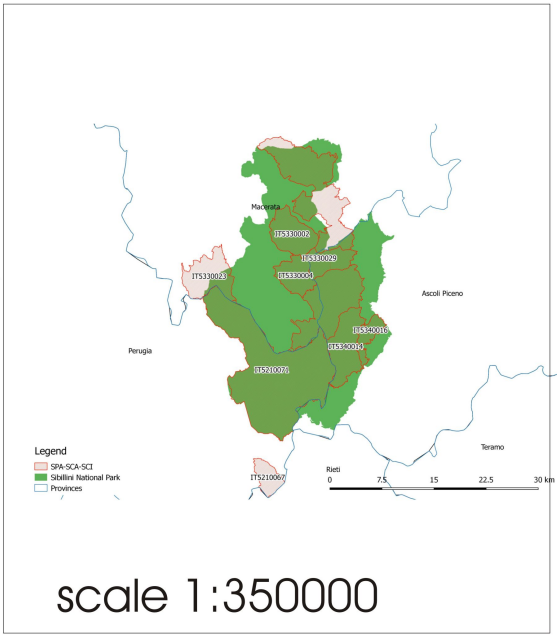
SCALE 1:7000000

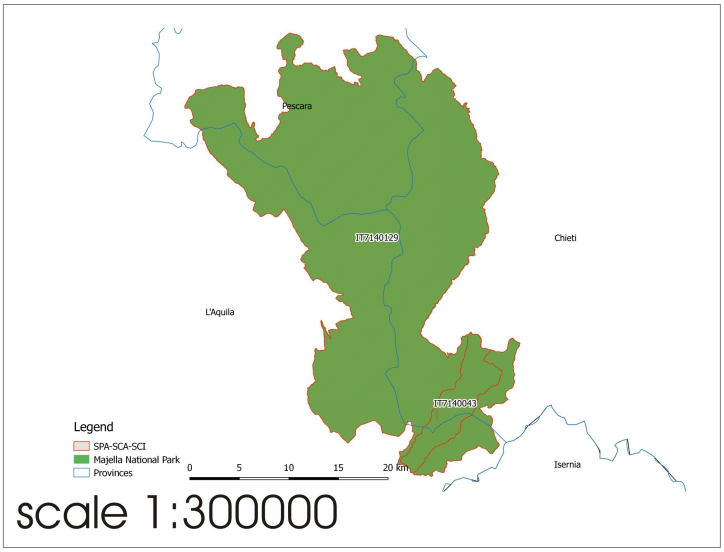


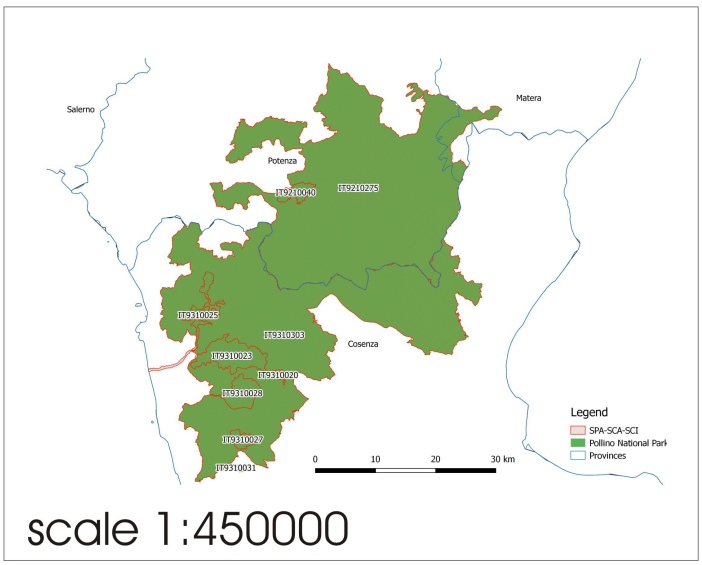












DESCRIPTION OF SPECIES / HABITATS / BIODIVERSITY ISSUES TARGETED BY THE PROJECT

Scientific name

In Italy the taxonomic status of the Mediterranean trout species complex is still controversial and several synonyms have been used: *Salmo macrostigma* Duméril, 1858, *Salmo cenerinus* Chiereghini, 1847; *Salmo cettii* Rafinesque, 1810; *Salmo ghigii* Pomini, 1941). In a recent taxonomic review, it has been proposed the use of the Latin name *Salmo cettii* to indicate the Mediterranean trout living in the rivers of the Italian peninsula. In the Annex II of Habitat Directive 92/43/EEC the Mediterranean trout is listed as *Salmo macrostigma,* while in the Italian III Report of Habitat Directive (ex Article 17) *Salmo cettii* is the valid scientific name for the same species (Genovesi et al., 2014: ISPRA Rapporti 194/2014). Therefore, according to the most recent scientific findings (e.g., Rondinini et al., 2013: Comitato Italiano IUCN e Ministero dell'Ambiente e della Tutela del Territorio e del Mare, Roma), the scientific name *S. cettii* will be adopted in the rest of the present application. The species is found in Corsica, Sardinia, Sicily, and in the Italian peninsula from the eastern Alps southward (streams in mountain areas and karstic springs in lowland areas) (Splendiani et al., 2016: DOI 10.1007/s10530-016-1149-7).

Population Size

According to Splendiani et al. (2016: DOI 10.1007/s10530-016-1149-7), *S. cettii* samples characterized by having only autochthonous genetic variants were from western Alps (20% of sites), Apennines (2.82%), Sardinia (50%) and Sicily (33.33%). The above data are consistent also with previously estimations reported in Rondinini et al. (2013). In fact, according to these authors the percentage of pure *S. cettii* populations in Italy (major islands excluded) is around 11%. In addition, non-hybridized populations usually persist in isolated and fragmented headwater habitats, where their long-term persistence is uncertain (Splendiani et al., 2019: https://doi.org/10.1007/s10592-018-1135-y). In the 2008 the IUCN indicated a population decline during the past three generations (10 to 12 years) by almost 30%, and it will continue in the future; in more recent years the Italian IUCN Committee considered "minimal" the abundance of the native populations and it is expected a further decrease of the consistency in the future by 80% (Rondinini et al., 2013: Comitato Italiano IUCN e Ministero dell'Ambiente e della Tutela del Territorio e del Mare, Roma).

Unfortunately, detailed quantitative data on the demographic status of the species in Italy are scarce or not well explored. Thanks to the preparatory action of the present project this gap will be filled. In fact, the acquisition of this information will be of crucial importance for the impact monitoring of the concrete conservation actions that will be adopted in STREAMS.

Conservation status

According to the Italian IUCN Committee the risk of extinction of *Salmo cettii* has been evaluated in Critical Endangered (CR), with a decreasing consistency expected in the future (Rondinini et al., 2013). According to the IUCN (2019) the conservation status is Near Threatened with a decreasing population trend, but this assessment was carried out in 2008 and does not take into account the scientific nomenclature changes of the species and the information gathered in more recent years. In the Italian III Report of Habitat Directive (ex Article 17) (Genovesi et al., 2014), the conservation status of *S. cettii* is described as "unfavorable bad" with a declining trend. The worrying estimation presented in the ex Article 17 Report is coherent with a recent review (see Splendiani et al., 2016: DOI 10.1007/s10530-016-1149-7, and references there in) showing that in the Apennine rivers, the genetically pure samples analyzed to date, resulted less than 3%.

For the International Union for the Conservation of Nature (IUCN, 2016, The IUCN Red List of Threatened Species) the main threats on *S. cettii are* water abstraction, dams, overfishing and stocking of non-native trout (because of hybridisation and competition phenomena).

Habitat alterations, combined with the presence of dams and weirs also reduce the trout's ability to adapt to climate changes. Because of the increase in water temperature, resulting from the decreased rainfall and the increase in water abstraction and evapotranspiration, some studies showed the tendency of the trout to move towards upstream along the longitudinal gradient of the rivers, trying to reach the thermal optimum. This range shift can be hindered by insurmountable barriers or by unsuitable environmental conditions.

CONSERVATION / BIODIVERSITY PROBLEMS AND THREATS & PREVIOUS CONSERVATION EFFORTS

Provide this information for those species / habitat types or biodiversity issue directly targeted by the project

As explained above the mains threats to the future persistence of native *S. cettii* populations are:

i) **the introgression phenomena** with the Atlantic genome of domestic origin, that in national parks, where restocking is prohibited, at present it is a probably consequence of illegal activities;

ii) **river habitat alterations with two main causes** a) the river fragmentation due to the presence of insurmountable barriers and b) the water abstractions from rivers, which reduces the habitat available for the trout, worsens the consequences of pollution due to reduced dilution of pollutants and impedes movement of aquatic fauna (hydrological barriers)by interrupting the fluvial continuity analogously to dams (physical barrier);

iii**) the lack of a wide strategy at national scale** to improve the conservation status of *Salmo cettii*.

The introgression phenomena: about this threat (i.e., genetic introgression) we can easily understand the severity of the problem if we keep in mind that along the Apennines only less than 3% of the populations genetically analyzed to date showed no traces of genetic introgression, and less than 8% showed only a mild level of introgression (e.g., Splendiani et al., 2016: DOI 10.1007/s10530-016-1149-7). The origin of the genetic hybridization and successive introgression between native trout and alien trout is mainly related with the traditional stocking activity carried out since the end of the nineteenth century using trout of domestic origin (Caputo et al., 2004: https://doi.org/10.1111/j.0022-1112.2004.00458.x). Although stocking practices with alien species is forbidden in protected areas, there is, the need to underpin concrete conservation action to contrast the spread of alien trout genome into residual pure or almost pure Mediterranean populations. To contrast this threat the present project foresees several preparatory and concrete actions aimed to: i) take a genetic and ecological characterization of the residual population in the project area in order to design properly concrete conservation actions location per location; ii) support native population characterized by low-medium level of introgression by the use of local or mobile hatcheries and with the implementation of several strategies; iii) remove alien trout by eradication activities (i.e., by electrofishing); iv) involve local anglers in innovative activities of selective fishing. To reduce the introgression with the non native genome, an action will also be taken in order to quantify the spread of illegal restocking and to fight it in the project area. The extent of illegal stocking has so far escaped all controls. Anyway, it is possible that the recent worsening of the introgressive hybridization rates in the wild Mediterranean trout populations is mainly due to this illegal practice, as demonstrated by recent scientific papers (Splendiani et al., 2018 doi.org/10.1007/s10592-018-1135-y) and by the results obtained within the LIFE+ TROTA 12 NAT/IT/000940. Given the difficulty of identifying the phenomenon, a wide-range strategy will be implemented based on stakeholders' awareness and engagement, improvement of territorial surveillance system, improvement of the regulatory and legislative framework.

River habitat alterations: simultaneously with actions to contrast the spread of alien trout and to reduce the level of hybridization in *Salmo cettii,* the habitat of the native trout populations will also be improved by removing the **physical** (dams, weirs, etc.) and **hydraulic** (river stretches with insufficient flows) **barriers** that interrupt the fluvial continuity. To give an idea of the importance of the problem, in the Umbria region every 5 km of river the presence of a weir was recorded within the potential range of the Mediterranean trout. Barriers affect the demographic size of the already natural low size of isolated native trout populations because limits the upward movement of adults and causes a reduction in the abundance of demes. This increases the risk of extinction of a population. On the other hand, in some cases barriers (natural or artificial) preserve the genetic integrity of isolated native population limiting the range expansion of alien species moving upstream from the sites where the restocking is a common practice (Spendiani et al., 2019: https://doi.org/10.1007/s10592-018-1135-y). For this reason, following the European Project Life+ TROTA (Trout populations recovery in Central Italy, LIFE12 NAT/IT/000940) the presence of barriers was a fundamental requirement for the selection of river stretches in which carry out the alien trout eradication activities. At the same time the selection of the barriers to be removed to restore the river continuity will be carefully evaluated considering all the knowledge acquired during the preparatory phase, including the genetic characteristics of trout populations occurring (and any other alien fish species) in the sites downstream and upstream the barrier. The improvement of the habitat availability for the brown trout, reached

through the restoration of river continuity and the increase in flow rates, will have positive effects also for the other native species occurring in the project area with which it shares the same habitat, some of which are listed in Annex II of the habitat directive such as *Cottus gobio* e *Austopotamobius pallipes.* Through habitat improvement it is possible to pursue one of the objectives of the Water Framework Directive (WFD-2000/60) / EC) and to mitigate the effects of the climate change in some of the aquatic ecosystems in the project area.

The lack of a wide strategy at national scale: one of the most important results of the project will be surely the draft of a National Guideline to regulate the management of *S. cettii* in Italy, according to the fundamental principles of biological conservation and using all the information and the experiences gathered during this and other projects, as the European Project Life+ TROTA (Trout populations recovery in Central Italy, LIFE12 NAT/IT/000940) and Life+ NAT.SAL.MO (Recovery of S.macrostigma: Application of innovative techniques and participatory governance tools in rivers of Molise, LIFE17 NAT/IT/000547). The scientific and technical aspects of the guidelines will be developed in those actions concerning the investigation of the following issues: genetic diversity, identification of conservation units, captive breeding and stocking strategy, limiting factors of the populations and the ecological requirements of the species. The draft of the Guidelines will imply the development of the following three main arguments: i) Guidelines for population management, ii) Guidelines for habitat management; iii) Adoption of the guidelines by the NATURE 2000 Managing Authorities.

Finally, we will transfer the main conservation actions to others protected areas in order to cover as much as possible the complete species range and to disseminate the outcomes of the project promoting a strong awareness of the conservation issues concerning the Mediterranean trout.

PREVIOUS CONSERVATION EFFORTS IN THE PROJECT AREA AND/OR FOR THE HABITATS / SPECIES TARGETED BY THE PROJECT

At European Community level, the following proposals have been presented:

i) LIFE03 NAT/F/000101 “Conservation of the macrostigma trout in Corsica” proposed by Federation de la Corse pour la Peche et la Protection des Milieux Aquatiques;

ii) LIFE12 NAT/IT/000940 “Trout population recovery in central Italy” proposed by Pesaro Urbino Province;

iii) LIFE17 NAT/IT/000547 “Recovery of *S. macrostigma*: Application of innovative techniques and participatory governance tools in rivers of Molise” proposed by University of Molise;

iv) INTERREG IIIA – Italy-France – “Identification, saving and rehabilitation of autochthonous trout populations in Valle d’Aosta and Haute-Savoie district”.

A genetic study on the stream trout aimed at detecting local stocks has been carried out within the interregional project. We will follow the management indications suggested at the end of this pivotal study. At National, and/or more regional scale, several conservation projects have been undertaken in the last twenty five years:

i) start date **1992**, Terni Province: “Project for the restoration and conservation of the native Mediterranean trout populations”. This project led to the creation of a first breeders stock selected by using morphological methods in 1996. An important result of this project consisted in the discovery and protection of a *S. cettii* population characterized by a very low level of genetic introgression;

ii) duration **2003-2011**, Genetic characterization of *S. cettii* populations from Magra River;

iii) duration **2009-2011**, Pesaro - Urbino and Ancona Provinces: “Projects for the retrieval of a native stream trout stock – genetic characterization of the salmonid populations in the river basins of the Marche Region (central Italy)”. Thanks to the development of this project, it has been possible to verify the presence of *S. cettii* populations at different degree of introgression in some streams of the central Apennine. In particular, the Pesaro Urbino Province, using the results collected throughout these genetic survey, created a partnership with Università Politecnica delle Marche (Ancona) and Università degli studi di Perugia in order to perform a genetic in-depth study of the salmonid populations status in

the freshwater areas under their own competence. A first, practical result of this action was the production at the "Centro Ittiogenico di Cantiano" (Pesaro and Urbino Province) of a genetically selected Mediterranean trout stock. Nowadays, the above aquaculture plant is involved in the project LIFE12 NAT/IT/000940;

iv) start date **2013**, signing of a memorandums of understanding between "Regione Autonoma della Sardegna, Assessorato della difesa dell'Ambiente" and the "Comunità Montana Sarcidano-Barbagia di Seulo CM-SBS" aimed to start protection and restoration activities for native *S. cettii* populations in Sardinian rivers.

v) duration **2016 to today**, Regione dell'Umbria: "Accordo di collaborazione, tra la Regione Umbria - giunta regionale, il Dipartimento di Chimica, Biologia e Biotecnologie, dell'Università degli Studi di Perugia e l'Universita' Politecnica delle Marche - Dipartimento di Scienze della Vita e dell'Ambiente, per la ricerca, selezione e produzione di trote mediterranee da ripopolamento". This research led to the creation of a first breed stock in the Umbria region selected by using genetic method developed and tested in the project previously described. A first important result of this project consisted in the detection and protection in the Umbria region of some new *S. cettii* population characterized by a very low level of genetic introgression and the reconversion of the "Regional Centro Ittiogenico di Borgo Cerreto" (Perugia) to conservation for the production of Mediterranean trout specimens

C.1 REPRODUCTION OF WILD PURE SPAWNERS

Beneficiary responsible for implementation: PARCO MAGRA

Responsibilities in case several beneficiaries are implicated: All parks + FORESTAS will perform all the actions and UNIVPM and UNIPG will support technicians through field counselling visits.

Description (what, how, where, when and why) (max 7,000 characters):

WHAT

The Action aims at producing the pure wild trout to be used for restocking and restoration of *S. cettii* populations: C.2.3 Stocking with native fry, C.2.4 Selective fishing and restocking, C.3.2 Restoration of pure native population.

It is foreseen the installation of mobile hatcheries (task C.1.1) (in PNFC improvement of the existing fixed plant) to be used for the artificial reproduction of pure wild trout (Task C.1.3).

The river segments with pure (or almost pure) populations where wild spawners will be captured by electrofishing and selected by the use of molecular markers (Task C.1.2) are identified according with the results of the preparatory actions A.3.

A detailed summary of the field activities, means and personnel foreseen for the action is reported in the table C.1b.

HOW

Task C.1.1 Hatchery installation

A total of 11 hatcheries will be used for captive breeding in the project sites, it will be installed 10 mobile hatcheries and used 1 aquaculture plants in PNFC already operative which will be adapted in order to guarantee the separation of breeder by the two basins selected by the park. This answers to the need to catch and produce trout in the same basin where they are restocked.

These structures, operative from 26th month is equipped with aquaculture accessories, external race ways for wild spawners storage, video surveillance system and removable fences.

The hatcheries installation will be realized requiring: 1) the hydraulic concession (R.D. 523/1904), 2) the assent for the Environmental Incidence Assessment (art. 6 of the P.D.12 March 2003, n. 120), and 3) the landscape authorization (L.D n. 42 of the 22 January 2004 "Code of Cultural Heritage and Landscape").

Task C.1.2 Genetic selection

The field activities will be carried in autumn, just before the mating season. In fact, a too early female captive rearing could have a negative impact on ovulation. The Parks technicians will carry out a series of two samplings for 2 years, aimed to catch the wild spawners to be used for the artificial reproduction.

It is expected to catch the wild spawners in 1 watercourse for each hatchery that will be installed. The number of wild spawners to be collected in each watercourse will be estimated on the basis of the demographic analyses carried out during the preparatory action A.3. After electrofishing collection, the wild spawners will be tagged (by Passive Integrated Transponder and elastomers) and photographed (digital camera) prior to any data collection. From each fish, a small fragment of adipose fin will be taken and biometric and morphological parameters will be recorded. The spawners will be transported through Pick up equipped with oxygenized thanks in their own hatcheries awaiting the results of genetic analysis. Successively, the adipose fin clips will be transferred to the selected genetic laboratory for DNA extraction and subsequent genetic analysis (LDH-C1* locus and 15 DNA microsatellite loci, see the ac A.3.3 for a comprehensive description of the molecular markers). Genetic analyses will be carried out on a total of 4400 wild native spawners (200 x years x each of the 10 mobile hatcheries + 400 in the PNFC plant where breeders of 2 basins will be hosted). The statistical treatment of data will be carried out by ISPRA to estimate: i) standard parameters of population genetics; ii) the level of genetic introgression of each trout; and iii) the effective population size (Ne) represented by the pure trout selected as pure spawners. Parks' technicians will record morphological (i.e., acquiring trout images for other purpose, see action C.2) and biometric parameters which will be stored and analysed by UNIPG for a complete characterization of the pure wild spawners. Only pure wild spawners

will be used for the artificial reproduction, that is, with a level of genetic introgression with alien genome null or almost null. Only the **translocation of fish from the same river basins will be permit.**

Task C.1.3 Artificial reproduction

Once the genetic analyses will have been completed, artificial reproduction will be carried out during the winter season (from December to March). UNIPG will join the first reproduction session in each Park. During this action a long time will be devoted to the ordinary care of fishes. However, after each reproductive season, pure wild spawners hosted in the mobile hatcheries will be returned into the wild. On the contrary, non-pure spawners will be translocated into fishing ponds. In order to avoid, as much as possible, unintended inbreeding depression phenomena, the river location for wild spawners collection will be slightly different from year to year. Finally, the wild spawners will be managed according to the exemption request from Article 16, clause 5 of the L.D. n. 148/08. It is expected that Parks' technicians and veterinarians will make an average of 8 site visits per year per hatchery.

WHERE

A priori details about the river basins where mobile hatcheries will be installed are provided in table C.1a. However, the outcomes of the preparatory action A.3 (task A.3.3) will be indispensables for a definitive choice of the best locations for mobile hatchery installation and for the choice of the more appropriate number of hatcheries to be used. It is also hypothesized the need to supply each hatchery by collecting wild spawners from at least 1 watercourse, for a minimum of 13 watercourses

WHEN

From project months 13th and 48TH

WHY

This action is crucial for achieving the goals of the supplementation strategies (see actions C.2 and C.3, tasks C2.3, C.2.4 and C.3.2). The use of mobile hatcheries is related with two aims: i) to avoid the deleterious effects of domestication of wild spawners and their descendants and ii) to locate these plants as much as possible close to the river locations hosting pure native spawners. Although mobile hatcheries will be located as much as possible close the river portion that will be used as source of pure wild spawners, the use of a pickup truck and tanks for fish transportation will be fundamentals during this action and for the successive action C.2. In addition, the use of a genetic screening is unavoidable mainly for two reasons: first, as explained above (see form B2d) the number of pure *S. cettii* populations is very scarce and as a consequence, there is the need for a genetic selection of wild spawners. Second, we have to avoid as much as possible the deleterious effects of inbreeding depression. For this latter point, the analysis of ten microsatellites will provide a tool for monitoring the effect of artificial reproduction making comparisons between genetic diversity of fry (born in captivity) and wild donor populations.

C2 SUPPLEMENTAL CONSERVATION STRATEGIES AND SELECTIVE FISHING

Beneficiary responsible for implementation: PNM

Responsibilities in case several beneficiaries are implicated: All parks + FOR will perform all the actions and ISPRA and UNIPG will support technicians for data collecting, data storing and on-going revision-updating fine-tuning of supplemental strategies. PNM coordinate and collect contribution of the other PARKS+ FOR for the production of the 3 yearly record on supplemental strategy quantitative results.

Description (what, how, where, when and why) (max 7,000 characters):

WHAT

Thanks to the ecological, demographic and genetic information collected in action A.3 and the artificial reproduction outcomes (C.1), different conservation genetic strategies to restore the native *S. cettii* genetic biodiversity will be applied. The comparison among the results obtained through the different measures and their integration will provide key data to optimize the choice between the different conservation strategies and the basic knowledge for the designing of the **National guidelines for *Salmo cettii* conservation** (C.5).

HOW

Task C.2.1 Translocation of wild native trout

The first strategy consists in the direct translocation of wild trout from “source sites” (i.e., river segments characterized by very low or null levels of genetic introgression; that is, approximate percentage of alien genes < 5%) into “sink sites” (sites characterized by moderate level of genetic introgression; i.e., percentage of alien genes between 30 and 60%). After the individuation of source populations, thanks to the action A.3, a number of wild native trout compatible with the demographic characteristics of the source population will be captured by electrofishing and transported to as many “sink” river segments. Sink and source populations will be from the same river basin. The trout translocation will be made through Pick up equipped with oxygenized thanks bought in action C.1.

The action foresees 2 translocations per each year per 3 years from source populations. Please refer to the enclosed table C.2 for a detailed summary of the foreseen interventions.

Task C.2.2 Selective fishing

The second strategy consists in the selection of river segments characterized by moderate level of genetic introgression (c. 50%) where selective fishing activity will be carried out.

Selective fishing will start at month 13th and will be performed for 3 years during project lifetime and it will continue after project end. It is foreseen an average of 15 anglers engaged in selective fishing action for each Parks + FOR.

However, before selective fishing activities begin, a two-days workshop will be organised (one workshop for each project administrative area) to train the anglers involved in this action. The training will explore the following themes: how to manage trout during fin clip, how to distinguish a native phenotype from an alien or hybrid phenotype, how to interpret the Park map indicating selective fishing sites, how to communicate information to Park guards, etc. The same workshop will be repeated each year to exchange opinions and advice about the action progress.

Then, at the end of the first workshop each angler will be equipped with a biopsy kit. In addition, the mobile application developed during the Task A.2 will be used for the field data storage (pictures of trout retained, geographical coordinates and n. of the tube with fin clip). All data collected will be transferred and recorded in the UNIPG central server for the further analyses by Technical Scientific Committee. Furthermore, a standard catch records card will be provided; it will contain all the useful information for the angler to practice selective fishing (map, fishing rules, pictures of native and alien trout, etc...). In this way, by using the above tools, selective anglers will have the task of communicating precise information concerning their fishing activities: the analysis of the annual catch record cards will provide an estimation of fishing effort intensity, fish harvested by anglers and trends on catch-per-unit effort (CPUEs), key parameters to assess the impact of sport fishing activities on native populations.

During the selective fishing the anglers will be asked to clip a small portion of fin which will be analyzed in the frame of action D.1 to assess the effectiveness of the action.

Task C.2.3 Stocking with native fry

Also in this task the conservation effort is directed to those river segments characterized by moderate level of genetic introgression. However, in this case, native population will be supplemented with native fry produced by the hatcheries of action C.1. At the end of the project the effects of this task will be monitored by the action D.1.
Stocking with native fry will start at the end of the first artificial reproductive season Month 32 and will be performed for the following year in April during project lifetime and it will continue after project end.
The action foresees two stocking actions per year per two years from each one of the installed hatchery. Please refer to the enclosed table C.2 for a detailed summary of the foreseen interventions.

Task C.2.4 Selective fishing and stocking with native trout

Here, the strategies C.2.3 and C.2.4 will be carried out on the same river stretches.
For both Tasks C.2.3 and C.2.4 the number of fry that will be released will depend on the efficiency of artificial reproduction action (Task C.1.3, see also expected results in form B). At the end of the project the effects of this task will be monitored by the action D.1.
This integrated strategy foresees two stocking with native fry with the same scheduling of the Task C.2.3, and three seasons of selective fishing with the same scheduling of the Task C.2.2; it is foreseen that also the integrated strategy will continue after project end. Please refer to the enclosed table C.2 for a detailed summary of the foreseen interventions.

WHERE
C.2.1: it is expected that at least 1 site per park will be selected
C.2.2: it is expected that at least 1 site per park will be selected
C.2.3: a total of 11 river segments (one for each hatchery) will be selected to implement this action.
C.2.4: it is expected that at least 1 site per park will be selected

WHEN
C.2.1: 3 translocation seasons May-Sept 2021-22-23
C.2.2: 3 selective fishing seasons March-Sept 2021-22-23
C.2.3: 2 stocking seasons Apr-July 2022-23
C.2.4: 3 selective fishing seasons March-Sept 2021-22-23 + 2 stocking seasons Apr-July 2022-23

WHY
The supplemental conservation strategies are tested in the different pilot areas in order to guarantee a decrease of the introgression level with alien genome of the *S. cettii* populations; furthermore, the comparison among the data related with the effectiveness of the different interventions will provide key input to establish the fitter methodologies and approach in the different ecological and genetic condition of the target populations. Those input will be gathered in order to design the Nationalguidelines (C4).
The conservation strategies is based on sound scientific evidences showing the effectiveness of direct translocation of wild spawners and stocking with native fry on other Mediterranean trout populations (see Gil et al., 2016, Rev Fish Biol Fisheries, 26: 1-11, and references therein). As well as the positive effects of angling activities for the conservation of native trout has been showed in Härkönen et al., 2014, Canadian Journal of Fisheries and Aquatic Science, 71: 1900-1909).

C.3 ALIEN SPECIES REMOVAL

Beneficiary responsible for implementation: PNFC

Responsibilities in case several beneficiaries are implicated: All parks + FOR will perform the actions foreseen activities in their own sites. PNFC coordinate and will collect contribution of the other PARKS+ FOR for the production of the 3 Yearly record on eradication quantitative results.

Description (what, how, where, when and why) (max 7,000 characters):

WHAT
Thanks to the results that will be obtained in the preparatory actions A.3, river segments potentially harmful for the genetic integrity of *S. cettii* will be selected for the implementation of an alien brown trout removal activities by electrofishing. Native populations will be restored at the end of the eradication phases.

HOW
Task C.3.1 Eradication
This conservation activity will be performed adopting two-pass electrofishing method. A priori, a total of 11 sites are foreseen (9 corresponding to the 9 mobile hatcheries installed and 2 for the fixed plant). The rational of the above number of sites is related with the potential of fry production of the two different type of hatchery (mobile and fixed plant) used in the project.
In case of no sites suitable for eradication, a different strategy will be adopted (e.g., selective fishing strategy as in C.2.2 or a stocking with native fry strategy as in C.2.3). A total of eight events per site in three years will be programmed between summer and autumn (3 events in the first and second year 2 in the third). Only streams of modest dimensions will be selected (e.g., no longer than 3 km). In this case the habitat characterization will be of crucial importance (see action A.3). For example, the detection of obstacles that prevent the spread from downstream of alien trout, the presence of environmental conditions suitable for *S. cettii*, the flow regime, etc. will play a key role in the selection of suitable sites in which carry out eradication activities.
It is foreseen the involvement of local sport fishing associations for supporting the eradication action. The local anglers will be engaged through an awareness raising action detailed in the Task E.1.2. The Parks and FOR will give a contribution to the local anglers association joining the activities.
During each field phases the demographic parameters (trout population density and biomass) will be collected for successive evaluations on the effectiveness of the removal activities (action D.1). Finally, alien specimens will be transferred (no profit) to small fishing lakes isolated from the river networks. The transfer will be carried out using trucks outfitted with tanks. Fore more details about number of eradication events and means involved see table C.3.

Task C.3.2 Restoration of native population
After the end of the third season of alien trout eradication, the corresponding river stretches will be involved in restoration activities.
The restoration phases will be carried out using the native fry produced in the hatcheries (C.1) and/or through translocation of wild native trout (Task C.2.1). Also in this case only translocation of fish from the same river basins will be allowed.
Within the project two restoration actions are foreseen in the period March – June of the last project year, the restoration action will continue after project end.
The ongoing monitoring implemented within the Task D.1.1 will allow to evaluate the possible anticipation of the restoration action after the end of the third eradication season; this evaluation will be based on the results of the eradication effectiveness and according to the availability of native fry produced in the hatcheries and/or wild native trout for translocation. Details on foreseen interventions are reported on the enclosed table C.3.

WHERE
This action will be carried out in 11 river stretches isolated from the rest of the basin (11 corresponding to the 11 mobile hatcheries installed and 2 for the fixed plant). These locations will be identified on the bases of the results of the action A.3.

WHEN
Eradication actions will be performed two times per year in the following periods: August – November 2021-2022-2023.
The restoration action will be performed two times in the period March – June 2023.

WHY
As explained above (see Form B2d), the major threat to *S. cettii* conservation is represented by the spread of genetic introgression rates with the alien Atlantic trout genome. Therefore, the removal of alien trout in those rivers hosting both relict and isolated *S. cettii* populations and alien trout appear an unavoidable conservation strategy. In addition, restoration of native trout in the above eradicated sites represents an important chance to improve *S. cettii* population size in each river basin involved.

C.4 FRESHWATER HABITAT IMPROVEMENT

Beneficiary responsible for implementation: UNIPG

Responsibilities in case several beneficiaries are implicated: Parks + FOR will perform all the actions. UNIPG will ensure technical scientific support for the whole action and coordinate and collect contribution of the other. PARKS+ FOR, in order to produce the scientific reports on Freshwater habitat improvement and the data reports on Morpho-hydraulic characterization.

Description (what, how, where, when and why) (max 7,000 characters):

WHAT: The aim of this action deals with the removal of barriers that fragment the river corridor and block the fish passage, in particular to damage the native trout populations.
Two types of barriers will be mainly investigated: 1) physical barriers (e.g. dams, floodgates, causeways and weirs) and 2) hydraulic barriers (e.g. areas of low flow). GIS information on other environmental barriers (e.g. alteration of bank, stream bed and riparian vegetation, dark tunnels or unnatural substrates created by pipes, and poor water quality) will be also gathered.
The results obtained with the implemented measures for physic and hydraulic barriers removal will provide the knowledge for the designing of the National guidelines for *S. cettii* conservation (Action C.5).

Task C.4.1 - Removal of physical barriers
The highest priority barriers (HPB) to remove will be identified on the basis of the mapping results of action A.3.2, with special reference to genetic and demographic characterization. Similar procedures will be followed in concrete conservation actions for detection and selection of eradication sites (Task C.3.1): In this case the barriers (natural or artificial) will be identified excluding the eradicated stretches that can be recolonized by the alien trout coming from the neighboring fluvial areas.
Moreover, the selection of barriers to remove will be planned in order to obtain a maximum defragmentation length with the minimum number of habitat management interventions. The restoration actions will be implemented to facilitate the movements of the highest number of native species, avoiding sites where the barriers removal can play the role of invasion corridors by alien species, in particular of non native trout.
A quantitative analysis of fish community up- and down-stream of the barrier will be conducted by the park technicians in July 2021 in each place where fish pass will be installed. The data will represent the baseline for the habitat status monitoring. (Task D.1.2.)
A fish pass outline design sketch will be developed for each HPB, taking into account its morphological and hydraulic characteristics. The fish passage implementation will be realized requiring: 1) the hydraulic concession (Royal Decree 523/1904), 2) the assent for the Environmental Incidence Assessment (art. 6 of the Presidential Decree 12 March 2003, n. 120), and 3) the landscape authorization (Legislative Decree n. 42 of the 22 January 2004 "Code of Cultural Heritage and Landscape"). According to the preliminary analysis a total number of 5 fish passes will be installed: two in PNMS and PNP, 3 for PNFC and 1 P.N.M. No fish pass will be installed in FOR and PMAGRA

Task C.4.2 - Removal of hydraulic barriers
Hydraulic barriers will be investigated by measuring the differences between the current flow and the minimum vital flow (MVF). Flow rates will be measured using the current-meter method. Discharge is computed as the product of the area and current speed. The measurement will be done by subdividing a stream cross section into segments, and by measuring the depth and current speed in a vertical within each segment. The total discharge is the summation of the products of the partial areas of the stream cross section and their respective average current speed. Together with the flow rates some other environmental parameters will be measured (dissolved oxygen, water temperature, pH, conductivity) in order to monitor the level of pollution. A stream data series will be created in each intervention site making periodic measurements 1 time x 2 years in each one of the 51 sites where concrete conservation actions will be carried out (C1 - 11, C2.1 - 6, C2.2. -6, C2.3 11, C.2.4 -6, C3 11). Flow data will be recorded by park technicians and saved on the web database.

The estimation of the minimum vital flow will be done using the hydraulic model adopted in the regional PTAs (Piano di Tutela delle Acque) and by the experimental model proposed within the Life+TROTA (LIFE12 NAT/IT/000940), specifically calibrated on the ecological requirements of the Mediterranean trout (MTModel)
The divergence between summer-autumn low flows and the MVF will highlight the high priority watercourses of each park where case by case will be decided and implemented the administrative and/or legal actions.

WHERE: Removal of physical barriers will be performed in the sites selected according to the Action A3 results in the following parks: PNP, PNMS, PNFC, PNM.
Removal of hydrological barriers will be performed in all the sites of the concrete conservation actions.

WHEN:
Removal of physical barriers will be finalized within October 2022
The MVF evaluation will be carried out in Aug-Sept 2021 and 2022

WHY: Native fish require unimpeded access along waterways in order to survive and reproduce. Barriers to fish passage prevent the migration of native fish species and can have severe implications for these populations, even as a consequence of environmental modifications foreseen in Italian watercourses related to climate-change. The most probable response of native specimens to the increasing water temperature will be the upstream migration, following their thermal optimum. In extreme cases, barriers can result in local extinctions, and the removal of barriers represents an effective way to increase the resilience of native fish populations to climate-related risks. In addition, the L.D. 152/2006, transposition of the Water Framework Directive, identifies the river continuity as one of the hydro-morphological elements supporting the classification of the ecological status of rivers, and the good ecological status can be achieved only where the impacts of anthropogenic activities on the continuity is limited, and the undisturbed migration of the animals is guaranteed.

D1 MONITORING OF PROJECT RESULTS

Beneficiary responsible for implementation: ISPRA

Responsibilities in case several beneficiaries are implicated: ISPRA in in charge of the monitoring of genetic data related with all the conservation strategy, UNIPG will be in charge of the monitoring of habitat status. PARKS+FOR collect and provide the field data. ISPRA and UNIPG are in charge of designing the Monitoring report on the respective field of expertise

WHAT This action aims to evaluate the impact of the concrete conservation actions in terms of population status (C.1, C.2, C.3) and habitat status (C.4), and to document the effectiveness of the project actions as compared to the initial situation, objectives and expected results. The data analysed and the information gathered during the action will be used for the updating of performance indicators table for the mid-term and final report and contribute to the drafting of the National Guidelines for *S. cettii* conservation developed within the Action C5.

HOW

D.1.1 Monitoring of population status

The outcomes of the actions C.1, C.2 and C.3 will be evaluated by genetic analysis carried out on the specimens collected during field actions by park technicians in the project phase
For each concrete conservation action specific expected results are foreseen and indicators and baseline identified. During the Action A.3 the baseline will be updated according with the results of the ecological and genetic characterization and with the data on habitat status
Here following the specific parameters adopted for each one of the concrete conservation actions:

Reproduction of pure wild spawners C.1 The analysis will be performed with quantitative and qualitative indicators:

1) qualitative: it will be performed an analysis on morphological characteristics of eggs and fry to verify the dimension and presence of malformation. The analysis will be made a sampling of 100 eggs and 100 fry photographed with a millimetric reference scale
2) quantitative: Numbers of fecundate eggs and fry produced yearly

The parks + Forestas technicians will gather and store the data for each hatchery and mating season and send data to UNIPG and ISPRA for processing

Translocation of wild native trout C.2.1 The impact of the action will be monitored in terms of:
1) reduction of introgression rate in the “sink population” through a sampling action carried out by the park + FOR technicians in the last project year. It will be analyzed 120 specimens 20 per 1 site x 6 parks.
2) the impact on the source populations age structure and abundance through a quantitative sampling action following the same methodology described in Ac A3. The parks + FOR technicians will gather and store the data whilw UNIPG and ISPRA will in charge of data processing and evaluation.

Selective fishing C.2.2 The impact of the action will be monitored in terms of:

1) reduction of introgression rate in target population through a sampling action carried out by the park + FOR technicians in the last project year. It will be analyzed 120 specimens (20 per 1 site x 6 parks)
2) capability of the anglers to recognize non- native trout by phenotype by analyzing the fins collected by the anglers in each selective fishing season. A random sample of 360 fin will be analysed (20 per 1 site x 6 parks x 3 years)

Stocking with native fry C.2.3 The impact of the action will be monitored in terms of reduction of introgression rate in target population through a sampling action carried out by the park + FORESTAS technicians in the last project year. It will be analyzed 220 specimens (20 X 11 sites – 1 per hatchery installed)

Selective fishing and stocking with native trout C.2.4 it will be used the same methodology described to monitor actions C.2.2. Selective fishing and C.2.3 Stocking with native fry

Eradication C3: aimed at assessing the effectiveness of alien trout removal actions through a one quantitative sampling (removal method) during the last eradication event of the project, with the aim to determine the abundance and the age structure of the alien trout populations. The parks + Forestas technicians will gather and store the data and UNIPG will in charge of data processing and evaluation

Task D.1.2 Monitoring of habitat status
The outcomes of the task will be evaluated by the effectivity of the fish passages. Two types of activities will be carried out:
1) ***Direct verification of fish passage usage.*** This control will be performed by capturing at least 150 specimens downstream of each fish passage and tagging them with visible implant elastomer (VIE). Following a mark-recapture protocol on the proportion of tagged fish migrating upstream a measure of fish passages effectivity will be estimated; a series of 3 intervention carried out by the Parks technicians are expected: the 1st aimed to tag the fish downstream of the passage, the 2nd and the 3rd (within one month of each other) in order to monitor the fish that will be moved upstream.

2) ***Verification*** **of effectiveness of removal of hydraulic barriers**
Data on stream flow will gathered in each intervention site (Action C4) while after the removal of hydraulic barriers a quantitative analysis on the demographic characteristics (abundance, age structure, etc…) of trout populations will be performed. The comparison between the data collected before and after the removal will allow effectiveness of removal of hydraulic barriers in improving the status of trout's populations. All these data will contribute to the drafting of the National Guidelines for *S. cettii* conservation developed within the Ac C5.

WHERE The specimens will be collected in the hatcheries and in the site of the concrete conservation actions.

WHEN From months 13th to 51th.

WHY Although supplementation management practices (e.g. 100% local, wild-origin brood stock) can successfully boost population size with minimal impacts on the fitness (see Hess et al., 2012, Molecular Ecology, 21: 5236-5250) of fish in the wild, the risk of introduction of undesirable effects (e.g.: bottleneck effects, low effective population size, inbreeding depression, etc.) during the artificial reproduction phase is concrete. For this reason, there is the need for a "before" and "after" genetic comparison between the genetic variability of the "source" population and the genetic variability of F1 native fry. About the C.2 supplemental strategies, also in this case very encouraging results has been observed (see Gil et al., 2016, Rev Fish Biol Fisheries, 26: 1-11, and references there in). However, the evaluation of the impact of the different supplemental strategies adopted in this project is fundamental to understand which strategy worked best. This information is very important to draft conservation management guidelines (see action C.5) and to plan the post Life activities. Finally, it is also evident the need for an evaluation of the impact of the fish passages. Although the task C.4.2 foreseen a preliminary study for fish passage installation, these structures could not work properly. Thank to the task D.1.2 the proper operation of fish passages will be evaluated and, in case, changing will be proposed. In fact, correct planning and implementation of fish passages is an essential part of any large-scale attempts to maintain the best possible biotic integrity in the aquatic systems and to increase the resilience of native fish populations to climate-related risks.

E.2 REPLICABILITY AND TRANSFERABILITY AT HORIZONTAL AND VERTICAL LEVEL

Beneficiary responsible for implementation:
PNM

Responsibilities in case several beneficiaries are implicated:
PNM is responsible for Task E.2.1, all the other partners except LEG will perform transfer action.
ISPRA is responsible for Task E.2.2, all the partners are involved.

Description and methods employed (what, how, where, when and why) (Maximum Characters: 7000)

WHAT: This action is aimed to ensure the replicability and transferability of the approach in terms concrete interventions on *S. cettii* populations and habitat restoration and, at the same time, it wants to pave the way for the development of National Plan for the management and conservation of the species in its native range.

The action foresees a double levels strategy:
- At horizontal level 11 protected areas' managing authorities, representing the major part of the whole species native range, will be objective of concrete transfer interventions aimed at providing the knowledge base regarding ecological and genetical status of *S. cettii* and the characterization of its habitat.
- At vertical level the National Authorities in charge of the planning and implementation of conservation policies of the species (Ministry of Environment, ISPRA, National and Regional parks, N2000 areas managing authorities) will be engaged in national table to discuss the key aspect for management of *S. cetti*.

This action is based on the transferring of the National guidelines for *S. cettii* conservation developed in the Action C5 and adopted by the 6 protected areas directly engaged in the project.

HOW
The action is divided into 2 different Tasks:
Task E2.1: Horizontal transfer: Guidelines application in NATURE2000 protected areas
According with the guidelines developed under the responsibility of ISPRA, the staff engaged in the concrete conservation actions in the six project pilots will be in charge of supporting the technicians of 11 identified transfer areas in the ecological and genetic characterization of sample sites and the analysis of the respective habitat status; in details:
- PARCO SIB: Umbria Region and Gran Sasso e Monti della Laga National Park
- PNM: Abruzzo, Lazio e Molise National Park, Sirente Velino Regional Park
- PNFC: Tosco Emiliano National Park e Parks and Biodiversity of Romagna managing authority
- PARCOMAGRA: Alpi Marittime Regional Park, Monviso Regional Park
- PNP: Cilento National Park, Aspromonte National Park
- FORESTAS: Corse Region

The process foreseen:
- Site selection. Each transfer area in agreement with the ecological and genetical available data will select a number of sites to perform field actions. It is envisaged to select an overall number of 66 sites in the 11 transfer areas. (Jan – Feb 2022)
- Training activity addressed to area technicians aimed to provide the technical knowledge to implement the National Guidelines. The training activities will be performed through e-learning utilizing the ISPRA e-learning platform (Mar-Apr 2022)
- Field action. In each of the transfer area will be carried out ecological and genetical characterization of an identified number of sample populations and respective habitat. In particular the technicians of the pilot areas will perform 2 field visit in each transfer area. (May-Sept 2022)
  The matching between the pilot areas and transfer areas is made in agreement with their geographical, geological and ecological conditions.

- Data Analysis. The data and specimens gathered in the field actions will be analyzed by ISPRA and UNIPG in order to design for each transfer area a comprehensive study regarding populations and habitat. (Sept-Dec 2022)
- Managing measures. In each transfer area the N2000 sites managing measures will be analyzed to identify needed updating in agreement with Guidelines and local study regarding populations and habitat. (Dec 22-Aug 23)

Task E2.2: Vertical transfer: Toward a National Conservation Strategy.
The aim of this task is to start up a process of negotiation with all the national bodies in charge of biodiversity management to design a National Plan for *S. cettii* conservation.
A national technical table will be organized and coordinated by ISPRA with the involvement of the Ministry of Environment and N2000 national, regional and local authorities, with the support of scientific communities.
The debate will be based on the project knowledge, lesson learnt, key actors and stakeholders input arise during the whole project implementation: National strategy for *S. cettii* conservation, N2000 managing measures, Stakeholders tables feedback, technical scientific knowledge.
The key topics discussed in the table's meetings will be:

- river fragmentation and habitat loss;
- water abstraction;
- genetic introgression and alien species introductions;
- overfishing and sport fishing management;
- integration between *S. cetti* conservation and WFD 2000/60 CE;
- adaptation on climate change.

All the input of the discussion of the round table will summarized in Policy recommendation presented in the Final Project Conference organized in Rome in the last project month.

WHERE:

Task E2.1 Horizontal transfer: The park where the transfer action will be implemented are:

- Abruzzo, Lazio e Molise NP
- Sirente Velino RP
- Alpi Marittime RP
- Monviso RP
- Tosco Emiliano NP
- Parks and Biodiversity of Romagna MA
- Umbria Region
- Gran Sasso e Monti della Laga NP
- Cilento NP
- Aspromonte NP
- Corse

Task E.2.2 The meeting of the technical table will be organized in ISPRA in Rome, the Final conference will be organized by Legambiente in Rome

WHEN: The action will be implemented from Jan 22 to Nov 23

WHY:

Task E.2.1: Horizontal transfer will ensure the updating of the N2000 sites managing measures in 11 transfer areas and it will allow to collect data and information to design a National Plan for *S. cettii* conservation.

Task E.2.2: Vertical transfer will develop a debate ensuring the adoption of a set of common policies at national level to mitigate the threats and to improve the conservation status of *S. cettii*.

TIMETABLE

Action		2019				2020				2021				2022				2023				2024			
Action numbe	Name of the action	I	II	III	IV	I	II	III	IV	I	II	III	IV	I	II	III	IV	I	II	III	IV	I	II	III	IV
A. Preparatory actions, elaboration of management plans and/or of action plans																									
A.1	PROJECT START UP AND PERMITS ISSUING			■	■																				
A.2	LIFE STREAMS PROJECT FIELD MANUAL AND TECHNICAL PERSONNEL TRAINING			■	■	■	■																		
A.3	POPULATION AND HABITAT CHARACTERIZATION						■	■																	
B. Purchase/lease of land and/or compensation payments for use rights																									
C. Conservation actions																									
C.1	REPRODUCTION OF WILD PURE SPAWNERS							■	■	■	■	■	■	■	■	■	■	■	■	■	■				
C.2	SUPPLEMENTAL CONSERVATION STRATEGIES AND SELECTIVE FISHING							■	■	■	■	■	■	■	■	■	■	■	■	■	■				
C.3	ALIEN SPECIES REMOVAL							■	■	■	■	■	■	■	■	■	■	■	■	■	■				
C.4	FRESHWATER HABITAT IMPROVEMENT							■	■	■	■	■	■	■	■	■	■	■	■	■	■				
C.5	NATIONAL GUIDELINES FOR SALMO CETTII CONSERVATION							■	■	■	■	■	■	■	■	■	■	■	■	■	■				
C.6	ACTIONS AGAINST ILLEGAL STOCKING PHENOMENON					■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■				
D. Monitoring of the impact of the project actions (obligatory)																									
D.1	MONITORING OF PROJECT RESULTS							■	■	■	■	■	■	■	■	■	■	■	■	■	■				
D.2	MONITORING ON SOCIO ECONOMIC IMPACT					■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■				
D.3	MONITORING ON ECOSYSTEM FUNCTIONS RESTORATION					■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■				
E. Public awareness and dissemination of results (obligatory)																									
E.1	COMMUNICATION, AWARENESS AND NETWORKING			■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■				
E.2	REPLICABILITY AND TRANSFERABILITY AT HORIZONTAL AND VERTICAL LEVEL													■	■	■	■	■	■	■	■				
F. Project management (obligatory)																									
F.1	PROJECT MANAGEMENT			■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■				
F.2	FINANCIAL MANAGEMENT AND AUDITING			■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■				
F.3	AFTER-LIFE CONSERVATION PLAN			■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■				