

RESEARCH PROGRESS STATUS

Title of the project: “Concrete conservation actions for the Etruscan chub *Squalius lucumonis* and the Italian nase *Protochondrostoma genei* in central Italy”

Target species: the Etruscan chub *Squalius lucumonis* and the Italian nase *Protochondrostoma genei*

Reporting period: November 2020 - April 2021

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In November 2020, the fish samplings aimed at the identification of suitable sites for *P. genei* restocking activities in the Esino river basin (Marche Region) were completed. Because of the third wave of coronavirus pandemic that invested central Italy, in the period between December 2020 and February 2021 it was not possible to carry out field activities, while laboratory and data processing activities continued. In March and April 2021, the eradication of alien species has been

carried out in the 2 previously selected sites on the Carpina and Argento streams. The eradication activities were accompanied by fish and environmental monitoring at the control sites (Table 1). As in the previous monitoring, for each sampling site the

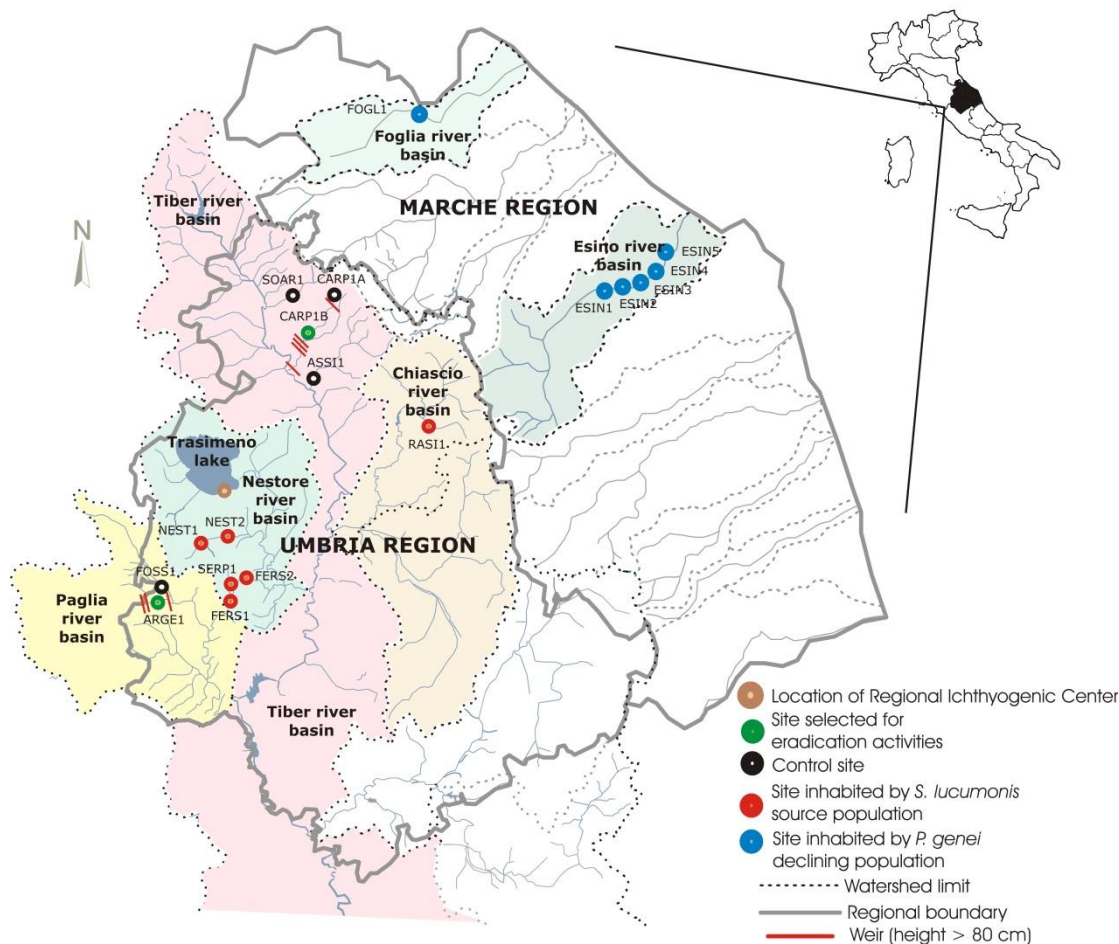


Figure 1. Location of the study area in central Italy. The main map includes the location of the sampling sites involved in the different conservation actions.

composition of the fish assemblages, and the density and standing crop for each population are being evaluated. For all the species caught, the age structure and growth are being estimated. At the same time as the electro-fishing activities, morphological, hydrological and chemical-physical parameters were measured. Environmental characterization have been essential to: i) ascertain the suitability of the eradicated sites for Etruscan chub restocking activities, ii) show the similarity with the control sites, and iii) discard the hypothesis that changes that have occurred over time may have influenced the results of eradication and restocking activities in the eradicated sites.

The Italian nase captured in the eradicated sites were stored at the regional ichthyogenic center of Sant'Arcangelo sul Trasimeno (Perugia, Italy); they were partly destined for the production of specimens to be used for the restocking of Slovenian watercourses within the concrete conservation actions of the LIFE FOR LASCA project (LIFE16 NAT/SI/000644). The location of the ichthyogenic center is showed in Figure 1.

Material and methods

Study area

In Figure 1 the location of the study area in central Italy and the location of the sampling sites is shown. Compared to the previous report, a new control site has been added on the Carpina creek (CARP1A); the site is located upstream of the weir that limits the eradicated site CARP1B; this addition to the study design was motivated by the need to verify the presence of alien species in the upper stream reaches of Carpina creek, as they could recolonize the eradicated stretch located downstream in the future.

Fish data collection

A total of 7 fish samplings were carried out from 05/11/2020 to 01/04/2021 (Table 1). The methodology used for fish sampling, laboratory activities and analysis of fish data (still in progress) is the same as described in previous reports.

WATERSHED	WATERCOURSE	SAMPLING SITE CODE	GEOGRAPHIC COORDINATES	DATE OF SAMPLING
Esino	Esino river	ESIN1	43° 21' 11" N; 12° 59' 56" E	05/11/2020
Tiber	Carpina creek	CARP1B	43° 22' 42" N; 12° 20' 52" E	16/03/2021
	Carpina creek	CARP1A	43° 23' 2" N; 12° 21' 85" E	22/03/2021
	Soara creek	SOAR1	43° 26' 53" N; 12° 17' 30" E	22/03/2021
Paglia	Argento creek	ARGE1	42° 52' 28" N; 11° 59' 56" E	25/03/2021
	Fossalto creek	FOSS1	42° 53' 24" N; 11° 59' 03" E	26/03/2021
Tiber	Assino creek	ASSI1	43° 17' 11" N; 12° 22' 02" E	01/04/2021

Table 1. List of sampling sites with their codes, geographical coordinates and date of fish sampling.

Environmental characterization

Environmental data were collected during the preliminary surveys at the same time as fish data collection. Two hydrological (average current speed, flow rate) and 5 physico-chemical parameters (water temperature, pH, conductivity, dissolved oxygen, percentage of oxygen saturation) were used to characterize the sampling locations. The flow rate was measured at the cross-sectional area of each sampling reach, using an OTT MF-pro electromagnetic current meter (ISO 748:2007 Hydrometry Measurement of liquid flow in open channels using current-meters or floats). Field measurements of specific conductivity, pH, water temperature and dissolved oxygen were carried out with a multiparameter probe by Hanna Instruments. Moreover, canopy cover, substrate size and shaded areas were estimated by visual observation as the percentage of the total surface. The lithology was determined from the Italian geo-lithological map 1:500000 (WMS Service - pcn.minambiente.it). Altitude was derived from digital maps (GIS).



Figure 2. Measurement of the water flow rate on the Fossalto stream.

Results

On the basis of the results obtained, the progress status for each action is described in detail below. Moreover, the number of specimens for each species and for each sampled site are reported. The results of the environmental characterization were also shown.

Progress status for each action

Action 1: Alien species eradication activities and Italian nase restocking actions.

The first round of eradication activities, together with the monitoring of control sites, has been completed. A total of 350 *P. genei* and 19 *P. bonelli* were removed.

CARP1A (control site) (Fig. 3): the results of the fish sampling confirmed the absence of *P. genei* in the river stretch located upstream the eradicated site; therefore, the possibility that the Carpina stream will be re-colonized by *P. genei* specimens coming from upstream can be excluded. Except for one specimen belonging to *P. bonelli*, all the other species caught (*Squalius squalus*, *Sarmarutilus rubilio*, and *Telestes muticellus*) were native. CARP1B (eradicated site) (Fig. 4): a total of 310 Italian nase and 19 Padanian gobies were caught and removed; all the other species were measured and then released in their natural environment. At the end of the field activities, all the Italian nase were transported to the Regional Ichthyogenic center of Sant'Arcangelo sul Trasimeno (Fig. 5), and stored until they are used for restocking programs.

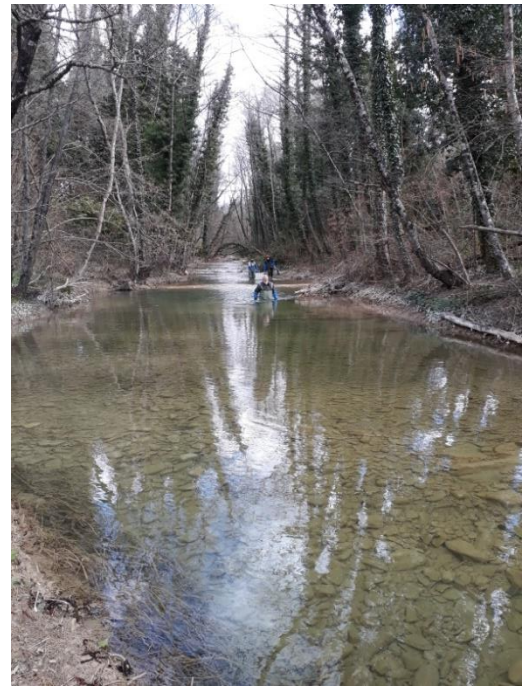


Figure 3. CARP1A: control site located on the Carpina creek upstream the insurmountable weir.



Figure 4. CARP1B: eradication activities on the Carpina creek downstream the insurmountable weir.



Figure 5. CARP1B: transfer operations of eradicated Italian nase.



Figure 6. Regional ichthyogenic center of Sant’Arcangelo sul Trasimeno: storage of eradicated Italian nase.

SOAR1 (control site): in this control site the fish sampling revealed the presence of 120 Italian nase and 14 Padanian gobies. All the other species were native. All specimens were measured and then released in their natural environment, in order not to influence the comparison with the eradicated sites (Fig. 7).

ASSI1 (control site): In this station the presence of the following 3 alien species has been detected: *Barbus barbus*, *Gobio gobio* and *Padogobius bonelli*, which was the dominant species in the fish community. As in the other sites, also in this case the native species are typical of the barbel zone: *Barbus tyberinus*, *Padogobius nigricans*, *Telestes muticellus*. and *Sarmarutilus rubilio*.

ARGE1 (eradicated site). Fourty *P. genei* individuals were caught and removed. All other species were native. The most represented species was *S. rubilio* with 321 specimens.



Figure 7. Release of fish into the river at the end of field activities.

Action 3: Identification of 5 P. genei declining populations in the Esino river basin (Marche Region). The fish sampling results revealed the total absence of *P. genei* on this site, where once there was an abundant population. Except for *Barbus tyberinus* whose origin is still uncertain, all the other species detected (*Alburnus arborella*, *Barbus plebejus*, *Padogobius bonelli*, *Sarmarutilus rubilio*, *Squalius squalus*, *Telestes muticellus*) were native. These results confirmed the suitability of the river stretch for the reintroduction of the species, which will be carried out at the end of April 2021.



Figure 8. Sampling site ESIN1 suitable for *P. genei* restocking activities.

Critical issues: As a consequence of the restrictions put in place by the Italian government to face the third waves of Coronavirus pandemic, it was not possible to carry out field activities in the period from December 2020 to February 2021. However, considering the project timeline (Table 3), we don't believe that the health emergency can determine significant changes in the project outcomes, as we will try to intensify the eradication and restocking activities and make up for lost time in the coming months.

	2019				2020												2021								
	Sep	Oct	Nov	Dec	Gen	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Gen	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep
Identification of Etruscan chub populations in good conservation status																									
Selection of suitable sites for eradication activities of the alien species																									
Identification of Italian nase declining populations																									
Request authorizations to the competent authorities																									
Field inspections																									
Eradication activities and Italian nase restocking actions																									
Etruscan chub traslocations																									
Progress reports																									

Table 3. Project timetable.

Added values for the project: We continued our collaboration within the European “LIFE for Lasca” project. Last April 14th, all the *P. genei* adult specimens collected from the eradicated sites have been transported to the Ticino National Park, where the adults will be used to produce juveniles for restocking activities in Slovenian streams (Fig. 5).



Figure 5. Eradicated Italian nase transfer operations.

In the annex below, for each site, the results of fish sampling, in terms of fish assemblage composition, and number of specimens caught for each species, and the outcomes of the environmental characterization are reported.

Esino river – ESIN1 – Site suitable for *P. genei* restocking activities

Species	Number of specimens
<i>Alburnus arborella</i>	22
<i>Barbus plebejus</i>	73
<i>Barbus tyberinus</i>	8
<i>Padogobius bonelli</i>	23
<i>Sarmarutilus rubilio</i>	8
<i>Squalius squalus</i>	82
<i>Telestes muticellus</i>	18

Environmental parameters	
Water temperature (°C)	14.01
Conductivity ($\mu\text{S cm}^{-1}$)	557
pH (units)	8.37
Dissolved oxygen (mg L^{-1})	10.74
Oxygen saturation (%)	105.5



Electrofishing on the Esino river



Protochondrostoma genei

Carpina creek – CARP1A – Control site

Species	Number of specimens
<i>Padogobius bonelli</i>	1
<i>Sarmarutilus rubilio</i>	36
<i>Squalius lucumonis</i>	36
<i>Squalius squalus</i>	1
<i>Telestes muticellus</i>	8

Environmental parameters	
Water temperature (°C)	4.21
Conductivity ($\mu\text{S cm}^{-1}$)	584
pH (units)	8.6
Dissolved oxygen (mg L^{-1})	14.89
Oxygen saturation (%)	120.6
Average current speed (m s^{-1})	0.177
Flow rate ($\text{m}^3 \text{s}^{-1}$)	0.289



CARP1A - Measurement of the water flow rate



Squalius lucumonis

Carpina creek – CARP1B – Eradicated site

Species	Number of specimens
<i>Barbus tyberinus</i>	42
<i>Padogobius bonelli</i>	19
<i>Protochondrostoma genei</i>	310
<i>Sarmarutilus rubilio</i>	63
<i>Squalius cephalus</i>	1
<i>Squalius lucumonis</i>	12
<i>Squalius squalus</i>	118
<i>Telestes muticellus</i>	5

Environmental parameters	
Water temperature (°C)	4.49
Conductivity ($\mu\text{S cm}^{-1}$)	582
pH (units)	8.4
Dissolved oxygen (mg L^{-1})	15.60
Oxygen saturation (%)	126.2
Average current speed (m s^{-1})	0.375
Flow rate ($\text{m}^3 \text{s}^{-1}$)	0.351



CARP1B

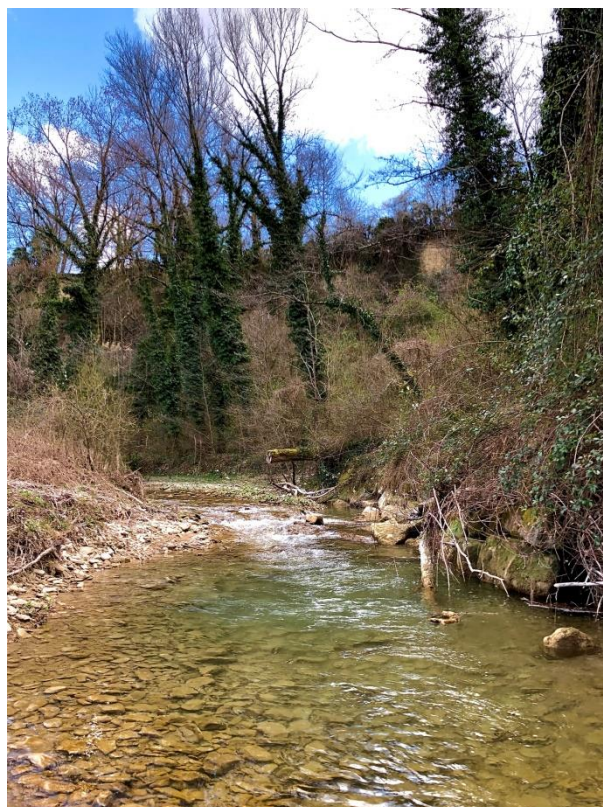


CARP1B - Measurement of the water flow rate

Soara creek – SOAR1 – Control site

Species	Number of specimens
<i>Padogobius bonelli</i>	14
<i>Padogobius nigricans</i>	5
<i>Protochondrostoma genei</i>	120
<i>Sarmarutilus rubilio</i>	63
<i>Squalius lucumonis</i>	16
<i>Squalius squalus</i>	93
<i>Telestes muticellus</i>	49

Environmental parameters	
Water temperature (°C)	6.99
Conductivity ($\mu\text{S cm}^{-1}$)	462
pH (units)	8.56
Dissolved oxygen (mg L^{-1})	13.53
Oxygen saturation (%)	117
Average current speed (m s^{-1})	0.341
Flow rate ($\text{m}^3 \text{s}^{-1}$)	0.297



SOAR1



Electrofishing on the Soara creek



Field activities

Argento creek – ARGE1 – Eradicated site

Species	Number of specimens
<i>Barbus tyberinus</i>	18
<i>Padogobius nigricans</i>	10
<i>Protochondrostoma genei</i>	40
<i>Sarmarutilus rubilio</i>	321
<i>Squalius lucumonis</i>	75
<i>Squalius squalus</i>	33

Environmental parameters	
Water temperature (°C)	10.5
Conductivity ($\mu\text{S cm}^{-1}$)	572
pH (units)	8.06
Dissolved oxygen (mg L^{-1})	12.29
Oxygen saturation (%)	113.6
Average current speed (m s^{-1})	0.049
Flow rate ($\text{m}^3 \text{s}^{-1}$)	0.146



Weir on the Argento creek



Electrofishing on the Argento creek

Fossalto creek – FOSS1 – Control site

Species	Number of specimens
<i>Barbus tyberinus</i>	2
<i>Padogobius nigricans</i>	10
<i>Sarmarutilus rubilio</i>	132
<i>Squalius lucumonis</i>	19
<i>Squalius squalus</i>	1
<i>Telestes muticellus</i>	42

Environmental parameters	
Water temperature (°C)	11.5
Conductivity ($\mu\text{S cm}^{-1}$)	551
pH (units)	8.18
Dissolved oxygen (mg L^{-1})	12.63
Oxygen saturation (%)	118.7
Average current speed (m s^{-1})	0.164
Flow rate ($\text{m}^3 \text{s}^{-1}$)	0.083



FOSS1

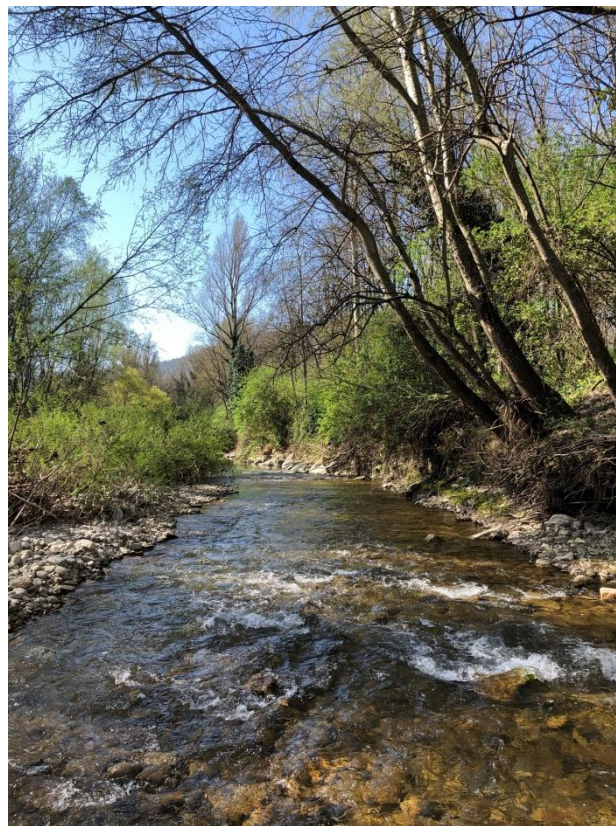


Padogobius nigricans - male

Assino creek – ASSI1 – Control site

Species	Number of specimens
<i>Barbus barbus</i>	3
<i>Barbus tyberinus</i>	13
<i>Gobio gobio</i>	3
<i>Padogobius bonelli</i>	30
<i>Padogobius nigricans</i>	2
<i>Sarmarutilus rubilio</i>	2
<i>Telestes muticellus</i>	42

Environmental parameters	
Water temperature (°C)	10.26
Conductivity ($\mu\text{S cm}^{-1}$)	540
pH (units)	8.43
Dissolved oxygen (mg L^{-1})	12.05
Oxygen saturation (%)	110.2
Average current speed (m s^{-1})	0.215
Flow rate ($\text{m}^3 \text{s}^{-1}$)	0.146



ASSI1



Barbus tyberinus