

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: April 2021 - November 2021

Project participants: Antonella Carosi, Silvia Carletti, Francesca Lorenzoni, Giovanni Tagliaferri, Daniele Pieracci, Alessandro Soresina, Massimo Lorenzoni.

The last part of the project, which took place in the period between April and November 2021, concerned the completion of the eradication activities of the alien species and the restocking activities in support of the declining populations of both target species, *P. genei* and *S. lucumonis*.

In April and May 2021, eradication activities of alien species continued in the selected sites on the

Carpina and Argento streams. As in the previous phases of the project, the eradication activities were accompanied by fish and environmental monitoring at the control sites (Table 1). For each sampling site the 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.

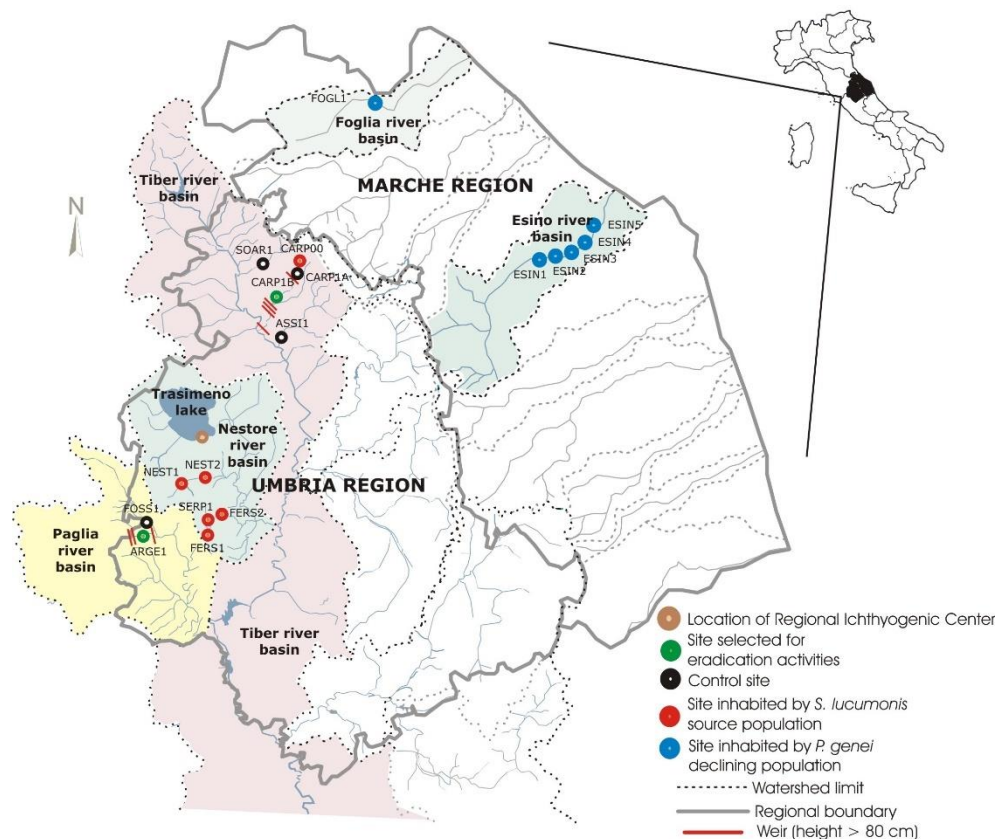


Figure 1. Location of the study area in central Italy. The main map includes the location of the sampling sites involved in the different conservation actions.

Part of the Italian nase captured in the eradicated sites stored at the regional ichthyogenic center of Sant'Arcangelo sul Trasimeno (Perugia, Italy) were used for restocking activities in sites inhabited by *P. genei* decreasing populations. On 20/05/2021, 3 kg of *P. genei* were used to repopulate the Esino River. The restocking activities and the picture of the selected site located in the Esino river were showed in Figures 2 and 3.



Figure 2. Restocking activities with Italian nase (20/05/2021).



Figure 3. Esino river. Site inhabited by decreasing Italian nase population (20/05/2021).

In November 2021, restocking activities were carried out to support populations of Etruscan chub inhabiting the eradicated sites, using specimens collected from source populations (Figures 4-7

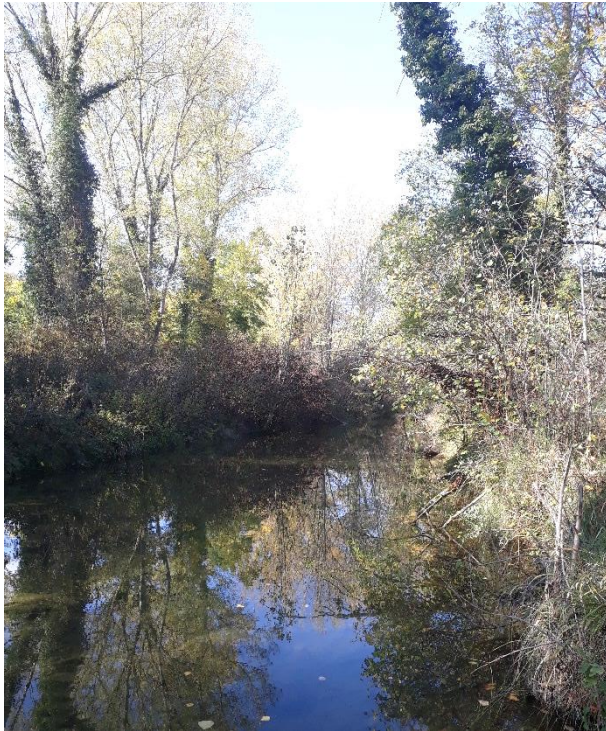


Figure 4. Fersinone creek: Etruscan chub source population site (18/11/2021).



Figure 5. Restocking of Etruscan chubs into the eradicated site on Argento creek (18/11/2021).

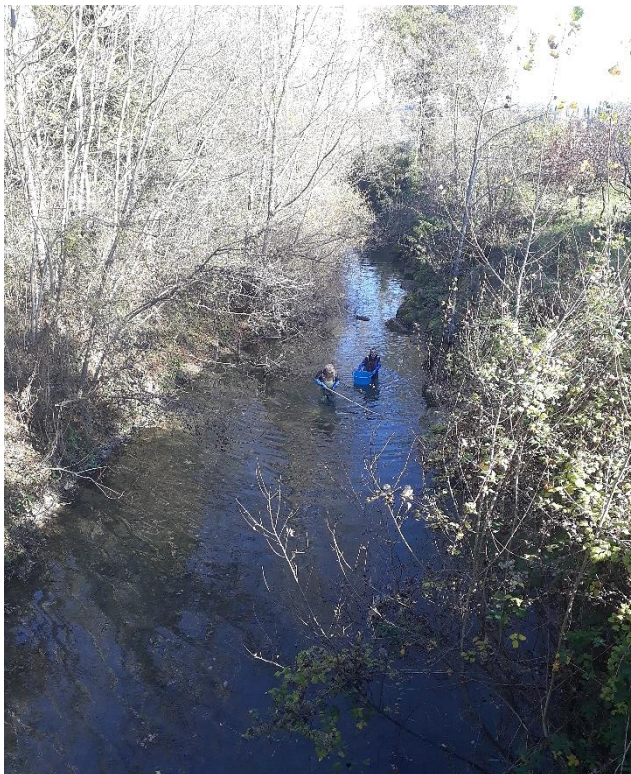


Figure 6. Etruscan chub collection from the source population on Carpina creek (24/11/2021).



Figure 7. Restocking of Etruscan chubs into the eradicated site (24/11/2021).

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 *S. lucumonis* source population site has been added on the Carpina creek (CARP00); the site is located upstream of the control site CARP1A; this addition to the study design was motivated by the need to replace the source population site located on the Rasina creek (Chiascio river basin) that in summer 2021 has completely dried.

Fish data collection

A total of 8 fish samplings and restocking activities were carried out from 22/04/2021 to 24/11/2021 (Table 1). The methodology used for fish sampling, laboratory activities and analysis of fish data (still in progress) was the same as described in previous reports.

WATERSHED	WATERCOURSE	SAMPLING SITE CODE	GEOGRAPHIC COORDINATES	DATE OF SAMPLING/RE STOCKING ACTIVITIES
Esino	Esino river	ESIN1 (<i>P. genei</i> restocking activities)	43° 21' 11" N; 12° 59' 56" E	05/11/2020
Tiber	Carpina creek	CARP1B (eradicated site- <i>S. lucumonis</i> restocking activities)	43° 22' 42" N; 12° 20' 52" E	22/04/2021 24/11/2021
	Carpina creek	CARP1A (control site)	43° 23' 2" N; 12° 21' 85" E	11/05/2021
	Carpina creek	CARP00 (source population)	43° 23' 40" N; 12° 22' 19" E	24/11/2021
	Soara creek	SOAR1 (control site)	43° 26' 53" N; 12° 17' 30" E	11/05/2021
Paglia	Argento creek	ARGE1 (eradicated site)	42° 52' 28" N; 11° 59' 56" E	04/05/2021
	Fossalto creek	FOSS1 (eradicated site- <i>S. lucumonis</i> restocking activities)	42° 53' 24" N; 11° 59' 03" E	18/05/2021
Nestore	Fersinone creek	FERS1 (source population)	42° 55' 11"N 12° 10' 04" E	18/11/2021

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

Environmental characterization

As in previous monitoring, environmental data were collected at the same time as fish data collection. The methodology used was the same as described in previous reports.

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 third round of eradication activities, together with the monitoring of control sites, has been completed. A total of 88 *P. genei* and 71 *P. bonelli* were removed.

CARP1A (control site) (Fig. 8): As in the previous monitoring, 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*, *Squalius lucumonis*, *Barbus tyberinus*, *Sarmarutilus rubilio*, and *Telestes muticellus*) were native.

CARP1B (eradicated site) (Fig. 4): a total of 73 Italian nase and 71 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, and stored until they were used for restocking programs.



Figure 8. CARP1A: control site located on the Carpina creek upstream the insurmountable weir (11/05/2021).

SOAR1 (control site) (Fig. 9): in this control site the fish sampling revealed the presence of 36 Italian nase and 40 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. The presence of the native crustacean *potamon fluviatile* should be noted (Fig. 10). The species is listed as near Threatened in the IUCN red list, where decreasing population is reported.

ARGE1 (eradicated site) (Figures 10-11). Fourteen *P. genei* individuals were caught and removed on this occasion. All other species were native. As in the previous monitoring, the most represented species was *S. rubilio* with 339 specimens. The presence of the alien crustacean *Procambarus clarkii* should be noted; even the specimens belonging to this species have been removed.

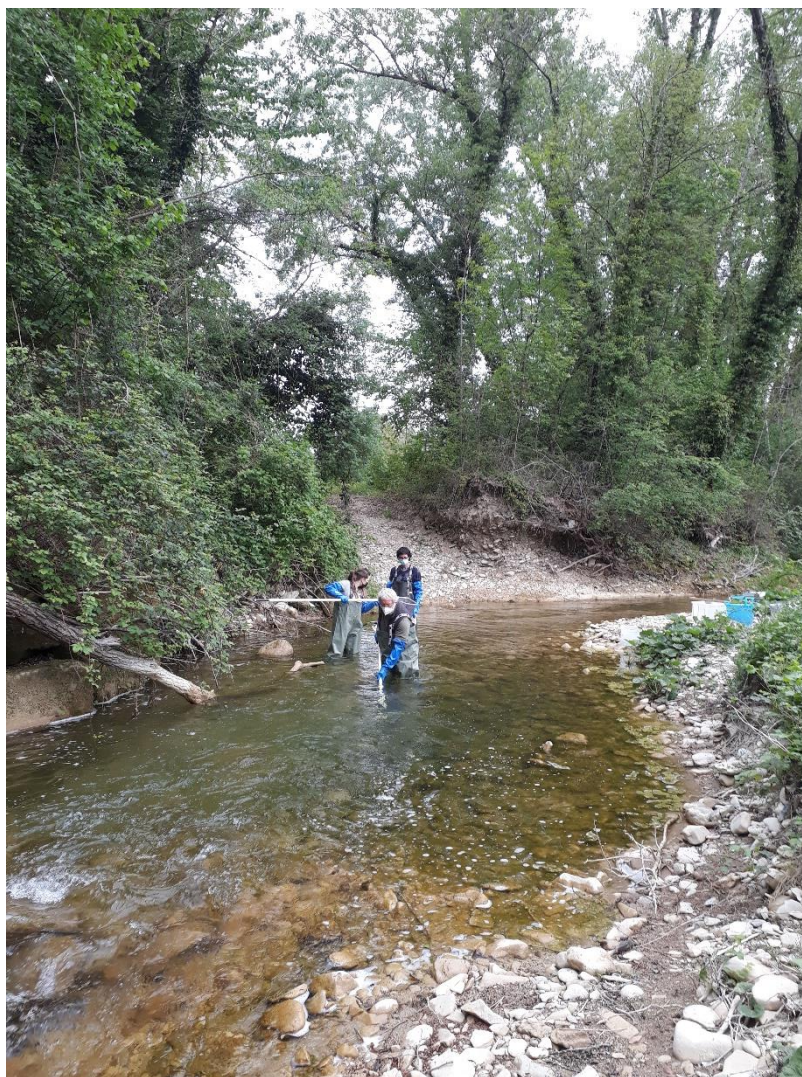


Figure 9. SOAR1: control site located on the Soara creek (11/05/2021).



Figure 10. *Potamon fluviatile* collected in the control site SOAR01 (11/05/2021).



Figure 11. *Procambarus clarkii* collected in the eradicated site ARGE1 (04/05/2021).

Action 4: Etruscan chub traslocations to improve the conservation status in the eradicated sites. Restocking activities have been carried out using selected wild populations from which wild *S. lucumonis* specimens were collected. In particular, on November 2021, 45 Etruscan chubs collected by electrofishing from the Fersinone river were introduced into the Argento creek, and 42 specimens were taken from a source population identified in the Carpina stream basin and placed into the eradicated stretch.

Etruscan chub conservation status improvement

The following histograms illustrate the trends in density and standing crop for the Etruscan chub over the sampling phases (Fig. 12). It is possible to observe that for both parameters increasing trends over time have been detected. These results, even if preliminary and require further verification, suggest a gradual recovery of the Etruscan chub populations subject to concrete conservation actions. Further samplings will also be conducted to verify the effectiveness of the restocking activities carried out using wild specimens collected from the source populations.

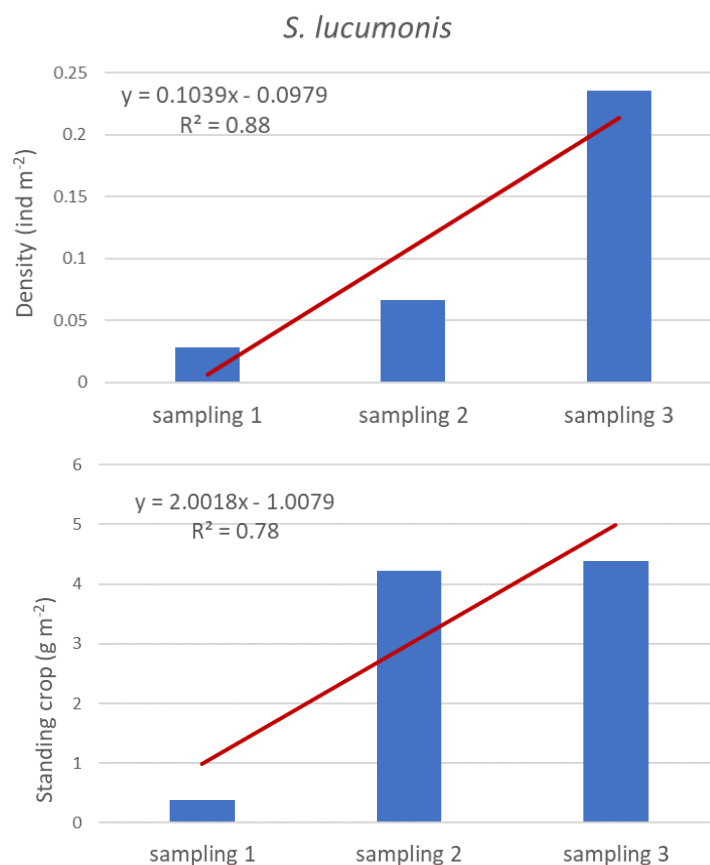


Figure 12. Mean density and standing crop values for the *S. lucumonis* total sample in the eradicated sites (CARP1B and ARGE1), broken down by the three fish samplings carried out between 22 May 2020 and 4 May 2021. Red lines indicate linear regression between mean values.

Action 5: Improvement of the Italian nase populations. Restocking activities using wild Italian nase individuals coming from eradicated sites were carried out to improve the population status in the Esino river. 20/05/2021, all the remaining Italian nase (for a total biomass of 3 Kg) stored at the Ichthyogenic center of Sant’Arcangelo sul Trasimeno, were translocated to the selected stretch for restocking activities of the Esino river.

Critical issues: Some setbacks did not allow us to complete within the scheduled time (May-September 2021) the action relating to the translocations of the Etruscan chubs from the source populations (i. e. from the sites with Etruscan chub populations in good conservation status) to the eradicated sites, where the alien species were removed. In particular, last summer was very drought in central Italy, and the sites identified for restocking activities were low in water. In autumn the environmental conditions were more favorable and the transfer activities were carried out during November 2021.

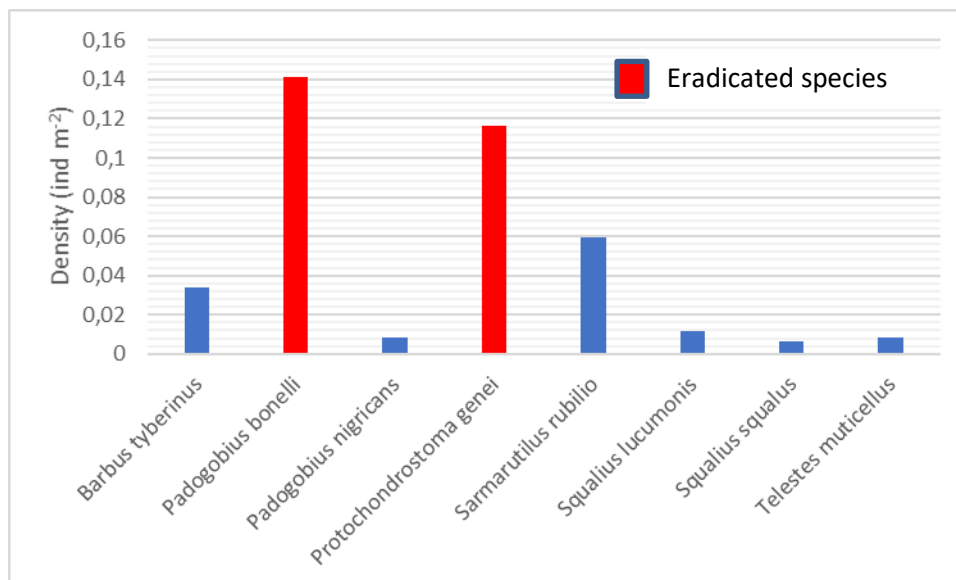
	Sep	Oct	Nov	Dec	Gen	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Gen	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
Identification of Etruscan chub populations in good conservation status																												
Selection of suitable sites for eradication activities of the alien species																												
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. Re-modulated project timetable.

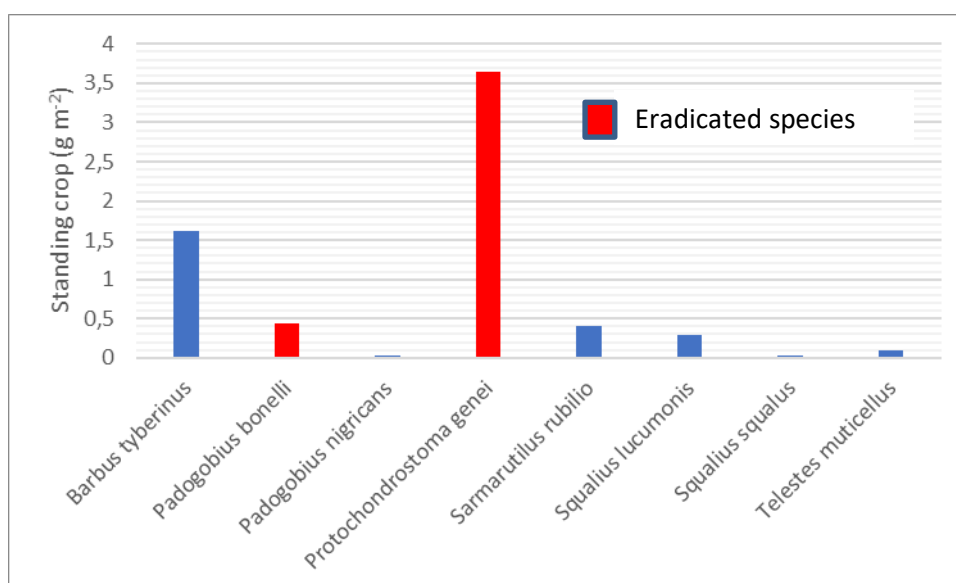
Future perspectives: Our intention is to continue monitoring in the next months to evaluate the effectiveness of the concrete conservation actions carried out within the project. We also plan to publish a scientific article on the results obtained.

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

Carpina river – CARP1B – Site suitable for alien species eradication and *S. lucumonis* restocking activities (22/04/2021).



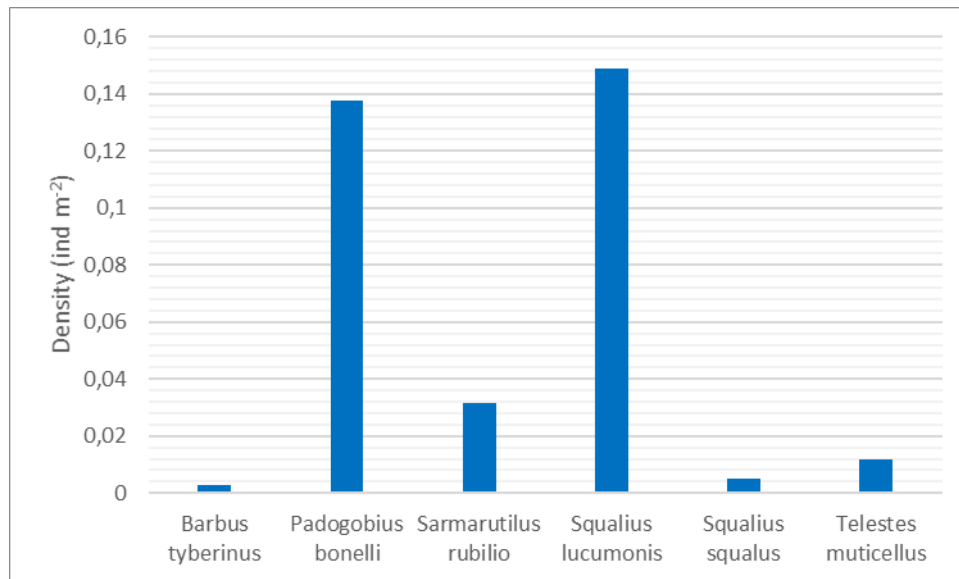
Fish species density



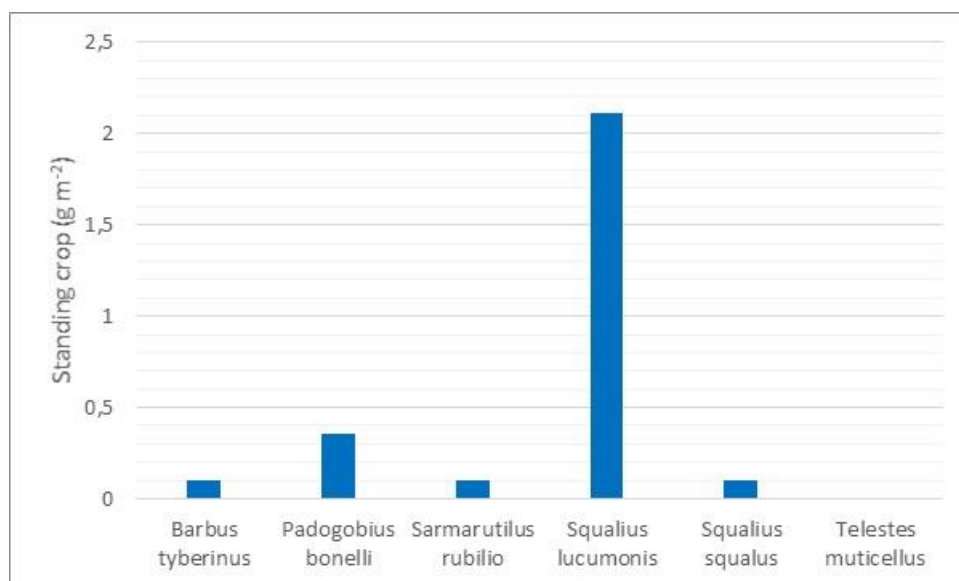
Fish species standing crop

Environmental parameters	
Water temperature (°C)	10.5
Conductivity (μS cm⁻¹)	556
pH (units)	8.74
Dissolved oxygen (mg L⁻¹)	13.2
Oxygen saturation (%)	122.7

Carpina creek – CARP1A – Control site (11/05/2021)



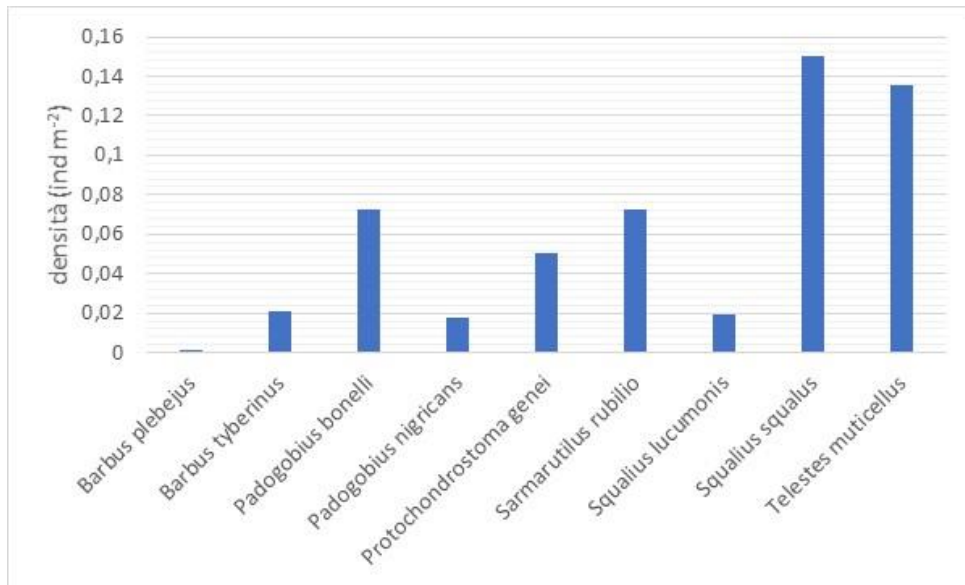
Fish species density



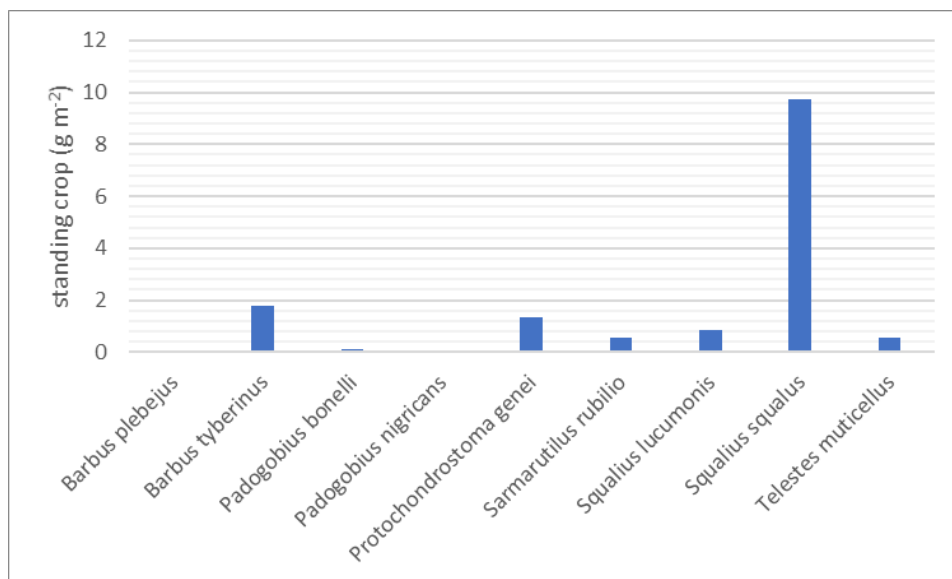
Fish species standing crop

Environmental parameters	
Water temperature (°C)	15.25
Conductivity (μS cm ⁻¹)	551
pH (units)	8.19
Dissolved oxygen (mg L ⁻¹)	11.05
Oxygen saturation (%)	116
Average current speed (m s ⁻¹)	0.177
Flow rate (m ³ s ⁻¹)	0.288

Soara creek – SOAR1 – Control site (11/05/2021)



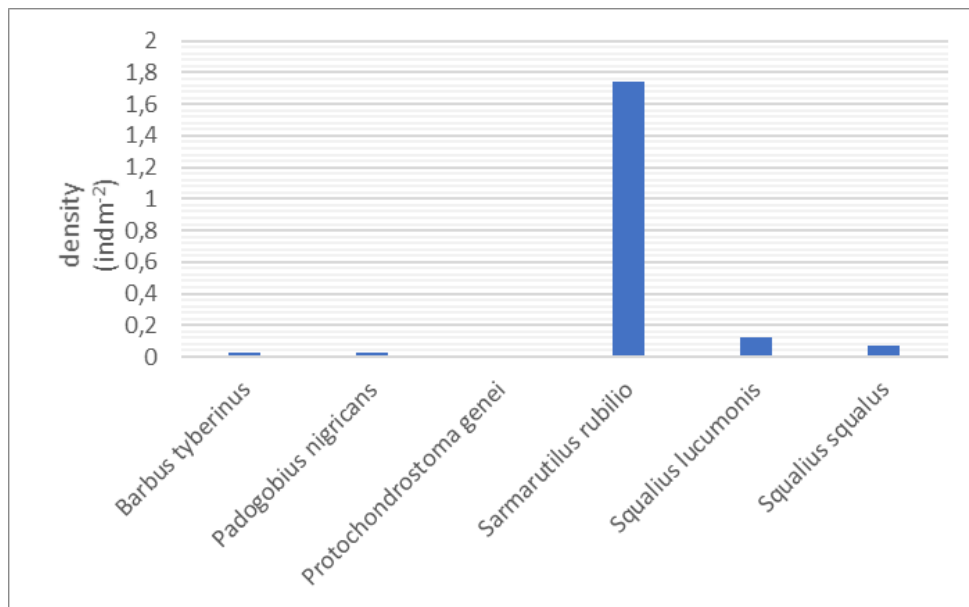
Fish species density



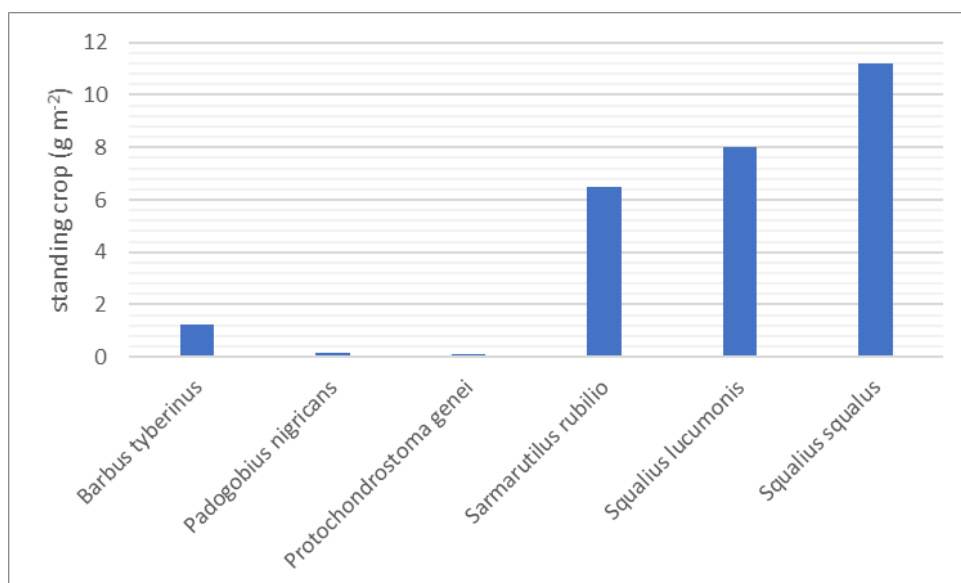
Fish species standing crop

Environmental parameters	
Water temperature (°C)	14.35
Conductivity (μS cm ⁻¹)	548
pH (units)	8.36
Dissolved oxygen (mg L ⁻¹)	11.80
Oxygen saturation (%)	119.4
Average current speed (m s ⁻¹)	0.274
Flow rate (m ³ s ⁻¹)	0.274

Argento creek – ARGE1 – Eradicated site (04/05/2021)



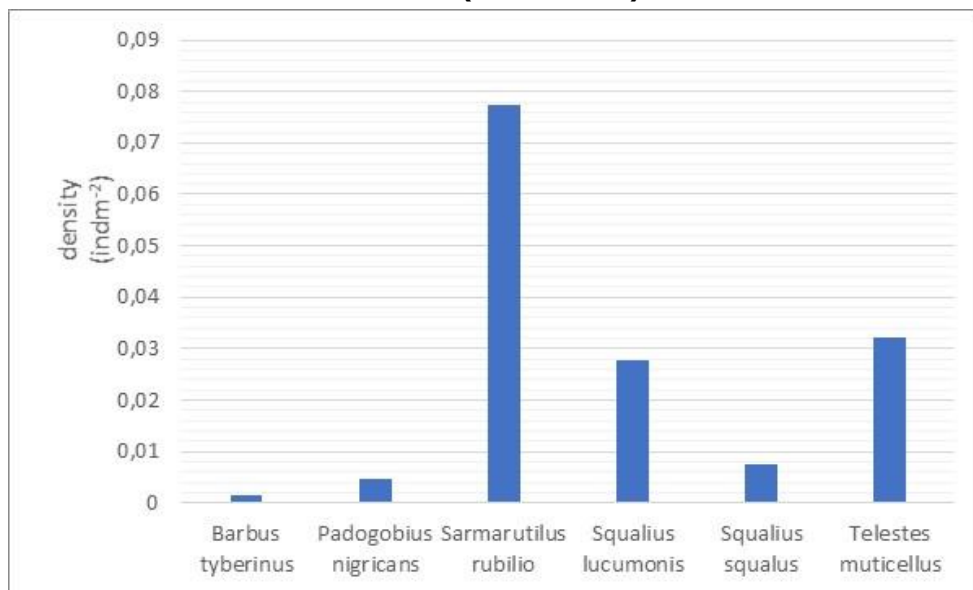
Fish species density



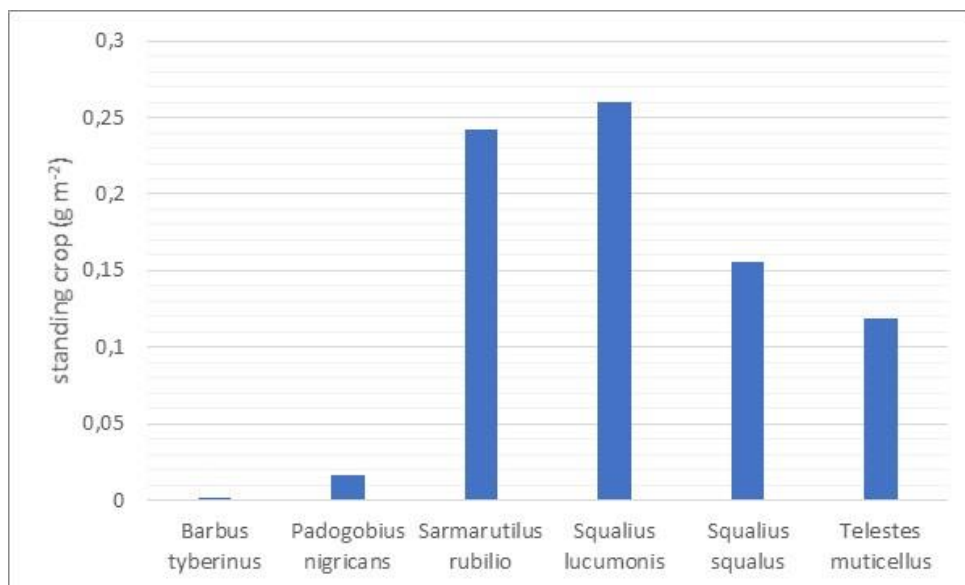
Fish species standing crop

Environmental parameters	
Water temperature (°C)	12.8
Conductivity (μS cm ⁻¹)	587
pH (units)	8.10
Dissolved oxygen (mg L ⁻¹)	11.7
Oxygen saturation (%)	113.8
Average current speed (m s ⁻¹)	0.05
Flow rate (m ³ s ⁻¹)	0.065

Fossalto creek – FOSS01 – Control site (18/05/2021)



Fish species density



Fish species standing crop

Environmental parameters	
Water temperature (°C)	17.9
Conductivity (μS cm ⁻¹)	574
pH (units)	7.81
Dissolved oxygen (mg L ⁻¹)	12.84
Oxygen saturation (%)	139.7
Average current speed (m s ⁻¹)	0.102
Flow rate (m ³ s ⁻¹)	0.042