

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: May - October 2020

Project participants: Antonella Carosi, Silvia Carletti, Alessandro Soresina, Francesca Lorenzoni, Massimo Lorenzoni.

Immediately after the end of the lockdown put in place by the Italian government to face the Coronavirus pandemic, it was possible to carry out field inspections and sampling activities. In particular, in order to analyse the ecological and demographic traits of fish populations, an extensive field activity was conducted between May and October 2020. For each sampling site, the composition of the fish assemblages, and the density and standing crop for each population were evaluated. In addition, for the Etruscan chub and the Italian nase, the age structure and growth were analysed. These information have been essential to ascertain the suitability of the sites chosen on the basis of the data reported in the Regional Fish Map. Moreover, the results obtained were needful to plan the concrete conservation actions, in terms of establishing how many Etruscan chub specimens to collect for restocking activities in the eradicated sites, and quantifying the presence of alien species that must be eradicated or, in the case of the Italian nase, transferred to their original range. Furthermore, at the same time as the sampling activities, the removal of two alien species (*Padogobius bonelli* and *Alburnus arborella*) began.

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.

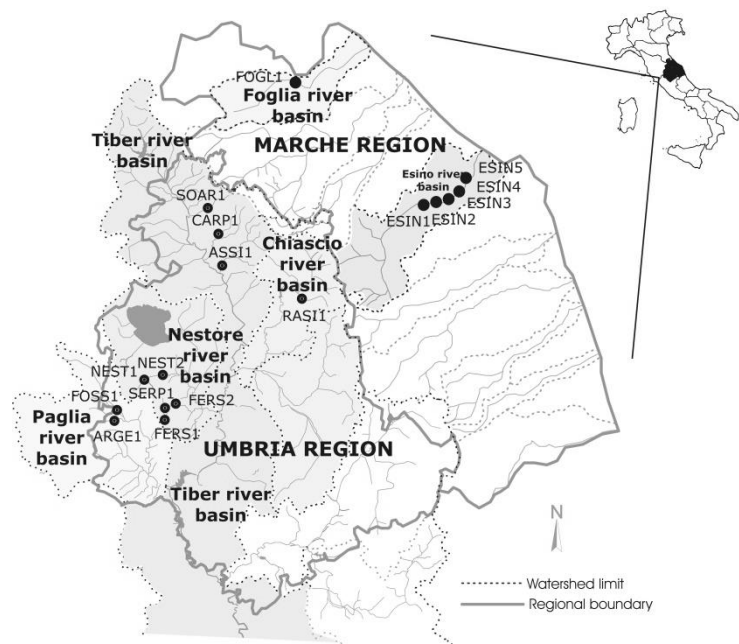


Figure 1. Location of the study area in central Italy. The main map includes watershed areas, hydrographic network within each basin, location of the sampling sites.

Fish sampling

A total of 12 fish samplings were carried out from 22/05/2020 to 09/10/2020 (table 1). The census of the fish fauna was carried out by electrofishing at each sampling location by two-pass electrofishing using the removal method (Moran, 1951; Zippin, 1956). The length of the sampling stretches, established as 10 times the wetted channel, was comprised within the range of 50–200 m. The two pass electrofishing method provided the sampling of the same river stretch (starting from downstream to upstream) for two consecutive times, applying the same fishing effort (Seber and Le Cren, 1967). Each specimen captured was anesthetized and then identified, the total length and weight was measured and a sample of scales was collected for age determination. At the end of the field activities, all captured fish (except for *P. bonelli* in the site CARP1 and for *A. arborella* in the site NEST1) were released into their natural environment. Age was determined in the laboratory using the scalimetric method.

WATERSHED	WATERCOURSE	SAMPLING SITE CODE	GEOGRAPHIC COORDINATES	DATE OF SAMPLING
Chiascio	Rasina creek	RASI1	43° 13' 53" N; 12° 39' 28" E	09/10/2020
Foglia	Foglia river	FOGL1	43° 44' 56" N; 12° 27' 07" E	26/08/2020
Nestore	Fersinone creek	FERS1	42° 54' 54" N; 12° 11' 41" E	01/07/2020
	Fersinone creek	FERS2	42° 55' 13" N; 12° 14' 53" E	30/09/2020
	Nestore river	NEST1	42° 57' 28" N; 12° 04' 16" E	30/09/2020
	Nestore river	NEST2	42° 59' 09" N; 12° 06' 02" E	30/09/2020
	Serpolla creek	SERP1	42° 55' 56" N; 12° 10' 37" E	24/06/2020
Paglia	Argento creek	ARGE1	42° 52' 28" N; 11° 59' 56" E	24/06/2020
	Fossalto creek	FOSS1	42° 53' 24" N; 11° 59' 03" E	24/06/2020
Tiber	Assino creek	ASSI1	43° 17' 11" N; 12° 22' 02" E	15/09/2020
	Carpina creek	CARP1	43° 22' 42" N; 12° 20' 52" E	22/05/2020
	Soara creek	SOAR1	43° 26' 53" N; 12° 17' 30" E	11/09/2020

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

Data analysis

For each population, the density (ind/m²) and standing crop (g/m²) were estimated. The population density was calculated by dividing the estimated number of fish belonging to each age group by the surface area of the sampling site. For *S. lucumonis* and *P. genei* the length-weight relationship (LWR) was calculated by the equation $W = a * TL^b$, where *W* is weight, *TL* is total length, *a* is the intercept on the Y-axis of the regression curve and *b* is the regression coefficient (Ricker, 1975).

Results

Fish census

A list of the species found is reported along with information about their Italian and global IUCN category (Table 2). 12 species of fish were found. 4 species are endemic to the Italico-peninsular ichthyogeographic district (IP): *B. tyberinus*, *P. nigricans*, *S. rubilio*, *S. lucumonis*). Other 4 species (*B. plebejus*, *P. bonelli*, *P. fluviatilis* and *P. genei*) are endemic to the Padano-venetian district (PV), and thus they are exotic in the Umbria Region since they were introduced in the Tiber river basin from other Italian basins. *S. squalius* and *T. muticellus* are native for both Italian ichthyogeographic districts. *Gobio gobio* is an alien species to Italy.

Scientific name	Common name	Origin	IUCN Italian category	IUCN global category
<i>Alburnus arborella</i> (Bonaparte, 1841)	Po bleak	PV	NT	LC
<i>Barbus plebejus</i> Bonaparte, 1839	Po barbel	PV	VU	LC
<i>Barbus tyberinus</i> Bonaparte, 1839	Tiber barbel	IP	VU	NT
<i>Gobio gobio</i> (Linnaeus, 1758)	Gudgeon	Alien		
<i>Padogobius bonelli</i> (Bonaparte, 1846)	Po goby	PV	LC	LC
<i>Padogobius nigricans</i> (Canestrini, 1867)	Arno goby	IP	VU	VU
<i>Perca fluviatilis</i> Linnaeus, 1758	Perch	PV		LC
<i>Protochondrostoma genei</i> (Bonaparte, 1839)	Italian nase	PV	EN	LC
<i>Sarmarutilus rubilio</i> (Bonaparte, 1837)	South Italian roach	IP	NT	NT
<i>Squalius lucumonis</i> (Bianco, 1983)	Etruscan chub	IP	CR	EN
<i>Squalius squalus</i> (Bonaparte, 1837)	Chub	Italy	LC	LC
<i>Telestes muticellus</i> (Bonaparte, 1837)	Italian riffle dace	Italy	LC	LC

Table 2. List of fish species, origin and their threatened status according to IUCN RED LIST criteria.

On the basis of the results obtained, the progress status for each action is described in detail below. Moreover, the demographic characteristics of the fish populations for each sampled site are reported. For the target species of the project (Etruscan chub and Italian nase), the age structure and growth in terms of length-weight regression are also shown.

Progress status for each action

Action 1: Identification of 5 Etruscan chub populations in good conservation status from which to collect wild specimens for restocking activities in the eradicated sites.

The NEST1, FERS1, FERS2 and RASI1 stations, identified on the basis of data reported in the Regional Fish Map of the Umbria Region, are confirmed as suitable sites for the collection of specimens of *S. lucumonis* as they were inhabited by fairly abundant populations. The SERP1 site was found to be unsuitable as it was totally dry at the time of sampling. In order to identify an alternative site, another sampling was carried out on the Nestore river (site NEST2), but also in this case the station was discarded due to the scarce presence of fish fauna. The location of the sites is reported in Figure 2.

Given the co-occurrence of non-native species (*A. arborella*, *P. bonelli* and *P. fluviatilis*) with *S. lucumonis*, it is considered appropriate to proceed with their removal simultaneously with the collection of the wild Etruscan chub specimens.

Action 2: Selection of 2 suitable sites for alien species eradication activities, and consequent improvement of the conditions of Etruscan chub populations occurring in these sites.

CARP1: the results of the fish sampling confirmed the presence of alien species (*P. bonelli* and *P. genei*) in the Carpina creek. The presence of weirs located downstream and upstream of the site has been ascertained (Figure 3). At the same time as the sampling activities, the removal activities of the alien *P. bonelli* started. A total of 164 specimens, accounting for a biomass of 380 g, were removed from the site.

ARGE1: in the Argento creek the fish sampling revealed the presence of only *P. genei* as alien species. Also in this case the presence of weirs located downstream and upstream of the site has been confirmed.



Figure 3. Weir insurmountable from the fish fauna located on the Carpina creek downstream of the sampling site.

An additional control site, coded as FOSS1, has been added to the study design. This site was chosen on the basis of proximity to the eradicated site ARGE1, and the similarity of morphological characteristics. The location of the 3 control sites is showed in Figure 2.

Action 3: Identification of 5 *P. genei* declining populations in the Esino river basin (Marche Region). In addition to the Esino populations, a further declining population of *P. genei* has been identified in the Foglia river (Marche Region). The site has been coded as FOGL1 (Figure 2).

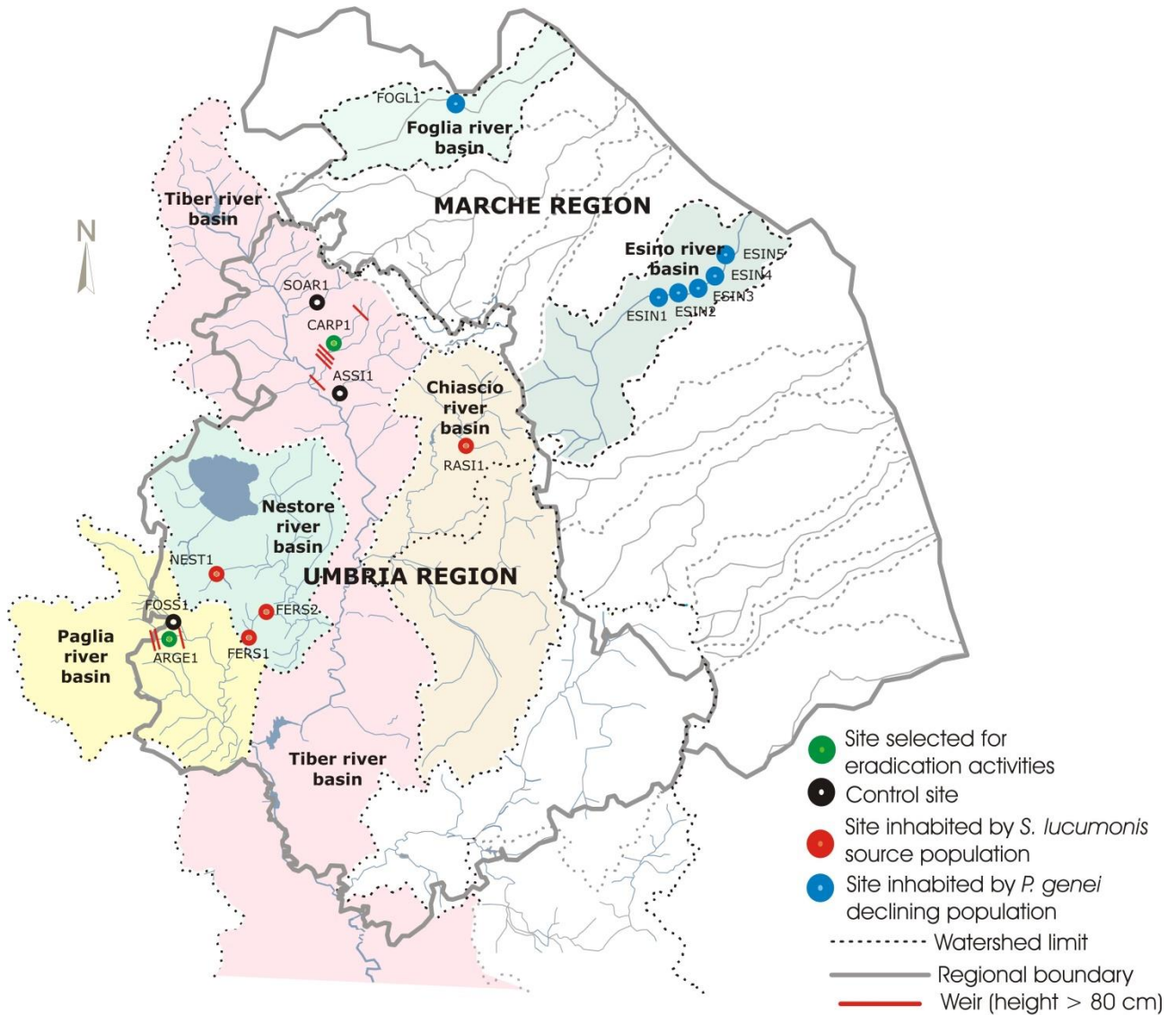


Figure 2. Location of the sampling sites involved in the different conservation actions identified on the basis of the demographic characterization of fish populations. Two sites were confirmed as suitable for eradication activities (green circles), six sites were selected for the presence of *S. lucumonis* source populations (red circles), and six sites for the presence of *P. genei* declining populations (blue circles). The location of the three additional control sites (black circles) is also shown.

Action 4: Request to the competent authorities the necessary authorizations to carry out the activities envisaged by the project.

The authorizations for fish samplings and restocking activities were obtained from the competent authorities (Umbria Region and Marche Region).

Critical issues: As a consequence of the restrictions put in place by the Italian government to face the Coronavirus pandemic, the expected times for the project activities have been slight re-modulated as reported in table 3. At the moment, considering the project timeline, we don't believe that the health emergency can determine significant changes in the project activities.

	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																												

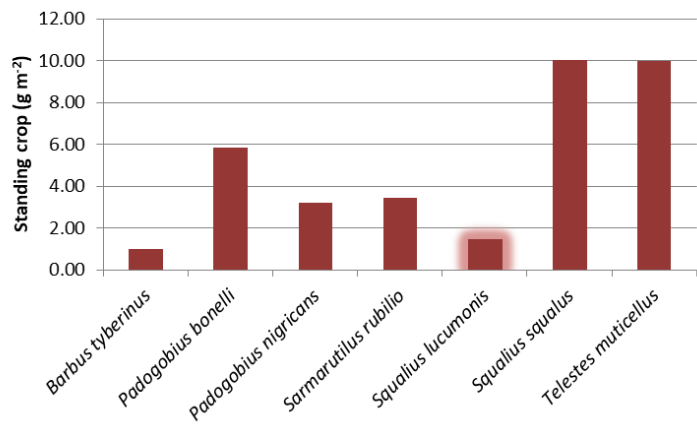
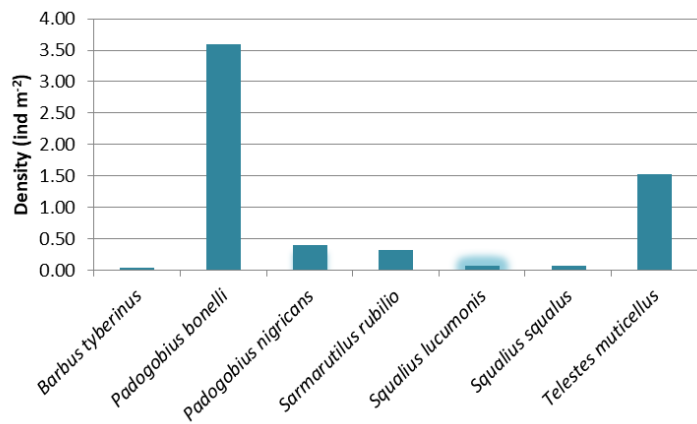
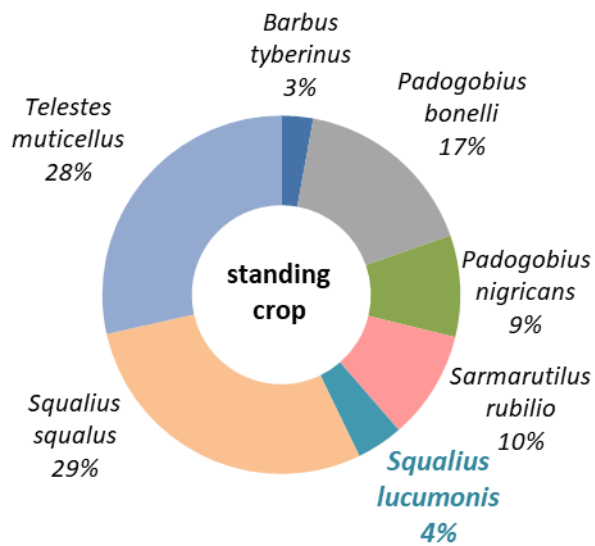
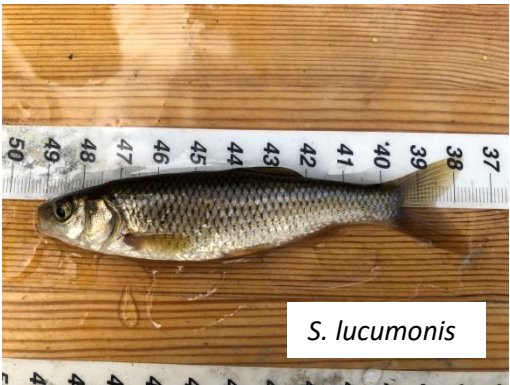
Table 3. Re-modulated project timetable.

Added values for the project: We continues our collaboration within the European "LIFE for Lasca" project. The genetic analysis results have shown that Tiber river basin vs. Po river basin *P. genei* samples do not differ amongst the two groups. This result allowed to plan *P. genei* transportation activities from the Tiber river basin to the native range. The collection of 1000 *P. genei* adult specimens to be transported to Slovenia has been scheduled for the next spring 2021.

In the annex below, for each site, the results of fish sampling in terms of fish assemblage composition and demographic characterization of fish populations are reported.

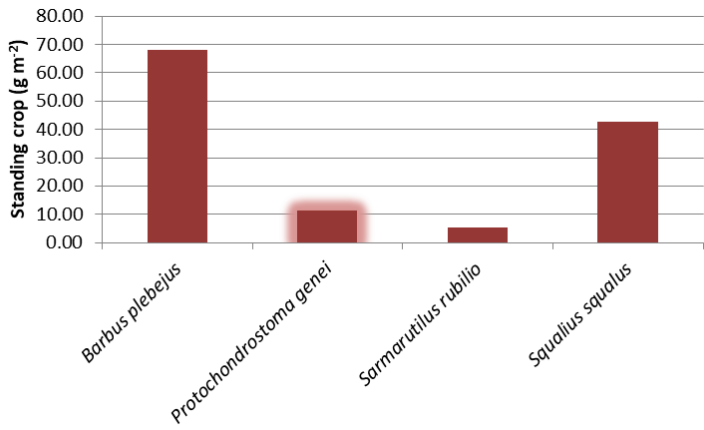
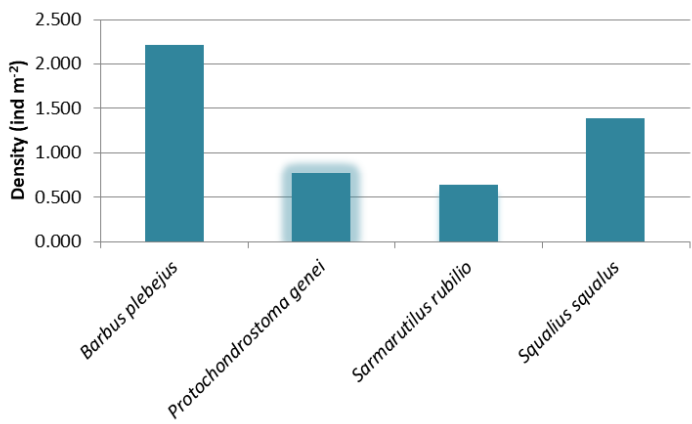
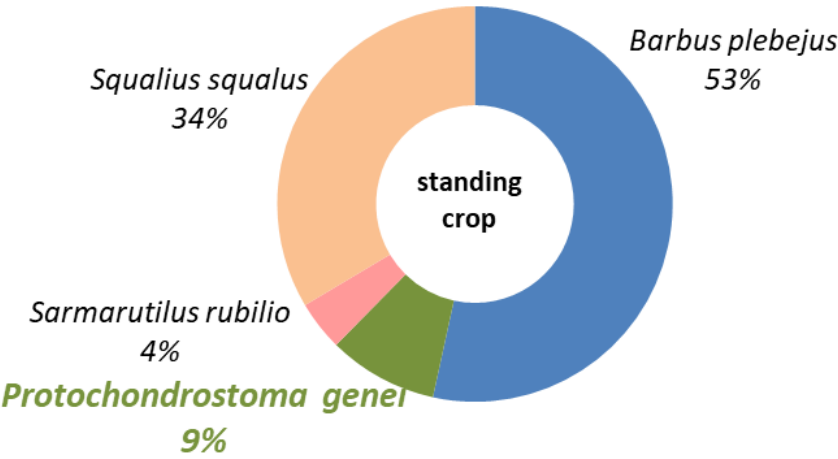
Rasina creek – RASI1

	Density (ind m ⁻²)	Standing Crop (g m ⁻²)
<i>Barbus tyberinus</i>	0.043	1.00
<i>Padogobius bonelli</i>	3.600	5.86
<i>Padogobius nigricans</i>	0.400	3.20
<i>Sarmarutilus rubilio</i>	0.326	3.43
<i>Squalius lucumonis</i>	0.064	1.47
<i>Squalius squalus</i>	0.067	10.01
<i>Telestes muticellus</i>	1.528	9.96



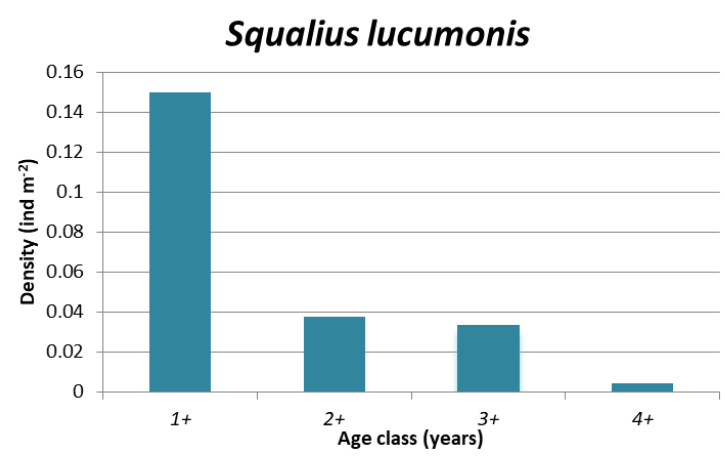
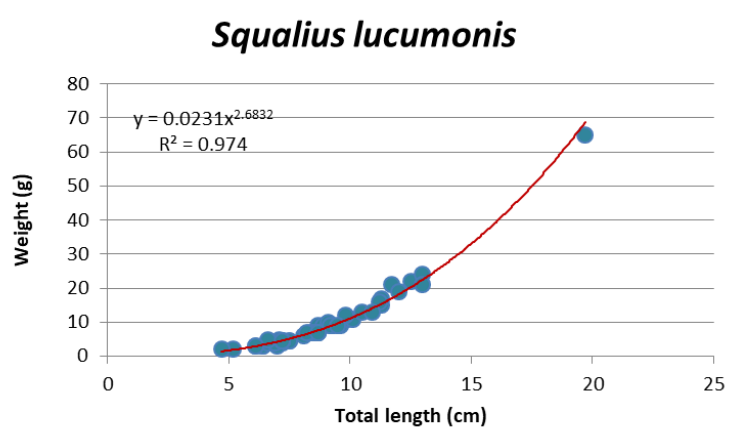
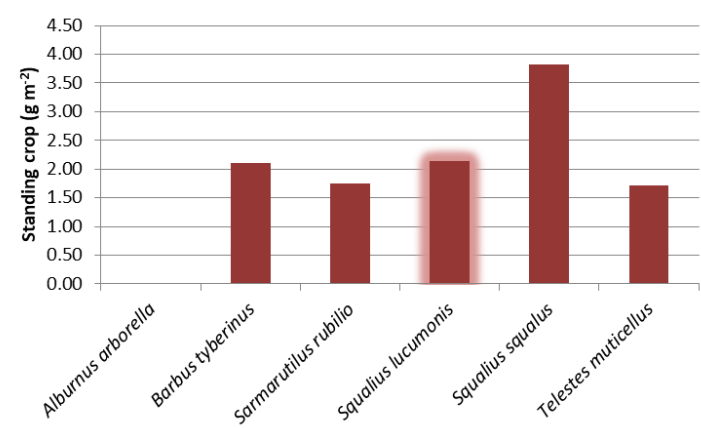
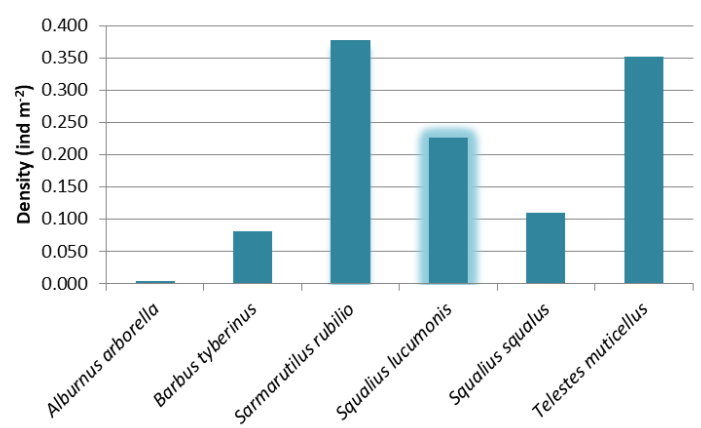
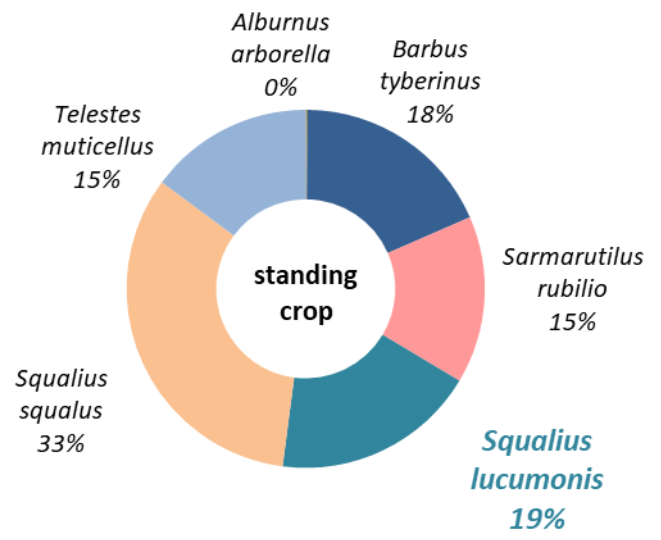
Foglia creek – FOGL1

	Density (ind m ⁻²)	Standing Crop (g m ⁻²)
<i>Barbus plebejus</i>	2.213	68.14
<i>Protochondrostoma genei</i>	0.773	11.43
<i>Sarmarutilus rubilio</i>	0.640	5.20
<i>Squalius squalus</i>	1.387	42.79



Fersinone creek – FERS1

	Density (ind m ⁻²)	Standing Crop (g m ⁻²)
<i>Alburnus arborella</i>	0.004	0.02
<i>Barbus tyberinus</i>	0.082	2.10
<i>Sarmarutilus rubilio</i>	0.376	1.74
<i>Squalius lucumonis</i>	0.225	2.13
<i>Squalius squalus</i>	0.110	3.81
<i>Telestes muticellus</i>	0.352	1.71

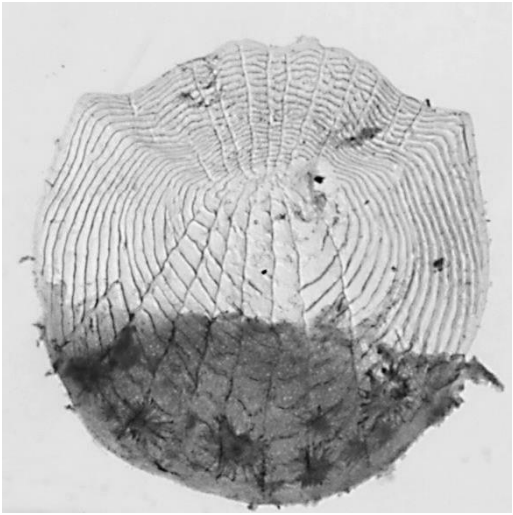
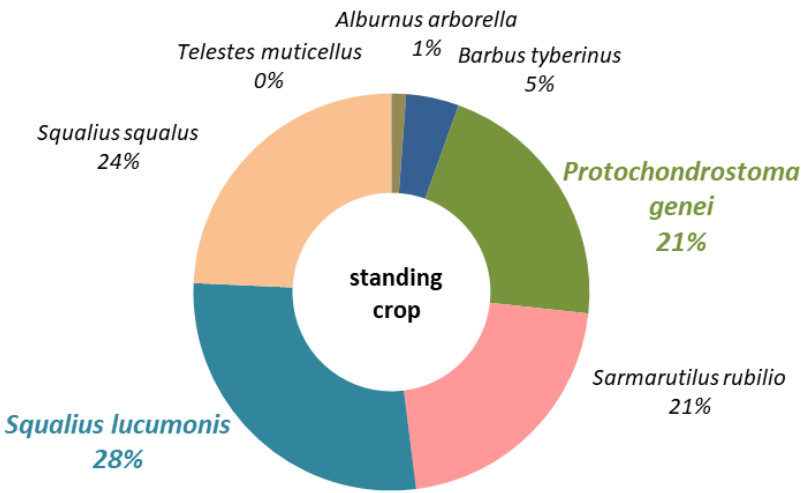


Fersinone creek – FERS2

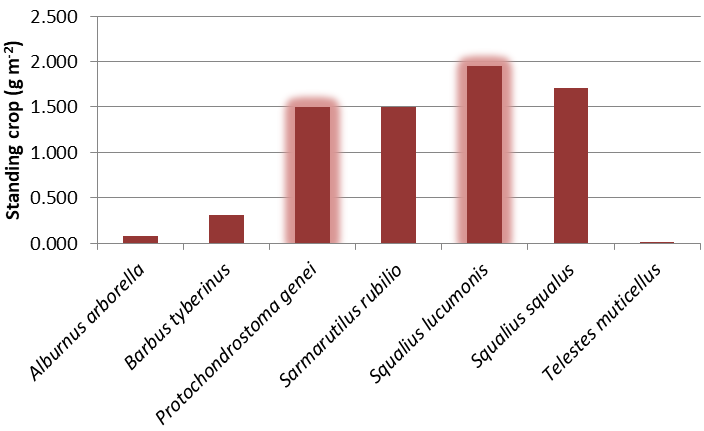
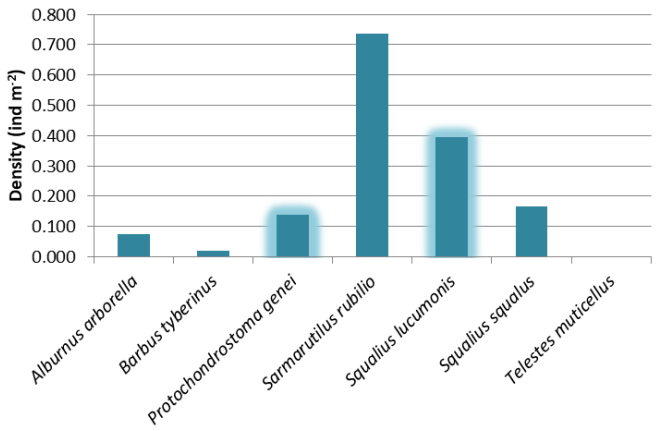
	Density (ind m ⁻²)	Standing Crop (g m ⁻²)
<i>Alburnus arborella</i>	0.074	0.084
<i>Barbus tyberinus</i>	0.020	0.305
<i>Protochondrostoma genei</i>	0.139	1.497
<i>Sarmarutilus rubilio</i>	0.738	1.503
<i>Squalius lucumonis</i>	0.395	1.957
<i>Squalius squalus</i>	0.166	1.712
<i>Telestes muticellus</i>	0.002	0.002



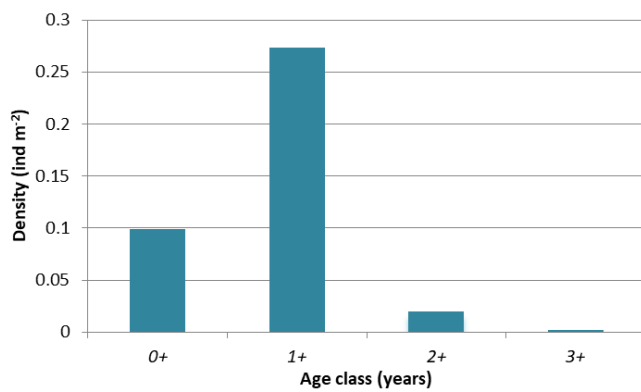
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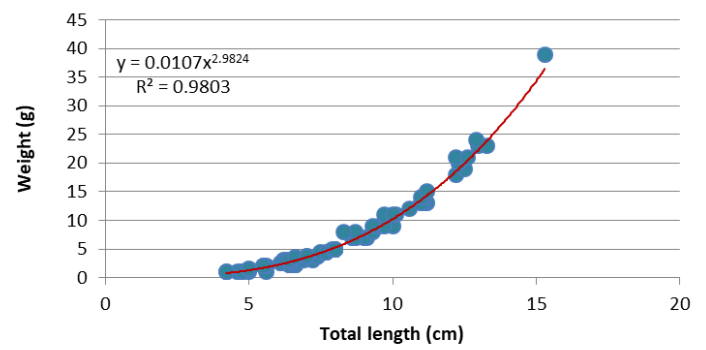
Etruscan chub scale for age determination



Squalius lucumonis



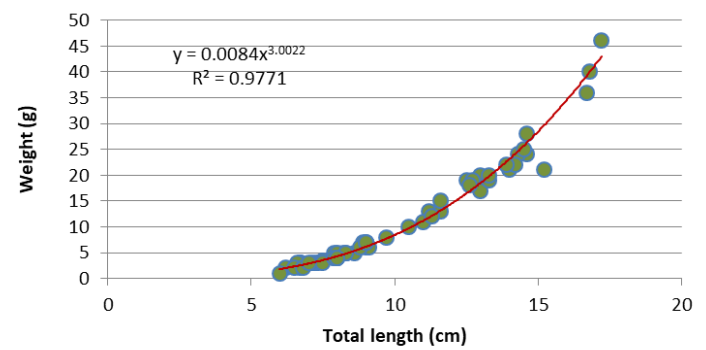
Squalius lucumonis



Protochondrostoma genei

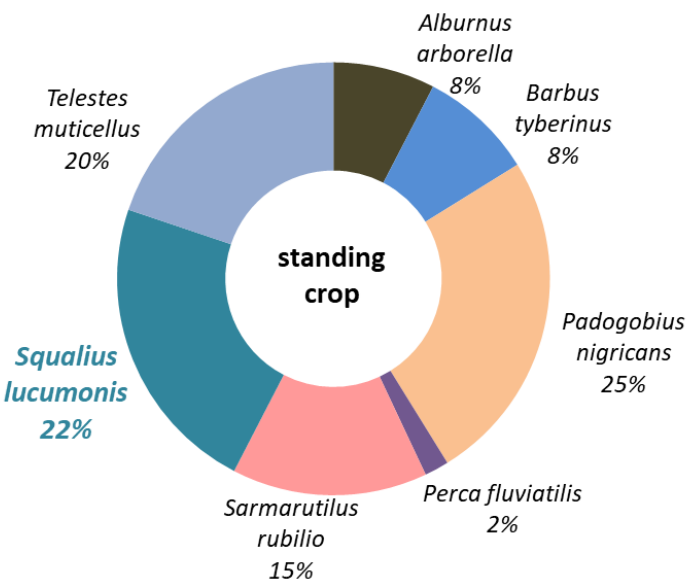


Protochondrostoma genei

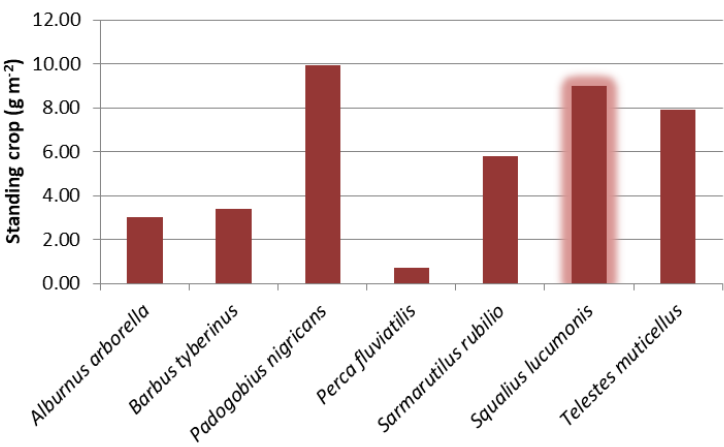
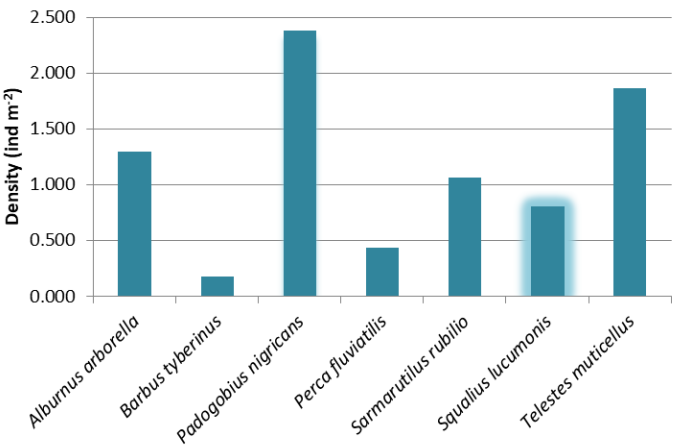
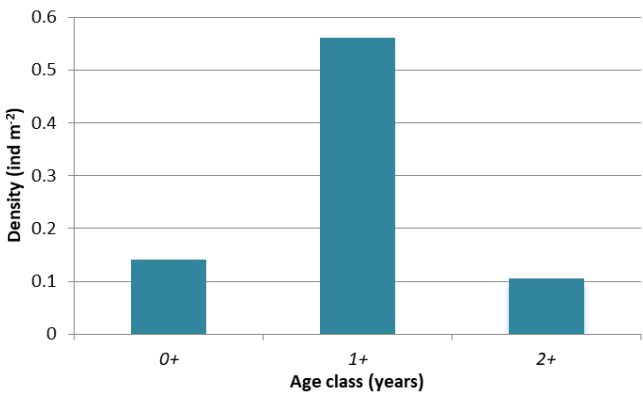


Nestore river – NEST1

	Density (ind m ⁻²)	Standing Crop (g m ⁻²)
<i>Alburnus arborella</i>	1.294	3.03
<i>Barbus tyberinus</i>	0.175	3.40
<i>Padogobius nigricans</i>	2.380	9.95
<i>Perca fluviatilis</i>	0.439	0.72
<i>Sarmarutilus rubilio</i>	1.061	5.81
<i>Squalius lucumonis</i>	0.807	8.98
<i>Telestes muticellus</i>	1.867	7.89

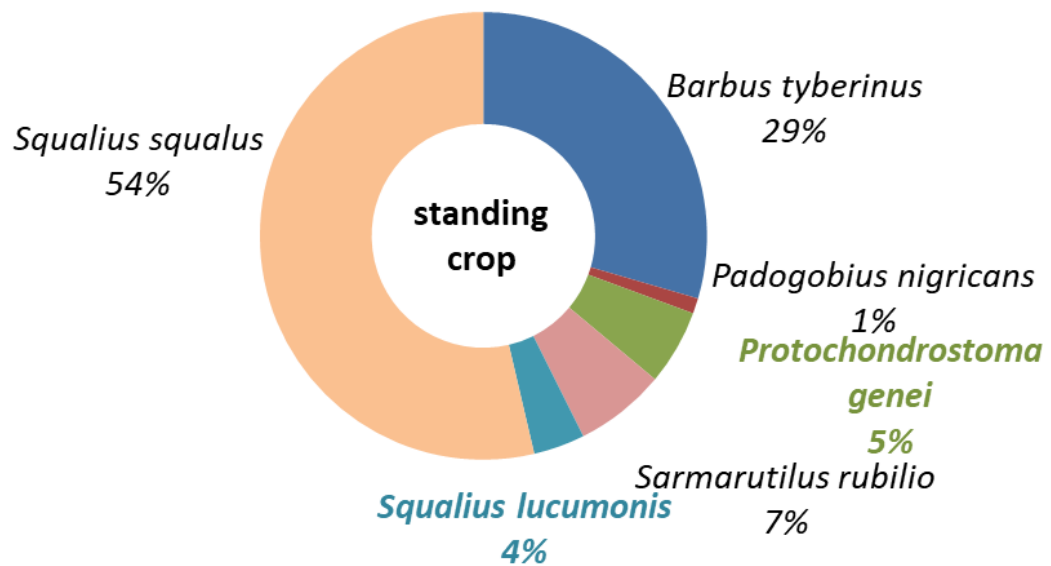


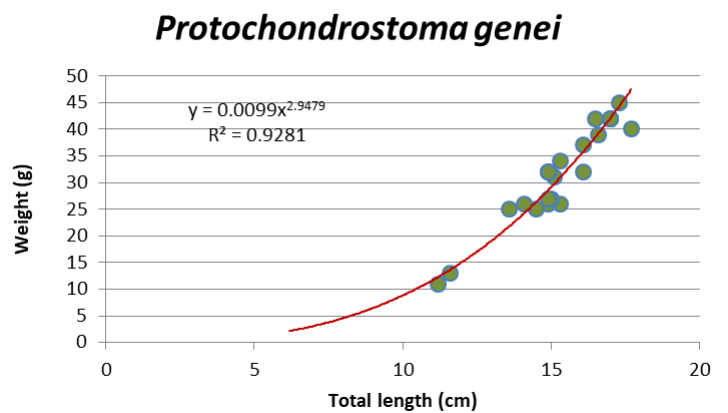
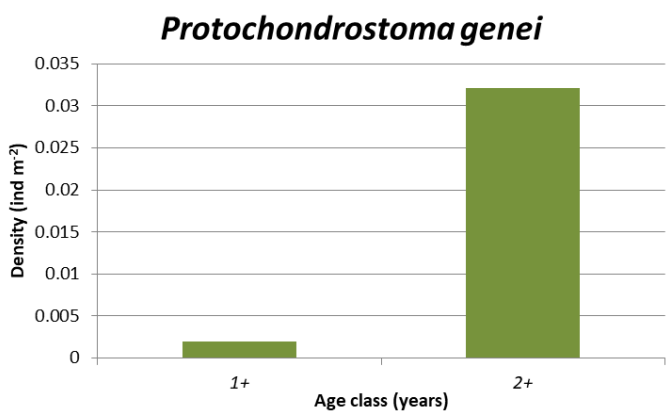
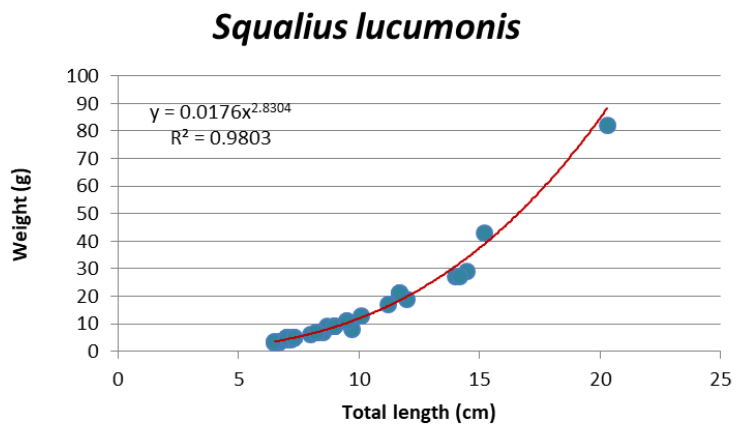
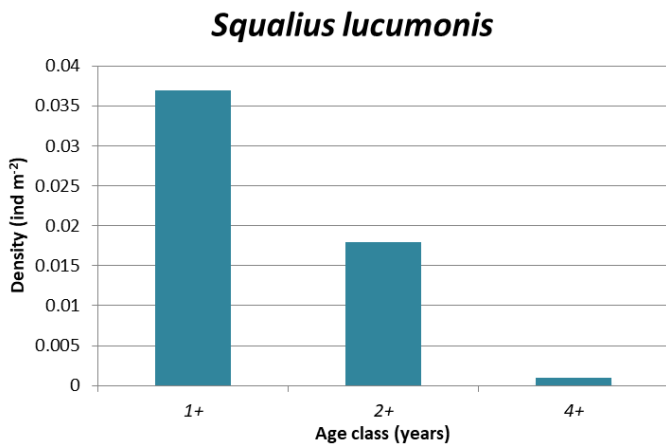
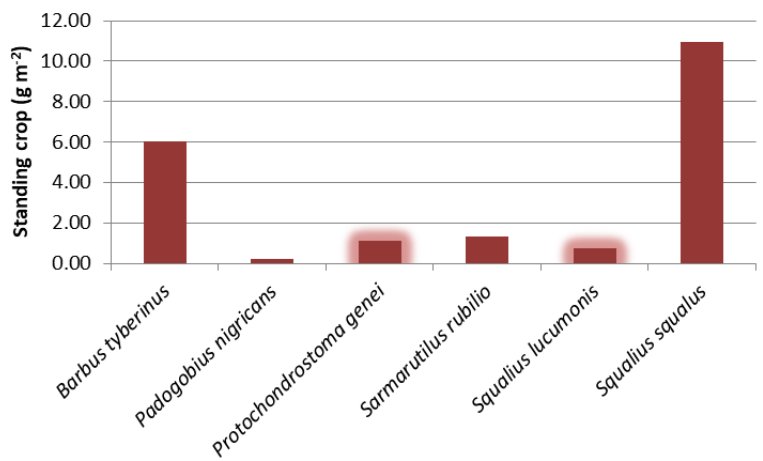
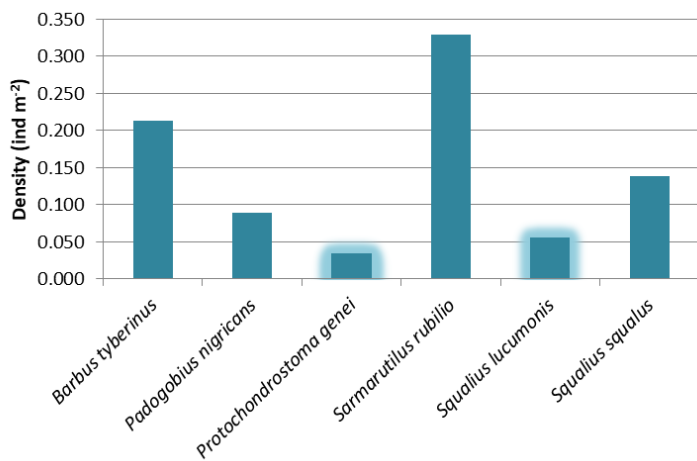
Squalius lucumonis



Argento creek – ARGE1

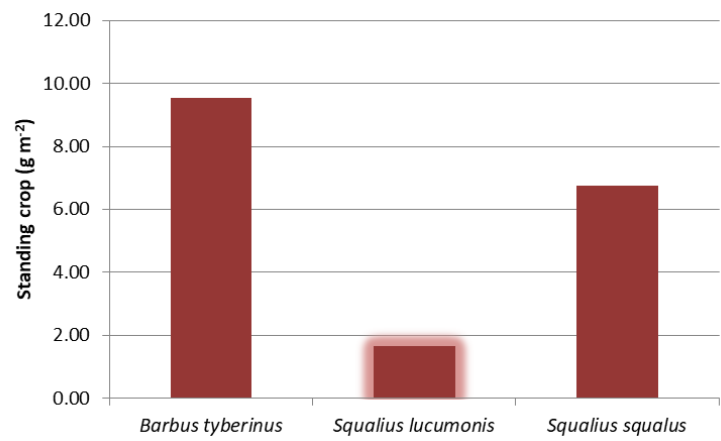
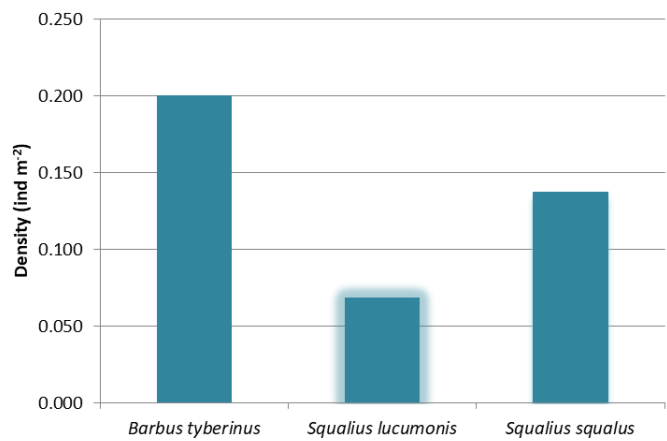
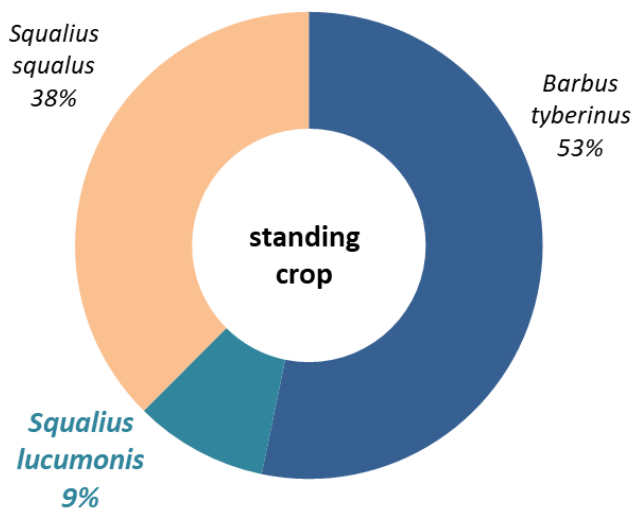
	Density (ind m ⁻²)	Standing Crop (g m ⁻²)
<i>Barbus tyberinus</i>	0.213	6.03
<i>Padogobius nigricans</i>	0.089	0.23
<i>Protochondrostoma genei</i>	0.034	1.11
<i>Sarmarutilus rubilio</i>	0.328	1.34
<i>Squalius lucumonis</i>	0.056	0.76
<i>Squalius squalus</i>	0.138	10.96





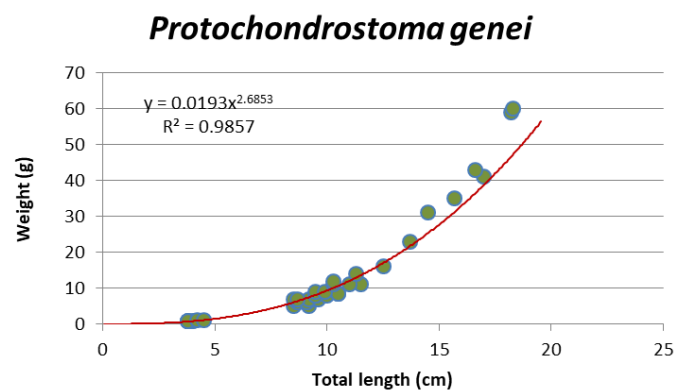
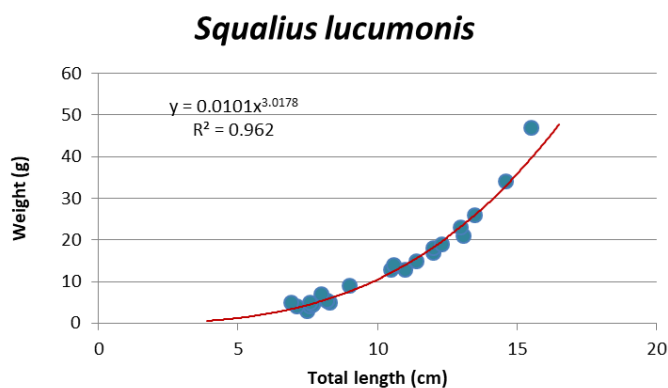
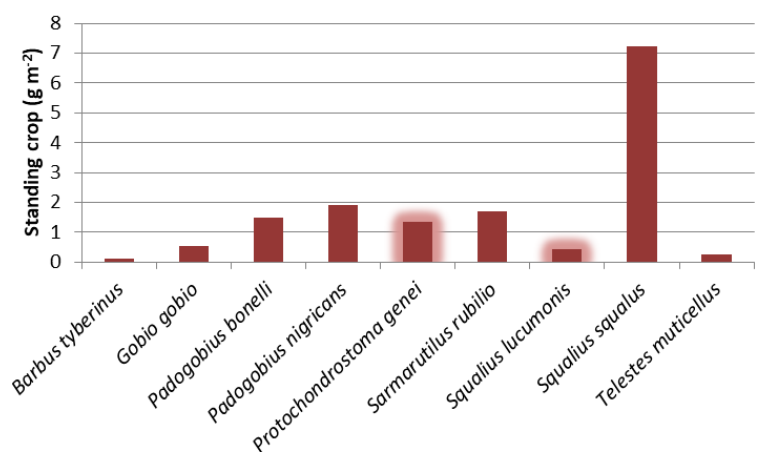
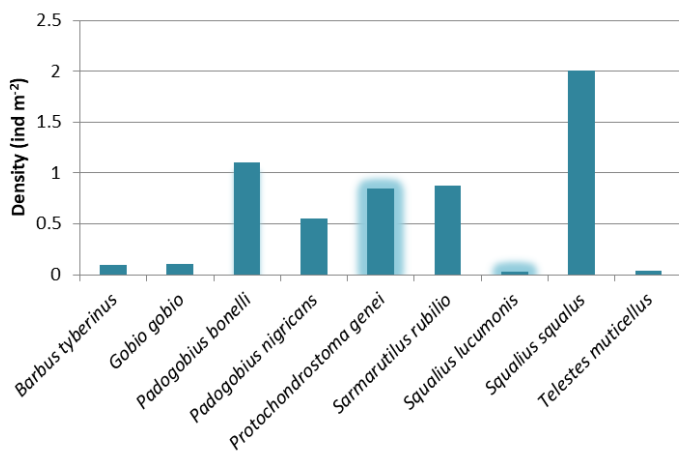
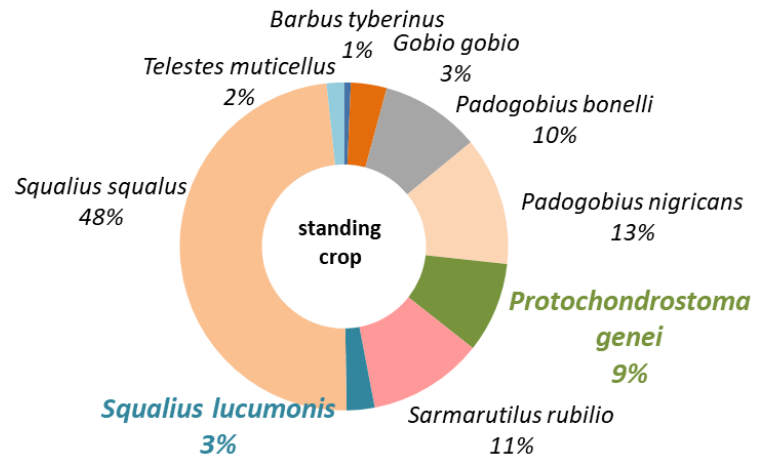
Fossalto creek – FOSS1

	Density (ind m ⁻²)	Standing Crop (g m ⁻²)
<i>Barbus tyberinus</i>	0.200	9.55
<i>Squalius lucumonis</i>	0.069	1.66
<i>Squalius squalus</i>	0.138	6.73



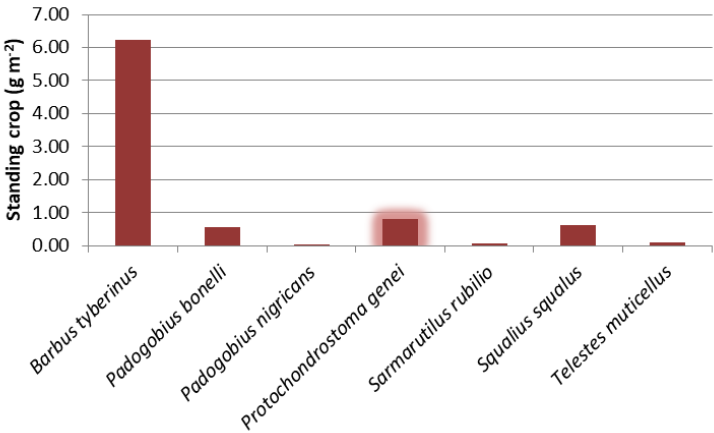
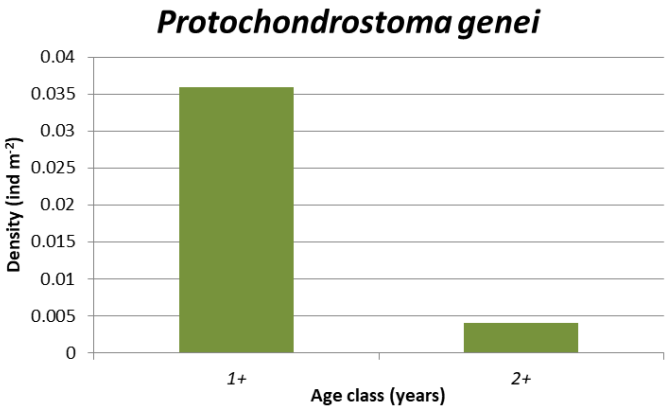
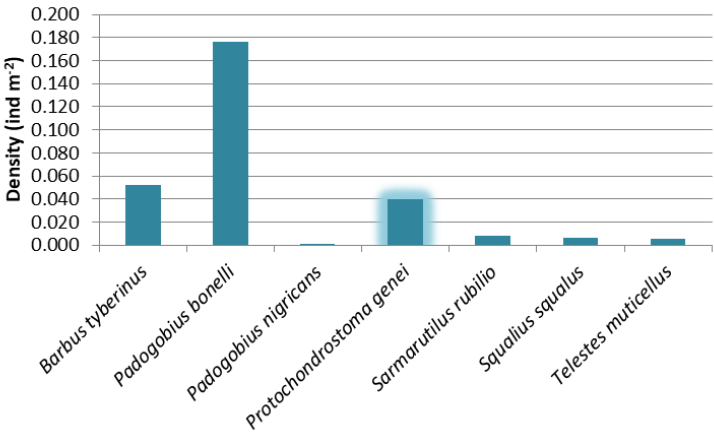
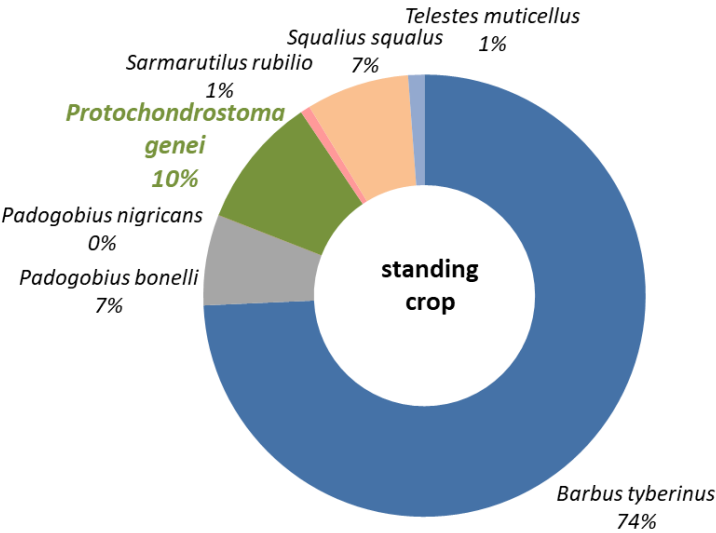
Assino creek – ASSI1

	Density (ind m ⁻²)	Standing Crop (g m ⁻²)
<i>Barbus tyberinus</i>	0.100	0.10
<i>Gobio gobio</i>	0.099	0.53
<i>Padogobius bonelli</i>	1.100	1.47
<i>Padogobius nigricans</i>	0.550	1.89
<i>Protochondrostoma genei</i>	0.842	1.33
<i>Sarmarutilus rubilio</i>	0.871	1.69
<i>Squalius lucumonis</i>	0.029	0.41
<i>Squalius squalus</i>	2.006	7.24
<i>Telestes muticellus</i>	0.038	0.25



Carpina creek – CARP1

	Density (ind m ⁻²)	Standing Crop (g m ⁻²)
<i>Barbus tyberinus</i>	0.052	6.24
<i>Padogobius bonelli</i>	0.176	0.56
<i>Padogobius nigricans</i>	0.001	0.00
<i>Protochondrostoma genei</i>	0.040	0.81
<i>Sarmarutilus rubilio</i>	0.008	0.06
<i>Squalius squalus</i>	0.007	0.63
<i>Telestes muticellus</i>	0.005	0.10



Soara creek – SOAR1

	Density (ind m ⁻²)	Standing Crop (g m ⁻²)
<i>Barbus tyberinus</i>	0.339	7.70
<i>Padogobius bonelli</i>	0.115	0.18
<i>Padogobius nigricans</i>	0.087	0.22
<i>Protochondrostoma genei</i>	0.427	3.51
<i>Sarmarutilus rubilio</i>	2.206	12.35
<i>Squalius lucumonis</i>	0.114	2.45
<i>Squalius squalus</i>	2.388	39.69
<i>Telestes muticellus</i>	2.489	12.48

