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PRIMARY RESEARCH PAPER

Genetic variability as expression of the invasion success: the case of the European catfish (*Silurus glanis*) in four Italian basins

Caterina Maria Antognazza · Martin Čech · Annamaria Nocita · Thomas Busatto · Jan Baer · Cesare Puzzi · Serena Zaccara

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Abstract *Silurus glanis* is considered a top-priority invasive fish out of its natural range, being recorded in Italian basins since the 1950s. In few decades, *S. glanis* expanded its distribution along the main Italian basins, picturing a scenario of invasion success. Its invasion success was here analysed throughout the assessment of the genetic variability detected in 15 populations of *S. glanis* collected from four Italian basins along north–south gradient (Po River basin in the northern, Arno River and Serchio River basin in the central, and Volturno River basin in the southern). Additionally, three populations from native areas of *S. glanis* in central Europe (Elbe and Rhine River basins) have also been added to the dataset. The genetic variability and structure were retrieved through: i) sequencing of the mitochondrial control region and ii) genotyping of nuclear DNA at 10 microsatellite loci. The genetic outcomes evidenced high levels of genetic variability in each basin, supporting a favourable trait for high colonization potential. Genetic structure inference suggested the presence of two genetic pools, distinctly recorded in adjacent basins of central Italian peninsula and in two distant basins located in norther and southern part of Italian peninsula, probably related to distinct origin of introduced fish.

Keywords *Silurus glanis* · Invasive alien species · Freshwater · Microsatellite · Control region · Management

Introduction

One of the major factors in the human-caused biodiversity crisis is biological invasions (Sala et al., 2000), able to alter and disrupt the ecosystem

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C. M. Antognazza (✉) · S. Zaccara
Department of Theoretical and Applied Sciences, University of Insubria, Via H. Dunant 3, 21100 Varese, Italy
e-mail: caterina.antognazza@uninsubria.it

M. Čech
Biology Centre of the Czech Academy of Sciences, Institute of Hydrobiology, Na Sádkách 7, 37005 České Budějovice, Czech Republic

A. Nocita · T. Busatto
Natural History Museum, The University of Florence, Via Romana 17, 50125 Florence, Italy

J. Baer
Fisheries Research Station of Baden-Wuerttemberg, Argenweg 50/1, 88085 Langenargen, Germany

C. Puzzi
G.R.A.I.A. S.R.L. Gestione E Ricerca Ambientale Ittica Acque, Via Repubblica 1, Varano Borghi, 21020 Varese, Italy

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functioning throughout changing of recipient community structure (Simberloff et al., 2013). Many non-native species have been introduced into freshwater environments because of their close ties to human activity, with fish being among the most introduced freshwater organisms (Copp et al., 2005).

This is the case of the European catfish, *Silurus glanis* (Linnaeus, 1758), a species that can reach over 2.7 m in length and achieve body weights of over 130 kg (Boulêtreau & Santoul, 2016), making it one of the largest freshwater fish species worldwide and an apex predator. Its native range includes the Baltic, Black, Caspian, and Aral Sea basins of Eastern Europe, with the most westerly extent being the southern part of the River Rhine drainage in Germany (Kottelat & Freyhof, 2007). Its potential predation pressure can have a negative influence on local biodiversity (Vejřík et al., 2017a), especially affecting local communities not evolved with such a large apex predator. This occurred to the Iberian Peninsula fish communities where the absence of other piscivorous joined to catfish predation has driven to the brink of extinction of endemic native small-bodied fish (Copp et al., 2009; Ferreira et al., 2019; De Santis et al., 2024). Due to its popularity with anglers and for different biomanipulation projects (Vejřík et al., 2017b), *S. glanis* has effectively spread outside its native range from east to west across nearly the entire Europe, including the British Isles (Verreycken, 2019). In Italy, *S. glanis* is currently listed as an invasive alien species (IAS) (i.e. evidence of impact) (Verreycken, 2019), and it is considered as a top-priority invasive fish showing in the last decades an invasion success along Italian peninsula (De Santis et al., 2024).

Although the first *S. glanis* detection dates to 1937, recorded in the Po River basin, specifically the Adda River, its introduction likely occurred in the 1950s (Manfredi, 1957 and then confirmed in the second half of the 1970s (Gandolfi & Giannini, 1979). It was primarily introduced by stocking, frequently input-and-take lakes, to enhance the recreational angling (Castaldelli et al., 2013; Cerri et al., 2018), and even to control other invasive species, acting as biocontrol agent (Pizzul & Specchi, 1994). The climate change has favoured a rapid general degradation of the freshwater environments, changing from further cold, fast-flowing rivers into a cascade of slowly flowing, warm flow-through lakes (Britton et al., 2010). The general degradation have probably contributed to the spreading and the flourish of European catfish in vast range of environments, like eutrophic, turbid, and still waters of canals, shallow lakes, large rivers, demonstrating an extraordinary ecological adaptability. Nowadays, *S. glanis* is spread across all the major basins in northern and central Italy, from Po River, Isonzo River, Arno River (Nocita, 2002; Lanzoni et al., 2018; Haubrock et al., 2020) up to Volturno River in southern Italian peninsula (De Bonis et al., 2015), it colonizes deep oligotrophic subalpine lakes, such as Lake Maggiore and Garda Lake (Confortini, 1995; Volta et al., 2018; De Santis & Volta 2021; Antognazza et al., 2022). Its presence is also documented in central Italian lakes, such as Lake Massaciuccoli and Lake Bolsena (Baldaccini & Ercolini, 2006; Ercolini 2015; Mancini et al., 2022).

In the last decades, the importance of studying the genetics of biological invasions has been recognized as an interesting tool for analysing invasion success, for planning focused management, prevention, and control strategies of IAS, and population dynamics studies through the applications of molecular genetics (Holland, 2000; Sakai et al., 2001). In fact, population genetics of invasive species has been recognized as a potential tool to offer opportunities to investigate rapid evolutionary processes (Lee, 2002) and to assess the importance of genetic diversity in biological invasion success (Mooney & Cleland, 2001). Through this application is possible to find out if the introduction and establishment of an invasive species can derive from a small number of individuals of a single invasive population or from multiple introductions of divergent invasive populations. These two processes produce distinct population genetic signals: the founder effect determines an overall loss of genetic diversity while admixture of multiple introductions promotes gene flow between genetically distinct populations and the increase in genetic diversity.

So, in this study the success of invasion was assessed by comparing the genetic signature of European catfish in four Italian basins selected along a north–south geographic gradient in order to emphasize a mix of different factors like the geographic extension of the basins and the invasion timing, from 1950s up to 2010s (Fig. 1). The genetic diversity and structure of *S. glanis* from four basins across the Italian peninsula were analysed through the application of two different molecular approaches: (i) sequencing of mitochondrial control region (D-loop) and (ii)

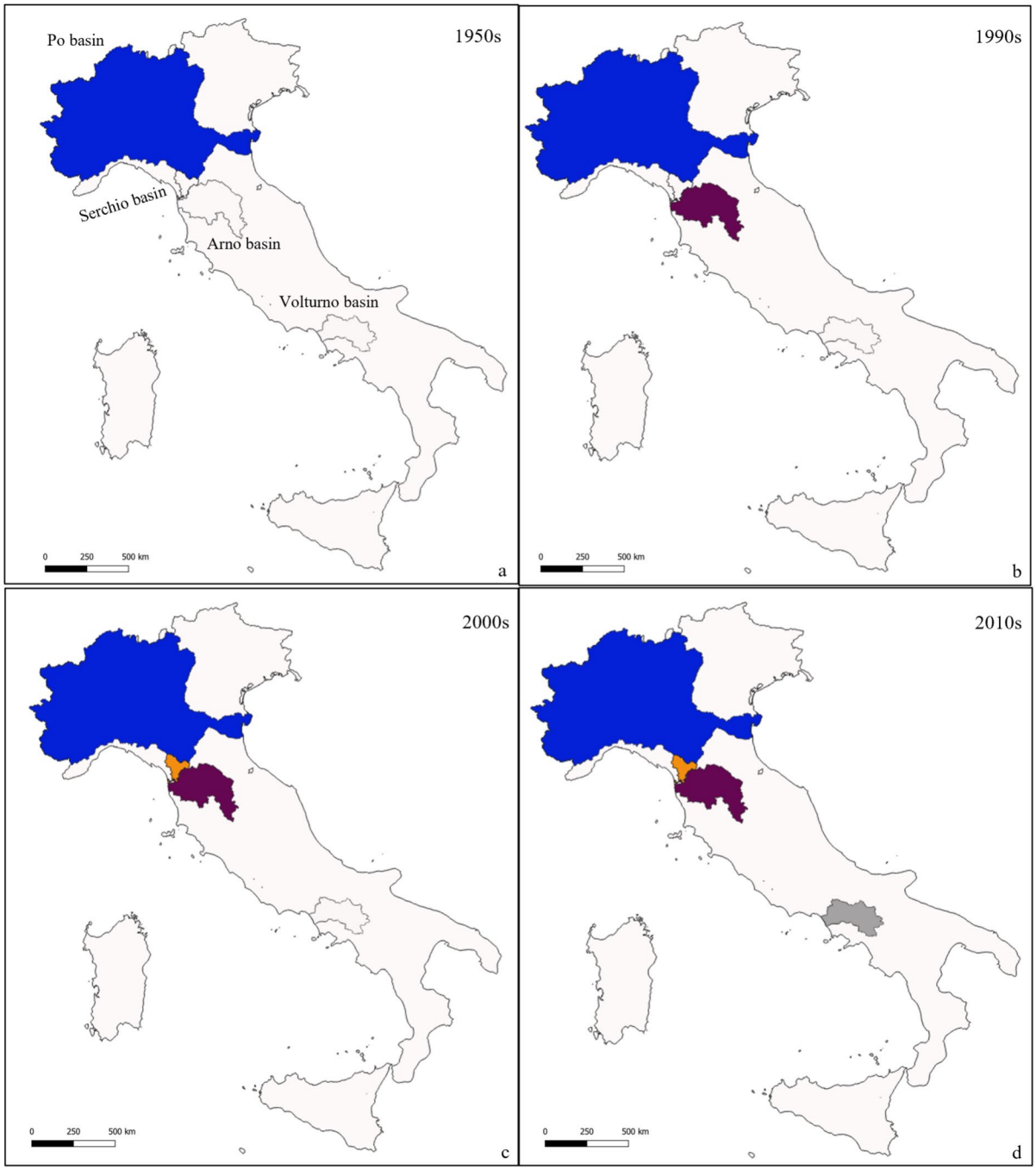

Fig. 1 *Silurus glanis* invasion history in the four Italian basins investigated in this study, **a** established presence within the Po basin (basin extension: 71,000 Km^2) in the 1950s (Gandolfi & Giannini, 1979), **b** established presence within the Arno basin (basin extension: 8,200 Km^2) in the 1990s (Lanzoni et al., 2018), **c** established presence within the Serchio basin (basin extension 1,500 Km^2) in the 2000s (Ercolini, 2015), and **d** established presence within the Volturno basin (basin extension 5,500 $km.^2$) in the 2010s (De Bonis et al., 2015)

genotyping of nuclear DNA at 10 microsatellite loci. To appreciate the signature of invaded populations, the genetic signature of *S. glanis* in two basins along its native range from central Europe (the Elbe and the Rhine basins) was also analysed.

Materials and methods

The fish DNA sampling took place between 2023 and 2024 on different types of water bodies (small/large rivers, lakes), mainly by electrofishing. Small fragments of pelvic fins were removed from each individual and stored in 96% ethanol. A total of 268 individuals were collected from 18 different sampling sites: i) three were in its native basins from central Europe and 15 were in the non-native area of catfish from 4 basins of the Italian peninsula (see Table 1).

Extraction of genomic DNA from fin clips was completed using a modified salt extraction method with proteinase *K* (Aljanabi & Martinez, 1997). DNA was eluted in 100 µL MilliQ water and stocked in freezer until next used. The genetic analyses then included (i) sequencing of the entire mitochondrial DNA control region (D-loop) and (ii) genotyping of at 10 loci (Supplementary Material S1).

The D-loop was amplified using the primer pair SGDF–SGDR (Bektaş et al., 2020). Polymerase chain reactions (PCRs) were performed using Multiplex PCR Master Mix (QIAGEN) according to the manufacturer's instructions, using about 30 ng of DNA and 0.25 µL of each primer pair (0.2 µM) in 12 µL total volume reaction. The following protocol was used after a preliminary denaturation at 95 °C for 15 min, each of the 35 cycles consisted of denaturation at 94 °C for 45 s, annealing at 53 °C for 45 s, and primer denaturation at 72 °C for 60 s, and a final extension at 72 °C for 10 min. The PCR products were purified before sequencing using EuroSAP (EuroClone, Pero, MI, Italy) enzymatic kit, following the manufacturer's instruction. The purified products were then sent to Macrogen Europe B.V. (Amsterdam, the Netherlands) for sequencing in both directions. Subsets of the 10 loci were combined in three "multiplexes" for PCR. The primer pairs for each locus and the annealing temperatures used in each PCR multiplex can be found in Supplementary Material S1. The Type-it® Multiplex PCR Master Mix (Qiagen,

Table 1 *Silurus glanis* Sampling sites. Population code (ID), basin, sub-basin, sampled water flow, locality (CZ = Czech Republic; DE = Germany, IT = Italy), status of *S. glanis* (range), geographic coordinates (WGS84), and number of samples (No) are indicated

ID	Basin	Sub-basin	Water flow	Locality	Range	Coordinates (N-E)	No
ZE	Elbe	Sazava	Želivka reservoir	Vojslavice (CZ)	Native	49.58666; 15.24055	13
LR	Elbe	Moldava	Lipno reservoir	Bližná (CZ)	Native	48.73500; 14.06777	11
Bod	Rhine	Rhine	Lake Constance	Gohren (DE)	Native	47.58523; 9.55763	16
CB	Po	Ticino	Lake Comabbio	Varano Borghi (IT)	Non-native	45.76955; 8.69411	19
E	Po	Oglio	Lake Endine	Madrera (IT)	Non-native	45.78943; 9.95025	7
TS	Po	Oglio	Lake Iseo	Torbiere del Sebino (IT)	Non-native	45.64134; 10.03489	26
G	Po	Mincio	Lake Garda	Peschiera del Garda (IT)	Non-native	45.44393; 10.68843	5
TR	Po	Ticino	Tresa	Luino (IT)	Non-native	45.98897; 8.76516	5
TC	Po	Ticino	Ticino	Ponte di Oleggio (IT)	Non-native	45.98897; 8.76516	10
L	Po	Ticino	Naviglio Grande	Lonate Pozzolo (IT)	Non-native	45.56830; 8.71492	8
BA	Po	Ticino	Bardello	Besozzo (IT)	Non-native	45.84224; 8.66158	20
AF	Po	Adda	Adda	Fara Gera d'Adda (IT)	Non-native	45.57416; 9.53470	20
ON	Po	Oglio	Oglio	Torre Pallavicina (IT)	Non-native	45.44913; 9.88447	21
MN	Po	Mincio	Mincio	Curtatone (IT)	Non-native	45.16185; 10.71871	24
SI	Arno	Arno	Sieve stream	Pescaia di Marino (IT)	Non-native	43.80858; 11.47255	19
SE	Serchio	Serchio	Serchio	Migliarino Pisano (IT)	Non-native	43.85654; 10.32025	9
MA	Serchio	Massaciuccoli	Lake Massaciuccoli	Massarosa (IT)	Non-native	43.85654; 10.32025	30
S	Volturno	Volturno	Calore Beneventano	Castel di Sasso (IT)	Non-native	41.14516; 14.29563	5
Total							268

Hilden, Germany) was used for all PCR according to the manufacturer's instructions in 10 µL total volume reaction using about 20 ng DNA and 0.25 µL of each primer pair (0.2 µM). The following protocol was used: after a preliminary denaturation at 95 °C for 15 min, each of the 30 cycles consisted of denaturation at 94 °C for 30 s, annealing at 56 °C for 30 s, and primer denaturation at 72 °C for 30 s, and a final extension at 60 °C for 30 min. Negative controls were included in each PCR to control for DNA contamination. Multiplexed PCR products were analysed by Macrogen Europe B.V. (Amsterdam, the Netherlands) and allele size was measured using Peak Scanner™ Software v.1.0 (Applied Biosystem 2006, Foster City, CA, USA).

Data analysis control region

Nucleotide sequences of the mitochondrial control region (D-loop) were trimmed and assembled in a multiple sequence alignment using BioEdit v.7.0.5 (Hall 1999). The number of haplotype (h), haplotype diversity (H_d), and nucleotide diversity (π) were computed in DnaSP version 6 (Rozas et al., 2017). To analyse the genealogical relationship within the dataset, a minimum spanning network (MSN) was built on the same alignment through a statistical parsimony criterion as available in TCS v1.3 software (Clement et al., 2000) and TCS beautifier (Santos et al., 2016). Hierarchical analysis of molecular variance (AMOVA) with 10,000 permutations was performed to test the population genetic structure using the software Arlequin v3.5.2.2 (Excoffier & Lischer, 2010).

Data analysis microsatellite

Genotyping artefacts were assessed using Microchecker version 2.2.3 (Van Oosterhout et al., 2004). Departure from the Hardy–Weinberg equilibrium (HWE), the linkage disequilibrium (LD), and inbreeding coefficients (F_{IS}) (Cockerham & Weir, 1984) was assessed for each locus–population combination and for each locus across all populations using Genepop 4.0.10 (Rousset, 2008) with 10,000 permutations of the data, using a level of significance for multiple tests of 1%. Levels of significance for Hardy–Weinberg equilibrium and linkage disequilibrium tests were corrected using the Bonferroni correction (Weisstein 2004).

Genetic diversity at population and basin level was assessed by calculating expected heterozygosity (H_E), observed heterozygosity (H_O), and number of alleles (N_A) using Arlequin v. 3.5 (Excoffier & Lischer, 2010), and two standardized indices of genetic diversity that allow comparisons between samples with unequal numbers of sampled individuals, i.e. allelic richness (AR, (Petit et al., 1998)) and private allelic richness (PA, (Kalinowski, 2004)). *AR* and *PA* measure the mean number of alleles across loci in a population, and the mean proportion of alleles only present in one population, respectively. *AR* and *PA* were calculated using the software ADZE v. 1.0 (Szpiech et al., 2008). Current effective population sizes (N_e) were estimated using the linkage disequilibrium method implemented in NeESTIMATOR v.2.1 (Do et al., 2014), assuming critical values equal to 0.1.

Genetic substructure was explored using Structure v. 2.3.2 (Pritchard et al., 2000; Falush et al., 2003), varying the number of clusters (K) between 1 and 20 (number of populations + 2), using the admixture model, without using locations as prior with default parameters. Ten replicates of structure were run per K using 150,000 MCMC generations as burn-in, followed by 750,000 MCMC replicates. The ΔK method (Evanno et al., 2005) and the outputs were visualized using StructureHarvester 0.6.93 (Earl & vonHoldt, 2012) and StructuRly 0.1.0 (Angelini & Criscuolo, 2020).

Results

Sequencing mitochondrial control region

A total of 13 haplotypes were identified in the 805 bp length multiple D-loop alignment that was obtained from the 207 analysed European catfish (25 from native range and 171 from non-native range) while for 59 samples the sequences failed. There were 21 variable nucleotide positions detected, one of which was singletons and 20 were parsimony informative sites; the resulting K value was 2.1. Among the sequences produced in this study, 11 haplotypes were recorded for the first time. Two haplotypes (Sl1 and Sl4), just retrieved in the literature (MW796042 and MT183644), respectively, were largely detected in this study (35% and 36%, respectively; Table 2).

Table 2 Haplotype distribution of *Silurus glanis* haplotypes identified in this study

Basin	ID	Sl1*	Sl2	Sl3	Sl4*	Sl5	Sl6	Sl7	Sl8	Sl9	Sl10	Sl11	Sl12	Sl13	Tot
Elbe	ZE	3	2	1	1										7
Elbe	LR				3	2	2	1							8
Rhine	Bod			4	5				1						10
Po	CB			4	13					1					18
Po	E	1			3						2				6
Po	TS	17										6			23
Po	G				5										5
Po	TR				5										5
Po	TC	4			4							2			10
Po	L	3			2						1	1			7
Po	BA				14										14
Po	AF	20													20
Po	ON	2			5							9			16
Po	MN	22			1				1						24
Arno	SI			3	9										12
Serchio	SE														–
Serchio	MA												16	1	17
Volturno	S				5										5
	Tot	72	2	12	75	2	2	1	2	1	3	18	16	1	207

*Sl1 = MW796042 (Hungary. Székvári. 2021); Sl4 = MT183644 (Bektaş et al.,2020)

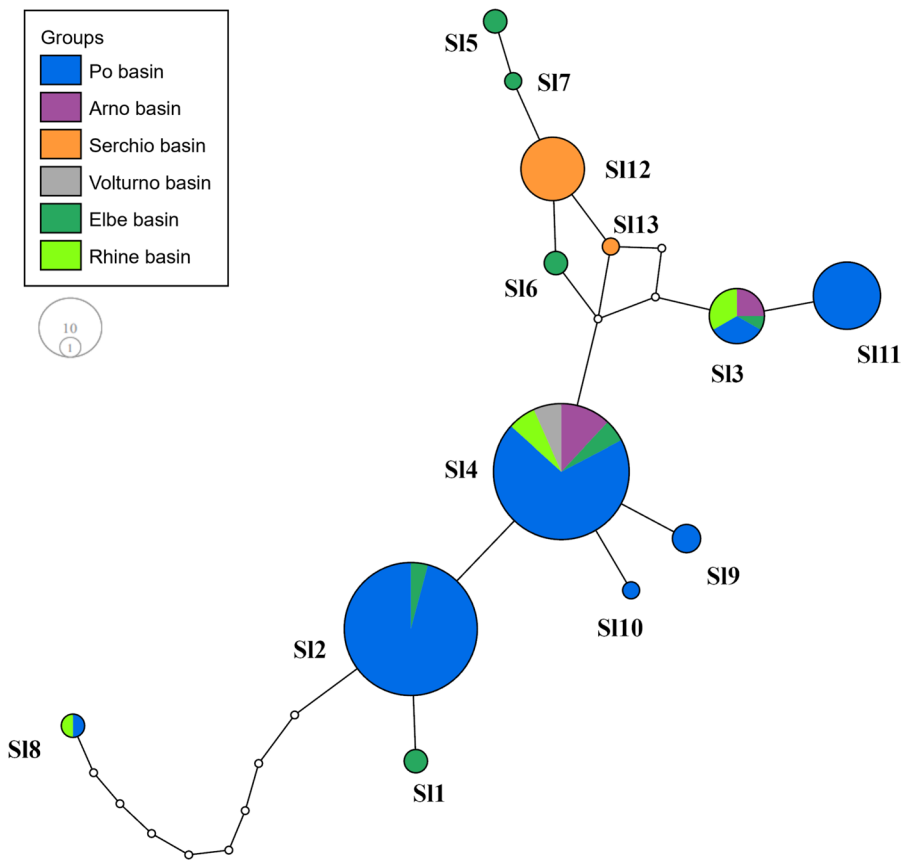

Fig. 2 Minimum spanning network based on 5' end mtDNA D-loop (805 bp length) representing the haplotypes detected in this study. Each circle represents one haplotype, and the sizes of circles are proportional to the number of individuals sharing the same haplotype (see scale). Blank circles represent internal haplotypes (mutational steps). *Green shades colour highlighted individuals recorded in* Silurus glanis *native range*

The evolutionary relations among haplotypes were shown in the minimum spanning network (Fig. 2). The two most widespread haplotypes (Sl1 and Sl4), linked by one mutation step, occupy the central positions of the network. Looking at their geographic distribution in non-native basins, Sl1 resulted exclusively widespread in Po River basin while Sl4 was dispersed in all Italian basin with the exclusion of the southern basin (Serchio River basin), characterized only by two private haplotypes (Sl12 and Sl13). From Sl1, Sl8 radiated with nine mutational steps, and it was recorded in the Po River basin (i.e. pop MN = Mincio River). On the other hand, from Sl4 radiated nine haplotypes: i) Sl10 and Sl9 directly with only one mutational step, while ii) seven haplotypes radiated in a subnetwork connected up to five mutational steps (Fig. 2). A total of four haplotypes were shared among native and non-native basins, specifically Sl1, Sl3, Sl4, and Sl8, while five haplotypes were exclusive in the non-native basins (Sl9-Sl13). In detail, the most abundant were Sl11 retrieved in four populations of Po River basins, while Sl12 only in Lake Massaciuccoli, in Serchio River basin.

In native populations, the genetic variability ranged between 0.64–0.82 (H_d) and 0.23–0.45% (π), while in non-native range the H and π values ranged between 0–0.81 and 0–0.29%, respectively (Supplementary Material S2).

Grouping native and non-native populations by basins, the values of genetic variability indices recorded in the Po river basin ($H_d = 0.65 \pm 0.02$ st.d. and $\pi = 0.21\% \pm 0.02$ st.d.) resulted similar to values detected in the native range ($H_d > 0.64$ and $\pi = 0.36\%$); on the other hand, the genetic variability showed decreasing values along north–south gradient up to the absence of genetic variability in Volturno basin (pop S) dominated by exclusively Sl4 haplotypes (Table 3; see Fig. 2).

The genetic variability among six basins was also tested through AMOVA, where significant results ($P < 0.001$) were found within populations and among population within groups but not among six basins (Table 4).

Nuclear DNA variability and microsatellite analysis

Given the lack of general evidence for significant errors or genetic disequilibria, all loci and all sites were considered for further analyses. The final genotype dataset included all ten microsatellite loci and 268 individuals (see Table 1). In detail, the estimation of genetic variation showed that allelic richness ranged from 1.69 to 1.82, while the observed heterozygosity (H_o) ranged between 0.98 to 1 (Supplementary Table S4). The number of alleles recorded for loci ranged from 3.4 to 8. Genetic variability

Table 3 Statistical tests of neutrality and demographic parameter estimated with *P*-value (in parenthesis) sampled basins. Sample size (*N*), number of haplotypes (*h*), haplotype diversity (H_d), nucleotide diversity (π)

Diversity index				
Basin	*N*	H	H_d	π%
Elbe	15	Sl1.Sl2.Sl3.Sl4.Sl5.Sl6.Sl7	0.89 ± 0.05	0.36 ± 0.11
Rhine	10	Sl3.Sl4.Sl8	0.64 ± 0.10	0.45 ± 0.19
Po	148	Sl1.Sl3.Sl4.Sl8.Sl9.Sl10.Sl11	0.65 ± 0.02	0.21 ± 0.02
Arno	12	Sl3.Sl4	0.41 ± 0.13	0.15 ± 0.05
Serchio	17	Sl12.Sl13	0.12 ± 0.10	0.02 ± 0.01
Volturno	5	Sl4	0.00 ± 0.00	0.00 ± 0.00
	207	13	0.73 ± 0.02	0.23 ± 0.02

Table 4 Results of analysis of molecular variance (AMOVA) test on D-loop based on the six different basins (two native and four non-native)

Source of variation	d.f	Sum of square	Variance components	Percentage variation	Fixation indices	
Among groups	5	56.32	0.40 *Va*	33.25	Φ_{CT}	Φ_{CT}
Among population within groups	11	43.16	0.27 *Vb*	22.12	Φ_{SC}	0.33*
Within populations	190	103.01	0.54 *Vc*	44.63	Φ_{ST}	0.55*
Total	206	202.49	1.21	–	–	–

*$P < 0.001$

recorded for each population evidenced as the native populations H_e and H_o were higher than 0.99 and 0.76, respectively, while in the non-native populations, the lowest values of H_o and H_e (0.98 and 0.65) were recorded in Lake Garda (pop G, Po River basin) (Supplemental Table S4). All F_{is} values resulted negative and significant ($P < 0.01$) for all populations (Supplemental Table S4). The analysis of effective population size $N_e \rightarrow \infty$ reported N_e exclusively for native populations, assuming any variation in genetic signature caused by genetic drift (normally promoted by a finite number of parents). On contrary, estimated N_e in eight non-native populations ranged from $N_e = 38.6$ to $N_e = 175.9$, suggesting genetic drift, while in the other seven non-native population with small sample size ($N < 10$) the N_e values was not supported (Supplemental Table S4).

Then, genetic variation estimated per basin evidenced as the observed (H_o) and expected (H_e) heterozygosity in native basins (Elbe and Rhine) were similar to values recorded in non-native basins (Po River, Arno River and Serchio River), with significant and negative F_{is} values, suggesting an excess of heterozygosity in all basins (Table 5). Looking at the effective population size, only the Po River basin and the Arno River basin showed $N_e = 99$ and $N_e = 43$, respectively (Table 5).

The most probable number of K populations in the Bayesian clustering analysis was 2 with $\mathrm{LnP}(K) = -9333.94$. The two main genetic pools clustered into: (i) native populations from Rhine and Elbe basins plus non-native populations from central Italian basins (Serchio and the Arno basins) and (ii) all 11 non-native populations from the Po basin and the population from Volturno basin, geographically located in the north and southern Italian peninsula, respectively (Fig. 3).

Discussion

Invasion is a multi-stage process, where introduced species overcome environmental and biological barriers, may establish and spread in a new, non-native habitat. One of the focal factors in the establishment success of the invasion process is directly related to the number of individuals introduced and to the frequencies of introductions (i.e. propagule pressure), generating a different impact on genetic diversity (Lockwood et al., 2005). Although non-native species introductions often create a founder effect, where a new population is established by a small number of individuals, leading to significantly reduced genetic diversity compared to the source population (Lawson Handley et al., 2011), paradoxically, many invaders succeed in invasion expansion. The genetic signature may track and explain invasion success by revealing the genetic variability and evolutionary potential of non-native species. As supported by several studies, admixture between populations acts as a significant process increasing genetic diversity and supporting invasion success (Wolfe et al., 2007; Dutech et al., 2012; Chapple et al., 2013). The occurrence of introduction events from distinct genetic sources may be a valid strategy to establish, spread, and rapidly adapt to new environments, bringing together distinct genetic pools, increasing genetic admixture, and reducing the risk of inbreeding depression. In Italian basins, the introduction has been prevalently promoted by a combination of intentional and unintentional introduction events, driven by anglers for sport fishing and

Table 5 Genetic variation estimated per basin of *Silurus glanis*.

Basin	n	H_e	H_O	N_A	A_R	A_{pr}	F_{IS}	N_e
Elbe	24	0.78	1.00	6.9	1.81	0.23	**−0.24**	∞
Rhine	16	0.76	1.00	6.9	1.79	0.28	**−0.28**	∞
Po	165	0.80	0.99	5.8	1.76	0.13	**−0.23**	99.3
Arno	19	0.78	1.00	8	1.81	0.28	**−0.24**	43.4
Serchio	39	0.77	1.00	4.4	1.78	0.31	**−0.28**	∞

Indicated are sample size (n), expected heterozygosity (H_e), observed heterozygosity (H_o), average number of alleles per locus (N_A), allelic richness (A_R), private allelic richness (A_{pr}), fixation index (F_{IS}) (significance level $P < 0.01$), and effective size (N_e) (critical values 0.1). *Volturno basin was not included due to the low sample size ($n = 6$). Significant values are in bold

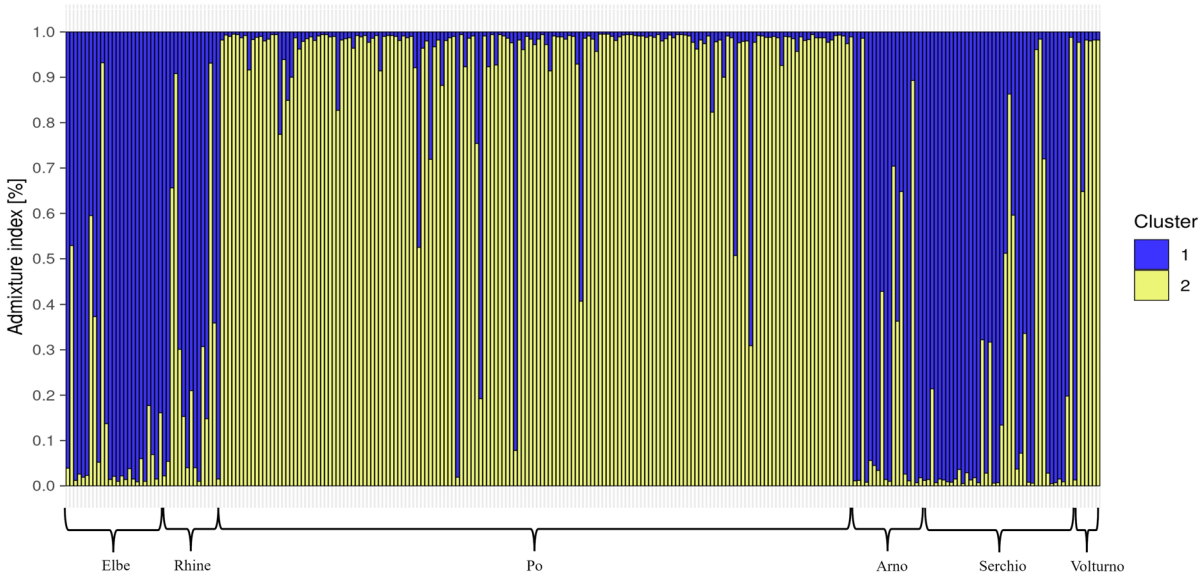

Fig. 3 Structure bar plot ($K=2$, highest likelihood run out of 10 repetitions)

accidental releases from private ponds, respectively (Castaldelli et al., 2013; Sicuro et al., 2016).

In this study, analysing the genetic signature of European catfish along the non-native Italian basins, it is supported as the occurrence of the multiple-source introduction may be the principal model of the actual invasion success. This assumption is endorsed by general high values of genetic diversity (H_d ranging from 0.12 to 0.65 and $\pi\%$ ranging from 0.02 to 0.21) joined to an high number of alleles (up to 6.9) and high level of observed heterozygosity (ranging from 0.98 to 1), recorded in both mitochondrial and nuclear markers, respectively. All F_{IS} values resulted significantly negative, typically indicating heterozygous excess caused by mixing genetically distinct individuals, supporting the genomic signature for multiple-source introduction as a principal model of invasion (Kolbe et al., 2004).

In detail, in the Po River basin the high genetic variability ($H_o=0.99$) joined to the high genetic diversity ($H_d=0.65\pm0.02$ and $\pi\%=0.21\pm0.02\%$) may be the signal of multiple sources of introduction. Even though the N_e value resulted below 100 parents, multiple sources of introduction is further supported by the robust presence of several private haplotype and the widespread presence of the haplotype Sl1 and Sl4, detected both in the native range (Elbe and Rhine basins) and in the Hungarian and Turkish basins (Bektaş et al., 2020; Székvári et al., 2021, respectively).

Further, for Tyrrhenian basins (Arno, Serchio, and Volturno) the hypothesis of *S. glanis* introduction from multiple sources can be advanced. For Arno and Serchio basins, high genetic variability ($H_o=1.00$) has been detected, jointly with F_{IS} values supporting outbreeding. Despite that the genetic pool of both Arno and Serchio basins clustered with the same pool recorded in Elbe and Rhine basins, and the genetic composition evidenced the relevant presence of Sl12 haplotype, previously never recorded and for which a distinct source may be supposed. Similar comments were promoted by Castagné et al., (2023), in which were reported introduction events of *S. glanis* from Bulgarian populations, specifically in the Arno basin and Sile basin (north-east Italy).

Although only one haplotype was detected in the Volturno basin (Sl4), outcome probably influenced by the low number of individuals analysed ($n=6$), it is surprising the high level of heterozygosity observed ($H_o=0.98$). This genetic outcome might be indicative of the ongoing invasion success also in Volturno basin, where European catfish was for the first time recorded in 2010s (Fig. 1).

The genetic pattern of high genetic variability, evidenced in this study, may drastically support the idea that *S. glanis* populations are well introduced and

adapted in the new habitat. This genetic diversity may have enhanced adaptability, on the one side, preventing depression from inbreeding depression and, on the other side allowing populations to adapt to changing environmental conditions like climate change. Indeed, it has been observed that with rising temperature, the European catfish becomes relatively active throughout the entire year (Encina et al., 2024; Gavioli et al., 2024), promoting the ability to boost reproduction and growth (Copp et al., 2009). Additionally, both extremely high fecundity (Gkenas et al., 2025) or very fast spreading through the river basin via the pelagic larval stage (Sajdlová et al., 2025) might increase the availability of new potential habitats for *S. glanis* expansion (Baer et al., 2025), favouring in the further way its invasion success.

Management actions towards the eradication

In light of the results obtained in this study, it is clear that the successful invasion and spread of the European catfish in Italian basins is also favoured by high genetic variability, combined with dietary plasticity and the continued natural expansion of its range associated with climate change. The invasion scenario affecting Italian basins demonstrates the need to intensify efforts to monitor the species' expansion and promote containment and eradication actions at both the local and basin levels.

At the beginning of the century, it was already clear that *S. glanis* was well adapted and abundant in the Po River basin, which offers habitats of slow-flowing lotic and lentic waters, both suitable for its growth (Copp et al., 2009). Its expansion has contributed to the loss of ecosystem stability, already severely altered by human pressures such as pollution, habitat, and hydraulic flow alterations. To limit the successful invasion and expansion of *S. glanis*, the first official eradication action has been launched in 2002 (LIFE2000NAT/IT/7268). Today, after two decades of containment efforts, population abundance shows no significant decline (Lanzoni et al., 2018), a finding ascribable to its high ecological adaptability, which may be associated with the high overall genetic variability recorded in this study. These factors may, in fact, have limited the effectiveness of the containment management strategy.

Furthermore, the results of this study showed a genetic variability similar to that recorded in populations from the Po basin. The recent introduction of *S. glanis* in the Arno basin (8,200 km^2) and the Serchio basin (1,500 km^2) showed a genetic variability similar to that recorded in populations from the Po basin, although the invasion began around the 1990s and 2000s, respectively. The genetic results highlighted the rapid success of the introduction and the great capacity of the species to adapt to a new habitat, underlining the need to integrate management with containment activities.

Regarding the Volturno basin, where the first establishment of *S. glanis* can be dated in the mid-2010s (De Bonis et al., 2015), there are a few data available to better understand its status. However, the few individuals investigated in this study showed a unique haplotype but a high level of observed heterozygosity. Considering the extension of its basin (around 5,500 km^2) and the presence of suitable growing habitat for *S. glanis*, it is possible to hypothesize that *S. glanis* populations could grow and expand its distribution range as it happened in the Po basin. As for the Serchio basin, management strategies of eradication action should be put in place. In other southern European lakes and reservoirs, new management strategies to prevent further spreading and future introductions are on the way (De Santis et al., 2024).

In conclusion, even if non-native fish removal programmes are challenging, considering the availability of funding compared to the efforts required to complete the eradication programme, the selection of a suitable programme (Rytwinski et al., 2018), these programmes can be effective (Britton et al., 2011). Electrofishing studies indicated successful eradication is possible but requires intensive effort and multiple treatments over several years (Rytwinski et al., 2018). Up to now in the Po River basin, these efforts have unsatisfactory results, probably because they were put in place not soon enough, hence, not intercepting the first phases of introduction and demographic expansion. Therefore, the unsuccessful results of the eradication strategy implemented in the Po basin suggest that an early start of the eradication strategy is imperative. It should also be remembered that rapid actions are urgently needed due to the positive effect of climate change on acclimatization and expansion of *S. glanis*, a factor that is hindering the success of containment and eradication programmes.

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Declarations

Conflict of interest The authors have no relevant financial or non-financial interests to disclose.

References

Aljanabi, S. M. & I. Martinez, 1997. Universal and rapid salt-extraction of high quality genomic DNA for PCR-based techniques. Nucleic Acids Research 25: 4692–4693.

Antognazza, C. M., T. Costantini, M. Campagnolo, et al., 2022. One year monitoring of ecological interaction of *Silurus glanis* in a novel invaded oligotrophic deep lake (Lake Maggiore). Water 14: 105. https://doi.org/10.3390/w14010105.

Baer, J., S. Hüsgen, M. Fromherz, et al., 2025. Drivers of the range expansion of the European catfish (*Silurus glanis*) within its native distribution. Journal of Fish Biology 107: 1030–1044.

Baldaccini, N. E, Ercolini, P., 2006. Il popolamento animale. Terra e acqua, una bonifica per lo sviluppo. Ed. Pacini, Pisa: 111–131.

Bektaş Y, Aksu I. K, G, Turan D.,2020) Low Genetic Diversity in Turkish Populations of Wels Catfish S*ilurus glanis* L., 1758 (Siluridae, Pisces) Revealed by Mitochondrial Control Region Sequences. Turkish Journal of Fisheries andAquatic Sciences 10: 767–776. https://doi.org/10.4194/1303-2712-v20_10_06

Boulêtreau, S. & F. Santoul, 2016. The end of the mythical giant catfish. Ecosphere 7: e01606.

Britton, J. R., J. Cucherousset, G. D. Davies, et al., 2010. Non-native fishes and climate change: predicting species responses to warming temperatures in a temperate region. Freshwater Biology 55: 1130–1141. https://doi.org/10.1111/j.1365-2427.2010.02396.x.

Britton, J. R., R. E. Gozlan & G. H. Copp, 2011. Managing non-native fish in the environment. Fish and Fisheries 12: 256–274.

Castagné, P., I. Paz-Vinas, S. Boulêtreau, et al., 2023. Patterns of genetic variation in native and non-native populations of European catfish *Silurus glanis* across Europe. Biodivers Conserv 32: 2127–2147. https://doi.org/10.1007/s10531-023-02596-w.

Castaldelli, G., A. Pluchinotta, M. Milardi, et al., 2013. Introduction of exotic fish species and decline of native species in the lower Po basin, north-eastern Italy. Aquatic Conservation: Marine and Freshwater Ecosystems 23: 405–417. https://doi.org/10.1002/aqc.2345.

Cerri, J., A. Ciappelli, A. Lenuzza, et al., 2018. Recreational angling as a vector of freshwater invasions in Central Italy: perceptions and prevalence of illegal fish restocking. Knowledge & Management of Aquatic Ecosystems. https://doi.org/10.1051/kmae/2018028.

Chapple, D. G., K. A. Miller, F. Kraus & M. B. Thompson, 2013. Divergent introduction histories among invasive populations of the delicate skink (*Lampropholis delicata*): has the importance of genetic admixture in the success of biological invasions been overemphasized? Diversity Distrib. 19: 134–146. https://doi.org/10.1111/j.1472-4642.2012.00919.x.

Clement, M., D. Posada & K. Crandall, 2000. TCS: a computer program to estimate gene genealogies. Molecular Ecology 9: 1657–1660. https://doi.org/10.1046/j.1365-294x.2000.01020.x.

Confortini, I., 1995. L'ittiofauna del lago di Garda, Provincia di Verona, Settore tutela faunistico ambientale.

Copp, G. H., P. G. Bianco, N. G. Bogutskaya, et al., 2005. To be, or not to be, a non-native freshwater fish? Journal of Applied Ichthyology 21: 242–262. https://doi.org/10.1111/j.1439-0426.2005.00690.x.

Copp, G. H., J. R. Britton, J. Cucherousset, et al., 2009. Voracious invader or benign feline? A review of the environmental biology of European catfish *Silurus glanis* in its native and introduced ranges. Fish and Fisheries 10: 252–282. https://doi.org/10.1111/j.1467-2979.2008.00321.x.

Criscuolo, N. G. & C. Angelini, 2020. StructuRly: A novel shiny app to produce comprehensive, detailed and interactive plots for population genetic analysis. PLOS ONE 15: e0229330. https://doi.org/10.1371/journal.pone.0229330.

De Bonis, S., A. Giorgio, F. Sirignano, et al., 2015. Presenza di *Silurus glanis* Linnaeus, 1758, nel bacino del fiume Volturno (Campania). Biologia Ambientale 29: 62–67.

De Santis, V., S. Brignone, M. Čech, et al., 2024. LIFE PREDATOR: prevent, detect, combat the spread of *Silurus glanis* in south European lakes to protect biodiversity. NeoBiota 93: 225–244. https://doi.org/10.3897/neobiota.93.105200.

Do, C., R. S. Waples, D. Peel, et al., 2014. NeEstimator v2: re-implementation of software for the estimation of contemporary effective population size (Ne) from genetic data. Molecular Ecology Resources 14: 209–214.

Dutech, C., B. Barrés, J. Bridier, C. Robin, M. G. Milgroom & V. Ravignè, 2012. The chestnut blight fungus world tour: successive introduction events from diverse origins in an invasive plant fungal pathogen. Molecular Ecology 21: 3931–3946. https://doi.org/10.1111/j.1365-294X.2012.05575.x.

Earl, D. A. & B. M. von Holdt, 2012. STRUCTURE HARVESTER: a website and program for visualizing STRU CTURE output and implementing the Evanno method. Conservation Genetics Resources 4: 359–361. https://doi.org/10.1007/s12686-011-9548-7.

Encina, L., A. Rodríguez-Ruiz, C. Orduna, et al., 2024. Impact of invasive European catfish (*Silurus glanis*) on the fish community of Torrejón reservoir (Central Spain) during a 11-year monitoring study. Biological Invasions 26: 745–756. https://doi.org/10.1007/s10530-023-03204-2.

Ercolini, P., 2015. Il siluro (*Silurus glanis* Linnaeus, 1758) nelle acque del Lago di Massaciuccoli: un rischio per la biodiversità. Biologia Ambientale 29: 35–38.

Evanno, G., S. Regnaut & J. Goudet, 2005. Detecting the number of clusters of individuals using the software structure: a simulation study. Molecular Ecology 14: 2611–2620. https://doi.org/10.1111/j.1365-294X.2005.02553.x.

Excoffier, L. & H. E. L. Lischer, 2010. Arlequin suite ver. 3.5: a new series of programs to perform population genetics analyses under Linux and Windows. Molecular Ecology Resources 10: 564–567. https://doi.org/10.1111/j.1755-0998.2010.02847.x.

Falush, D., M. Stephens & J. K. Pritchard, 2003. Inference of population structure using multilocus genotype data: linked loci and correlated allele frequencies. Genetics 164: 1567–1587. https://doi.org/10.1093/genetics/164.4.1567.

Ferreira, M., J. Gago & F. Ribeiro, 2019. Diet of European catfish in a newly invaded region. Fishes. https://doi.org/10.3390/fishes4040058.

Gandolfi, G., Giannini, M ., 1979. La presenza di *Silurus glanis* nel fiume Po (osteichthyes siluridae).

Gavioli, A., G. Castaldelli, S. Trasforini, et al., 2024. Global warming and fish diversity changes in the Po river (Northern Italy). Environments. https://doi.org/10.3390/environments11100226.

Gkenas, C., V. Sequeira, D. Ribeiro, et al., 2025. Reproductive traits of the European catfish, *Silurus glanis*, during the early stages of invasion. Journal of Vertebrate Biology 74: 24122–24131.

Hall, T. A., 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucleic Acids Symposium Series 41: 95–98.

Haubrock, P. J., M. Azzini, P. Balzani, et al., 2020. When alien catfish meet - resource overlap between the North American *Ictalurus punctatus* and immature European *Silurus glanis* in the Arno River (Italy). Ecology of Freshwater Fish 29: 4–17. https://doi.org/10.1111/eff.12481.

Holland, B. S., 2000. Genetics of marine bioinvasions. Hydrobiologia 420: 63–71. https://doi.org/10.1023/A:1003929519809.

Kalinowski, S. T., 2004. Counting alleles with rarefaction: private alleles and hierarchical sampling designs. Conservation Genetics 5: 539–543. https://doi.org/10.1023/B:COGE.0000041021.91777.1a.

Kolbe, J. J., R. E. Glor, L. Rodríguez Schettino, et al., 2004. Genetic variation increases during biological invasion by a Cuban lizard. Nature 431: 177–181. https://doi.org/10.1038/nature02807.

Kottelat, M. & J. Freyhof, 2007. Handbook of European Freshwater Fishes, Kottelat Cornol, Switzerland and Freyhof, Berlin, Germany:

Lanzoni, M., M. Milardi, V. Aschonitis, et al., 2018. A regional fish inventory of inland waters in Northern Italy reveals the presence of fully exotic fish communities. The European Zoological Journal 85: 1–7. https://doi.org/10.1080/24750263.2017.1415384.

Lawson Handley, L. J., A. Estoup, D. M. Evans, et al., 2011. Ecological genetics of invasive alien species. BioControl 56: 409–428. https://doi.org/10.1007/s10526-011-9386-2.

Lee, C. E., 2002. Evolutionary genetics of invasive species. Trends in Ecology & Evolution 17: 386–391.

Lockwood, J. L., P. Cassey & T. Blackburn, 2005. The role of propagule pressure in explaining species invasions. Trends in Ecology & Evolution 20: 223–228. https://doi.org/10.1016/j.tree.2005.02.004.

Mancini, E., F. Tiralongo, F. C. Coccia, et al., 2022. The wels catfish *Silurus glanis* Linnaeus, 1758 (Actinopterygii Siluriformes) in Italian waters: a review with first report in the Bolsena lake (Italy). Biodiversity Journal 13: 673–684.

Manfredi, P., 1957. Cattura di un *Silurus glanis* nell'Adda presso Lecco. Natura 48: 28–30.

Mooney, H. A. & E. E. Cleland, 2001. The evolutionary impact of invasive species. Proceedings of the National Academy of Sciences 98: 5446–5451. https://doi.org/10.1073/pnas.091093398.

Nocita, A., 2002. Carta ittica della Provincia di Firenze.

Petit, R. J., A. El Mousadik & O. Pons, 1998. Identifying populations for conservation on the basis of genetic markers. Conservation Biology 12: 844–855. https://doi.org/10.1111/j.1523-1739.1998.96489.x.

Pritchard, J. K., M. Stephens & P. Donnelly, 2000. Inference of population structure using multilocus genotype data. Genetics 155: 945–959. https://doi.org/10.1093/genetics/155.2.945.

Rousset, F., 2008. genepop'007: a complete re-implementation of the genepop software for Windows and Linux. Molecular Ecology Resources 8: 103–106. https://doi.org/10.1111/j.1471-8286.2007.01931.x.

Rozas, J., A. Ferrer-Mata, J. C. Sánchez-DelBarrio, et al., 2017. DnaSP 6: DNA sequence polymorphism analysis of large data sets. Molecular Biology and Evolution 34: 3299–3302. https://doi.org/10.1093/molbev/msx248.

Rytwinski, T., J. J. Taylor, L. A. Donaldson, et al., 2018. The effectiveness of non-native fish removal techniques in freshwater ecosystems: a systematic review. Environmental Reviews 27: 71–94. https://doi.org/10.1139/er-2018-0049.

Sajdlová, Z., T. Jůza, P. Blabolil, et al., 2025. Pelagic distribution and night foraging of early juvenile European catfish (*Silurus glanis* L.). Journal of Fish Biology 106: 1893–1898. https://doi.org/10.1111/jfb.16072.

Sakai, A. K., F. W. Allendorf, J. S. Holt, et al., 2001. The population biology of invasive species. Annual Review of Ecology and Systematics 32: 305–332. https://doi.org/10.1146/annurev.ecolsys.32.081501.114037.

Sala, O. E., F. Stuart Chapin, J. J. Armesto, et al., 2000. Global biodiversity scenarios for the year 2100. Science 287: 1770–1774.

De Santis, V., Volta, P., 2021. Spoiled for Choice during Cold Season? Habitat Use and Potential Impacts of the Invasive *Silurus glanis* L. in a Deep, Large, and Oligotrophic Lake (Lake Maggiore, North Italy). Water 13.18: 2549. https://doi.org/10.3390/w13182549

Santos AM, Cabezas MP, Tavares AI, et al., 2016. tcsBU: a tool to extend TCS network layout and visualization. Bioinformatics: 32.4: 627-628. https://doi.org/10.1093/bioinformatics/btv636

Sicuro, B., M. Tarantola & E. Valle, 2016. Italian aquaculture and the diffusion of alien species: costs and benefits. Aquaculture Research 47: 3718–3728. https://doi.org/10.1111/are.12997.

Simberloff, D., J. Martin, P. Genovesi, et al., 2013. Impacts of biological invasions: what's what and the way forward. Trends in Ecology & Evolution 28: 58–66. https://doi.org/10.1016/j.tree.2012.07.013.

Specchi M, Pizzul E., 1994. First observations of *Silurus glanis* (L. 1758) in the streams of the Isonzo basin (Northeastern Italy (Osteichthyes, Siluridae). Gortania; Atti del Museo Friulano di Storia Naturale di Udine: Udine, Italy 213–216

Székvári, K., Z. Szabolcsi, B. Kutasy, et al., 2021. New complete mitogenome datasets and their characterization of the European catfish (*Silurus glanis*). Data in Brief 38: 107418. https://doi.org/10.1016/j.dib.2021.107418.

Szpiech, Z. A., M. Jakobsson & N. A. Rosenberg, 2008. ADZE: a rarefaction approach for counting alleles private to combinations of populations. Bioinformatics 24: 2498–2504. https://doi.org/10.1093/bioinformatics/btn478.

Van Oosterhout, C., W. F. Hutchinson, D. P. M. Wills, et al., 2004. micro-checker: software for identifying and correcting genotyping errors in microsatellite data. Molecular Ecology Notes 4: 535–538. https://doi.org/10.1111/j.1471-8286.2004.00684.x.

Vejřík, L., I. Vejříková, P. Blabolil, et al., 2017a. European catfish (*Silurus glanis*) as a freshwater apex predator drives ecosystem via its diet adaptability. Scientific Reports 7: 1–15. https://doi.org/10.1038/s41598-017-16169-9.

Vejřík, L., I. Vejříková, L. Kočvara, et al., 2017b. Thirty-year-old paradigm about unpalatable perch egg strands disclaimed by the freshwater top-predator, the European catfish (*Silurus glanis*). PLoS One 12: e0169000.

Verreycken H., 2019. Invasive alien species native to parts of the EU: The European catfish (*Silurus glanis*). Technical note prepared by IUCN for the European Commission.

Volta, P., E. Jeppesen, P. Sala, et al., 2018. Fish assemblages in deep Italian subalpine lakes: history and present status with an emphasis on non-native species. Hydrobiologia 824: 255–270. https://doi.org/10.1007/s10750-018-3621-0.

Weir BS, Cockerham CC., 1984. Estimating F-statistics for the analysis of population structure. evolution:1358–1370. https://doi.org/10.1111/j.1558-5646.1984.tb05657.x

Weisstein EW., 2004. Bonferroni correction. https://mathworld.wolfram.com/.

Wolfe, L. M., A. C. Blair & B. M. Penna, 2007. Does intraspecific hybridization contribute to the evolution of invasiveness?: an experimental test. Biol Invasions 9: 515–521. https://doi.org/10.1007/s10530-006-9046-0.