A new possible breeding site of *Alosa fallax* (Lacépède 1803) (Actinopterygii: Clupeiformes: Alosidae) on the Tyrrhenian coast of Central Italy

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Abstract - The River Mignone (Tarquinia, Viterbo, central Italy) is one of the best preserved watercourses in Lazio. Here, in April and May of the years 2020-2023, a conspicuous contingent of *Alosa fallax* (Lacépède 1803) was reported. The frenetic behaviour of the individuals observed, some of which showed swollen bellies and considerable size, suggested that this watercourse may represent a potential new breeding station for this threatened migratory euryhaline fish species. The correct recognition of the sex of *A. fallax* in the field, problematic during the breeding season, is crucial for understanding its population structure as indicators of possible reproduction. To this end, statistical analysis (Mann-Whitney U-test) of 20 morphometric measurements made on individuals captured and subsequently released revealed significant differences between the sexes for seven morphometric characters, confirming a sex ratio compatible with reproduction. However, the presence of a transverse dam with no fish ladder and the dispersion of biocides from farmland in the final stretch may represent a serious threat to effective reproduction.

Key words: *Alosa fallax*, Clupeiformes, new breeding site, conservation.

Riassunto - Un nuovo possibile sito riproduttivo di *Alosa fallax* (Lacépède 1803) (Actinopterygii: Clupeiformes: Alosidae) sulla costa tirrenica dell'Italia Centrale.

Il fiume Mignone (Tarquinia, Viterbo, Italia centrale) è uno dei corsi d'acqua meglio conservati del Lazio. Lungo questo corso d'acqua, nei mesi di aprile e maggio 2020-2023, è stata segnalata la risalita di un cospicuo contingente di *Alosa fallax* (Lacépède 1803). Il comportamento frenetico degli individui osservati, alcuni dei quali mostravano il ventre gonfio e dimensioni considerevoli, ha suggerito che questo sito possa rappresentare una nuova potenziale stazione riproduttiva per questa specie ittica eurialina migratrice minacciata. Il corretto riconoscimento del sesso di *A. fallax* sul campo, problematico anche durante il periodo riproduttivo, è fondamentale per comprenderne la struttura di popolazione e il comportamento come indicatori di una possibile riproduzione. A tal fine, l'analisi statistica (Mann-Whitney U-test) di 20 misure morfometriche effettuate su individui catturati e successivamente rilasciati ha rivelato differenze significative tra i sessi per sette caratteri morfometrici, confermando un rapporto sessi compatibile con la riproduzione. Tuttavia, la presenza di una diga trasversale priva di scala di risalita per i pesci e la dispersione di biocidi dai terreni agricoli possono rappresentare una seria minaccia per l'effettivo concretizzarsi della riproduzione.

Parole chiave: *Alosa fallax*, Clupeiformes, conservazione, nuovo sito riproduttivo.

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INTRODUCTION

Alosa fallax (Lacépède 1803), a clupeiform fish, is a polymorphous gregarious species with a still controversial systematics, with sedentary populations (called agons) and anadromous euryhaline migratory populations (Chiesa & Nonnis Marzano, 2016) known as twaite shad (Quignard & Douchement, 1991). In Italy, both anadromous and landlocked populations are present. Recent genetic evaluations have confirmed their belonging to the same phylogenetic lineage (Chiesa *et al*., 2014), but also a certain adaptive ecological plasticity in landlocked populations (Sabatino *et al*., 2022).

Agons, which usually live in the pelagic zone of some large inland pre-alpine lakes, move to the littoral zone for spawning. Adult twaite shads, after spending 3-5 years in pelagic and coastal marine areas, once they reach sexual maturity, return to fresh waters, travelling tens or even hundreds of kilometres along watercourses, until they reach ecologically suitable stretches, where they spawn (Aprahamian *et al*., 2003).

In this species, the migration begins in April and continues until May-June, ending with the spawning, which takes place during the night, between the end of May and the beginning of July (Quignard & Douchement, 1991). Sexual dimorphism is not evident, except that mature females are slightly larger than males due to the expansion of the ovarian cavity.

A. fallax has a wide geographical distribution, ranging from the Baltic Sea to Morocco, via the coasts of Great Britain and Ireland, the Atlantic coasts of Western Europe and the Mediterranean, where it is most frequent in Gree-

ce, Turkey and Egypt (Nile Delta); the species is rarer in the Black Sea (Whitehead, 1984).

Being an anadromous species, it can be found in most European Rivers. Twaite shads are present in all Italian seas and are used to moving up all the main waterways of the peninsula and the major islands to reproduce. Today, spawning populations are found in the Po river up to the Isola Serafini weir, and in the Taro, Ombrone and Magra rivers (Bianco *et al*., 2013). However, in recent decades, the populations of this species have undergone considerable decrease mainly for the presence of transversal dams, and the pressure of overfishing on migrating spawners (Zerunian, 2007; Rondinini *et al*., 2022). Some relevance can be attributed also to water pollution and to climate change acting on the chemical and physical parameters of water (Chiesa & Nonnis Marzano, 2016).

Alosa fallax is a species of conservation concern (Annexes II, V - Habitats Dir. 92/43/EEC; Annex III Bern Convention; 'Endangered' category in the Italian Red List of native freshwater fishes): as a matter of fact, the species is in sharp decline in Italy, with few residual populations and most of the reproductive stocks now extinct (Bianco, 2014; Rondinini *et al*., 2022).

Within the framework of a long-term study on the faunal biodiversity of the Mignone River Valley initiated in 2016, which also includes a citizen science project, the 'Gruppo Naturalistico della Maremma Laziale' encouraged anglers to share reports and photographic documentation on the catch of infrequent fish species (Crescia *et al*., 2020; Ferri *et al*., 2021). In this context, the non-occasional presence of *Alosa fallax* in this river is discussed.

MATERIALS AND METHODS

The study area is included in the Special Area of Protection IT6010035 'Fiume Mignone (lower course)', within the S.A.P. 6030005 'Tolfetano-Cerite-Manziate' (Fig. 1). The total length of Mignone river is 62 km (from the Sabatini Mountains to the Tyrrhenian Sea). The overall contribution of the tributaries is low and the hydraulic behaviour is variable and typical of a torrential regime. The catchment area (560 km^2) is hilly and shows some elevations with steep slopes that correspond to the valley sides eroded by water. Altitude averages 233 m a.s.l. (range: 6-618 m a.s.l.). Geologically, the Mignone river is characterized by volcanic rocks in the upper basin, while flysch, sands, conglomerates, clays, and alluvial deposits characterize the lower basin (Della Seta *et al*., 2006).

Fish community includes ten species: Italian bleak *Alburnus alborella*, European eel *Anguilla anguilla*, horse barbel *Barbus tyberinus*, Italian chub *Squalius squalus*, Italian spined loach *Cobitis taenia bilineata*, Padanian goby *Padogobius bonelli*, South European nase *Protochondrostoma genei*, rovella *Sarmarutilus rubilio*, the allochthonous stone moroko *Pseudorasbora parva*, and the recently recorded sea lamprey *Petromyzon marinus* (Ferri *et al*., 2021).

In April 2020, an angler (A. Olini) reported an unexpected catch. During a morning spinning session, he caught a big individual of *Alosa fallax*, using a fishing rod and a single barbless hook baited with an artificial bait, in the same stretch of river where the presence of the sea lamprey had previously been reported (Ferri *et al*., 2021). The site is located approximately 6 km from the river mouth and just downstream of the Le Mole dam, an apparently impassable obstacle for the species. There the river presents a gravel bottom and sustained current.

In the following months, three more morning spinning sessions were carried out, with the same fishing gear, in the same stretch of river. At the time of the captures, the river was about 10 m wide and between 20 and 60 cm deep (Fig. 4), with moderate current and turbid water. Water temperature ranged 12-15 °C.

The casual captures involved experienced people with regular sport fishing permits (article 8, paragraph 3, of the L.R. Lazio no. 87/1990). Given the fragility of this species as per resistance to capture stress, the time taken from capture to release, including weight measurement and photographic documentation, was kept to a minimum (Figs. 1 and 2; Table 1). Weight (W) was measured in grams with a digital scale with an error of ±1 g and a maximum load capacity of 7 kg. Some scales were also collected for age determination, in anticipation of their future use for study. Sex determination was estimated visually during capture following Quignard & Douchement (1991). Correct sex recognition in this species, which exhibits a low degree of sexual dimorphism even during the breeding season, is nonetheless important in order to know the social structure and behaviour during mating (Chiesa & Nonnis Marzano, 2016). Sexual dimorphism in fishes can be assessed using morphometric methods with fairly accurate results (e.g., Ulicevic *et al*., 2018). To avoid further manipulation stress, the caught individuals were photographed left-side near to a ruler or to an object of known size for comparison, trying to place the fish as horizontal as possible and the camera lens parallel to the shooting plan (Fig. 2; Suryaningsih *et al*., 2014). Linear measurements (mm) were then carried out on the photographs using the Snakemeasurer© software (Penning *et al*., 2013), freely available as Snake Measure Tool Version 2.1.1. ©2011 Marcin Stefanski (http://serpwidgets.com/main/measure).

Using the method described above, the following measurements were taken on each captured individual: total length (TL), standard length (SL), head height (HH), head length (HL), eye diameter (ED), the distance between the mouth and the eye or periorbital distance (POD), pre- and post- dorsal distance of dorsal fin (PDDF, PDAF), maximum and minimum body height (BHa, BHb), distance between dorsal and caudal fin (BAF), dorsal fin height and length (HDF, DFL), pectoral fin height and length (HPF, PFL), ventral fin height and length (HVF, VFL), anal fin height and length (HAF, AFL). Data were subsequently analysed performing a Mann-Whitney U-test and the *results box-plotted. T*o describe the relationship between total length and weight we utilized an Ordinary Least Square Regression. All analyses were achieved by the PAST software (Hammer *et al*., 2001). The results of the statistical tests were interpreted with $\alpha = 0.05$.

Fig. 1 - One of the caught individuals of *Alosa fallax* along Mignone river (North Latium). It was retained within seconds only for photographic documentation and was immediately released on site. / Uno degli individui di *Alosa fallax* catturati lungo il fiume Mignone (Lazio settentrionale). È stato trattenuto pochi secondi solo per documentazione fotografica ed è stato immediatamente rilasciato sul posto.

Fig. 2 - Morphological characters of *Alosa fallax* considered in the study: total length (TL), standard length (SL), head height and length (HH, HL), eye diameter (ED), the distance between mouth and eye or periorbital distance (POD), pre- and post- dorsal distance of dorsal fin (PDDF, PDAF), maximum and minimum body height (BHa, BHb), distance between dorsal and caudal fin (BAF), dorsal fin height and length (HDF, DFL), pectoral fin height and length (HPF, PFL), ventral fin height and length (HVF, VFL), anal fin height and length (HAF, AFL). / Caratteri morfologici di *Alosa fallax* considerati nello studio: lunghezza totale (TL), lunghezza standard (SL), altezza e lunghezza della testa (HH, HL), diametro dell'occhio (ED), distanza tra bocca e occhio o distanza periorbitale (POD), distanza pre- e post-dorsale della pinna dorsale (PDDF, PDAF), altezza massima e minima del corpo (BHa, BHb), distanza tra pinna dorsale e caudale (BAF), altezza e lunghezza della pinna dorsale (HDF, DFL), altezza e lunghezza della pinna pettorale (HPF, PFL), altezza e lunghezza della pinna ventrale (HVF, VFL), altezza e lunghezza della pinna anale (HAF, AFL).

RESULTS

The first capture of *A. fallax* took place on 13 April 2020, the last on 3 May 2020: altogether, 12 individuals were caught in 2020. In 2021 (22 April-14 May), eight catches of this species were carried out. In 2022 (22 April-10 May), five catches were made. In 2023 (13 April-2 June), five catches were obtained by A. Olini and P. Crescia. All in all, 30 individuals were caught and then released in good health conditions.

Table 1 shows the averages for each biometric measurement, divided between males and females, of the *A. fallax* individuals for which measurements could be taken (N=11).

As expected, total length and weight were directly correlated ($r=0.75$, $R^2=0.57$, $p<0.001$), but these measures were not significantly different when comparing sexes (Figs. 3-4).

There were no significant differences between males and females also for HL, POD, EDF, PDDF, PDAF, HDF, HPF, PFL, VFL, FL. Instead, the following measurements showed very significant differences between sexes even when visually sexed females are not perceptibly bearing eggs: HH ($p=0.03$), BAF ($p=0.01$), BHa ($p=0.004$), BHb ($p=0.014$), DFL ($p=0.002$), HVF ($p=0.008$), HAF ($p=0.0005$; Fig. 5).

Table 1 - Averaged values of biometric measurements of *Alosa fallax* individuals caught in the Mignone river. Legenda: $\bar{X}$ = arithmetic means; SD = standard deviation; Min = minimum and Max = maximum values; TL= total length; SL = standard length; HH, HL= head height and length; ED = eye diameter; POD = the distance between mouth and eye or periorbital distance; PDDF = predorsal distance of dorsal fin; PDAF = predorsal distance of adipose fin; BHa, BHb = maximum and minimum body heights; BAF = distance between dorsal and caudal fins; HDF, DFL = dorsal fin height and length; HPF, PFL = pectoral fin height and length; HVF, VFL = ventral fin height and length; HAF, AFL = anal fin height and length (HAF, AFL). All length measures are in mm. W = Weight, in grams. / Valori medi delle misure biometriche di individui di *Alosa fallax* catturati nel fiume Mignone. Legenda: $\bar{X}$ = medie aritmetiche; SD = deviazione standard; Min = valori minimi e Max = valori massimi; TL= lunghezza totale; SL = lunghezza standard; HH, HL= altezza e lunghezza della testa; ED = diametro dell'occhio; POD = distanza tra bocca e occhio o distanza periorbitale; PDDF = distanza predorsale della pinna dorsale; PDAF = distanza predorsale della pinna adiposa; BHa, BHb = altezza massima e minima del corpo; BAF = distanza tra pinna dorsale e caudale; HDF, DFL = altezza e lunghezza della pinna dorsale; HPF, PFL = altezza e lunghezza della pinna pettorale; HVF, VFL = altezza e lunghezza della pinna ventrale; HAF, AFL = altezza e lunghezza della pinna anale (HAF, AFL). Tutte le misure di lunghezza sono espresse in mm. W = peso, in grammi.

Morphometric character	Males (N=11)				Females (N=7)			
	$\bar{X}$	Min	Max	SD	$\bar{X}$	Min	Max	SD
TL	462.00	402	515	34.25	505.14	469	531	19.33
SL	408.45	352	449	31.15	444.71	410	476	22.68
HH	81.90	62.8	94.6	11.06	92.16	77.8	99	6.77
HL	88.42	76.1	99	7.31	91.20	79.6	103	8.42
POD	18.46	15.5	21.8	1.94	0.23	17.7	24.1	2.49
ED	14.27	11.4	16.6	1.66	14.59	12	16.2	1.50
PDDF	177.55	151	201	17.03	183.00	162	200	13.71
PDAF	236.82	204	262	19.75	252.29	215	273	20.31
BAF	134.00	119	152	11.33	152.57	122	163	14.37
BHa	93.81	74.9	106	10.84	108.00	94	114	7.09
BHb	66.85	59.1	74.3	5.59	78.37	57.3	91.4	10.63
HDF	49.25	40.2	55.6	5.11	54.21	30	68.4	16.44
DFL	59.72	49.2	66.6	6.13	71.64	65	76.3	5.18
HPF	55.11	48.7	64.4	4.67	54.73	45	67.5	8.24
PFL	31.38	24.7	39.5	4.56	30.37	20.8	45.2	9.45
HVF	31.73	22.8	40.1	5.29	41.70	32	52.6	6.77
VFL	30.02	26.6	33.5	2.52	27.66	20	36.7	5.89
HAF	26.10	16.8	33	5.00	27.01	23	30.2	2.44
AFL	67.25	53.2	77.2	8.29	69.87	56	83.4	11.55
W	569.64	491	693	62.33	896.57	801	1024	79.42

DISCUSSION

The number of mature individuals recorded, the presence of egg-bearing females about to lay eggs and their concentration in a limited stretch of the river allow us to hypothesise the existence of an unknown new twaite shad breeding site in Italy.

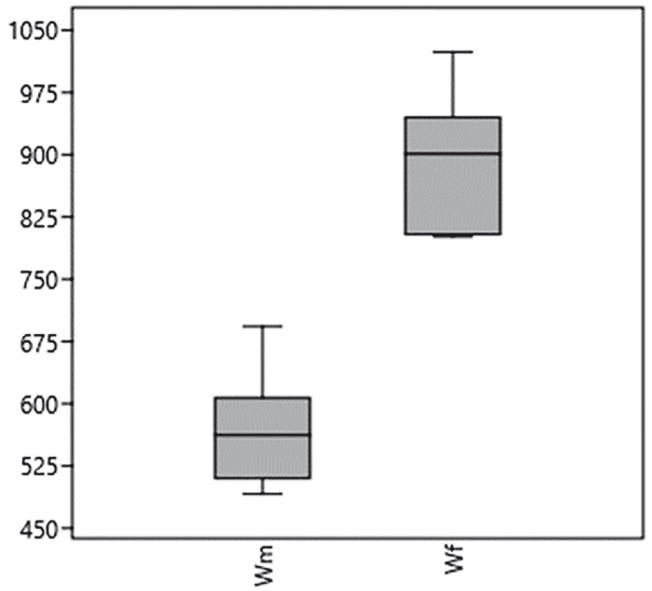

Fig. 3 - Box-plot based on the results of the Mann-Whitney U test for comparison of Weight (Z=0.344, p<0.001), of individuals of *Alosa fallax* caught: Wm = males weight; Wf = females weight. / Box-plot basato sui risultati del test U di Mann-Whitney per il confronto del Peso (Z=0,344, p<0,001), degli individui di *Alosa fallax* catturati: Wm = peso dei maschi; Wf = peso delle femmine.

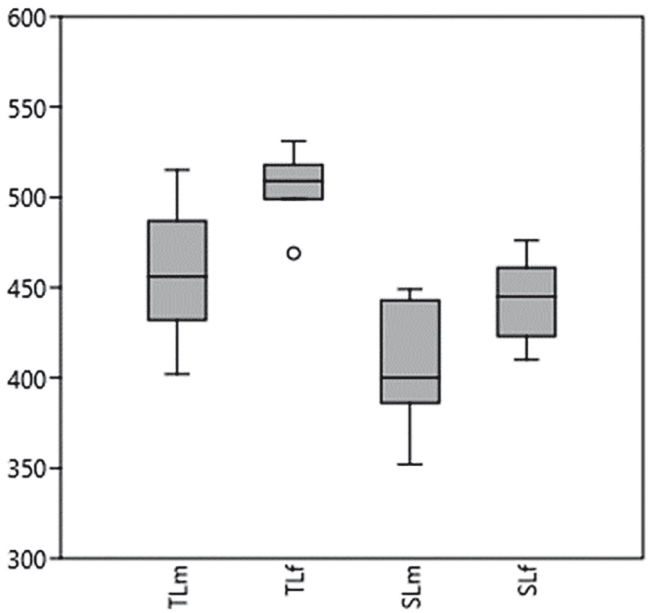

Fig. 4 - Box-plot based on the results of the Mann-Whitney U test for comparison of total and standard length (TL: p = 0.086; SL: p = 0.17), of *Alosa fallax* individuals caught in the Mignone River; TLm and SLm=males; TLf and SLf=females. / Box-plot basato sui risultati del test U di Mann-Whitney per il confronto tra la lunghezza totale e quella standard (TL: p = 0,086; SL: p = 0,17), degli individui di *Alosa fallax* catturati nel fiume Mignone; TLm e SLm=maschi; TLf e SLf=femmine.

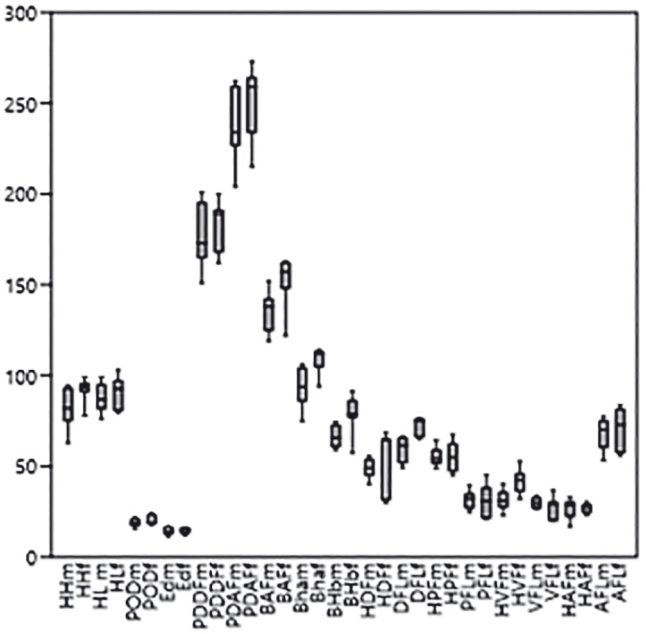

Fig. 5 - Box-plots based on the results of the Mann-Whitney U test for comparison between males and females of *Alosa fallax* biometric measurements. The differences in measurements were significant in: HH, BAF, BHa, BHb, DFL, HVF and HAF. / Box-plot basati sui risultati del test U di Mann-Whitney per il confronto tra maschi e femmine delle misurazioni biometriche di *Alosa fallax*. Le differenze nelle misurazioni erano significative in: HH, BAF, BHa, BHb, DFL, HVF e HAF.

In recent years, the Mignone river has been the subject of several research and monitoring campaigns in its middle stretch (Tancioni & Cataudella, 2009; Bianco & Santoro, 2011; Tancioni, 2011; Sarrocco *et al*., 2012). After the recent discovery of the presence of the sea lamprey (Ferri *et al*, 2021), the river returned another important result in the context of ichthyofaunal diversity and conservation. The number and sex composition of this twaite shad population, indicates a breeding migration purpose and confirms old reports for this stretch of the Tyrrhenian coast after many years of an apparent absence of the species (Cataudella, 1977). On the other hand, the finding is in contrast to the sharp decline trend observed for this species in Lazio (Sarrocco *et al*., 2012).

The correct recognition of sex in this species, which does not present evident sexual dimorphism even in the reproductive period, is important to know its social structure and behaviour during mating (Aprahamian *et al*., 2003). Analyses showed that a number of external morphological characters can be used to sex the species with good approximation, namely: head height (HH), distance between dorsal and caudal fins (BAF), maximum body height (BHa), minimum body height (BHb), dorsal fin length (DFL), ventral fin height (HVF), and anal fin height (HAF). These initial data are important for future conservation strategies, but certainly further studies on morphology and reproduction will have to be conducted in the future to refine the method.

According to Chiesa & Nonnis Marzano (2016) sport fishing does not cause a substantial impact, being mostly based on the “catch and release”, and catching is already

prohibited in the spawning season (from 15 May to 30 June). In the Mignone river and in other watercourses where the reproduction is suspected, this prohibition should also be extended to the pre-reproductive period, i.e. starting from mid-April, while putting in place more controls to stop illegal mass captures. These actions could not be undertaken without simultaneously activating an awareness-raising action among anglers, the main users of the river.

Even though to confirm this river as an effective breeding site for *Alosa fallax* it is essential to continue the monitoring in order to find evidence of the presence of fry and/or juveniles, it is obvious that the presence of the Le Mole Dam on the river precludes access to the extensive and almost intact upstream sector, reducing the river stretch potentially available for spawning. For this reason, a management plan should be implemented for the realization, after a proper feasibility assessment, of a fish ladder for this and the other threatened fish species reported in the river. Also, an awareness-raising campaign among farmers should be carried out for a limitation of the use of biocides on farmland in the Mignone river basin. In addition, to safeguard and preserve the interesting ichthyofauna of this watercourse, the Mignone river connectivity should be carefully restored taking care to avoid the spread of alien species.

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