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Rapid Communication

New records of the spiny-cheek crayfish *Faxonius limosus* (Rafinesque, 1817): expansion in subalpine lakes in North-western Italy

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Abstract

We report the first occurrence record of the spiny-cheek crayfish *Faxonius limosus* in two North-western Italian lakes: Orta and Mergozzo. We also confirm the occurrence of the species in Lake Maggiore. *Faxonius limosus* is native to the east coast of the USA. Since the end of the 19th century, it was introduced to Europe, where it rapidly spread in Poland, Russia, France, and Germany. In 1991, it was recorded for the first time in Italy in Lake Iseo (Lombardy region; NW Italy), and soon it has spread in Northern and Central Italy. Although *Faxonius limosus* is included in the list of European Union Concern, only two reports suggested the occurrence of this species in lakes Maggiore, Orta and Mergozzo, but these were never confirmed by experts. The aim of the present paper is to shed light on the distribution of *F. limosus* along the coasts of the three North-western Italian lakes. To this end, we used visual census and trapping to detect the occurrence of *F. limosus*, providing also georeferenced and environmental information of its habitat. We discovered 31 new occurrence localities (18 municipalities) for the species, scattered across the three lakes. These new findings highlight the urgency of implementing monitoring and management programs to prevent *F. limosus* spread and to mitigate its potential ecological impacts.

Key words: freshwaters, invasive alien species, Lake Maggiore, Lake Orta, Lake Mergozzo

Introduction

Invasive alien species (IAS) are important global drivers of biodiversity loss. In 2020, the high economic costs for managing IAS reached up to 116.61 billion euros in Europe only (Gallardo et al. 2016; Haubrock et al. 2021). Crayfish are some of the most widely introduced species in the world (Lodge et al. 2012). After the introduction and acclimatization of crayfish in a new habitat, they can sharply increase in abundance and significantly impact the local biodiversity by feeding on benthic invertebrates, macrophytes, algae, and sediment communities (Gherardi 2007).

Presently, at least twelve alien crayfish species occur in European freshwaters, including four (namely, *Faxonius limosus, Pacifastacus leniusculus, Procambarus clarkii* and *Procambarus virginalis*) of European Union Concern (EU Regulation 1143/2014; European Commission – Environment 2019). All of them are native to North America, and were introduced to Italy to supply market demand for human food, fish forage, and bait (Gherardi 2011). The consequences of their introduction have ecological and socioeconomic impacts being reported in Lodge et al. (2012), Madzivanzira et al. (2020), and Kouba et al. (2022).

Of high concern is the impact of these invasive species on native crayfish: *Austropotamobius pallipes* (Lereboullet, 1858), *A. torrentium* (Schrank, 1803) and *Astacus astacus* (Linnaeus, 1758), which are presently limited to very few water bodies in Europe (Jussila et al. 2021).

Faxonius limosus is considered among the worst invasive species globally due to the impacts caused to the colonized environments (Invasive Species Compendium 2022). It was introduced to Europe since the 19th century, spreading rapidly through waterways in Poland, Russia, France, and Germany (Crandall and de Grave 2017). In Italy, the first record of *F. limosus* was in 1991 in Lake Iseo (Lombardy region-NW Italy). Afterward, the species rapidly spread in northern (Piedmont, Lombardia, Trentino-Alto Adige, Veneto) and central (Emilia-Romagna, Tuscany, Latium, Umbria, Sardinia) Italian regions (Aquiloni et al. 2010; Morpurgo et al. 2010).

In Piedmont, very few information is available on the distribution of *F. limosus*. Available information suggests *Faxonius limosus* was released in 1997 near Baldissero d'Alba (Cuneo Province, southern Piedmont; Delmastro 1999). Two technical reports (Bazzoni 2006; GRAIA 2009) hypothesized its occurrence in lakes Maggiore, Orta and Mergozzo, but both studies lack taxonomic confirmation. Since 2016, *F. limosus* was included in the list of IAS of European Union concern (EU Regulation 1143/2014) on the prevention and management of the introduction and spread of invasive alien species, with implications and restrictions on its importation, bred, transport, commercialization or release into the environment.

To best of our knowledge, there is no recent updates on the distribution of invasive crayfish in Europe, and above all in Italy. Therefore, the present study aims at providing a first picture on the expansion of *F. limosus* in three main Italian North-western piedmontese lakes (namely, lakes Maggiore, Orta and Mergozzo) by providing up-to date and georeferenced information.

Materials and methods

Study area

The three studied lakes are: Maggiore (46°5.88′N; 08°42.88′E), Orta (45°48.77′N; 08°23.73′E), and Mergozzo (45°57.33′N; 08°28′E) (Figure 1). While the first two lakes are large and deep Northern Italian lakes (Capotondi

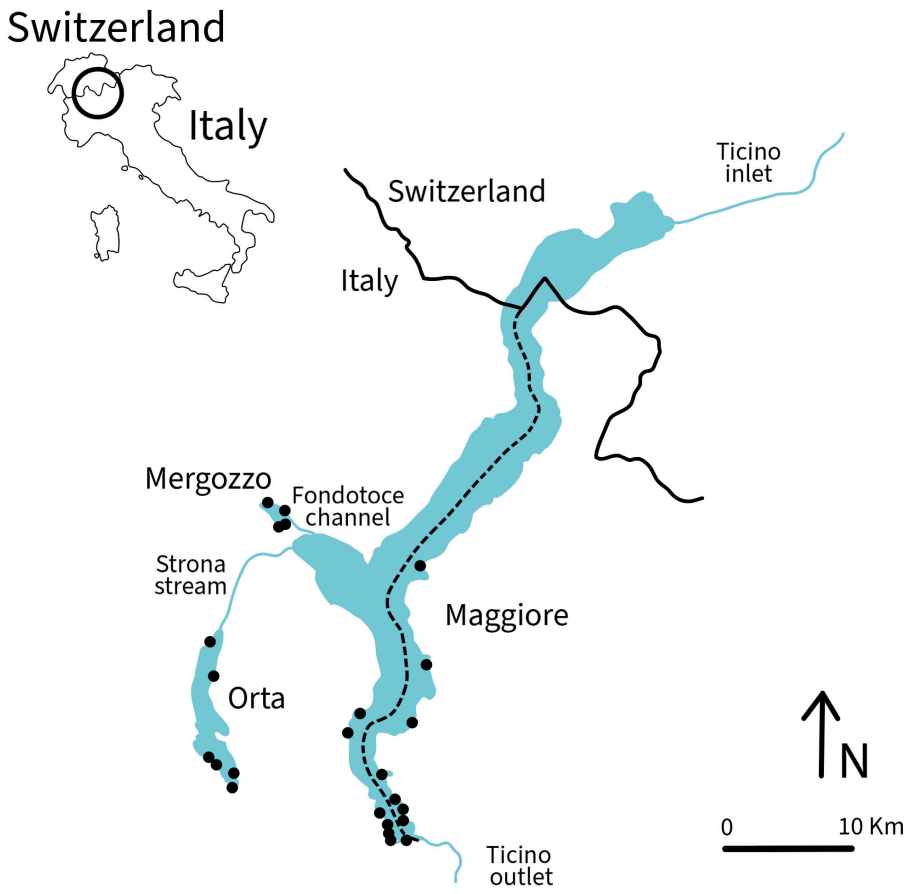

Figure 1. Map of the studied lakes with their river network. Black dots: sites of occurrence of *Faxonius limosus*.

et al. 2021), the third one is the fifth largest lake in Piedmont following lakes Maggiore, Orta, Viverone and Candia.

The three lakes under question in this study are interconnected through different waterways. River Strona connects Lake Orta to Lake Maggiore, and a small non-navigable artificial channel (Fondotoce channel) connects Lake Mergozzo to Lake Maggiore (Figure 1).

All the lakes have a fluvio-glacial origin; their coasts are characterised by steep slopes and by gentler areas where tourism and recreational activities have flourished.

Along the littoral of the studied lakes, several municipalities and natural sites such as Natura 2000 sites (see lakes Maggiore and Orta, Italy) and Emerald zones of protection (see Lake Maggiore, Switzerland) are concentrated. Lake Mergozzo shows only the municipality of Mergozzo and a special protection area (ZPS) "Lago di Mergozzo and Mont'Orfano" (IT1140013). Within the Long Term Ecological Research Network (LTER), lakes Maggiore and Orta are included in the Subalpine lakes macrosite (LTER 2022) and are regularly monitored for water chemistry and biological components (plankton and fish) since the 1980s (Table 1; Rogora et al. 2016, 2021).

In the past century, Lake Maggiore catchment area has been subjected to intense urbanization and industrialization, which contributed to the

Table 1. Main morphometric (above) and chemical (below) characteristics of the three studied lakes. Chemical characteristics are expressed as weighted averages on late winter mixing volumes.

Variable	Unit	Lake Maggiore	Lake Orta	Lake Mergozzo
Altitude	m a.s.l.	193	290	194
Catchment area	km^2	6599	116	10.4
Lake area	km^2	212	18.14	1.82
Max. lenght	km	64.4	12.4	2.32
Max. width	km	10	2.5	1.1
Max. depth	m	372	143	73
Volume	km^3	37	1.3	0.08
pH		7.27	6.98	6.89
Conductivity	$\mu S\ cm^{-1}$ at 20 °C	151.0	74.5	48.5
Alkalinity	meq L^{-1}	0.83	0.31	0.27
$P\text{-}PO_4$	$\mu g\ L^{-1}$	11	4	3
P-tot	$\mu g\ L^{-1}$	13	5	5
$N\text{-}NO_3$	$\mu g\ L^{-1}$	788	964	567
N-tot	mg L^{-1}	0.89	1.11	0.71
R-Si	mg L^{-1}	1.81	1.62	1.11

eutrophication of the lake, although the lake shifted to the present oligotrophic status (Rogora et al. 2021).

Lake Orta is a well-studied example of how a strong water acidification due to copper and ammonium sulphate discharges could collapse a whole biocoenosis in a lake (Bonacina 2001). Following its chemical recovery, accelerated by a liming intervention (Calderoni et al. 1991) carried out by the Italian Institute of Hydrobiology in Pallanza (currently Water Research Institute, CNR-IRSA), the lake has gradually been repopulated, with the re-appearance of new species. Thirty years later, the lake still stands out from the other subalpine lakes, due to its poor biodiversity even if its water quality returned to a pre-pollution chemical oligotrophic state (Rogora et al. 2016).

Finally, Lake Mergozzo is oligotrophic because no direct inputs of anthropogenic origin exist along the lake-shores and therefore very low concentrations (average P values between 3 and 5 $\mu g\ L^{-1}$ at mixing, Rogora *pers. comm.*) and low intake of phosphorus from the catchment area are present. Since the 1980s, this condition was maintained both by the domestic sewage discharges diversion from the lake into the nearby River Toce, and by the interdiction of the use of motorboats. Generally speaking, the modifications in the ecological status of lakes with time, together with local climate changes are favouring not only the arrival and acclimatization of IAS in areas with low water quality (Ricciardi 2001), but also, we speculate, their spread in environments with good ecological status such as Lake Mergozzo, impairing it.

Methods

During 2017–2022, we carried out extensive surveys on lakes Maggiore (2017–2018), Orta and Mergozzo (2021–2022) shores (Figure 1) to monitor the occurrence and population distribution of alien crayfish. The morphometric descriptions and physical-chemical variables of the sampled lakes are

summarised in Table 1. A total amount of 75 sampling stations were surveyed in one year in Lake Maggiore, 15 in Lake Orta and 5 in Lake Mergozzo.

Preliminary inspections to characterize the environmental features of the shorelines (type of substrate, riparian and shore dominant vegetation, crayfish traces like burrows and remains, or live individuals or of other alien) were carried out on the first surveys on all lakes as suggested by Boggero et al. (2019). We created a specific information form to be filled in during preliminary inspection of sites where all items present in the supplementary materials table are listed (Supplementary material Table S1). Table S1 revealed that 57% of the total number of the littoral sites where *F. limosus* occurs do not have a tributary river, but presented artificial benches and showed a very gentle slope (< 5°). The substrate type was mostly sand (43%) and/or pebbles (37%), while mud and gravel are rare (< 15%). Beaches were surrounded by *Phragmites australis* (Cav.) Trin. ex Steud. in 60% of the cases with few single trees scattered on the bench.

Two different sampling methods were used in all the studied lakes: i) visual encounter (modified from Bonk et al. 2019) which involves the use of hand or a hand-net to capture crayfish during the day and waders boots for walking over the rough sediment and ii) trapping (modified from Garzoli et al. 2020) which involves the use of cylindrical bite traps (30 × 60 cm). Twenty grams of cat food per trap were used to attract crayfish.

For the visual encounters, crayfish were visually surveyed along the lake-shoreline for 20 minutes recording the species found. The baited traps were placed at each sampling site at night when crayfish are more active, at a distance of about 2–3 m between them and retrieved in the following morning (soak time of approximately 12 h). On retrieval, the number of crayfish caught in each trap was recorded and by-catch of incidental non-target native species released. In the laboratory, only invasive crayfish were anesthetized by freezing at about −20 °C, then thawed for further biometric analyses (total body length, cephalotorax length, total weight, single chelae weight).

Results

Morphological identification

The crayfish *Faxonius limosus* has relatively small dimensions (< 10 cm in length; Hamr 2002). The head has a rostrum with almost parallel edges, prominent lateral teeth and no median ridge. The carapace is characterized by an areola between branchiocardiac grooves, a couple of post-orbital ridges and the presence of prominent spines on each side of the carapace in the anterolateral region (Figure 2). The abdomen shows distinct red to brown-red transversal bands across abdominal segments. Claws have hooked tip with an orange band, regular and smooth internal margin (without teeth) and presence of a prominent and curved spine on the carpus (Holdich et al. 2006).

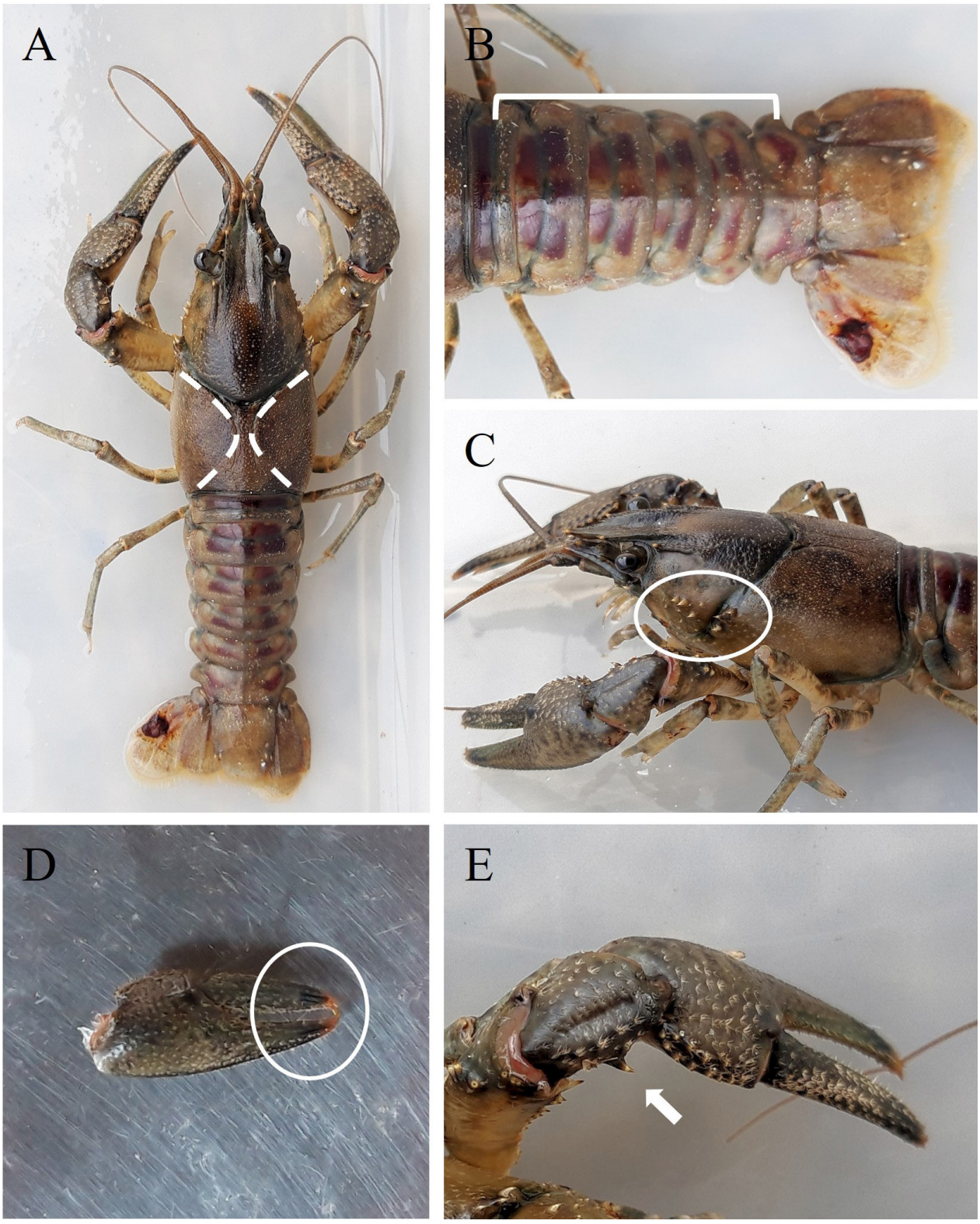

Figure 2. Main features characterising *Faxonius limosus* body shape A: separation between branchiocardiac grooves; B: striped abdominal segments; C: carapace spines; D: claws with orange tip; E: carpus spine. Picture and styling C. Croci.

Occurrence of Faxonius limosus

We report for the first time the occurrence of the species in lakes Orta and Mergozzo, and we confirmed its establishment in Lake Maggiore reported previously by Garzoli et al. (2020).

Table 2. *Faxonius limosus* occurrence in the three lakes in NW Italy, with number of measured individuals (n. ind), mean Catches Per Unit Effort (CPUE), body length and male/female ratio (M/F) per lake. Data derived from trapping only. SD: Standard Deviation; wo: without outlier.

Lakes	n. ind	mean CPUE	Body length (cm)			sex ratio M/F
			min	max	mean ± SD	
Maggiore	238	1.14	3.04	10.57	6.71 ± 1.35	2.61
$Maggiore_{wo}$	237	1.14	3.04	9.88	6.70 ± 1.33	2.59
Orta	80	1.03	3.94	7.56	5.70 ± 0.82	1.29
Mergozzo	56	1.08	4.02	9.15	7.08 ± 1.20	3.00

The three lakes were differently invaded by *F. limosus* (Figure 1): alive specimens were recorded in 20 out of 75 surveyed sampling stations in Lake Maggiore, in 6 out of 15 sampling stations in Lake Orta, and in 4 out of 5 sampling stations in Lake Mergozzo. In Lake Maggiore, the occurrence of *F. limosus* was limited only to the central-southern part of the lake, while in Lake Orta the species was mainly distributed along the southern and northern less steep shores where beaches and *Phragmites australis* occurred. In Lake Mergozzo *F. limosus* occurred almost everywhere, along the south-eastern and northern sides of the lake. Also in Lake Mergozzo, the discovery of *F. limosus* occurred in beaches surrounded by densely vegetated areas (*Phragmites australis)*. No traces of individuals or remains had ever been observed in both lakes Orta and Mergozzo in all other investigated sites (9 in Lake Orta, and 1 in Lake Mergozzo).

Direct visual encounter surveys allowed to record *F. limosus* specimens in Lake Maggiore where they were easily visible and active at any time of day, while during the day no alive specimens were visible in lakes Orta and Mergozzo. Indeed, the total absence of native *A. pallipes* crayfish individuals in the studied sites is noteworthy, because it is usually confined to tributaries of River Toce (one of the main tributaries of Lake Maggiore) and Val Grande National Park (IdroLIFE project LIFE15NAT/IT/000823, and protected area staff personal communication).

Faxonius limosus trapping findings pertaining to each lake are shown in Table 2 with main biometric characteristics. The mean values of the Catches Per Unit Effort (CPUE) highlighted that Lake Maggiore manifested the highest abundance, followed by lakes Mergozzo and Orta (Table 2).

The results also revealed that maximum total body length is highest in Lake Maggiore compared to the other two lakes. The sex ratio [Males (M) / Females (F)] in all lakes was biased toward males, even if the M/F ratio in Lake Orta was less skewed than those in lakes Maggiore and Mergozzo.

Discussion

EU Member States are required to take actions for managing invasive species within their territories. Therefore, it is of crucial importance for all Member States to acquire constantly up-to date distribution information in order to trace the spreading of IAS and to map their distribution all over Europe. Nonetheless, other than for *Procambarus clarkii,* there is a general

lack of updates on the situation of the invasive crayfish in Europe, with the last updates of *Faxonius limosus* distribution map dated back to 2014 (Kouba et al. 2014).

Here, we reported for the first time the occurrence of *F. limosus* in Lake Orta, where it coexists with *Procambarus clarkii*, although the former has lower abundances as compared to *P. clarkii*. *Faxonius limosus* is still the only one invasive crayfish in Lake Mergozzo and its abundance is similar to the one of Lake Maggiore. On the contrary, the species is the dominant among the three invasive crayfish in Lake Maggiore (Garzoli et al. 2020). The lack of native crayfish in the three lakes is likely to ease the establishment success of these invasive crayfish as there will not be any biotic resistance by resident crayfish or native analogues (see South et al. 2020 for biotic resistance).

Faxonius limosus was observed on soft-bottom and silty sediments or on beaches with sandy/gravel substrates and drifting algal patches in evidence (see Hamr 2002; Garzoli et al. 2020 for comparative data on auto-ecology). But the present dataset allow us to state that the species is easily found on beaches with gentle slopes, dominated by *Phargmites australis* and sand/pebbles sediments, while it was less frequent on silt soft sediments (< 15%).

In Lake Maggiore, during our diurnal activities, the species was visible and hidden under the few pebbles and boulders present, while no individuals were observed in the daytime in the other two lakes. Thus, it is likely that *F. limosus* burrows deeper in lakes Orta and Mergozzo where waters are more stable and cover permanently the sediment layers, and slight temperature fluctuations occurs. Moreover, our trapping activity during the winter suggested that the species seemed to overwinter in the deeper waters where it likely escape shoreline drought and sharp variations in water temperature and abrupt cold and freeze that can strike it. This is in line with its ecological preferences: the species is known to adapt to different environmental conditions and to tolerate low water temperatures, calm and deep waters (Souty-Grosset et al. 2006) such as those of the three studied lakes (see morphological characteristics in Table 1 and Figure S1).

We assume also that the species has been probably spreading from Lake Maggiore to the other two lakes through the river network connecting them. However, population-level molecular analyses would be needed to confirm the hypothesis that Lake Maggiore is the potential invasion hub for the other two lakes.

In North America, Momot (1988) found a maximum size of *F. limosus* in the range 50–54 mm (approximately 5 cm), which is half of the observed maximum total body length of Lake Maggiore (10.6 cm) (Table 2). The maximum size reached in Lake Mergozzo (max value: 9.15 cm, mean value: 7.8 ± 1.2 cm) was higher than the one reported by Momot (1988) and exceeding the values reached in Lake Orta (max value: 7.56 cm, mean

value: 5.7 ± 0.8 cm). However, 99% of all crayfish caught in the three lakes were below the commercial size on the European market (i.e., 10 cm) (Holdich and Black 2007). Hence, although these environments seems to offer more favourable conditions for *F. limosus* growth than those found in America, the species size does not reach the values required by the market. This is particularly advantageous because, i) the species cannot be commercialized, bred, transported, or intentionally released in nature (EU, 2019/1262), and ii) especially in the case of Lake Orta and its peculiar history of sediment contamination (Rogora et al. 2016; Vignati et al. 2016), no doubts will be raised on its consumption and on the related health risks.

However, the introduced crayfish are a food resource for many native and invasive fish such as *Silurus glanis* (De Santis and Volta 2021) and numerous birds inhabiting or using the lake shores, with possible consequences on their population dynamics that still needs to be assessed. This is important for future research considering the bioaccumulation of toxic and harmful chemicals (such as mercury, lead, PFAS, PCBs) in *F. limosus* that could impact conservation strategies of species protected under the Habitat Directive (92/43/EEC).

Conclusions

The present work reports the first record of *Faxonius limosus* in lakes Orta and Mergozzo and provide the environmental characteristics of the colonized areas where the species was observed.

We believe that, in order to get a complete picture of the structure and composition of the population assemblage of the species and on its evolution in lakes Maggiore, Orta and Mergozzo, it is essential to collect more samples and to implement long-term, extensive and comprehensive monitoring programs, including water chemistry analyses. Future studies could also explore more the autoecology of the species in this area, including at which depth *Faxonius limosus* can spread in each lake, also depending on lake characteristics or on lake sediment composition (texture).

Due to globalisation of trade, a further expansion of *F. limosus* in Italy seems inevitable. Indeed, the current situation in many regions may be different from that reviewed in the introduction. Such a situation will hopefully improve as better-quality data are collected and made available to scientific community and other stakeholders.

The occurrence of *F. limosus* poses real threats to the native crayfish populations at national and international levels. Hence, there is an urgent need to prevent its expansion through both national and regional measures, following, for example the development of a guide for IAS containment as it was done in Lombardy region (AA VV 2019), and through management actions shared between neighbouring countries.

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Authors' contribution

ABo: research conceptualization, sample design and methodology, data analysis and interpretation, funding provision, writing – original draft; LK, LG: sample design and methodology, investigation and data collection, writing – review and editing; CC, AZ, SZ, DP, TB, ABu: investigation and data collection, writing – review and editing; AO: investigation and data collection; CC, SB: photograph collection.

Ethics and permits

The present work did not required any ethics. Catch permits were requested and obtained from: Italy, Commissariato italiano per la Convenzione italo-svizzera sulla Pesca (Cooperation agreement n. 769/2019 – permits 87/2017 e 83/2022), Provincia di Novara (permits 1172/2021, 2110/2021), Provincia Verbano-Cusio-Ossola (permits 1494/2021, 1891/2021); Switzerland, Canton Tessin – Ufficio Federale dell'Ambiente (permits 30/10/2017, 28/05/2018).

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Supplementary material

The following supplementary material is available for this article:

Table S1. Main geographic and environmental characteristics of each surveyed lake site.

Figure S1. Photographs taken on March 23, 2023 which attest the occurrence of crayfish and its burrows at depths of about 11–12 m. Pictures S. Beatrizzotti.

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