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Research Article

An integrated evaluation of the invasiveness risk posed by non-native crayfish in Lake Maggiore (Northwest Italy)

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Abstract

Risk analysis of non-native species invasions is one of the main challenges currently faced by both scientists and environmental managers. In this study, the three risk screening toolkits *Harmonia*+, Aquatic Species Invasiveness Screening Kit (AS-ISK) and Canadian Marine Invasive Screening Tool (CMIST) were used in conjunction to evaluate the risk of invasiveness of eight non-native crayfish species (three extant already present in the risk assessment area and five horizon, not yet reported, but likely to arrive in the near future) for Lake Maggiore (Northwest Italy). Based on the toolkit-specific risk scores for each species and their final ranking according to the thresholds set for each toolkit: 1) *Harmonia*+ ranked five species with a medium-risk level of invasiveness and three with a low-risk level; 2) AS-ISK ranked all species as high risk; 3) CMIST ranked six species as high risk and two as medium risk. By combining the risk scores from the three toolkits and setting an *ad hoc* threshold, extant horizon calico crayfish *Faxonius immunis*, spinycheek crayfish *Faxonius limosus*, signal crayfish *Pacifastacus leniusculus*, red swamp crayfish *Procambarus clarkii* and marbled crayfish *Procambarus virginalis* were ranked as high risk, whereas horizon Australian red claw crayfish *Cherax quadricarinatus*, yabby *Cherax destructor* and Danube crayfish *Pontastacus leptodactylus* were ranked as medium risk. It is anticipated that the findings of this study will help inform managers about the proper implementation of non-native species management strategies for the Lake Maggiore watershed.

Key words: invasive species, risk identification, Harmonia+, Aquatic Species Invasiveness Screening Kit (AS-ISK), Canadian Marine Invasive Screening Tool (CMIST)

Introduction

With the global surge in trade, travel and tourism, the intentional and unintentional movement of species worldwide has become increasingly common to the point that the biogeographical barriers that once separated species and ecosystems have lost their primary ecological role (Meyerson and Mooney 2007; Ricciardi 2007, 2013; Hulme 2009; Capinha et al. 2015). Consequently, the prevention of biological invasions and mitigation of the impacts caused by non-native invasive species have become the main cores of relevant policies at both the international and national level (e.g. Regulation EU 1143/2014; Italian Legislative Decree 230/2017). However, in order to identify, manage and control non-native species, it is not only crucial to understand the environmental biology of potential invaders and the threats they pose to native species but also to study the environmental characteristics of the recipient ecosystem that can favour non-native species colonisation (Pyšek et al. 2010; Boggero et al. 2014; Colangelo et al. 2017). Yet not all ecosystems are prone to invasion due to their characteristics, surrounding habitats, level of human-induced impact and potential effects of climate change (Catford et al. 2012). In this respect, freshwater ecosystems are among the most vulnerable to biological invasions (Francis and Chadwick 2012; Gallardo et al. 2016). This is because their invasibility is associated not only with the high connectivity of the hydrographic system of inland waters, which acts as a corridor for the spread of non-native species, but also to the elevated level of human-induced disturbance and habitat modification (Rinaldo et al. 2020; Guareschi and Wood 2021; Paganelli et al. 2021).

The combination of the above abiotic factors with the fact that freshwater invaders are highly adaptable to new environments and usually difficult to identify timely is the cause of a global increase in biological homogenisation (Olden 2006). Furthermore, the impacts of non-native freshwater species are often less evident, more difficult to predict in the long term and, consequently, difficult to manage due to the frequent failure and costs of preventative actions compared to the impacts by non-native terrestrial species, which are generally more evident (Moorhouse and Macdonald 2015; Simberloff 2021). At the same time, whilst current EU legislation restricts or prohibits the use of non-native species (Commission Implementing Regulation EU 2022/1203), these laws have come into force only recently, hence there is still no control over their implementation. For these reasons, it is crucial to define the risk of invasiveness of non-native freshwater species and evaluate the impacts associated with their occurrence (Rejmánek et al. 2005).

The number of available decision-support tools for the risk screening and assessment of non-native species has been increasing rapidly in recent years (Srėbalienė et al. 2019), but no single method has proven universally applicable. The screening of non-native species is the first step in the risk analysis process that aims to identify which species are likely to be invasive and can then be subject to follow-up risk assessment (Copp et al. 2016). In turn, risk screening and risk assessment methods for non-native species are designed to support risk management and communication – the third step in the risk analysis process (Copp et al. 2016). At the same time, risk analysis is a dynamic process that should be constantly updated, especially with new findings on the impacts exerted by the non-native species under study (see Vilizzi et al. 2021).

In a recent review of risk and impact assessment protocols (Roy et al. 2018), the screening tools *Harmonia*+ (D'hondt et al. 2015) and Aquatic Species Invasiveness Screening Kit (AS-ISK: Copp et al. 2016, 2021) were found to satisfy all 14 "minimum standards" against which a protocol should be evaluated within the context of the invasion process and related management approaches. The minimum standards were derived from the context of Regulation EU 1143/2014 "On the prevention and management of the introduction and spread of invasive alien species". To satisfy the minimum standards, a protocol should include: (i) a brief overview of a species with a description of its distribution, spread, likelihood of invasion and impacts; (ii) an assessment of the introduction pathways; (iii) an assessment of impacts on biodiversity (e.g. on threatened or protected species or habitats), ecosystems services and socio-economic sectors; and (iv) an assessment of the effects of climate change on the assessed species. Such a protocol should be completed even when there is a lack of information and should include a listing of relevant literature sources, an evaluation of uncertainty in the assessment, a summary and a quality assurance backup (Roy et al. 2018). AS-ISK and *Harmonia*+ are two comparable decision-support tools as their results are semi-quantitative and they consider both the bio-ecological traits and (potential) impacts of the non-native species under screening (Srėbalienė et al. 2019). An additional protocol, namely the Canadian Marine Invasive Screening Tool (CMIST: Drolet et al. 2016), albeit not evaluated against the minimum standards, complements the "suite" of currently available screening tools.

Crustacean decapods are among the most successful groups of aquatic invaders that are responsible for a wide range of impacts including disruption of ecosystem function as freshwater engineers (Emery-Butcher et al. 2020), changes in trophic chain, impacts on ecosystems services (e.g. decreased water quality, reduced macrophyte biomass) and potential pathogenicity (Alderman and Polglase 1986; Imhoff et al. 2012; Kouba et al. 2014). Invasive crayfish are *r*-strategist or pioneer species as they are able to switch to "unusual" reproductive strategies (i.e. hermaphroditism and parthenogenesis) that allow them to colonise more easily and rapidly new environments (Holdich 2002; Yazicioglu et al. 2016). Crayfish are characterised by high fecundity (700–800 eggs per female) and a generally short life cycle, with females able to reproduce from the first year of age and with a high generation turnover (Lindqvist and Huner 1999). Their impacts are therefore expected to increase with an increasing number of invasive crayfish species and a higher density of their populations (Gherardi and Acquistapace 2007). This becomes especially relevant when non-native invasive crayfish are assessed relative to the objectives of the European Water Framework Directive (2000/60/CE).

In this study, eight non-native crayfish species already reported in Italy were evaluated for their present and future risk of invasiveness in Lake Maggiore (Northwest Italy). Risk screening was undertaken using jointly the three

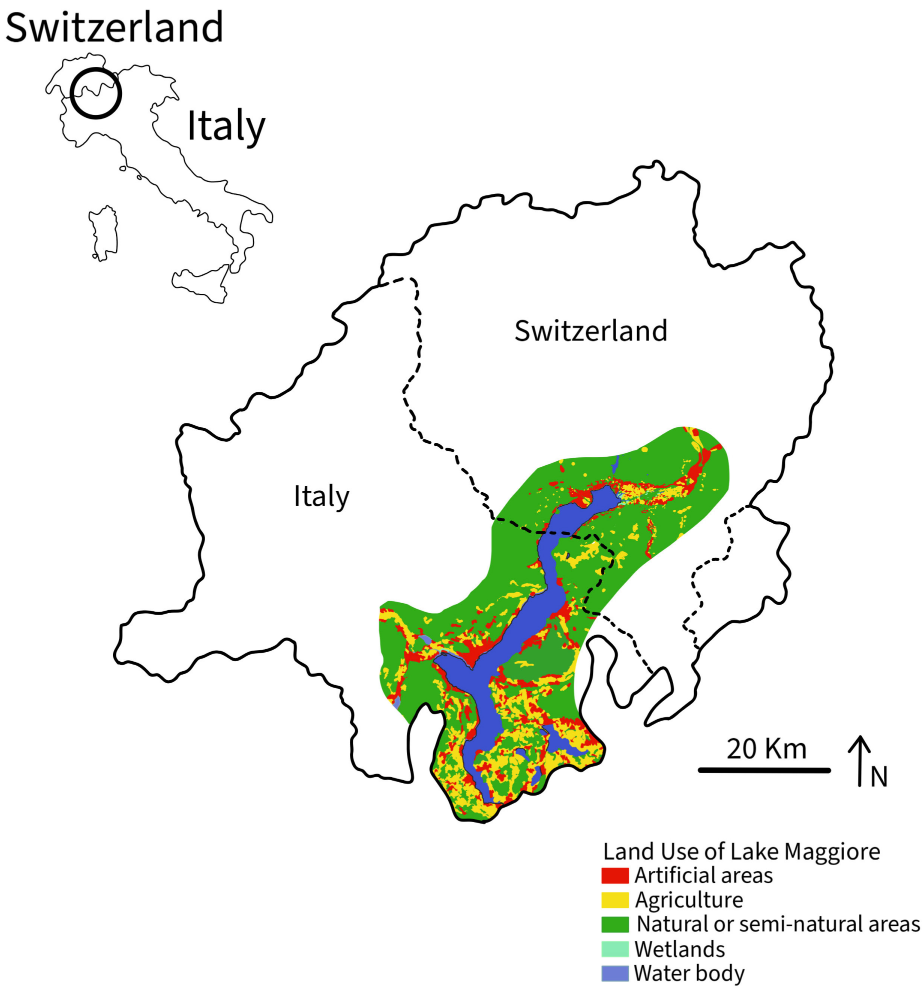

Figure 1. Lake Maggiore (Northwest Italy) catchment area used as risk assessment area for eight non-native crayfish species. Distribution of aggregated land cover classes is according to CORINE Land Cover (CORINE Land Cover 2018, vector/raster 100 m, Europe, 6-yearly) and is focused only on the area surrounding the lake.

toolkits *Harmonia*⁺, AS-ISK and CMIST. It is expected that the outcomes of this study will provide scientifically defensible guidelines to improve the management and conservation of the native ecosystem of Lake Maggiore given the occurrence of already invasive established crayfish species and the possible (future) introduction of additional ones, and after accounting for predicted climate change conditions.

Materials and methods

Study area and species selection

Lake Maggiore (the risk assessment area) is the second deepest and largest subalpine lake in Northern Italy, a part of which is shared with Switzerland. This water body has been impacted by tourism, recreational activities, public and private navigation, professional and sport fishing, hydroelectric production and water abstraction for potable use (Figure 1). The lake's water quality has been heavily affected by the above impacts (Guilizzoni et al. 2012) and climate change is exacerbating the decrease in its ecological quality (Fenocchi et al. 2018; Morabito et al. 2018). The pressures exerted upon the lake waters and littoral areas coupled with the increase in trade of aquarium species have facilitated the arrival and risk of entry of non-native species (Mosello and Lami 2014). This involves harmful consequences on the littoral zone, serious threats to the native biodiversity and an overall decline in the lake's ecological quality.

In Europe, 12 non-native crayfish species are currently reported: yabby *Cherax destructor* (Clark, 1936), Australian red claw crayfish *Cherax quadricarinatus* (Von Martens, 1868), calico crayfish *Faxonius immunis* (Hagen, 1870), Kentucky River crayfish *Faxonius juvenilis* (Hagen, 1870), spinycheek crayfish *Faxonius limosus* Rafinesque, 1817, virile crayfish *Faxonius virilis* (Hagen, 1870), signal crayfish *Pacifastacus leniusculus* (Dana, 1852), Danube crayfish *Pontastacus leptodactylus* (Eschscholtz, 1823), white river crayfish *Procambarus acutus* (Girard, 1852), Everglades crayfish *Procambarus alleni* (Faxon, 1884), red swamp crayfish *Procambarus clarkii* (Girard, 1852) and marbled crayfish *Procambarus virginalis* Lyko, 2017.

In Italy, *C. destructor*, *C. quadricarinatus* and *P. leptodactylus* are widely reported (Chiesa et al. 2006; Scalici et al. 2009), whereas *F. immunis* is sporadic. *Procambarus virginalis* has been found only in Sardinia (Tricarico and Zanetti 2021), Tuscany (Nonnis Marzano et al. 2009) and Veneto Regions (Vojkovská et al. 2014). The other species *F. limosus, P. clarkii* and *P. leniusculus* are considered main invaders of Italian freshwaters with established reproductive populations (Aquiloni et al. 2010).

In Switzerland, only *F. limosus* has been reported in Canton Tessin with a stable population, whereas *P. leniusculus* and *P. leptodactylus* have been found as isolated individuals between 1997–2007 (Maddalena et al. 2009). Since 2017, *P. leniusculus* and *P. clarkii* have been observed in the river network of Lake Maggiore, along its coasts and in the Bolle di Magadino Nature Reserve (Boggero et al. 2018; Maddalena *pers. comm.*). Of the remaining five species, only *C. destructor* and *P. leptodactylus* occur in Switzerland, although data refer to the years before 2000 without more recent information. As *F. juvenilis*, *F. virilis*, *P. acutus* and *P. alleni* are not found in the wild in either country, they were not included in this study.

Four of the above species (i.e., *P. clarkii*, *P. leniusculus*, *F. limosus*, and *P. virginalis*) are also included in the EU list of Union Concern (Commission Implementing Regulation EU 2022/1203). Although Switzerland is outside the European Community, it is trying to keep up to date and follow European legislation on the topic. In particular, the presence of invasive crayfish is regulated by the Federal Law 21/1991 on Fisheries (LFSP) and related Ordinance 24/1993 (OLFP), with a national action plan for their monitoring and eradication (Stucki and Zaugg 2011).

For the risk screening in Lake Maggiore, eight of the above-mentioned non-native crayfish species were therefore selected. Of these species, *F. limosus*, *P. leniusculus* and *P. clarkii* were classified as extant (i.e. already present in the risk assessment area) since they have already been recorded in Lake Maggiore: *F. limosus* and *P. clarkii* on the Italian side of the lake's shores and *P. leniusculus* on the Swiss side (Boggero et al. 2018, 2023; Garzoli et al. 2020). *Cherax destructor*, *C. quadricarinatus*, *F. immunis*, *P. leptodactylus* and *P. virginalis* were classified as horizon (i.e. not yet reported but likely to enter the risk assessment area in the near future).

Risk screening

The information used for answering the toolkits questions on the biology and ecology of the non-native crayfish species under screening was collected using specialised databases including the Global Invasive Species Database (GISD) and the Centre for Agriculture and Bioscience International Invasive Species Compendium (CABI ISC). Additionally, a detailed literature search on the ecological, environmental and socio-economic impacts of the selected crayfish was done using both Web of Science and Google Scholar. For the searches, the following combined string was used: ALL = ((“name of each selected crayfish species”) AND (“freshwater*”) AND (“ecological impact*”) AND (“environmental impact*”) AND (“socio-economic impact*”) AND (“biotic response*”)).

Invasiveness was considered from a management perspective and also in light of the operational projects in progress, namely the “Invasive species within Lake Maggiore watershed” and the “Pilot project for the monitoring of native and invasive benthic macro- and meio-fauna in Lake Maggiore using morphological and molecular approaches” (Garzoli et al. 2020; Zaupa et al. 2022).

Data analysis

For *Harmonia*+, the default settings were used so as not to introduce any further variability in the risk outcomes and the thresholds proposed by Lemmers et al. (2021) were applied: High risk, species with Overall risk score > 0.660; Medium risk, between 0.330 and 0.660; Low risk, < 0.330. Additionally, the Environmental impact and Socio-economic impact scores, with the latter calculated as the sum of the scores from the Plant, Animal, Human and Other target modules, were used for comparative purposes with the other two screening toolkits AS-ISK and CMIST.

For AS-ISK, given the limited number of screened species, the generalised threshold of 13.25 for freshwater invertebrates as defined in Vilizzi et al. (2021) was used (see also Vilizzi et al. 2022a, b) and for comparative purposes only the BRA (Basic Risk Assessment) scores were considered – though the BRA+CCA (Climate Change Assessment) scores and related risk ranks were also computed for completeness and reference for future studies (see Vilizzi and Piria 2022). Additionally, the Environmental and Commercial sector scores were used for comparative purposes with the other two screening toolkits *Harmonia*+ and CMIST. To this end, the scores for the BRA (ranging from −20 to 70), Environmental sector (−4 to 17) and Commercial sector (−5 to 23) were re-scaled to 0–1.

For CMIST, the approach by Brown and Therriault (2022) was followed to calculate the Likehood, Impact and Overall risk scores using package CMISTR in R v4.2.1 (R Development Core Team 2023), with a species ranked as high risk if scoring at least 2 for both Likelihood and Impact. Additionally, the sum of the scores for Questions 10–17 (as a proxy for Environmental impacts) and the score for Question 9 (as a proxy for Socio-economic impacts) from the Impact of invasion section were used for the screened species for comparative purposes with the other two screening toolkits *Harmonia*+ and AS-ISK. To this end, the Overall scores (ranging from 1 to 9) and the Environmental impact (8 to 24) and Commercial sector (1 to 3) scores were re-scaled to 0–1.

Following screening, a combined risk score for each species was computed as the average of the *Harmonia*+ Overall risk score, the AS-ISK BRA score and the CMIST Overall score. Following the approach of Britton et al. (2011), an *ad hoc* threshold was then defined. This threshold, which is heuristically determined based on the number of screened species and their corresponding risk scores, provides for a categorisation that enables a greater degree of species prioritisation according to their invasiveness in view of follow-up risk assessment. In setting the *ad hoc* threshold, the species with the higher risk scores are highlighted from the remaining ones (see Vilizzi et al. 2024 for a review of AS-ISK applications).

Results

Faxonius immunis, *F. limosus, P. leniusculus* and *P. clarkii* obtained the highest Overall risk scores (> 0.500) in *Harmonia*$^+$ (Table 1, Appendix 2) as a result of their Invasion and Impact scores and scored the same in terms of Environmental impact. *Procambarus virginalis* was the next species with a high score followed by *C. destructor* and *C. quadricarinatus*, with the latter two species obtaining identical scores for all components. *Pontastacus leptodactylus* was the lowest scoring species and the only one with a lower Socio-economic impact. All other species scored the same in terms of Socio-economic impact. Based on the Overall risk scores, five out of the eight species were ranked as medium risk (i.e. *F. immunis*, *F. limosus*, *P. leniusculus*, *P. clarkii* and *P. virginalis*) and the remaining three as low risk (i.e. *C. destructor*, *C. quadricarinatus* and *P. leptodactylus*). Confidence level ranged from medium to maximum for all species (Appendix 2).

Faxonius immunis, *F. limosus*, *P. clarkii* and *P. virginalis* were the highest scoring species in AS-ISK (Table 2, Appendix 3) for the BRA (> 40) and with *P. leniusculus* also for the BRA+CCA (> 50). *Cherax destructor* and *C. quadricarinatus* had lower (albeit identical) scores for both the BRA

Table 1. Results of the *Harmonia*+ risk screening protocol for eight non-native crayfish species in Lake Maggiore (Northwest Italy) – the risk assessment area. For each component: 0 = lowest level of invasiveness; 1 = highest level. The risk rank (based on the Overall risk score) is defined using the thresholds proposed by Lemmers et al. (2021): high risk, species with Overall risk score > 0.660; medium risk, between 0.330 and 0.660; low risk, < 0.330. Species are sorted from higher to lower Overall risk score (and then alphabetically). For each species, the status (extant or horizon) in the risk assessment area is indicated.

Species	Status	Invasion score	Impact score	Overall risk score	Risk rank	Environmental impact	Socio-economic impact
Faxonius limosus	Extant	0.693	0.833	0.577	Medium	0.833	0.833
Pacifastacus leniusculus	Extant	0.693	0.833	0.577	Medium	0.833	0.833
Procambarus clarkii	Extant	0.693	0.833	0.577	Medium	0.833	0.833
Faxonius immunis	Horizon	0.630	0.833	0.525	Medium	0.833	0.833
Procambarus virginalis	Horizon	0.500	0.750	0.375	Medium	0.750	0.833
Cherax destructor	Horizon	0.470	0.625	0.294	Low	0.625	0.833
Cherax quadricarinatus	Horizon	0.470	0.625	0.294	Low	0.625	0.833
Pontastacus leptodactylus	Horizon	0.437	0.542	0.237	Low	0.542	0.333

Table 2. Results of the AS-ISK risk screening protocol. BRA = Basic Risk Assessment; CCA = Climate Change Assessment, risk rank, sectors affected (C = Commercial; E = Environmental). Species with a score ≥ 13.25 are classified as high risk (threshold after Vilizzi et al. 2021). Species are sorted from higher to lower BRA score (and then alphabetically).

Species	Status	BRA		BRA+CCA		Sectors affected	
		Score	Risk rank	Score	Risk rank	C	E
Procambarus clarkii	Extant	46.5	High	58.5	High	18	16
Procambarus virginalis	Horizon	43.5	High	55.5	High	16	16
Faxonius immunis	Horizon	42.5	High	54.5	High	15	16
Faxonius limosus	Extant	42.5	High	54.5	High	15	16
Pacifastacus leniusculus	Extant	38.5	High	50.5	High	15	13
Cherax destructor	Horizon	37.0	High	49.0	High	14	15
Cherax quadricarinatus	Horizon	37.0	High	49.0	High	14	15
Pontastacus leptodactylus	Horizon	29.5	High	39.5	High	12	12

and BRA+CCA and *P. leptodactylus* was the lowest-scoring species. All screened species, except for *P. leptodactylus*, achieved the highest increase (+12 points) in the BRA+CCA score relative to the BRA. Based on the generalised threshold for freshwater invertebrates, all screened species were ranked as high risk. For all species, the scores for the sectors affected, reflected the corresponding BRA and BRA+CCA scores: *P. clarkii* scored highest in the Commercial sector and, together with *F. immunis*, *F. limosus* and *P. virginalis*, in the Environmental sector, and *P. leptodactylus* scored lowest in both sectors. Confidence factor was high for all species (Appendix 3).

Faxonius immunis, *F. limosus*, *P. leniusculus*, *P. leptodactylus*, *P. clarkii* and *P. virginalis* had scores > 2 in CMIST (Table 3, Appendix 4) for both. Likelihood and Impact of invasion and were therefore ranked as high risk; whereas *C. destructor* and *C. quadricarinatus* scored < 2 for Likehood of invasion (but > 2 for Impact of invasion) and were ranked as medium risk. The scores for the Environmental impact were similar across all species, and those for the Socio-economic impact were equal. Confidence level was medium to high for all species (Appendix 4).

Table 3. Results of the CMIST risk screening protocol. Species are sorted from higher to lower Score.

Species	Status	Likelihood of invasion	Impact of invasion	Score	Risk rank	Environmental impact	Socio-economic impact
Faxonius limosus	Extant	2.291	2.778	6.365	High	22	3
Procambarus clarkii	Extant	2.279	2.778	6.331	High	22	3
Pacifastacus leniusculus	Extant	2.162	2.125	6.005	High	22	3
Procambarus virginalis	Horizon	2.106	2.667	5.616	High	21	3
Faxonius immunis	Horizon	2.039	2.744	5.597	High	22	3
Pontastacus leptodactylus	Horizon	2.038	2.592	5.284	High	20	3
Cherax quadricarinatus	Horizon	1.811	2.778	5.031	Medium	22	3
Cherax destructor	Horizon	1.806	2.778	5.016	Medium	22	3

Based on the combined (average) risk scores obtained from the three screening protocols and an *ad hoc* threshold of 0.5, extant *P. clarkii*, *F. limosus* and *P. leniusculus* and horizon *F. immunis* and *P. virginalis* were ranked as "overall high risk" (in order of decreasing scores), and *C. quadricarinatus*, *C. destructor* and *P. leptodactylus* as "overall medium risk" (again in order of decreasing scores).

Based on the combined (average) risk scores obtained from the three screening protocols and an *ad hoc* threshold of 0.5, extant *P. clarkii*, *F. limosus* and *P. leniusculus* and horizon *F. immunis* and *P. virginalis* were ranked as "overall high risk" (in order of decreasing scores), and *C. quadricarinatus*, *C. destructor* and *P. leptodactylus* as "overall medium risk" (again in order of decreasing scores) (Figure 2).

Discussion

Risk outcomes

Of the eight crayfish species screened in this study, five were estimated to carry an overall high risk of invasiveness (the extant *F. limosus*, *P. leniusculus*, *P. clarkii* and the horizon *F. immunis* and *P. virginalis*) and three (the horizon *C. destructor*, *C. quadricarinatus* and *P. leptodactylus*) a medium risk of invasiveness for Lake Maggiore. These results are in line with the global risk screening study with AS-ISK by Vilizzi et al. (2021), *Harmonia+* (Lemmers et al. 2021) and CMIST (Brown and Therriault 2022).

Across Europe it is known that *F. immunis*, *F. limosus*, *P. leniusculus* and *P. clarkii* have exerted substantial negative environmental impacts (i.e. Gherardi and Barbaresi 2000; Lewis 2002; Gherardi and Acquistapace 2007; Kozubíková et al. 2011; Lodge et al. 2012; Garzoli et al. 2014; Nishijima et al. 2017), which have resulted mainly in the reduction of plant biodiversity due to the species' burrowing activity (Cronin et al. 2002) and native fauna (Geiger et al. 2005; Ficetola et al. 2011). Additionally, it is well documented that *P. clarkii* and *P. virginalis* cause negative impacts on agriculture, especially on rice

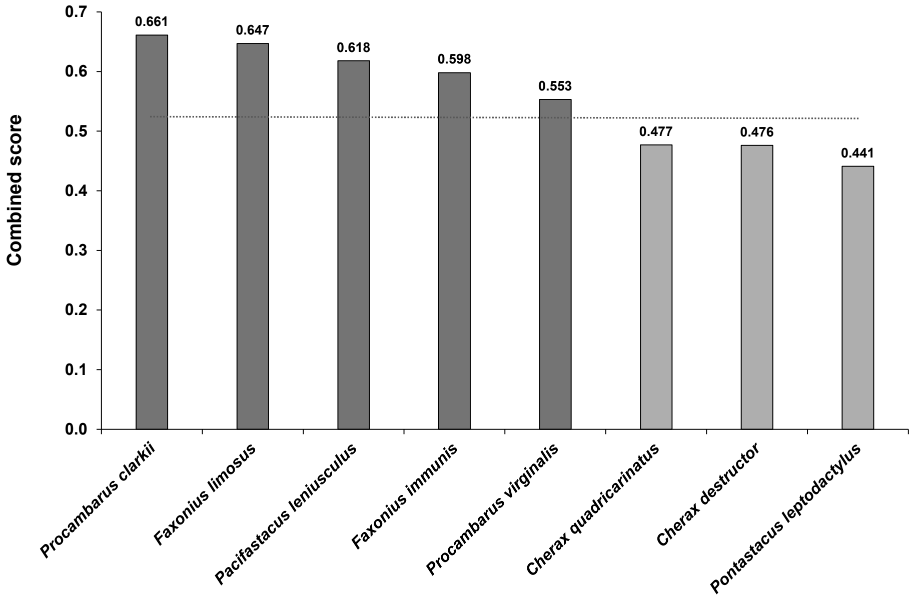

Figure 2. Combined (average) risk scores for the non-native crayfish species screened for Lake Maggiore with *Harmonia*+, AS-ISK and CMIST. Dark grey bars: score ≥ 0.50; Light grey bars = scores < 0.50.

cultivations (Jones et al. 2009; Faulkes et al. 2012; Souty-Grosset et al. 2016). However, studies on socio-economic impacts of these species are more limited as they are focused on related financial aspects (Bacher et al. 2018; Kouba et al. 2022), hence without considering negative impacts on other ecosystem services (Paganelli et al. 2024). Despite their high risk ranking, in Lake Maggiore direct effects of the extant non-native crayfish species *F. limosus*, *P. leniusculus* and *P. clarkii* on both macrophyte and macroinvertebrate assemblages have not been reported, possibly because they may have gone unnoticed in the absence of long-term and regular monitoring.

With regard to the medium-risk crayfish species, driving factors that may have contributed to their overall lower scores can be related to: (i) the species not having reached high densities in the wild (i.e. *C. destructor* and *C. quadricarinatus*); and (ii) the species not causing substantial environmental impacts (i.e. *P. leptodactylus*) despite possessing all characteristics to do so (see Scalici et al. 2009 for *Cherax* spp.). The lack of native crayfish (e.g. river crayfish *Austropotamobius pallipes* (Lereboullet, 1858)) along the shorelines of Lake Maggiore is due to their preference for running waters of tributaries of the River Toce and of the streams of the Val Grande National Park (*pers. comm.* from protected area staff and IdroLIFE project LIFE15NAT/IT/000823).

Socio-economic impacts were found to be high and similar for all crayfish screened in this study, even though in Lake Maggiore the impacts of the established non-native crayfish are unknown because monitoring activities have not been conducted. However, the risk of invasive crayfish establishment is unlikely to impact agriculture or aquaculture activities (both through the introduction of pathogens and parasites or by direct predation on fish). This is because of the lack of plant cultivations and to the presence of isolated aquaculture facilities in the risk assessment area. Only tourism has a positive and important socio-economic impact, whereas any other type of economic activity is scarce and infrequent. Conversely, the potential impacts on human health due to host-pathogens and parasites was estimated in this study to be very low because marketing based on non-native crayfish is not authorised, hence unlikely to happen (Longshaw 2011). In this respect, following EU Regulation 2019/1262, non-native crayfish species cannot be commercialised, bred, transported or intentionally released in nature. The same applies to Switzerland where Federal laws involve non-native invasive crayfish eradication. Despite Switzerland not being part of the European Community, it complies with rules and decrees to align with European requests to avoid undermining the monitoring and containment activities of the neighbouring countries.

Management considerations

The Lake Maggiore area is already compromised by the consequences of extreme climate events and increased population and tourism activity, which affect the demand for good water quality. The lake also suffers from increased water temperatures, which concern especially the upper lake layers (epilimnion). This has resulted in a progressive reduction of maximum winter mixing depths and an increase in thermal stability (Fenocchi et al. 2018; Jane et al. 2021), a decrease in oxygen concentration due to reduced intrusion of cold oxygenated waters from the tributaries (Dresti et al. 2023), and a higher frequency of algal blooms, especially cyanobacteria (as observed in other lakes worldwide: see Ho et al. 2019). All these factors have profound effects when evaluated against the objectives of the Water Framework Directive (2000/60/CE) for the ecological assessment of Lake Maggiore, with severe consequences on the use of the lake's waters for drinking purposes. From the date of entry into force of the Water Framework Directive, the deterioration of any water resource as evaluated through the assessment of its ecological status (i.e. using biological, physico-chemical and hydro-morphological indicators for water quality) is no longer allowed.

As a result of its geographic position, environmental characteristics and human-induced impacts, Lake Maggiore is highly vulnerable to biological invasions. Considering the bio-ecological traits of the non-native crayfish screened in this study combined with the effects of climate change, it is quite likely that the risk of bio-invasions will become even higher (Mosello and Lami 2014; Boggero et al. 2019). For the non-native crayfish species already well-established in Lake Maggiore (i.e. *F. limosus*, *P. leniusculus* and *P. clarkii*), the likelihood of their introduction and spread elsewhere may be underestimated. However, according to European, Italian and

Swiss laws it is unlikely that these crayfish species (which are included in the EU list of concern) will be released either intentionally or accidentally into the environment for economic purposes as they have no market value. The other five crayfish species evaluated in this study and not yet reported in Lake Maggiore (i.e. *C. destructor*, *C. quadricarinatus*, *F. immunis*, *P. leptodactylus* and *P. virginalis*) may represent a potential threat if introduced. *Cherax destructor* and *C. quadricarinatus*, which possess the same set of bio-ecological traits, may pose a similar level of risk in terms of socio-economic impacts as extant *F. limosus* and *P. clarkii*. In this regard, non-native species providing services such as aquaculture are more likely to be introduced than non-native species not providing such services, hence more prone to becoming invasive (Yessoufou and Ambani 2021).

Overall, the distinction between non-native species *vs* invasive species is relevant in view of their management. Before starting any actions to prevent their biological invasion, it is crucial to understand the potential impacts of new species on the recipient environment and possible mitigation actions and related costs to contrast their spread (Andreu et al. 2009; Diagne et al. 2020). To this end, risk assessment represents the second step in the management strategy (cf. risk analysis) of non-native species following their screening and categorisation as high (or even medium) risk. Further, risk assessment tools are useful for scientists to inform decision-makers and any other interested parties not only about the threats posed by non-native invasive species but also about the priority actions to be taken to mitigate their impacts. Within such a framework, and despite some intrinsic differences, the three risk screening toolkits employed in this study have proved to be useful for evaluating the potential impact of the eight non-native crayfish species under study.

Methodological insights

The joint use in this study of the three toolkits *Harmonia*+, AS-ISK and CMIST to screen a set of non-native species for a defined risk assessment area (i.e. crayfish for Lake Maggiore) and the computation of a combined risk score based on the toolkit-specific scores represent, to the best of the authors' knowledge, a novelty relative to previous risk screening applications, including those reliant on more than one toolkit. With regard to the latter: Drolet et al. (2016) compared the risk scores and outcomes from CMIST and the Marine Invertebrate Invasiveness Scoring Kit (MI-ISK, part of the -ISK family of toolkits: Copp 2013); de Camargo et al. (2022) those from the Fish Invasiveness Screening Kit (FISK, also part of the -ISK family of toolkits) and the "lesser-known" Fish Invasiveness Screening Test (FIST, which was not included in the present study because of the scarcity of background information on its implementation and its being almost fully qualitative); Paganelli et al. (2022) those from *Harmonia*+ and AS-ISK; and Tomanić et al. (2022) those from the Marine Fish Invasiveness Screening Kit (M-FISK, also part of the -ISK family of toolkits) and AS-ISK. However, in all of the above studies evaluation of the risk of invasiveness of the screened species was limited to a comparison of the corresponding toolkit-specific scores. Conversely, it is argued that the meta-analytical computation of an Overall risk score as achieved in the present study has provided a more accurate categorisation of the risk ranks of the screened species following the identification of an *ad hoc* threshold (see also Britton et al. 2011).

Authors' contribution

AB, DP, LV: research conceptualization, sample design and methodology, data analysis and interpretation, writing – original draft; LK, LG, SZ: sample design and methodology, investigation and data collection, writing – review and editing; AB: funding provision.

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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:

Appendix 1. Toolkits summary description.
http://www.reabic.net/journals/mbi/2025/Supplements/MBI_2025_Boggero_etal_Appendix_1.pdf

Appendix 2. *Harmonia+* reports for the eight non-native crayfish species screened for Lake Maggiore.
http://www.reabic.net/journals/mbi/2025/Supplements/MBI_2025_Boggero_etal_Appendix_2.pdf

Appendix 3. AS-ISK reports for the eight non-native crayfish species screened for Lake Maggiore.
http://www.reabic.net/journals/mbi/2025/Supplements/MBI_2025_Boggero_etal_Appendix_3.xlsx

Appendix 4. CMIST reports for the eight non-native crayfish species screened for Lake Maggiore available at https://zenodo.org/records/13375346