Title page

Causes and consequences of crayfish extinction: stream connectivity, habitat changes, alien species and ecosystem services

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Running title

Stream connectivity and extinctions

Keywords

alien, invasive, stream, ecological barriers, *Orconectes limosus*.

Summary

1) Given the ongoing decline of many species, it is important to perform multifactorial analyses of conservation status, and to assess the effects of species extinction on ecosystem services.

2) In this study we used long-term surveys to assess the influence of habitat change, landscape alteration and invasive species on extinction risk of the native crayfish *Austropotamobius pallipes*. We reviewed the existing literature to assess which ecosystem services are threatened by local extinction of *A. pallipes* and replacement-with alien crayfish.

3) We sampled 196 streams and wetlands in northern Italy. Of these, 117 received multiple surveys over a 13-year period (2004-2017), thus allowing accurate measurement of extinction rate.

4) Thirty-four percent of *A. pallipes* populations underwent extinction between 2004 and 2017. The occurrence of alien crayfish in the catchment basin and urban growth in the landscape surrounding streams were associated with *A. pallipes* extinction. The probability of persistence was significantly higher in populations close to stream springs and with physical barriers (especially waterfalls) separating them from basins with alien crayfish.

5) Extinction of native crayfish alters community structure and impairs regulating services such as detrital breakdown and pest regulation. Replacement by alien crayfish (*Procambarus clarkii* and *Faxonius limosus*) also threatens supporting and regulating services by altering nutrient cycling, food webs, sediments and erosion.

6) The implementation of management practices that control river connectivity using selective barriers is needed to prevent further local extinction of native species. Integrating information on extinction with knowledge of impacts on ecosystem services is essential in developing more effective conservation policies.

Introduction

Biodiversity conservation is pivotal for ecosystem functioning and is essential for human societies that depend on them (Apostolopoulou & Adams, 2017; Ebner *et al.*, 2016). However, dramatic collapses of species on the global scale suggest that a sixth mass extinction may be imminent and pose serious challenges to ecosystem conservation and sustainable human development (Barnosky *et al.*, 2011; Briggs, 2017). Analyses of conservation status and species trends are ongoing for the majority of vertebrates (Hoffmann *et al.*, 2010), but assessments are lacking for many invertebrate taxa, which constitute >95% of extant animal species (Mora *et al.*, 2011). Furthermore, the mere description of species decline is not enough. On the one hand, it is essential to understand the threatening factors to set up appropriate conservation strategies. Ideally, such knowledge requires complete datasets that report both species and environmental changes over time. Unfortunately, these data are rarely available, thus complicating an objective and quantitative assessment of threatening factors. On the other hand, understanding the consequences of species declines and the changes in ecosystem services after species extinction helps to identify conservation priorities and to increase public awareness (Butchart *et al.*, 2010). However, the multiple effects of species extinction on ecosystem services are rarely assessed (Gascon *et al.*, 2015), and few studies combine analyses of the factors determining species decline with evaluations of consequences of extinction at ecosystem levels.

Freshwater environments are among the ecosystems most threatened by human activities. They cover less than 1% of the Earth's surface, but support some 10% of the world's species (Collier, Probert & Jeffrie, 2016). Freshwater ecosystems provide a variety of valuable services, but industrial development and human population growth have increased the impact on freshwater resources, thus leading to a crisis in freshwater biodiversity (Abell, 2002; Cumberlidge *et al.*, 2009). While knowledge on the conservation status and ecological determinants of freshwater taxa is lower than in terrestrial species, there is growing evidence that freshwater organisms are suffering strong declines and extinctions (Collen *et al.*, 2012). The assessment of conservation status on 12,621 invertebrate

species has shown that freshwater species suffer the highest levels of threat, with significantly more threatened species than either terrestrial or marine invertebrates (Collen *et al.*, 2012). Freshwater invertebrates play a key functional role in food webs and river regulation (Collier, Probert & Jeffrie, 2016), and many species also furnish goods highly valued by humans, including food resources (e.g., several crustaceans and molluscs), medicinal and ornamental products (Elliot & Dobson, 2015).

Maintaining connectivity is often assumed to be pivotal for freshwater conservation. River systems are closely connected habitats that are quite vulnerable to disturbances at network scale, such as damming and fragmentation (Cooper *et al.*, 2017). The positive role played by river connectivity has been pointed out for the conservation of several taxa including fish, amphibians and macro-invertebrates. For instance, many fish require multiple habitats to complete their life cycles, and barriers can seriously threaten entire populations (Laffaille *et al.*, 2004; Van Looy, Tormos & Souchon, 2014). Barriers can also limit the spread of invasive species, thus in some circumstances isolation can help the conservation of native species, as in the case of dams that prevent alien fish species from moving upstream (Beatty *et al.*, 2017). A correct evaluation of the role of connectivity for freshwater biodiversity is complex and requires taking into account conservation priorities and the major threats occurring at the drainage level.

Crayfish are often considered good indicators of freshwater ecosystem quality and functioning (Nardi *et al.*, 2005; Grandjean, Momon & Bramard, 2003). They play a fundamental role in maintaining structure and equilibrium within benthic communities (Weinlander & Fureder, 2011), especially considering their large biomass (Richman *et al.*, 2015). Freshwater crayfish are found in a wide variety of habitats and have significant economic importance in some areas (Jones *et al.*, 2006; Westman, 1998). Recent declines in numerous freshwater crayfish species have highlighted the need for appropriate conservation actions and policies (Richman *et al.*, 2015). Crayfish decline is caused by multiple factors, including expansion of human settlements and activities that determine habitat modifications and water pollution, the invasion of alien species and the spread of infectious diseases (Chucholl, 2016). Given the complexity of factors, analyses integrating multiple potential threats are

essential to identify the ongoing processes and proposing management actions. However, until now quantitative analyses of crayfish decline have focused on one single or a few stressors.

The European white-clawed crayfish, *Austropotamobius pallipes* (Lereboullet, 1858) species complex (Souty Grosset *et al.*, 2006), is among the most threatened crayfish species in Europe (Chucholl, 2016). *A. pallipes* has a wide native distribution throughout Europe; Montenegro is the eastern limit, whilst Spain and Scotland are the southern and northern limits respectively. Although the white-clawed crayfish may still be abundant in some restricted areas, population densities have declined dramatically, and its distribution has been drastically abridged (Kozak *et al.*, 2011; Mazza *et al.*, 2011). The decline of this crayfish is caused by the joint action of multiple factors. In the last decades, pollution has caused strong declines (Nardi *et al.*, 2005) but, more recently, the spread of invasive crayfish species has posed an additional threat (Chucholl, 2016; Scalici *et al.*, 2009). Besides interspecific competition, invasive crayfish native to North America (e.g. *Procambarus clarkii* and *Faxonius limosus*) can spread the crayfish plague pathogen (*Aphanomyces astaci*), which has caused dramatic extinctions of European crayfish populations (Bonelli, Manenti & Scaccini, 2017). Some works suggest that *P. clarkii* and *F. limosus* have difficulties in spreading in northern Italy streams showing a well-structured and unpolluted habitat, and that physical barriers in streams may limit the extinction of native crayfish populations (Kerby *et al.*, 2005; Manenti *et al.*, 2014). However, until now no studies have explicitly tested these hypotheses.

The aims of this paper are 1) to assess the role of multiple drivers in the extinction of the white-clawed crayfish; 2) to test whether the ecological role of river connectivity for the conservation of populations may change when invasive species are present and 3) to analyze the threats to ecosystem services caused by the extinction of native crayfishes and the invasion of nonindigenous species. Our work proposes practical management strategies to deal with the spread of invasive competitors and diseases.

Material and Methods

Study area

We studied 117 sites (112 streams and creeks, and 5 ponds) in Lombardy, Northern Italy, within the catchment basin of the Po River (Fig. 1). The study sites were selected on the basis of previous freshwater crayfish surveys that confirmed the occurrence of *A. pallipes* in 2004, the period in which the spread of invasive freshwater crayfish started in the study area (Fea *et al.*, 2006; Manenti, 2006). Sites belonged to 45 different drainages. Some sites were located along the same stream, but the minimum distance between nearby sites was 900 m. A water quality assessment, obtained in 2004 through application of the Extended Biotic Index (EBI) (Solimini *et al.*, 2000), was available for these historical sites.

Besides the 117 sites with historical data, we surveyed 79 additional supplementary sites in tributaries, collectors, and affluents connected with the catchment basins of the study area in which *A. pallipes* occurrence has been observed at least once. The aim of supplementary sites was to detect the presence of alien crayfish near the historical *A. pallipes* sites. *A. pallipes* lives mostly in oligotrophic streams, while in our study area the alien crayfish have different habitat requirements and live preferentially in ponds, small lakes and rivers (Manenti *et al.*, 2014). Therefore, the additional sites were randomly selected among 390 sites already visited in 2004 and in which we did not detect the native species. These additional sites were in the nearby ponds, small lakes and streams most likely to host populations of *F. limosus* and *P. clarkii*.

Surveys

We surveyed each site at least 3 times between September 2016 and November 2017 to assess the distribution of *A. pallipes* and invasive freshwater crayfish. We used two independent nocturnal visual encounter surveys to maximize the probability of crayfish encounters (Reynolds *et al.*, 2006). All nocturnal surveys were performed between 9:00 p.m. and 01:00 a.m. For every stream we surveyed

transects of at least 150 m, while for ponds we surveyed the entire perimeter. To avoid the spreading of crayfish plague, we performed nocturnal surveys from upstream to downstream and at each survey we disinfected boots and material with 10% sodium hypochlorite when moving between different localities.

During daytime surveys we evaluated water quality and recorded the presence of barriers downstream of each site. To evaluate water quality, we applied EBI protocol modified for Italian streams (Ghetti, 1997): we sampled the macroinvertebrates for 10 minutes. Collected macrobenthos was classified and, based on the type and number of taxa collected, we assigned a score ranging from 1 (lowest quality: poor communities including very tolerant species) to 13 (maximum quality: the richest communities, including stenoecious species).

To assess the occurrence of barriers we considered the stretches downstream of each site for a maximum distance of 300 m and noted the occurrence of physical barriers (i.e. natural or artificial vertical falls >1 m that invasive crayfish cannot climb over (Dana *et al.*, 2011)), piped stretches (artificial subterranean stretches >10 m, which are generally unsuitable for crayfish) and polluted stretches (the presence of very abundant periphyton indicating high organic pollution) (Maitland, 1990; Moss, 1998). We visually assessed periphyton abundance using a rank scale (1: periphyton absent or < 5% of the stream bottom; 2: 5% ≤ periphyton cover < 40%; 3: 40% ≤periphyton cover < 60%; 4: 60% ≤ periphyton cover < 80% and 5: periphyton covering ≥ 80% of stream bottom; (Manenti, Ficetola & De Bernardi, 2009). We considered stream stretches with periphyton covering ≥80% of the stream bottom for ≥200 m as pollution barriers (Manenti *et al.*, 2014).

Finally, using GIS software (ArcGIS 10) we assessed three features of the landscape surrounding each site: the distance from the spring (linear length in metres), the change of urban cover over the last 13 years in a 250 m circular buffer around each study site (total surface; m^2) and the length of roads occurring in a 1 km circular buffer around each study site (total linear length in metres). The urban cover and the road lengths together identified the landscape alteration; they were calculated using the land use and cover data from the DUSAF (Land Use of Agricultural and Forest

Land) by the Regional Geoportal of Region Lombardy Authority, which has a ground-resolution of ~3m. Datasets were available for years 2005 (Dusaf 2.0) and 2015 (Dusaf 5.0; data available at www.cartografia.regione.lombardia.it).

Statistical analysis

We used PRESENCE 5.5 (Hines, 2006) to perform occupancy modelling on the two nocturnal sampling occasions of each site and to assess probability of *A. pallipes* detection. In all the historical sites, *A. pallipes* was present in past surveys (Fea et al., 2006; Manenti 2006). We calculated also the detection probability of *P. clarkii* and *F. limosus*,

Given the high detectability of *A. pallipes* (see results), we considered the species extinct in sites in which no individuals were found in the 2016/2017 surveys. To assess potential causes of extinction, we built generalized linear mixed models (GLMMs; binomial error distribution) with extinction / persistence of *A. pallipes* as a dependent variable. We considered six independent variables: increase of urban area and road extension in the surrounding landscape, distance from spring, change in EBI score, occurrence of alien crayfish in the catchment basin, and number of barriers occurring downstream. The number of barriers was the number of barrier typologies recorded for a stretch of 100 m downstream to each site (if we found polluted stretches in this range, we verified if periphyton cover was regularly ≥ 80 % of the stream bottom for more than 200 m). Change in EBI score was calculated as the 2016/2017 score minus the score reported by past studies (Fea *et al.*, 2006; Manenti, 2006); this parameter was considered as a measure of habitat change. We included stream identity and the catchment basin of each site as random factors to take into account spatial non-independence of observations. All the independent variables were log-transformed except the occurrence of alien crayfish (binary variable). We built models representing all possible combinations of independent variables and we used the Akaike Information Criterion for small samples (AICc) values to select the minimum adequate model, which is the model explaining the largest amount of variation using the smallest number of variables (Rolls, 2011). Variance inflation factor (VIF) was

calculated within each model and only models with a VIF value < 5 were considered. To evaluate the relative role of the different typologies of barriers, we also used GLMMs, considering the extinction of *A. pallipes* as a dependent variable and the three barrier typologies (polluted stretch, physical falls and piped stretches) as independent variables. The independent variables were log-transformed. We performed all the combinations of models and we selected the best model on the basis of AICc values. In all GLMs, we used conditional R_2 ($R_{2\,c}$) to assess the amount of variation explained by the best model, and assessed the significance of the variables composing the best model using a Wald Chi-square test. Analyses were performed in R environment (version 3.3.2) using the packages nlme, multcomp, car, MuMIn and lmerTest.

Ecosystem services

Threats to the provision of freshwater ecosystem services (ES) were evaluated by analysing the ecological impacts of the invasion of nonindigenous crayfishes on the ecosystem reported in the scientific literature, grey literature (e.g. technical reports) and directly observed in the study area by the authors. To define ES, we used the standard classification proposed by the Millennium Ecosystem Assessment (Millennium Ecosystem Assessment, 2005). For each ES category, we examined whether an impact on ecosystem services has been reported and if that impact was positive, negative or not well studied. The results were organized in a table summarizing which ES's are impacted, divided by the three different cases observed in our study area. Case 1): local extinction of the indigenous crayfish *A. pallipes* population in absence of replacement by alien species; case 2): local extinction of *A. pallipes* with replacement by the alien *P. clarkii;* case 3): replacement by *F. limosus*. This will allow us to understand the differences in threats to the ES caused by different degrees of alien species invasion, and to identify the impacts that still must be assessed to understand their full effect on freshwater ES provision.

Results

Drivers of Crayfish extinction

With our study methods, per-visit detection probability for *A. pallipes* was very high (0.98), therefore two surveys allowed detection of this crayfish with very high levels of confidence. We detected *A. pallipes* in 77 of the 117 sites investigated, thus the species was considered extinct in 40 sites where it occurred in 2004. We detected two alien freshwater crayfish species: *P. clarkii* and *F. limosus* (per-visit detection probabilities respectively 0.99 and 0.91). We never detected alien crayfish in sites where *A. pallipes* persisted. Alien crayfish (*P. clarkii*) were detected only in four of the sites in which *A. pallipes* was extinct. Furthermore, *P. clarkii* occurred in 45 of the 79 supplementary sites investigated, constituted mainly by ponds, lakes and artificial reservoirs. *Faxonius limosus* occurred in four sites only of the 79 supplementary sites. We did not detect *A. pallipes* in any of the 79 supplementary sites.

On average, the sites in which the native crayfish was extinct were 1074 m ± 250 m distant from the spring, while those in which the crayfish persisted were closer to the spring (average 550 m ± 69 m; Fig. 2A). Considering environmental features, the surveys conducted showed a general decrease in water quality. Water quality decreased also in the sites in which the native crayfish persisted. We detected an average ± SE decrease of -0.4 ± 0.13 of the score, with peaks of -8 (Fig. 2B).

The best-AICc mixed model showed that crayfish extinction was related to increase in urban cover, absence of barriers, occurrence of an alien crayfish species within the same drainage and decrease of EBI score (Table 1). Furthermore, extinctions were less likely in sites close to the stream spring. The best-AICc model explained the variation very well ($R^2_c = 0.80$).

When we compared the effect of different barrier typologies, the Presence of physical barriers, such as artificial and natural falls, was the only variable included in the best model, while the presence of pollution barriers or piped stretches were not included in the model and showed no significant effects. Probability of extinction was negatively related to the number of physical barriers (B = -27.5, χ^2_1 = 12.99, P < 0.001; R^2_c = 0.65).

Changes in ecosystem services

The literature on the ecological impacts of alien crayfish invasions documented several threats to ecosystem services as a consequence of the extinction of native crayfish and invasion by alien species. For the extinction of *A. pallipes*, about 30% of the services considered are undescribed in literature because specific studies are missing. Since in our study area different levels of biological invasion occurred, different impacts on provision of ecosystem services were expected (Fig. 3, Table S1). Comparing the three situations (extinction of *A. pallipes*, replacement by *P. clarkii* and replacement by *F. limosus*) the major threats to ecosystem services occurred with the invasion by *P. clarkii* (particularly as concerns supporting and regulating services). Lower threats were reported for *F. limosus*, possibly because fewer studies addressed the issue for this species. When local extinction of *A. pallipes* occurs without replacement by invasive species, ecosystem functions and related ecosystem services are threatened to a lesser extent, especially as concerns regulating and supporting services. In case of replacement of *A. pallipes* by alien crayfish species, positive effects, mainly related to an increase in provisioning services (food), an increase of abundance of crayfish predator taxa (water birds) and recreational services (fishing) have also been reported. In general, the positive impacts on ecosystem services provision by the presence of *A. pallipes* are poorly studied. Considering the extinction of *A. pallipes,* no studies were available to evaluate the impact on five ecosystem services considered in our study (medicinal, effect on soil, sediment and erosion, natural hazard protection, water regulation and recreation); similarly, the effect of the invasion of *F. limosus*

on six ecosystem services (from provisioning, supporting and regulating services) is lacking (Table S1, S3).

Discussion

Freshwater crayfish extinction drivers

The multi-temporal analysis of factors at both the local and landscape scale led to an understanding of the main drivers of the extinction of an endangered species within an area of high anthropic impact. Our approach, which considered the variation both of the occurrence of target species and environmental features over 13 years, highlighted a dramatic decline of the white-clawed crayfish, and suggested that impact of alien species, urbanization and freshwater pollution can jointly affect this crayfish, with highly detrimental on ecosystem services.

Considering the role of invasive species, *A. pallipes* extinction was closely linked to the occurrence of two alien freshwater crayfish species (*P. clarkii* and *F. limosus*) in at least one site within the same catchment basin. When data on *A. pallipes* were collected in 2004, alien crayfish had not yet spread into the study area (Fea *et al.*, 2006). Colonization of alien crayfish generally occurs from downstream to upstream (Cruz, Rebelo & Crespo, 2006), and lower extinction rate of populations near springs suggests that isolation may increase the persistence of *A. pallipes* populations. On the contrary, populations occurring downstream are more likely to become extinct by coming into contact with an alien crayfish population, but also because of disease spreading. *Procambarus clarkii* and *F. limosus* tend to occupy substantially different environments with respect to *A. pallipes* populations, with native crayfish restricted to small oligotrophic streams and alien species mostly occurring in wetlands with a slower current, such as eutrophic lakes, ponds and rivers (Chucholl, 2016; Gil-Sanchez & Alba-Tercedor, 2006; Manenti *et al.*, 2014). *Procambarus clarkii* prefers ponds, lakes or the lower part of streams where there are eutrophic conditions linked to organic

pollution and *F. limosus* prefers larger and deep subalpine lakes or mesotrophic rivers with important flow and depth (Siesa *et al.*, 2011; Souty Grosset *et al.*, 2006). Co-occurrence between native and the two alien species is extremely rare because the North American crayfish are vectors of the freshwater crayfish plague that causes massive mortality on *A. pallipes* before than interactions may be established (Bonelli, Manenti & Scaccini, 2017; Gherardi, 2006; Stucki, 1997; Weinlander & Fureder, 2012). Due to the plague spreading, the impact of alien crayfish may also occur over extents larger than the range of their establishment. In fact, we detected several cases of extinction without replacement, especially in unpolluted and undammed streams flowing into invaded ponds or rivers. In these cases, the plague was likely spread upstream in absence of alien species, for instance by native crayfish living in the stream mouth, by fish or by human-mediated movements of contaminated soil or water (Oidtmann, 2012).

Among habitat features, the increase of urban cover was a strong driver of extinction of *A. pallipes*. Urbanization poses a significant challenge to freshwater ecosystems (Liyanage & Yamada, 2017), since it is related to the decrease in stream flow, to alteration of connectivity and the reduction in functionality of stream river banks which generally follow home building along watercourses (Collier, Probert & Jeffrie, 2016; Janse *et al.*, 2015). Furthermore, humans can actively spread alien crayfish, and might even displace sediments with pathogens. The conservation of freshwater biodiversity requires preservation of robust buffer zones around wetlands, and our results further stress the importance of environmental policies that integrate stream conservation with landscape planning (Chucholl & Schrimpf, 2016; Ficetola, Padoa-Schioppa & De Bernardi, 2009; Riley *et al.*, 2005). Moreover, the increase of urban cover usually determines an increase of pollutants and sewages that reach the surrounding hydrographic basins (Ficetola *et al.*, 2011a; Moss *et al.*, 2005). Oganic pollution can have a huge impact on biodiversity in small streams and creeks (Dauba *et al.*, 1997). We detected a general decrease in water quality throughout the study area (Fig. 2c), that was an additional, significant driver of extinction. *A. pallipes* is linked to freshwater habitats with good

water quality, and our results confirm that anthropogenic disturbance through stream pollutions may be high detrimental for populations survival (Reynolds & Demers, 2006; Schulz, Smietana & Schulz, 2002).

Local-scale studies performed in pristine environments showed that *A. pallipes* populations can be organised as metapopulations, each stream being habitat patch where local extinctions and colonization can occur, thus allowing the long-term persistence of the crayfish(Neveu, 2009). However, at larger scales and in human-dominated environments populations are often isolated, likely representing the remnants of a previously continuous population, fragmented by loss of suitable habitat. Under heavy fragmentation, theory predicts that recolonization of habitat patches after local extinctions is unlikely (Hanski & Gaggiotti, 2004; Frankham, Ballou & Briscoe, 2002). In fact, we re-surveyed 30 sites without alien crayfish that were not occupied by *A. pallipes* in 2004. *A. pallipes* was unable to colonize any of those unoccupied sites, confirming that most of sites currently are isolated, and highlighting that local extinctions are very unlikely to be compensated by new colonisations.

The challenging role of freshwater connectivity

Barriers occurring downstream were associated with persistence of native crayfish populations. Physical barriers such as natural and artificial falls were particularly effective. The possible positive role played by barriers has already been suggested by other studies on crayfish conservation (Gil-Sanchez & Alba-Tercedor, 2006; Manenti *et al.*, 2014). Here we explicitly tested the relationship between stream barriers and extinction, considering both the number of different typologies of barriers, and the relative role of each typology. Our results challenge the idea that hydrological habitat connectivity always has positive effects on native biodiversity. Profound habitat modifications have altered the way natural components interact (Crutzen, 2006), thus in human-dominated landscapes conservation requires reassessment of traditional management strategies to

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meet emerging challenges (Kueffer & Kaiser-Bunbury, 2014). Waterfalls can hamper the spread of alien crayfish species and also limit contact between downstream and upstream native crayfish, thus preventing the spread of diseases. Species distribution models predict that in future decades the overall distribution of the European crayfish will be suitable for at least one invasive crayfish species (Capinha *et al.*, 2013). Our study suggests that the surviving native crayfish populations can escape major threats such as alien species and crayfish plague if natural or artificial barriers occur. The usefulness of barriers for crayfish conservation strongly complicates stream management. Reestablishing streams connectivity by removing dams and polluted stretches can reconstitute metapopulation dynamics and provide valuable ecological benefits for fish and aquatic invertebrates (Jackson & Pringle, 2010). At the same time, the removal of barriers to allow fish recolonization may favor upstream movements of invasive crayfish (Dana *et al.*, 2011), and remounting fish may also spread the crayfish plague and other diseases (Oidtmann, 2012). A broad framework of knowledge is necessary to understand the ecological effects of enhancing or reducing hydrologic connectivity in landscapes deeply shaped by human activities (Jackson & Pringle, 2010). For these reasons we suggest that management actions should a) favor connectivity of non-invaded areas to avoid isolation between native populations, b) promote the isolation of native populations from those of alien crayfish. Further studies should also consider the pattern of direct human introduction of the alien crayfish, as their direct introduction may result in extinctions even in apparently isolated areas (Bonelli, Manenti & Scaccini, 2017).

Threats to ecosystem services

Conservation of freshwater biodiversity is partly impeded by an inadequate understanding of the value of freshwater species and the services they provide (Darwall *et al.*, 2012; Richman *et al.*, 2015). Crayfish are often intentionally introduced because people derive value from them (Lodge *et*

al., 2012). However, their introduction often has negative impacts on key ecosystem services, and the overall outcomes of these actions are not yet well known (Hobbs & Lodge, 2010).

The extinction of *A. pallipes* will have multiple impacts on provisioning services. When only indigenous crayfish extinction occurs, the strongest impacts are expected to occur on supporting services (Fig. 3; Table S1), such as the reduction of species directly related to *A. pallipes* (ectosymbiotic worms), possible top-down effects on crayfish prey, and impact on competitors. These effects on the biotic community may in turn impact food webs, nutrient cycling and primary production which are basic (supporting) ecosystem services that support all the other services. In absence of any crayfish, detritus decomposition becomes less efficient, while some alien species like *P. clarkii* determine a reduction in organic matter content and an increase in phosphorous and nitrogen. The extinction of *A. pallipes* also reduces scavenging, which alters the natural regulation of the spread of diseases and pests (Table S1).

In principle, the negative effect on food provisioning caused by *A. pallipes* extinction can be partially compensated when species replacement occurs, especially when alien species reach high densities (e.g. *P. clarkii*; (Barbaresi & Gherardi, 2000). This can have positive effects on activities such as fishing, with related cultural benefits due to social interactions and cohesion. However, *P. clarkii* and *F. limosus* are Invasive Alien Species of European Union concern and according to EU regulation 1143/2014 their harvest is not allowed. Therefore, fishing activities for these alien species are likely to become restricted. Moreover, it should be remarked that the consumption of *P. clarkii* as food is controversial, as this crayfish can accumulate toxins and heavy metals (Gherardi, 2006). Increase in food availability may have direct effects on other species that feed on aquatic invertebrates such as otters and water birds, with consequent alteration in biotic community structure (increase in some groups at the expense of others). At the same time, high densities of invasive crayfish negatively impact the lower trophic levels (Matsuzaki *et al.*, 2009). For instance, in the study area the alien crayfish consumes freshwater macroinvertebrates and amphibian tadpoles, thus causing the decline of multiple native taxa (Ficetola *et al.*, 2012; Ficetola *et al.*, 2011b). *Procambarus clarkii* also

increases erosion which modifies benthic geomorphology through mechanical disturbance (foraging, burrowing and locomotor activities). Similarly, *F. limosus* impacts sediments through digging of deep burrows (Tables S2 and S3). As a consequence, both *P. clarkii* and *F. limosus* are responsible for major alterations in water quality through an increase in suspended sediments.

Our study identified multiple services that are threatened by indigenous crayfish extinction and replacement, which should thus be quantified at the local scale to understand the overall impact at the ecosystem scale. Nevertheless, several knowledge gaps remain, as there are services for which the impact is unclear and require further investigations (Tables S1-S3). We are still far from a complete understanding of the whole impact of these species on freshwater services, especially considering local variation. Moreover, the existence of trade-offs among different ecosystem services (i.e., gain in one service and loss in another) need careful analysis to support appropriate management choices (Lodge *et al.*, 2012). Focusing attention on this issue, and explicitly assessing species' contribution in maintaining services provision would be pivotal in strengthening conservation of threatened species.

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Tables

Variables	B	SE	χ^2	P
EBI score change	-5.695	1.176	23.5	**<0.001**
Urban cover increase	14.623	4.164	12.3	**<0.001**
N of barriers	-56.456	15.092	15.7	**<0.001**
AC occurrence	48.944	9.544	25.4	**<0.001**
Distance from spring	30.299	5.884	26.5	**<0.001**

Table 1. Variables included in the best model assessing the relationship between *Austropotamobius pallipes* extinction, environmental features and presence of alien species. EBI: Extended biotic index. The analysis was performed on 117 sites in which *A. pallipes* occurred in 2004. N of barriers= numbers of barriers occurring downstream of each site. AC occurrence = occurrence of alien crayfish in the catchment basin of each site. SE: standard error of the regression coefficient.

Figures captions

Figure 1. Study area. The map shows Lombardy region (northern Italy). Zoomed rectangles show the surveyed sites (indicated with black triangles) that belong to three areas of Lombardy such as the districts of Como –Lecco, Brescia and Pavia.

Figure 2. Box-whisker plots showing the relationship between *Austropotamobius pallipes* extinction and environmental variables. A) relationship between extinction and distance from the spring. B) relationship between extinction and extended biotic index (EBI) score variation between 2006 and 2017. C) relationship between extinction and urban cover change between 2006 and 2017.

Figure 3. Impacts on ecosystem service provision considering only *Austropotamobius pallipes* extinction and extinction and replacement by *Procambarus clarkii* and *Faxonius limosus*.