

## Mountain protected areas as refuges for threatened freshwater species: the detrimental effect of the direct introduction of alien species

Marco Bonelli, Raoul Manenti & Davide Scaccini

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### Abstract

One of the main threats to native European crayfish species is presented by the spread of invasive ones, which are vectors of the crayfish plague *Aphanomyces astaci*. In particular, the *Austropotamobius pallipes* complex is suffering extensive decline in its whole distribution range. In this paper, we describe a case of extinction of a native population of the *A. pallipes* complex driven by the direct human introduction of the alien species *Orconectes limosus*, in a mountain protected area in northern Italy. The local extinction event of the native crayfish population is reported, and the factors that drove it are reconstructed. Our results help to better understand the threats to native crayfish species occurring even in protected areas, as well as to establish proper prevention and management actions.

### Profile

Protected area

Monte Barro Regional

Park

Mountain range

Alps, Italy

### Introduction

Mountain protected areas (PAs) are usually considered crucial for the conservation of several biodiversity elements. Although only a limited number of these areas are designed or managed with the specific goal of conserving freshwater biodiversity (Chessman 2013), protected mountain areas generally may be important refuges for highly endangered aquatic species (Schauer et al. 2012). Native crayfish species (Decapoda: Astacidae) are currently among the most threatened organisms of the European freshwater fauna. While in the past they were widespread in all the river networks from springs to estuaries, currently they are often confined to mountain and hilly areas in the upper reaches of first-order creeks and streams (Westman et al. 2002; Nardi et al. 2005; Manenti et al. 2014).

Native crayfishes are threatened by habitat loss, fragmentation and alteration, pollution, overharvesting, climate change, and the introduction of alien species and epizootic diseases (Sala et al. 2000; Edgerton et al. 2004; DAISIE 2009; Holdich et al. 2009; Hulme 2009; Capinha et al. 2013; Chucholl 2013; Markovic et al. 2014).

The native white-clawed crayfish *Austropotamobius pallipes* complex has been included in the IUCN (International Union for Conservation of Nature) Red Data List as *Endangered (EN)* (Füreder et al. 2010). Populations of the white-clawed crayfish have declined enormously in the past few years and the species' range has been drastically reduced, such that conservation strategies have become mandatory (Kozák et al. 2011). In several territories in Italy, the white-clawed crayfish is considered to be on the verge of extinction (Fea et al. 2006; Brusconi et al. 2008). An understanding of the factors that cause a native species to become extinct is important for the devel-

opment of an effective and appropriate conservation strategy for the future.

A key issue for the conservation of native crayfish species is to avoid the spread of alien ones. Most of the non-native crayfish species currently established in European waters have apparently been introduced by anthropogenic activities, e.g. for aquaculture purposes or, to lesser extent, via the aquarium trade (Gozlan et al. 2006; Holdich et al. 2009). The spread of alien species by human activity often has a serious impact on native species (Strayer 2010), and PAs are expected to play a key role in the preservation of the native ecosystem.

In particular, three American freshwater crayfishes are significantly harmful to native European ones: *Pacifastacus leniusculus* (Dana, 1852) (Decapoda: Astacidae), *Procambarus clarkii* (Girard, 1852) and *Orconectes (Faxonius) limosus* (Rafinesque, 1817) (Decapoda: Cambaridae). Moreover, they also have a strong negative impact on freshwater communities generally, affecting both aquatic and semi-aquatic organisms (Gherardi 2007; Strayer 2010; Ficetola et al. 2011; Manenti et al. 2014).

These three American freshwater crayfish species are vectors of the crayfish plague pathogen *Aphanomyces astaci* Schikora, 1906 (Oomycetes: Saprolegniales) (Oidtmann 2012), which is listed in the top 100 of the world's most invasive species by the IUCN (Lowe et al. 2000; Gerlach et al. 2014). *A. astaci* was first recorded in Europe in the 1860s, in the Po Valley (northern Italy) (Cornalia 1860; Martinati 1861; Ninni 1865). Since the second half of the nineteenth century, the pathogen has affected the populations of native European crayfish extensively, causing several extinction events (Alderman 1996; Holdich et al. 2009).

All the North American crayfish species investigated are potential carriers of the plague (Söderhäll &

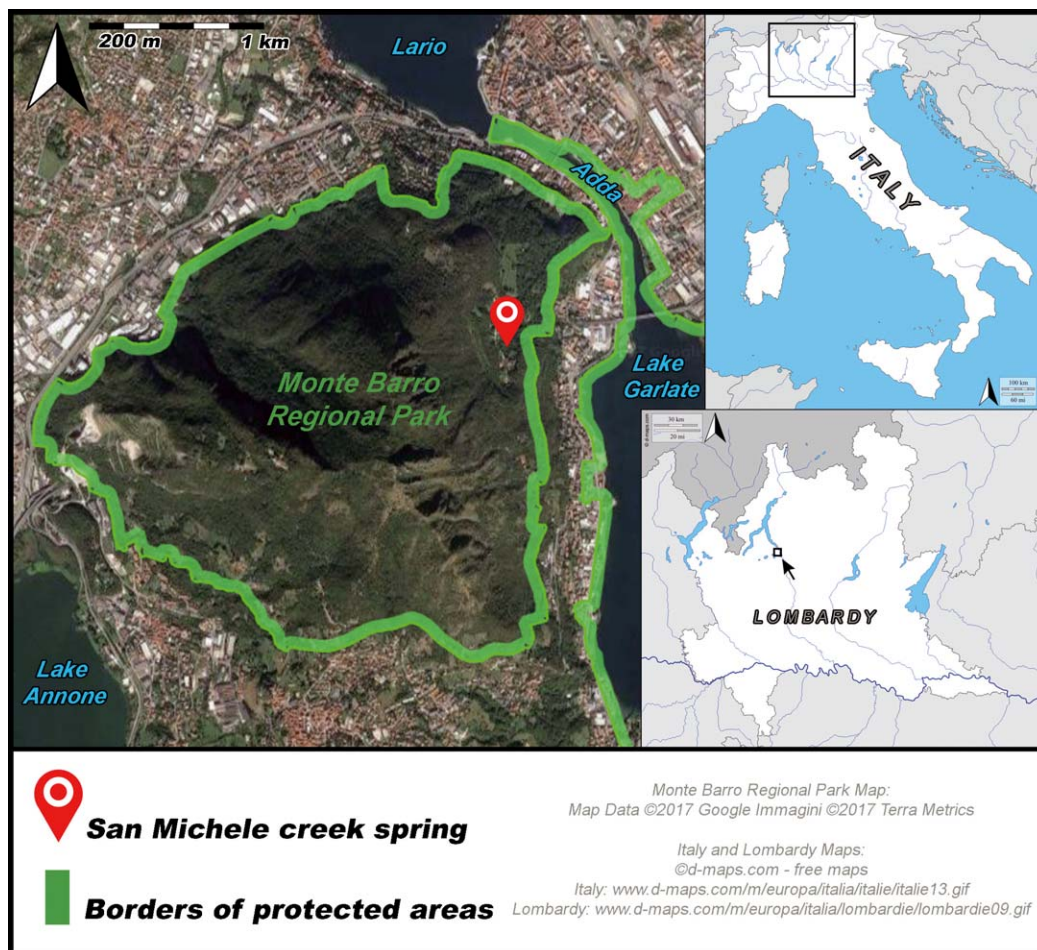


Figure 1 – Location of the study site; the marker indicates the location of the San Michele creek spring in the Monte Barro Regional Park.

Cerenius 1999; Oidtmann 2012). The *Old NICS* (Old Non Indigenous Crayfish Species; Holdich et al. 2009) in particular, *P. leniusculus*, *P. clarkii* and *O. limosus*, are often *A. astaci*-hosts.

The aim of this paper is to describe a case of extinction of a native population of the *A. pallipes* complex in a mountain PA in Lombardy (NW Italy) and to identify the factors that caused this event. Our results demonstrate that, after the introduction of the spiny-cheek crayfish *O. limosus* in the creek, *A. astaci* plague brought about the extinction of the native species. In addition, we tried to uncover how the alien species had been introduced into the area. Our results evidence the importance of including programmes of supervision for small freshwater habitats in the management plans of mountain PAs.

### Material and methods

Our research is focused on the San Michele creek situated in the Monte Barro Regional Park (Figure 1), in the Lecco province (NW Italy). The park is an Alpine PA (ALPARC 2016) and a Site of Community Importance (SCI), as defined by the European Commission Habitats Directive (92/43/EEC). The Monte

Barro is a mountain of the Lugano preAlps (Marazzi 2002).

The San Michele creek rises from a spring located in the area of the same name (situated in Galbiate Municipality, Figure 2), on the eastern side of the Monte Barro (WGS84/UTM32N, E530192, N5076023, 340 m a.s.l.). The flow rate of the spring is estimated to be 8 l per minute. Riparian vegetation consists of both deciduous trees and understorey plants, with a large number of ferns. A few metres downstream from the spring, a pool with permanent water occurs (Figure 3). The creek is perennial only in its initial stretch, then runs into a 60-metre high waterfall and meets permeable substrates, where the water is present only during periods of high rainfall. Finally, the creek flows into Lake Garlate and the river Adda. Both the presence of the waterfall and, downstream from it, the temporary nature of the presence of water acted as barriers for the spread of the invasive crayfish *O. limosus*, which was already established in the Lario (also known as Lake Como), which is nearby, in 2006 if not earlier (Manenti 2006). The San Michele creek hosted a well-structured population of the *A. pallipes* complex, which has been surveyed periodically since 2003 (Manenti 2006, and unpublished data).



From March to June 2013, we carried out night-time visual encounter surveys of native crayfishes occurring in the creek, estimating the Catch per Unit Effort (CPUE) index (Zimmerman & Palo 2011). From 26 July 2013, when *O. limosus* were first recorded in the creek, we started to remove individuals of the allochthonous species, taking at least three samplings per night.

Samplings were carried out on 26 and 30 July 2013, and monthly from August to October 2013. Periodical surveys of the creek were performed also from March to October 2014, and from March to October 2015. The samplings were taken between 21:00 to 01:00, by the same observers.

## Results

From March to June 2013, the population density of the *A. pallipes* complex was fairly constant, with relatively high CPUE values (mean value  $\pm$  SEM:  $3.5 \pm 0.26$ ). In May, we also observed throughout the entire stretch of the creek the presence of numerous new-born native crayfishes. However, on 26 July 2013 we recorded several dead native crayfishes (Figure 4), and one dying individual (Figure 5). This moribund specimen showed features typical of *A. astaci* infection (Oidtmann 2012), especially behavioural and physiological changes such as uncoordinated movements, reducing the tail escape reflex, and progressive paralysis and instability. Moreover, on the same occasion many *O. limosus* individuals were recorded in the creek. No further individual of the *A. pallipes* complex was recorded after July 2013.

Immediately following the first recording of the spiny-cheek crayfish, we started to perform a first sampling for the removal of *O. limosus* individuals, collecting 32 specimens (25 males and 7 females; sex ratio 3.57:1) (Figure 6). Their lengths varied from 70 to 110 mm. Attached between its gonopods, one of the males showed a zebra mussel *Dreissena polymorpha* (Pallas, 1771) (Veneroida: Dreissenidae) (Figure 7), a mollusc inhabiting standing waters or slow running rivers, which is common in the Lario. In the following three surveys (30 July, 15 August and 22 September 2013), 15 other *O. limosus* specimens (10 males and 5 females; sex ratio 2.00:1) in the same size range were collected. In total 47 specimens (35 males and 12 females; sex ratio 2.92:1) were removed from the stream. After these surveys, from October 2013, we did not observe any further *O. limosus* individual in the creek.

## Discussion

We report the sudden extinction of a population of the *A. pallipes* complex in a mountain PA. We hypothesize that the extinction of this species complex was immediately successive to the illegal introduction of the spiny-cheek crayfish *O. limosus*, and that the pathogen *A. astaci* was responsible for the mass



Figure 2 – The San Michele creek spring. © M. Bonelli 2016



Figure 3 – The pool with permanent water, San Michele creek. © M. Bonelli 2016

mortality of all the native crayfishes present in the isolated creek. The similarity in size of the recorded *O. limosus* individuals, the occurrence of a typical lake species such as *D. polymorpha* attached between the gonopods of a male, and the sudden appearance of the invasive species could indicate that a deliberate introduction occurred. Some individuals of *O. limosus* were 100 mm long or more (Figure 8). Based on their size, various individuals were considered to be of reproductive age.

It has already been demonstrated that infected individuals of the spiny-cheek crayfish pose a dramatic





Figure 4 – Dead white-clawed crayfish, San Michele creek, 26 July 2013. © M. Bonelli 2013



Figure 5 – Moribund white-clawed crayfish, San Michele creek, 26 July 2013. © M. Bonelli 2013



Figure 6 – Some individuals of the spiny-cheek crayfish, collected in the San Michele creek, 26 July 2013. © M. Bonelli 2013

threat for the native European species (Holdich et al. 2009; Oidtmann 2012). Indeed, the transmission of the crayfish plague pathogen can occur via the movement of infected specimens, or through contaminated water or equipment (Oidtmann 2012). The pathogen can also be transported through the digestive tracts of cold-blooded animals (Svoboda et al. 2016). In addition, crayfish predators (mammals, birds and fishes) can carry infected hosts to other water bodies (Evans & Edgerton 2002; Svoboda et al. 2016).

*O. limosus* shows a high dispersal rate (Puky & Schád 2006; Pârvolescu et al. 2012), influenced by water temperatures (Gherardi 2002; Buřič et al. 2009) and population densities (Taugbøl et al. 1993; Vrålstad et al. 2006). It is known that artificial and natural barriers can prevent crayfish movements and the upstream spread of the crayfish plague (Light 2003; Dana et al. 2011; Frings et al. 2013; Rahel et al. 2013; Kozubíková-Balcarová et al. 2014; Manenti et al. 2014; Benejam et al. 2015; Collas et al. 2016). In San Michele creek, the 60-metre high waterfall and the irregular presence of water can hinder the upstream movement of the alien species. Indeed, possibly due to the presence of these natural barriers, the invasive crayfishes were never reported in the San Michele creek before the 2013, despite their occurrence in the river Adda (Manenti 2006, and pers. obs.).

To understand the role played by users of the PA in the freshwater crayfish extinction that occurred in our study locality, information was collected by talking with local people. People in the area knew of the presence of the white-clawed crayfish, and the occurrence of the invasive crayfish species *P. clarkii*. In fact, some of them spoke of a red crayfish (possibly referring to the red-swamp crayfish, *P. clarkii*) as the only terrible and invasive crayfish species that was hitting the native one. However, they erroneously reported the presence of the autochthonous species in the river Adda, and the possibility of bringing specimens up to the San Michele creek in a homemade population restoration. Therefore, it is possible that the good faith of locals caused the spread of the spiny-cheek crayfish in the upper part of the creek, causing the extinction of the native crayfish population. It is probable that *O. limosus* specimens were found in the Adda, misidentified as the native species, and then introduced to the San Michele creek by locals.

Our report highlights the strong impact that humans may have as vectors of alien species, even in isolated mountain PAs. Moreover, this study emphasizes how prompt action following the introduction of alien crayfish species may be successful. Our results show that the alien *O. limosus* was completely eradicated within two months of its introduction. This successful eradication paves the way for further studies to evaluate the feasibility of a reintroduction of the white-clawed crayfish *A. pallipes* complex in this area, with insights of potential interest for other European mountain PAs. In fact, earlier studies have reported the possibility of restoring native freshwater crayfish populations after a minimum three-year absence of the host (Smith & Söderhäll 1986; Taugbøl et al. 1993; Souty-Grosset et al. 2006).

The extinction of an endangered aquatic species in a SCI in a regional park which we report here underlines the need for the careful management of freshwater habitats in mountain PAs. As the populations of different native crayfish species are becoming more and more isolated and confined to spring zones and head-

waters, mountain areas are likely to become the last refuges for several threatened species. Moreover, our study shows that in mountain parks users may release dangerous allochthonous species in pools and streams, even when they are isolated from the surrounding hydrographic networks. In this paper, we underline the need to ensure adequate surveillance of aquatic ecosystems in mountain PAs. The risks of public access to water bodies where endangered aquatic species occur should be carefully evaluated.

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Figure 7 – Spiny-cheek crayfish male with *D. polymorpha*, San Michele creek, 26 July 2013. © M. Bonelli 2013



Figure 8 – An individual (100 mm long) of the spiny-cheek crayfish, San Michele creek, 30 July 2013. © M. Bonelli 2013

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## Authors

### Marco Bonelli

is a PhD student in Environmental Sciences at the Department of Biosciences, Università degli Studi di Milano (Italy), where he is working in the field of insect physiology. He is also interested in anthecology and astacology. Department of Biosciences, Università degli Studi di Milano, Italy. Email: [marco.bonelli@unimi.it](mailto:marco.bonelli@unimi.it)

### Raoul Manenti

has a PhD in animal biology; he carries out research in various fields of zoology, from developmental biology to herpetology, biospeleology and conservation. Department of Biosciences, Università degli Studi di Milano, Italy. The Mohamed bin Zayed Species Conservation Fund, project no. 162512949. Email: [raoulmanenti@gmail.com](mailto:raoulmanenti@gmail.com)

### Davide Scaccini – Corresponding author

has a degree in Agricultural Sciences from the Università Cattolica del Sacro Cuore, Piacenza (Italy). He is interested in entomology, astacology, ecology, and terrestrial slugs as crop pests. Associazione WWF Lecco, Località Camporeso, Galbiate, Lecco, Italy. Email: [davide.scaccini@alice.it](mailto:davide.scaccini@alice.it)