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Rapid Communication

First record of a marbled crayfish *Procambarus virginalis* (Lyko, 2017) population in France

Frédéric Grandjean[1,*], Marc Collas[2], Magali Uriarte[3] and Marion Rousset[4]

[1]*Laboratoire Ecologie et Biologie des Interactions – UMR CNRS 7267 Equipe Ecologie Evolution Symbiose, Bâtiment B8-B35, 6, rue Michel Brunet, TSA 51106, 86073, Poitiers, France*

[2]*French Office of Biodiversity, Department section in Vosges, 22-26 Avenue Dutac, 88000, Epinal, France*

[3]*Federation of AAPPMA of Moselle, 4, rue du Moulin, 57 000 Metz-Magny, France*

[4]*French Office of Biodiversity, Department section in Moselle, Chemin du Longeau – Rozérieulles - 57160 Moulins-les-Metz, France*

Author e-mails: *frederic.grandjean@univ-poitiers.fr (FG), marc.collas@ofb.gouv.fr (MC), technique-fd57@orange.fr (MU), marion.rousset@ofb.gouv.fr (MR)*

**Corresponding author*

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Abstract

Here we report the first record of marbled crayfish *Procambarus virginalis* in France. In total, 34 individuals were found in a pond close to the Moselle River, coexisting with the invasive *Faxonius limosus* and the native *Astacus astacus*. Their presence seems limited to one pond of the seven located in this area, probably due to a very recent human introduction. Molecular analyses based on COI mt gene confirmed the morphological identification of captured specimens as marbled crayfish. The risk of spread to the Moselle is very high particularly during flood periods. Management recommendations are given.

Key words: invasive species, parthenogenetic crayfish, coexisting crayfish species, mitochondrial DNA

Introduction

Biological invasions are one of the biggest threats to the biodiversity of freshwater ecosystems around the world (Higgins and Vander Zanden 2010). When invaders are ecosystem engineers, they can have dramatic impacts on the ecosystem. Invasive freshwater crayfish are a good example, causing significant damage to both plant and animal communities, especially when they occur in high density (Larson and Olden 2013). Among the several invasive crayfish species reported in Europe, *Procambarus virginalis* (Lyko, 2017), the marbled crayfish, seems to be a serious threat to native communities and ecosystems because of its hardiness, omnivory, fast growth and reproduction by parthenogenesis (Scholtz et al. 2003).

This species is listed as an invasive alien species of concern under European Union Regulation 1143/2014. Some of the species characteristics are its ability to found large populations from a single individual (Feria and Faulkes 2011; Gutekunst et al. 2018) and to directly compete with, and act

as a vector of disease (aphanomycosis) towards native crayfish species (Chucholl and Pfeiffer 2010; Jimenez and Faulkes 2011; Keller et al. 2014). Linzmaier et al. (2018) have also shown that marbled crayfish were more aggressive than spiny-cheek crayfish, even against larger opponents, and may supplant *F. limosus*.

Its first occurrence in the wild was reported in 2003, in a water body near Karlsruhe (Marten et al. 2004). Since then, its distribution seems to have rapidly expanded. According to Vogt et al. (2018), the marbled crayfish is present in 11 European countries, Madagascar and Japan. In Madagascar, in ten years, the species has spread over 100 000 km² resulting from human and active dispersion (Lyko 2017). More recently, the species was recorded in Malta (Deidun et al. 2018). In continental Europe, its presence is reported in Germany (Chucholl et al. 2012; Chucholl 2016; Lyko 2017), the Netherlands (Soes and van Eekelen 2006), Italy (Marzano et al. 2009; Vojkovská et al. 2014), Sweden (Bohman et al. 2013), Ukraine (Novitsky and Son 2016), Czech Republic (Patoka et al. 2016), Slovakia (Lipták et al. 2016, 2017), Hungary (Lőkkös et al. 2016), Croatia (Cvitanić 2017), Romania (Pârvulescu et al. 2017) and Estonia (Ercoli et al. 2019).

In this paper, we report the first record of invasive marbled crayfish in reservoirs close to the mainstem of the Moselle River in France. The species is likely to pose additional threats to native communities in French freshwater ecosystems, particularly to the native white-clawed crayfish *Austropotamobius pallipes* (Linnaeus, 1758) and the noble crayfish *Astacus astacus* (Linnaeus, 1758).

Materials and methods

Field surveys

The study site is composed of 7 ponds which have resulted from the extraction of aggregates for the development of the metropolitan area of Metz in 1990 (Figure 1). These ponds are located on the right bank of the Moselle River (tributary of the Rhine). These are gravel pits with no outlet or drainage system. Today, they are fed by rainwater, runoff, the alluvial aquifer of the Moselle and its overflows during floods.

The first captures of the marbled crayfish were reported in March 2019 in one pond during a field trapping operation to check the success of reintroduction of the noble crayfish (*Astacus astacus*) in these reservoirs. A total of 67 marbled crayfish were captured with traps from March to May 2019 (Zion T. *pers. comm.*).

A field survey was conducted for this study in July and August 2019, in 6 of the 7 ponds. Crayfish surveys were carried out using cylindrical traps (70 cm long, with a cone-shaped funnel entry at each end, and containing a rectangular piece of mesh approximately 40 × 11 mm in size) baited with dog food. Traps were set for one night and lifted the following morning.

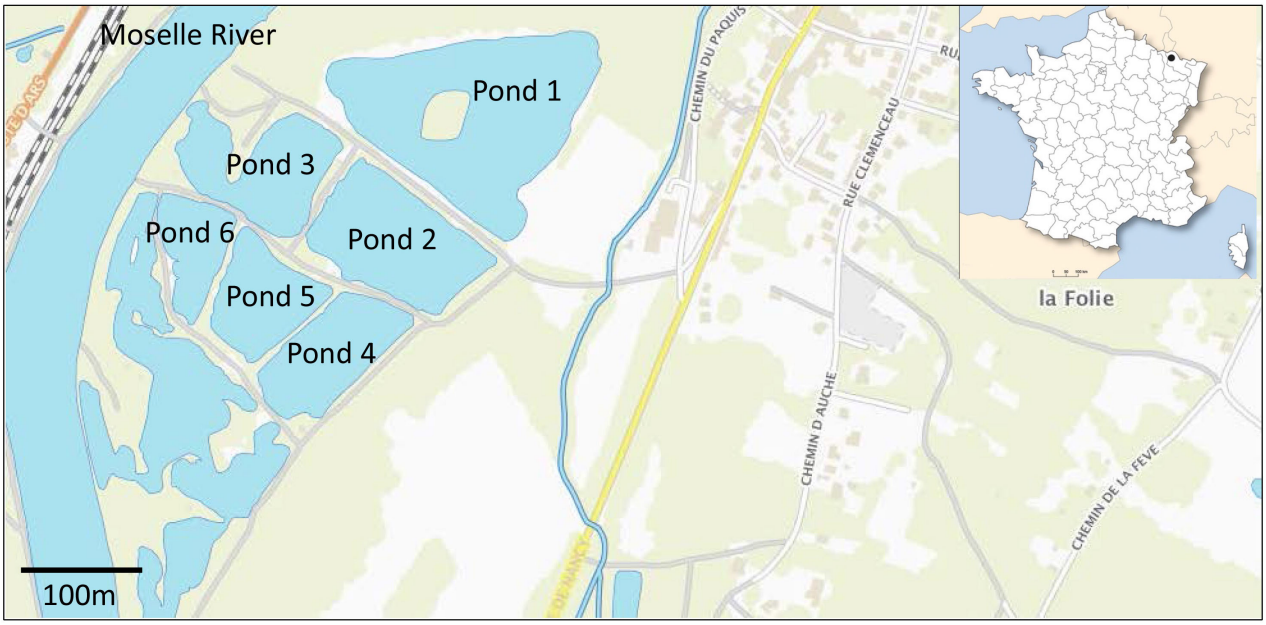

Figure 1. Ponds close to Moselle River located in northeastern France.

Table 1. Surface area of ponds (ha), with the total number of traps used per pond and the number of individuals caught per crayfish species, during July/August 2019.

Sites	Area (Ha)	No. of traps	*P. virginalis*	*F. limosus*	*A. astacus*
Pond 1	4.69	47	34	4	8
Pond 2	2.39	23	–	7	–
Pond 3	1.77	14	–	–	–
Pond 4	1.54	12	–	1	–
Pond 5	1.37	12	–	–	–
Pond 6	0.92	10	–	–	–

Only one field session was done per pond, except for ponds 1 and 2, the largest ones, with three field sessions. The number of traps per pond varied between 10 and 20 per session depending on their surface area (Table 1). Trapped crayfish were identified to species level, sexed, measured and counted.

Genetic identification of marbled crayfish

DNA was extracted from leg muscle tissue of 4 individuals using the Qiagen DNeasy Blood and Tissue kit following the manufacturer's instructions. COI fragments were amplified by PCR using the universal primer pair LCO1490/HCO2198 (Folmer et al. 1994) for species determination. The amplification was carried out in 25 µL volumes in a reaction mix containing a polymerase chain reaction (PCR) buffer with 1.5 mM $MgCl_2$ (Promega, Madison, Wisconsin), 400 µM dNTPs, 1 µM of each primer, 0.025 units of Taq polymerase (Promega), and 0.5 µL of the template (50 ng DNA). The PCR programme was as follows: an initial 1 min denaturation at 95 °C, 35 cycles of denaturation at 95 °C for 1 min, annealing at 55 °C and elongation at 72 °C for 1 min and 30 s. A final elongation step of 5 min at 72 °C was added at the end of the PCR. PCR products were purified by exonuclease 1

Figure 2. *Procambarus virginalis* trapped in pond 1. Photograph by Marc Collas.

and subsequently sequenced according to the Big Dye terminator method (PE Applied Biosystem, USA) on an ABI PRISM 3130 automatic DNA sequencer (PE Applied Biosystem, USA). For each individual we sequenced both forward and reverse fragments. All sequences were aligned with Geneious 10.1.3 (https://www.geneious.com) and blasted in GenBank to confirm the identity of each specimen (https://blast.ncbi.nlm.nih.gov/).

Results

Three crayfish species were caught in the Moselle ponds: *Faxonius limosus* (Rafinesque, 1817), *Astacus astacus* and *P. virginalis* (Table 1). The three species co-occurred in pond 1 (Figure 1). At this pond, *Procambarus virginalis* (Figure 2) was the most abundant, with 34 trapped individuals, compared to *F. limosus* and *A. astacus* with 4 and 8 trapped individuals respectively. *P. virginalis* and *A. astacus* occurred only in pond 1, whereas *F. limosus* was present in two further ponds (2 and 4). The size of *P. virginalis*, *F. limosus* and *A. astacus* caught ranged from 68 to 104 mm, 65 to 88 mm and 80 to 140 mm, respectively. No berried females were caught.

Four specimens of marbled crayfish analysed shared the same COI haplotype. It showed 100% similarity with the previously uploaded *P. virginalis* sequence (MK439899) and confirmed the presence of *Procambarus virginalis* in this area.

Discussion

This is the first report of *P. virginalis* in France. The circumstances of its introduction in Moselle, near Metz, remain unknown, but most probably result from a human translocation. This species is sought after by

aquariophilists due to its marbled appearance and its particular mode of reproduction. Although French and European law prohibit live transport of this exotic species, it is relatively easy to buy them on the internet (Faulkes 2015).

As the capture efficiency of crayfish by traps is highly dependent both on population size and crayfish activity, the eDNA technique, recently available for *P. virginalis*, could be very useful to check on its presence in other ponds and in the Moselle (Mauvisseau et al. 2019).

This species, which has a high dispersal rate, seems currently limited to the largest pond in the area. This is probably due to a recent introduction, coupled with the presence of two additional crayfish species, which could be limiting its reproductive rate by potential predation. Nevertheless, these first results have already shown that *P. virginalis* seems to be the most abundant crayfish species in the area. The co-occurrence of *P. virginalis* and *F. limosus*, both potential vectors of the crayfish plague, with *A. astacus*, a European crayfish species sensitive to this disease, suggests that *F. limosus* and *P. virginalis* are free of plague in this area.

Thus, this introduction of *P. virginalis*, a species with a high invasive potential, is a major preoccupation for the national "Office Français de la Biodiversité" (OFB). As the ponds examined here are close to the Moselle River, the risk of rapid expansion through the hydrographic network is high. Prompt actions should be taken to eradicate this species before its spread into the open water system. Intensive trapping sessions complemented by introductions of predatory fish into colonized ponds such as pike and perch are recommended. In addition, a technique to contain the species within the occupied pond must be applied to avoid its spreading. At the same time, although the transport of live exotic crayfish is prohibited by French legislation, an information and awareness campaign should be carried out jointly with the Moselle fishermen's federation, with owners and managers on the site.

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