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Rapid Communication

A new invader in freshwater ecosystems in France: the rusty crayfish *Faxonius rusticus* (Girard, 1852)

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Abstract

Crayfish are of major ecological importance within freshwater ecosystems, due to their central position in the trophic niche, acting as regulators of food webs. However, the introduction of exotic crayfish species threatens native species. Indeed, these introduced crayfish often outcompete local species and can even disseminate pathogens (i.e. exotic American species with the crayfish plague, *Aphanomyces astaci* Schikora, 1906), leading to rapid population losses. We report here the first record of rusty crayfish *Faxonius rusticus* in the wild in France and in Europe so far. The situation is alarming because *F. rusticus* is native to North America, potentially carrier of *A. astaci*, and classified among the most invasive species of crayfish in the world, due to its impressive dispersal capacities. This study reports high densities of individuals from a pond along the Inières Brook,where all size classes are represented, both adults and juveniles. A total of thirty-seven specimens were also observed over 2.5 km of the Inières brook as well as the Briane (main stream) in 2019 where this species co-occurs with another invasive crayfish species, the signal crayfish *Pacifastacus leniusculus*. In these sites, all specimens of both species were positive for crayfish plague presence with some individuals showing high level of infection (A5) (on an A0 to A7 scale), rarely observed in specimen of other invasive crayfish species. Since these first observations, the situation seems to have worsened, with 847 individuals captured, in the pond along the Inières brook, during survey sessions in 2021.

Key words: invasive crayfish, crayfish plague, co-occurrence, *Pacifastacus leniusculus*

Introduction

The situation of native crayfish in France has become alarming in recent decades due to various factors, among them habitat destruction and degradation of water quality but also increasing of introductions of non-native crayfish species in recent times (Kouba et al. 2014; Mrugała et al. 2017; Baudry et al. 2020; Grandjean et al. 2021).

Until recently, six North American species and one Australian species have been reported from the wild in France. Three American species were introduced several decades ago to reinvigorate the aquaculture and are now considered as “Old Non-Indigenous Crayfish Species”: the spiny-cheek crayfish *Faxonius limosus* (Rafinesque, 1817), the signal crayfish *Pacifastacus leniusculus* (Dana, 1852) and the red swamp crayfish *Procambarus clarkii* (Girard, 1852). They are widespread in France, having colonized all the hydrographic basins (Kouba et al. 2014; Préau et al. 2019). In spite of the French law prohibits the sale of live crayfish in pet trades and in the absence of crayfish aquaculture, an increase of introductions has been noted in the last decade with additional four alien crayfish species established (Chucholl 2013): the Kentucky River crayfish *Faxonius juvenilis* (Hagen, 1870) with one population in the east of France (Souty-Grosset et al. 2006), the Calico crayfish *Faxonius immunis* (Hagen, 1870) present in three affluents of the Rhine catchment (Collas 2020) and more recently, the parthenogenetic marbled crayfish *Procambarus virginalis* (Lyko, 2017) in a pond network in Moselle department located in North-east of France (Grandjean et al. 2021) and the Australian yabby *Cherax destructor* (Clark, 1936) in a catchment in Brittany (Collas 2020). These six American crayfish species are non-symptomatic carriers of *A. astaci*, causing melanized spots, eroded carapace or lost limbs to its native hosts, sometimes leading to death, depending on physiological conditions and different molt cycle of the considered specimen (Aydin et al. 2014). But the damage is greater on non-American crayfish species, for example in Europe and Japan, where this oomycete was responsible for numerous local extinctions of native crayfish populations (Filipová et al. 2013; Grandjean et al. 2014; Collas et al. 2016; Mrugała et al. 2017).

In this paper, we report the first record of the invasive rusty crayfish *Faxonius rusticus* (Girard, 1852) in France and in Europe. The species was discovered accidentally by a fisherman in 2019 in a pond in Aveyron department in Southern part of France. The circumstances of its presence in this location remain unknown, but undoubtedly result from an intentional human release as with most crayfish introductions in Europe. Yet, European law prohibits the sale and live transport of all exotic crayfish since 1983 (Regulation EU 1143/2014). This species is native to southwestern Ohio, northern Kentucky, southeastern Indiana and northern Tennessee in the Ohio River watershed (Perry et al. 2001).This species has been translocated by humans and now it is present throughout the northeastern United States up to southern regions of Canada (Reid and Nocera 2015). It is considered as one of the most invasive crayfish because of its aggressive behavior and ability to out-compete and displace native crayfish, when introduce outside its native range (Taylor and Redmer 1996; Olden et al. 2006). Thus, its presence in Europe is a real threat for local biodiversity

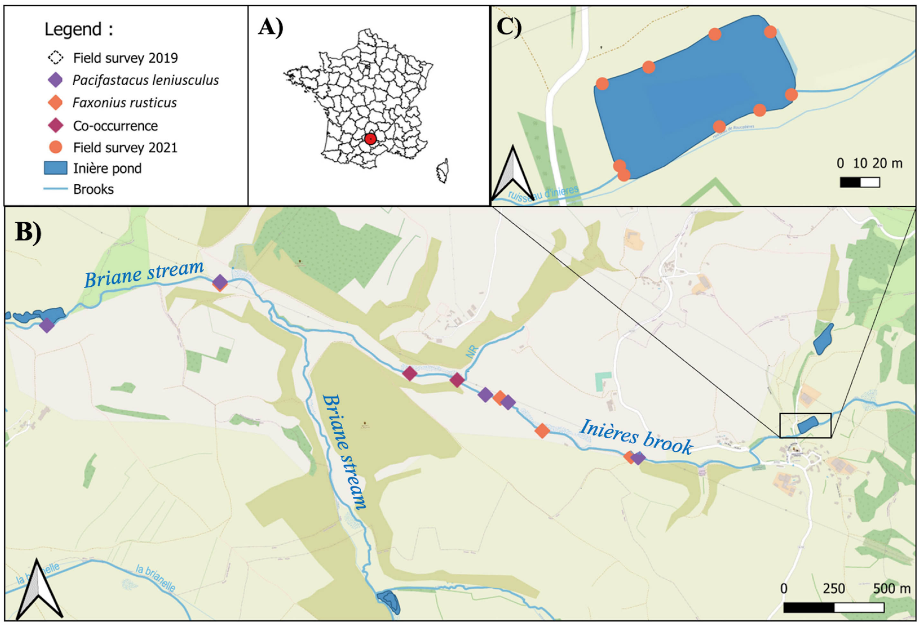

Figure 1. Location of the pond corresponding to the first occurrence of *Faxonius rusticus* along the Inières brook and the Briane (main stream) in Aveyron (France) (A). Diamonds represents the occurrence of *F. rusticus* (orange), *P. leniusculus* (purple) and both of them (red) (B). The orange circles in the pond correspond to the location of the 9 baited traps used for the survey in 2021 (C).

(Bobeldyk and Lamberti 2008), particularly towards white-clawed crayfish *Austropotamobius pallipes* (Linnaeus, 1758) which shares similar habitat in the head of water systems (Souty-Grosset et al. 2006).

Materials and methods

Ethical statements

All experimental procedures and animal manipulations, as well as field sampling, were performed according to French legislation. The sampling material was provided by the French Office for Biodiversity (OFB) and the Federation of Aveyron for fishing and protection of the of the aquatic environment. All captured specimens were euthanized in accordance with the French law on invasive species (law n° 2016–1087 of August 8, 2016).

Field surveys

The field survey carried out after fisherman brought live crayfish for taxonomic identification to the Federation of Aveyron for fishing and protection of the aquatic environment in 2019. The study areawas composed of a private pond supplied from an adjacent brook (Inières), and another stream (Briane), joining Inières 2 km further downstream from the pond, located in the department of Aveyron in south of France (Figure 1, Table 1).

Several adapted methodological approaches (baited traps, electrofishing, night light session) were used. Trapping was done using FIAP 1683 plastic traps (FIAP Gmbh, Ursensollen, Deutschland) (61 cm length × 31 cm width × 25 cm height, with a mesh of 1 cm × 2 cm and a double cone-shaped

Table 1. Number of crayfish trapped and observed in the successive surveys during the period September–October 2019 and April–August 2021 by the different methods usedin this survey.

Site	Sampling methods	Date	*F. rusticus*		*P. leniusculus*
			Number	Size range (mm)	Number
Inières Pond	Baited traps	25/09/2019	37	54–93	–
Inières Brook	Fishing nets	02/10/2019	3	–	–
	Fishing nets	10/10/2019	6	53–92	–
	Fishing nets	10/10/2019	8	79–95	–
	Baited traps	06/10/2019	1	63	4
	Baited traps	09/10/2019	6	83–104	6
	Light	09/10/2019	12	–	23
	Electrofishing	17/10/2019	1	50	–
Briane	Baited traps	08/09/2019	–	–	–
	Baited traps	09/09/2019	–	–	7
	Light	10/10/2019	1	–	–
	Light	16/10/2019	–	–	55
Inières Pond	Baited traps	20/04/2021	14	59–97	–
	Baited traps	27/04/2021	15	69–98	–
	Baited traps	04/05/2021	10	54–95	–
	Baited traps	18/05/2021	42	60–101	–
	Baited traps	01/06/2021	61	60–100	–
	Baited traps	08/06/2021	57	55–96	–
	Baited traps	15/06/2021	117	50–106	–
	Baited traps	29/06/2021	71	64–103	–
	Baited traps	16/07/2021	84	64–109	–
	Baited traps	28/07/2021	71	62–101	–
	Baited traps	30/07/2021	89	65–103	–
	Baited traps	17/08/2021	87	41–100	–
	Baited traps	27/08/2021	92	46–101	–

inlet), baited with fish meat (fresh sardines). Traps were deposited overnight and during the day, for a total of 12 to 16 hours in the water.

Two field survey periods were conducted. The first occurred between September 2019 and October 2019, following the discovery of *F. rusticus* in the Inières pond, by prospecting the Inières and the Briane brooks, situated next to the invaded pond. Trapping was carried out as described above and completed using a search light to chase and detect crayfish, during two-successive nights, to observe the invasion range of this species, along the two brooks (Figure 1). Electrofishing was also carried out in the Inières brook: this method is widely used to assess freshwater assemblages (crustaceans and fish) and consists of a power source producing alternating current with 300 volts, generating an electrical field when electrodes are placed in the water (Bryant et al. 2012).

Then, a second survey was conducted, between April and September 2021, after obtaining the owner's permission, aiming at realizing a robust population monitoring. For that, eachsampling event consisted of setting out 9 baited traps in fixed positions all around the pond (Table 1, Figure 1), repeated 13 times during the trapping season (i.e. April to September, when the crayfish activity—reproduction and moulting—is higher because of higher water temperature). Visual observations by light were also performed alongside the two adjacent brooks (as in 2019), to investigate the invasion front of *F. rusticus*. All captured individuals were identified (to the species level, as also other crayfish species can be caught), sexed, and measured (total body length) to the nearest mm.

Genetic identification of F. rusticus

One walking leg was collected from two individuals and stored in 90% ethanol, for further genetic analysis to confirm the taxonomic status of crayfish by COI barcoding. DNA was extracted from leg muscle tissue, using the Qiagen DNeasy Blood and Tissue kit following manufacturer's instructions. A fragment of the mitochondrial gene for the cytochrome c oxidase subunit I (COI) was sequenced (Folmer et al. 1994). Both forward and reverse fragments were assembled and aligned using Geneious TM (https://www.geneious.com) and blasted in GenBank to confirm the identity of each specimen (https://blast.ncbi.nlm.nih.gov/).

Screening for Aphanomyces astaci *infection*

The presence of *A. astaci* pathogen was tested on 10 specimens of each species (*F. rusticus* and *P. leniusculus*), sampled in the brook and its tributary in 2019 and stored in absolute molecular-grade ethanol. Twenty additional *F. rusticus* specimens, sampled in August 2021 were also analyzed. Before the dissection, a visual checking of the presence of black melanized spots were noted. Tissue from one half of the soft abdominal cuticle, one uropod, and any melanized spots were collected in a 1.5 mL tube and stored at −20 °C. Then, a DNA extraction was performed with a modified protocol of DNeasy tissue kit (Qiagen) (Filipová et al. 2013). Next, the presence of the pathogen *A. astaci*, ITS1 gene was tested by quantitative TaqMan MGB real-time PCR (Vrålstad et al. 2009), using a LightCycler 480 Instrument (Roche). Each run was composed of one pure sample and one 1/10 diluted sample, four standards and two negative controls. The infection rate was quantified with four standards of 3*4^10, 3*4^8, 3*4^4, 3*4^2, expressed as PCR forming units (PFU) (Vrålstad et al. 2009). The relative levels of infection were calculated and samples with agent levels A2 and higher were considered infected with *A. astaci* (Vrålstad et al. 2009). We estimated the prevalence of *A. astaci* in the two studied species and its 95% confidence interval, using the function "epi.conf" from the epiR library for the statistical package R v 4.0.5 (Filipová et al. 2013).

Results

Species identification

Two crayfish species were caught during the survey: *F. rusticus* and *P. leniusculus*. Both species were identified by morphological traits. *Faxonius rusticus* is characterized by black bands on the tips of its claws and an oval gap between the claw fingers when closed, a smooth, pinched and distinctly concave rostrum and rusty patches on each side of the shell (Figure 2)

Figure 2. *Faxonius rusticus* trapped in the Inières pond. This species shows dark "rusty" spots on either side of the carapace and claws generally smooth, with a dark band on the proximate end of the cheliped. Photo by Maud Laffitte.

(Wetzel et al. 2004). *Pacifastacus leniusculus* is characterized by the absence of spine on the cephalothorax, with a white or turquoise colored patch at the base of each claw and a red coloration on the underside of claws (Larson et al. 2012).

The presence of *F. rusticus* was confirmed by COI barcoding. Only one haplotype was obtained from COI sequencing from the 2 specimens analyzed. They were identical over a 660 base pair stretch with previously identified *F. rusticus* sequences (available on GenBank Accession number: MN902184) originating from the Lower Susquehanna watershed of south central Pennsylvania (Di Domenico et al. 2021).

Biometry and population description of F. rusticus

During the first survey session (2019), 37 individuals were captured in the Inières pond, using traps. This led to extending the search to the adjacent brooks, Inières brook and Briane brook. Thus, 12 individuals were observed by visual search in the Inières brook and 25 were captured (1 by electrofishing, 17 by fishing nets and seven by baited traps), where as only 1 individual was observed in the Briane brook.

Following the two survey periods (2019 and 2021), only *F. rusticus* was recorded in the Inières pond, with 847 individuals trapped (Table 1). The total length of *F. rusticus* caught ranged from 41 to 109 mm (Figure 3), with a size range between 50 and 109 mm for females and 41 and 106 for males, respectively. The overall female ratio (female/total individuals) of all captured crayfish, between April and September in 2021, was 0.36 but some variation occurred during the trapping season. No females were caught during the first month (April) of the campaign. However, there was a progressive increase in the number of females caught from May until August,

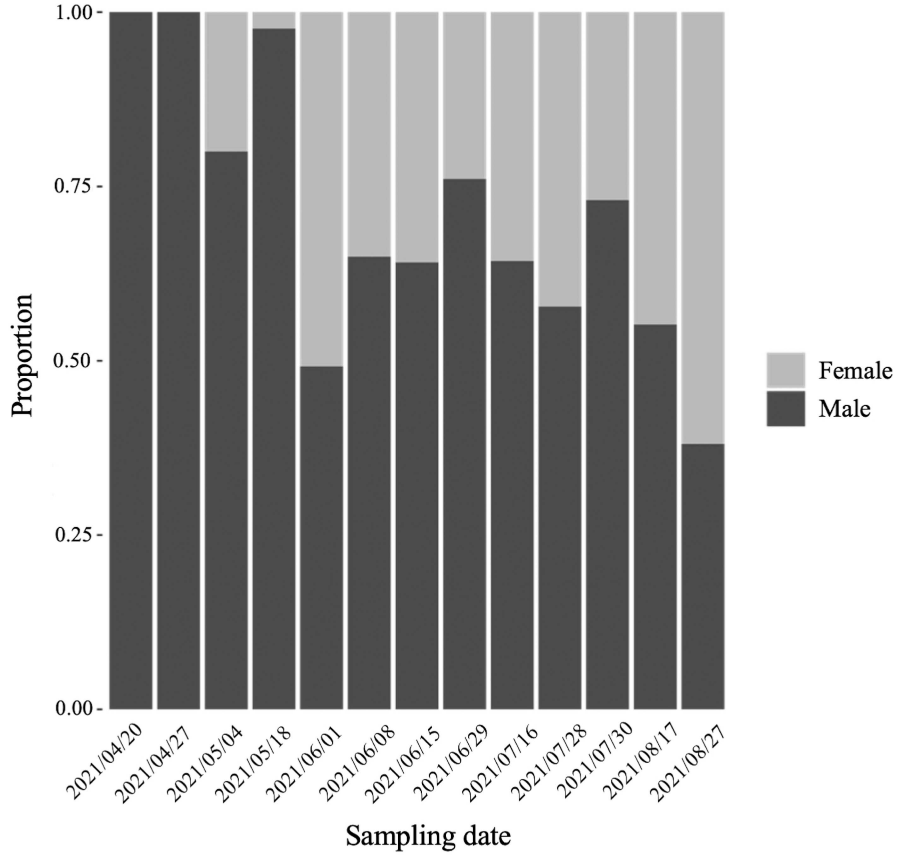

Figure 3. Number of crayfish trapped in the pond, by size class (total length in millimeters) and by sex (females in light grey and males in dark grey), following the two survey periods (2019 and 2021).

when the females became dominant (Figure 4). Furthermore, one female caught in May was egg-carrying.

The pond only hosts *F. rusticus*, whereas in both the Inières brook and the Briane (main stream), this species co-occurs with *P. leniusculus*. The colonization front of *F. rusticus* along the Inières was around 3 km downstream from the pond. One specimen has been observed in the Briane closed to the join with Inières. Additionnally, *P. leniusculus* was found to be the most abundant species in the Inières and the Briane (Table 1).

Prevalence of A. astaci *in the population*

All individuals from both species were infected with level of infection ranging from A2 to A5. In total, 10 *F. rusticus* (seven from 2019 survey period and three from 2021) were A2 infected, one (from 2019) at A3 level and two (from 2019) at A5 level. Among the 10 *P. leniusculus* analyzed from the pond in 2019, eight were infected by *A. astaci* with level of infection A2 and two at A5 level (Table 2).

Discussion

This study reports the first detection of the invasive crayfish *F. rusticus* in France, and so far in Europe. The population density appears to be very high

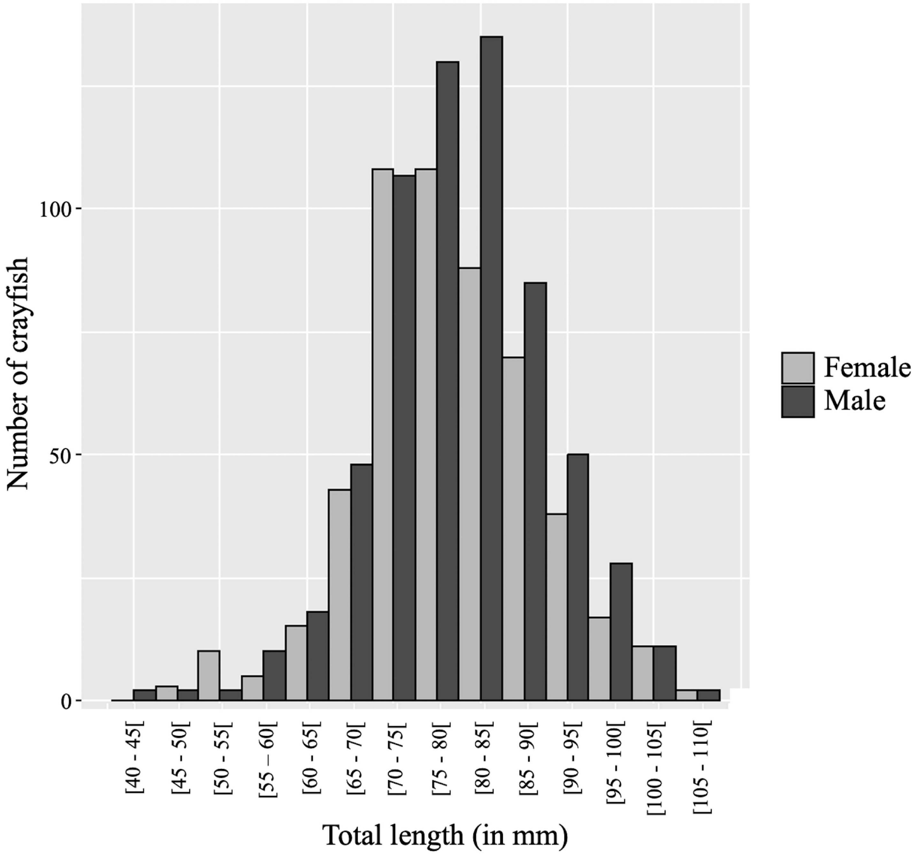

Figure 4. Sex-ratio of crayfish trapped (light grey bars for females and dark grey bars for males) for each sampling event carried out between April 2021 and August 2021.

Table 2. Results of *Aphanomyces astaci* detection in the *Faxonius rusticus* and *Pacifastacus leniusculus* from the pond and Inières brook.The prevalence represents the percentage of infected crayfish with 95% confidence intervals. Agent level corresponds to the infection level in Vralstad et al. (2009).

Species	Sampling year	Locality	Infected / Analyzed	Prevalence (95% CI)	Agent level
F. rusticus	2019	Inières brook	10/10	100% (69–100%)	A2 (7), A3 (1), A5 (2)
F. rusticus	2021	Pond	3/20	15% (3–38%)	A2 (3)
P. leniusculus	2019	Inières brook	10/10	100% (69–100%)	A2 (8), A5 (2)

in a private pond in the Aveyron French Department, with more than 800 individuals caught only in 2021. The situation is alarming because this species is known to be very invasive, with a high dispersal potential and some individuals were already observed in the adjacent brooks, three kilometers downstream of the pond. Evidence of reproduction was constated (all size classes represented and capture of an egg-carrier female) and survey session highlighted an increase of the capture probability of female, from May to August. This is certainly linked to the increase of crayfish activity during summer, as showed for signal crayfish (Dunn et al. 2017). Additionally, all the specimen captured were tested positive to the crayfish plague, with some individuals infected at a high level (A5), representing a dramatical risk for native crayfish.

As this is the first time that this species has been reported in Europe, no direct data on its invader potential in European freshwater networks exists, but some studies have described its high ability to outcompete crayfish congeners (Hill and Lodge 1999; Olden et al. 2006). For example, this crayfish was introduced in the Great Lakes region in US more than a century ago and now it is the most widespread crayfish among the thirteen species occurring in all five Great Lakes (Peters et al. 2014). This ability to replace native congeners has been also reported from Wisconsin lakes (U.S.A.), where this species is replacing the native *F. virilis* (Hagen, 1870), and even *F. propinquus* (Girard, 1852), an invader that arrived there earlier. The distribution of *F. rusticus* outside its native area has expanded rapidly due to introductions by human activities, including ornamental trade and aquaculture. *Faxonius rusticus* is reported in 20 states within the US (Taylor and Redmer 1996) and also in Canada (Peters et al. 2014). Its invasion success is attributed to its high dispersal rate (Wilson et al. 2004; Jansen et al. 2009), broad habitat niche (Freeman and Byers 2006), and its aggressive behaviour (Hill and Lodge 1999).

The presence of *F. rusticus*in France is therefore alarming for the preservation of *A. pallipes*, considered by IUCN as vulnerable, for three main reasons. Firstly, this species seems to share similar habitat preferences as *A. pallipes* in the head waters, with clear well oxygenated waters and available shelters (Gunderson 2008; Trouilhé et al. 2007). Secondly, this species seems to be present in dense populations and to show particularly aggressive behavior that could enhance its invasion capacity (Olden et al. 2006; Reid and Nocera 2015). As a result of its aggressiveness and its competitiveness, *F. rusticus* might eventually outcompete the signal crayfish *P. leniusculus*, historically present in the Inières brooks and currently co-occurring with *A. pallipes* in some regions (Souty-Grosset et al. 2006). Thirdly, *F. rusticus* could then become the dominant crayfish species in certain catchments in France, effectively dispersing a high-contagious crayfish plague strain. Indeed, all tested specimens in 2019 and 2021 (including *P. leniusculus* individuals) were infected (100% of prevalence) and someone at a high infection level (A5). These results are alarming because the crayfish plague was already known to be one of the most virulent pathogen known for crayfish (Becking et al. 2022; Jussila et al. 2021) but never in previous studies reported a combination of such high levels of infection in such large proportion of individuals been infected topics (Vrålstad et al. 2011; Kozubíková et al. 2011; James et al. 2017). For example, recent studies reported a low total infection prevalence in some U.S.A populations of around 11%, but some individuals could have high levels of infection (A5), according to the scale of Vrålstad et al. (2009) (Panteleit et al. 2019) or, inversely, a high prevalence of infected crayfish (87%), but at a medium level (A3) (Strand et al. 2013). Filipova et al. (2013) have reported on the signal crayfish, a pathogen prevalence ranged from 0% to 80% among the 43 populations with only 2 individuals from 513 harbored an infection rate of A4 level. More recently, in the same species, Grandjean et al. (2017)

reported only 5 individuals on 1131 with A5 infection level. Our result indicates that *F. rusticus* is a high-risk crayfish plague vector if nothing is done for its eradication.

For all of the reasons (e.g. aggressivity, reproduction ability, *A. astaci* infection rate), this species seems to be worse than other invasive species such as *P. leniusculus* and *F. immunis* present in headwater system in France (Collas et al. 2016). Actions, such as pond draining and intensive trapping effort, are done to eradicate this population before its spread to major French catchments.

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Author contributions

All authors contributed to the final proofreading of the manuscript. ML and FG wrote the first version of the manuscript, after performing the laboratory experiments (with CD). Fieldwork was done by MG, TA, NP, TD, MS and MC. The corrections of the manuscript were carried out by TB, ML and, and the conceptualization of the study as well as its supervision were ensured by FG and BM.

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