

## Research Article

## Distribution and population structure and dynamics of the red swamp crayfish *Procambarus clarkii* (Girard, 1852) in the eastern Po Valley and its Delta (northeastern Italy)

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### Abstract

*Procambarus clarkii* is among the “100 of the worst” invasive aliens in Europe. The geographic distribution and population dynamics of the species were studied for the first time in inland waters in the eastern Po Valley and its Delta, an area for which such information was absent. The geographic distribution of *P. clarkii* was assessed by sampling, in summer 2017, 12 geographically distant water bodies representative of an area of 3,000 km<sup>2</sup>. Population dynamics was studied from 2017 to 2018, in a canal running through the town of Ferrara. Growth and longevity were estimated using the von Bertalanffy growth function. *Procambarus clarkii* was found in 100% of the water bodies sampled, indicating that red swamp crayfish has now completely colonized the eastern Po Valley, where interconnected field irrigation systems have allowed crayfish to spread almost everywhere. From the analysis of the polymodal frequency distributions, 5–6 growth cohorts were observed, each cohort corresponding to 1 year of age. Sex-ratio was 1:1, and no appreciable between-sex differences were found in growth patterns of crayfish population, except for the asymptotic length for which females approach higher values. Differences in life history parameters with other Italian populations may be due to latitudinal and different ecological conditions at our study sites.

**Key words:** alien species, Louisiana crayfish, Po River Delta

### Introduction

The introduction and spread of non-indigenous species (NIS) are an issue of growing concern, being a major cause of biodiversity loss and impairment of inland and marine waters (Leppäkoski et al. 2002). Powered by globalization, in aquatic ecosystems the number of introduced NIS continues to increase (Galil et al. 2018), and, in addition to the loss of indigenous populations, they threaten ecosystem processes, economic interests, public health, as well as the sustainability of many ecosystem services (Simberloff et al. 2013). It has been recognized that aquaculture and related activities (e.g. sport fishing, fishery stock enhancement, ornamental trade) have been important drivers of NIS in Europe: as many

as 600 NIS (together with their parasites and associated biota) have been introduced into inland and marine waters of Europe as a result of stocking activities and aquaculture (Olenin et al. 2008). For example, the freshwater decapods *Procambarus clarkii* (Girard, 1852) and *Pacifastacus leniusculus* (Dana, 1852) were initially introduced to restore fisheries in southern and northern Europe, respectively, in order to find an alternative to declining native crayfish populations. *Astacus leptodactylus* (Eschscholtz, 1823) was introduced into western Europe from easterly countries for the same reasons (Savini et al. 2010).

The red swamp crayfish *Procambarus clarkii* (Decapoda, Astacidea, Cambaridae), is one of the freshwater decapods inducing the most negative impacts worldwide. Native from north eastern Mexico and south-central USA (Hobbs et al. 1989), *P. clarkii* was introduced for commercial and nutritional purposes in Europe in the seventies of last century (Gherardi and Acquistapace 2007). It is the most diffuse and commercial decapod species in the world (Hobbs et al. 1989), also because it is particularly invasive, exhibiting r-selected features with rapid growth rates (Scalici and Gherardi 2007). Moreover, the species is tolerant to the environmental conditions due to highly plastic biological cycle (Gutiérrez-Yurrita and Montes 1999). Its invasiveness may be increased by its overland spreading potential. In fact, thanks to its desiccation resistance and aerial respiration, *P. clarkii* is able to cover long distances out of water, up to 17 km in 4 days (Gherardi and Barbaresi 2000; Gherardi et al. 2000), with a walking speed on dry land of 90 m h<sup>-1</sup> (Banha and Anastácio 2014). Where present, red swamp crayfish becomes an important component of the aquatic fauna, frequently being the largest invertebrate predator in their habitats. It is considered an ecosystem engineer (Statzner et al. 2003). It can also act as a vector of parasites (Vogelbeine and Thune 1988) and disease, as it may carry fungi like *Aphanomyces astaci* Schikora, 1922, responsible for the crayfish plague and lethal to native European crayfish populations (Souty-Grosset et al. 2016), and *Batrachochytrium dendrobatidis* Longcore, Pessier and Nichols, 1999, the pathogenic agent of amphibian chytridiomycosis (Skerratt et al. 2007).

In recent decades, *P. clarkii* have invaded natural habitats and established stable populations in many ponds, lakes and streams across Europe, and particularly Spain and central Italy (Anastácio and Marques 1995; Gherardi et al. 1999; Coignet et al. 2012; Souty-Grosset et al. 2016). *Procambarus clarkii* is included among the “100 of the worst” invasive aliens in Europe (DAISIE 2017) and in the list of invasive alien species of Union concern (EU 2017/1263) for which effective management measures are required. The distribution of *P. clarkii* in Italy is mostly concentrated to the central parts of the peninsula (Gherardi et al. 2000; Chiesa et al. 2006; Aquiloni et al. 2010; Scalici et al. 2010; Dörr and Scalici 2013), but there are

also recent records in northern (Piscia et al. 2011; Peruzza et al. 2015; Donato et al. 2018) and southern (Cilenti et al. 2017; Deidun et al. 2018) regions. In the inland water system of the Po River Delta (Northeastern Italy) the occurrence and distribution of the red swamp crayfish *P. clarkii* is poorly known (Rossi et al. 2006).

Inland aquatic habitats of the Po River Delta have been profoundly altered in the past decades by pollution, drainage and irrigation, and the (intentional/unintentional) introduction of NIS has harmed the already embattled native biota. In those habitats, *P. clarkii* results in both structural and biological impact. On the structural side, the activity of burrowing may result in damaging agricultural areas (e.g., rice plantations) and canals, as they may destabilize the banks. On the biological side, *P. clarkii* exhibits competitive superiority over indigenous species, possibly leading to local extinction (Gherardi and Acquistapace 2007). Once established, the eradication of crayfish populations becomes extremely difficult or even improbable. Consequently, biological and ecological data, including population structure and dynamics, become useful in developing procedures for an effective maintenance management. The present study is the first attempt to describe the population dynamics of *P. clarkii* in the Po River Delta, from where no previous information on the red swamp crayfish was available. We also aimed at identifying the current distribution of the species in the inland water system of the Po Delta, the widest deltaic area in Italy and one of the largest in the Mediterranean basin.

## Materials and methods

### *Geographic distribution*

The geographic distribution analysis of red swamp crayfish *P. clarkii* in the eastern Po Valley and its Delta was carried out from June to July 2017. A total of 12 canals in the Provinces of Padua (n = 1), Rovigo (n = 3), Ferrara (n = 6) and Ravenna (n = 2), representative of an area of 3,000 km<sup>2</sup>, were sampled for crayfish (Supplementary material Table S1). At the sampling time, water depth varied between 60 and 120 cm, and banks were vegetated. In each canal, four cylindrical fish-traps (60 cm long, 30 cm diameter, 4 mm mesh and 2 entrances) baited with Caperlan Carp-fishing Boilies (strawberry flavor), were set at four different sampling sites, 500 m apart from each other. Sampled water bodies were all irrigation and drainage canals of comparable morphology (width: 4–6 m; depth: 1–2 m; vegetated banks), and characterized by the same degree of land-use intensity (agriculture, mainly corn). Each canal was sampled once, for a 24 hours period. Captured crayfish were brought to the laboratory and measured for carapace length (CL), and sex.

### *Population structure and dynamics*

Specimens of *P. clarkii* were collected in May (M17), July (J17), September (S17), November 2017 (N17), January (J18) and March 2018 (M18) at four different sampling sites (500 m apart from each other) in the canal Gramicia, running along the NE side of the town of Ferrara. At each sampling site, crayfish were caught by means of four cylindrical traps (60 cm long, 30 cm diameter, 4 mm mesh and 2 entrances), baited with Caperlan Carp-fishing Boilies (strawberry flavor). All traps were recovered after 24 hours. Once in the laboratory, crayfish were sacrificed by hypothermia, and for each specimen we recorded sex, and carapace length (CL) (from the tip of the rostrum to the posterior margin of the cephalothorax), measured with a caliper within 0.25 mm accuracy. The reproductive stage of captured males was determined according to Taketomi et al. (1996). At each sampling date, water temperature was measured with a YSI digital thermometer.

CL was used to generate polymodal frequency distribution histograms that were analyzed with the FAO-ICLARM Stock Assessment Tools (FiSAT-II) computer program (Gayanilo et al. 1996). Red swamp crayfish, divided per sex, were grouped by sampling month ( $n = 7$ ) and frequency distributions were obtained, according to Fidalgo et al. (2001), from 2-mm CL interval size classes. Length-frequency distributions were decomposed into their Gaussian components by means of Bhattacharya's (1967) method, and growth parameters were evaluated through the Von Bertalanffy equation:

$$L_{(t)} = L_{\infty} [1 - \exp^{-k(t - t_0)}]$$

where  $L_{(t)}$  is the length at the age  $t$ ,  $L_{\infty}$  is the asymptotic length (equal to  $L_{\max}/0.95$ , where  $L_{\max}$  is the maximum recorded length),  $k$  is the curvature parameter,  $t_0$  is the initial condition parameter (mathematically when specimens have  $CL = 0$ ). Von Bertalanffy dynamic parameters were assessed using Electronic Length Frequency Analysis (ELEFAN), a routine of FiSAT-II. The expected longevity ( $t_{\max}$ ) was then assessed through:

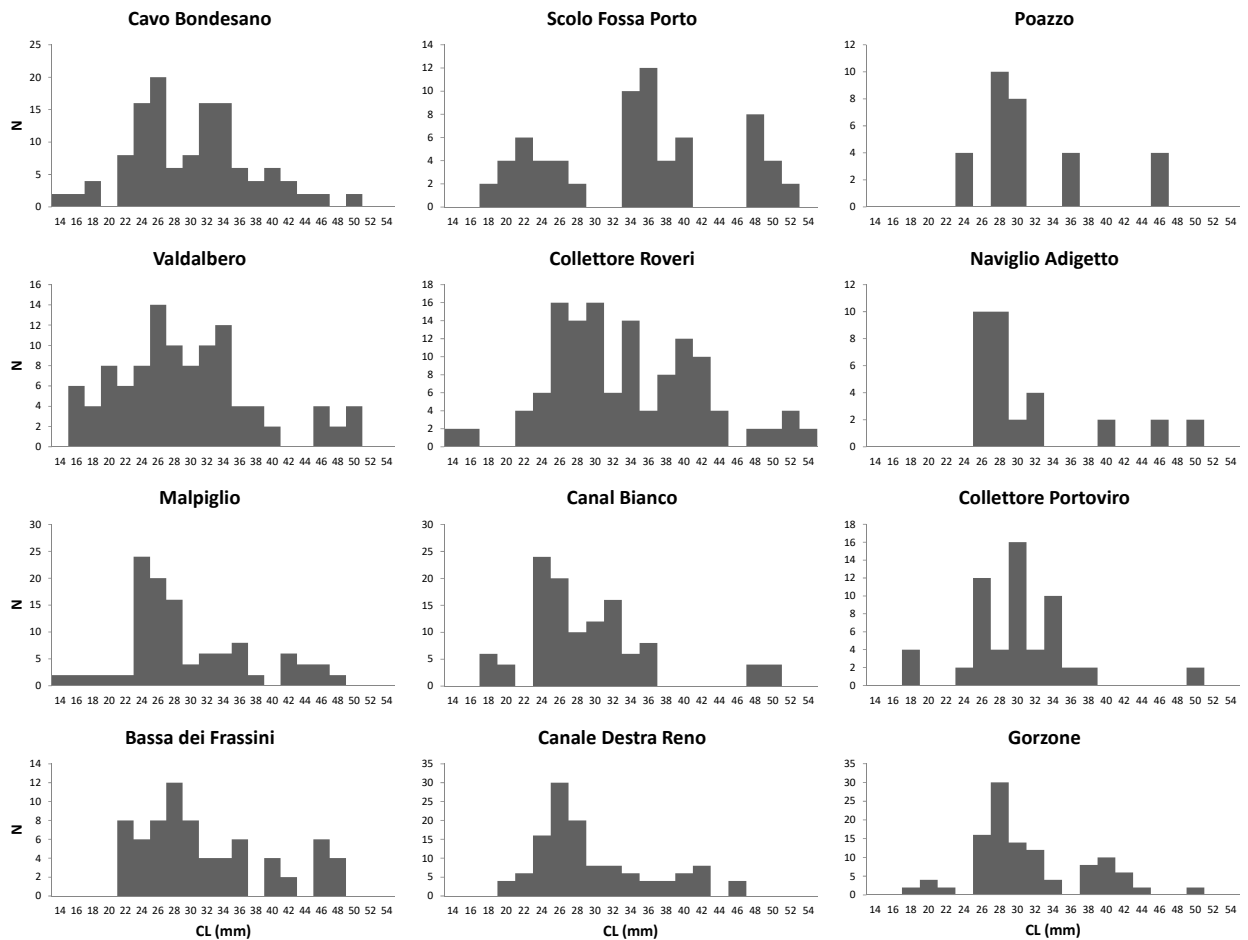
$$t_{\max} = (3/k) + t_0$$

Finally, the total mortality ( $Z$ ) was assessed through the Powell-Wetherall Plot equation (Wetherall et al. 1987) that allows estimation of  $L_{\infty}$  and  $Z/k$  using the length-frequency data imported in the FiSAT routine.  $Z$  is the total mortality, i.e. the sum of natural mortality and the mortality due to fishing. In this study,  $Z$  is equal to the natural mortality as *P. clarkii* in the study area is not subject to fishing.

## **Results**

### *Geographic distribution*

*Procambarus clarkii* specimens were captured in all the sampled canals. Catches varied between 30 and 128 specimens, with number of females

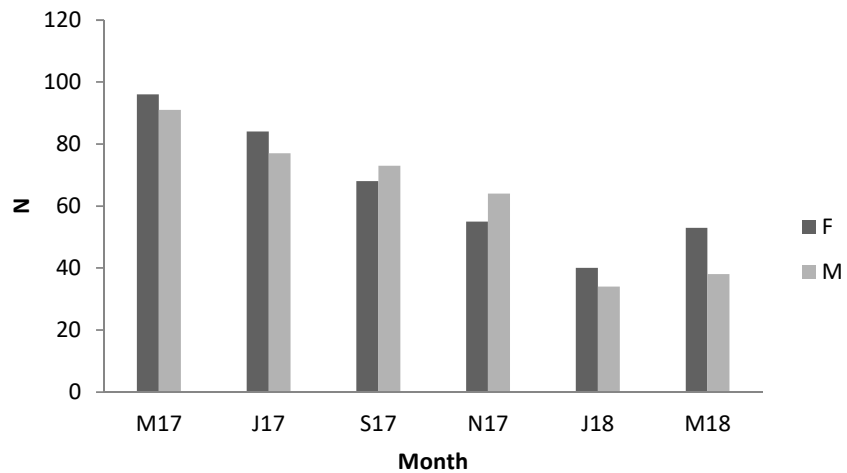


**Figure 1.** Size distribution of crayfish populations sampled in Po Delta inland waters.

about equaling that of males everywhere ( $\chi^2$  test, all  $p > 0.05$ ). In Figure 1, length frequency distributions of crayfish (females + males) at the 12 sampled canals are shown. The most represented size classes were those between 24 and 34 mm CL.

#### *Population structure and dynamics*

A total of 773 crayfish were collected during the sampling sessions in Gramicia canal: 377 males and 396 females (Figure 2). Number of specimens caught was highest at May ( $n = 187$ ) and July ( $n = 161$ ) sampling dates, and lowest at January ( $n = 74$ ). *Procambarus clarkii* specimens were captured at each sampling month, indicating that water temperatures during the sampling period (May 2017–March 2018) were never too low to inhibit the activity of crayfish. In the study period, water temperature varied between 10 and 27 °C (Figure 3). Sex ratio for the whole population was 1.05:1 in favor of the females. The latter were significantly more abundant in March 2018, which probably is the period following the hatching of the juveniles. The carapace length varied between 14.2 and 58.2 mm in males (average:  $33.9 \pm 2.5$  mm), and 12.2 and 60.4 mm



**Figure 2.** Number of individuals (N) caught in the six sampling sessions (sum of four sites) divided per sex.



**Figure 3.** Water temperature at Gramiccia canal (see text for x-axis acronyms).

in females (average:  $35.7 \pm 2.9$  mm). A between-sex comparison of log-transformed carapace length data per sampling date did not show any significant difference (ANOVA:  $p > 0.05$ ). Male crayfish exhibiting the copulatory organ (CL ranging from 26.60 mm to 56.90 mm) were found with higher numbers at spring-summer sampling dates (July and September: almost 80% of males), and decreased during the other months.

All CL measurements were used to generate size frequency distributions for the six sampling dates. The Bhattacharya's method on size frequencies, allowed us to classify the crayfish collected into several age classes (from 0+ to 4+) for both sexes in the study population. Some classes (e.g., 0+), however, were often composed of a few or zero individuals. From the analysis of the polymodal frequency distributions, 5–6 cohorts were observed in both sexes. The von Bertalanffy parameters ( $k$  and  $L_{\infty}$ ) are given in Table 1, together with others calculated on introduced *P. clarkii* populations from Italian freshwater habitats.

**Table 1.** Growth parameters of Italian *Procambarus clarkii* populations divided per sex. All study sites, except Po River Delta, are located in Central Italy.

Site	Sex	$L_{\infty}$	k	Z	Reference
Po River Delta	F	63.0	0.60	2.48	This study
	M	58.8	0.54	2.10	
Palude Torre Flavia	F	74.6	0.32	3.11	Scalici et al. 2010
	M	68.3	0.33	2.88	
Lake Trasimeno	F	73.7	0.58	5.10	Dörr and Scalici 2013
	M	69.4	0.59	5.50	
Lake Trasimeno	F	81.3	0.89	5.20	Dörr et al. 2006
	M	79.4	0.79	4.97	
Circeo National Park	F	64.3	0.70	4.07	Chiesa et al. 2006
	M	63.3	0.66	3.83	
Padule di Fucecchio	F	65.5	0.68	4.94	Scalici and Gherardi 2007
	M	62.7	0.69	3.19	

Longevity ( $t_{\max} = 5.54$  and  $4.98$  yrs for males and females, respectively) reflected quite adherent the number of modal classes for each sex. The total mortality (Z) was low compared with other studies (Table 1).

## Discussion

In Italy, *P. clarkii* spread in the wild in the 1990s, probably escaping from aquaculture facilities in Piedmont and Tuscany (Gherardi et al. 1999). Actually, the species is well established mostly in Central Italy (Gherardi et al. 2000; Chiesa et al. 2006; Scalici et al. 2010; Dörr and Scalici 2013), in Piedmont (Piscia et al. 2011; Donato et al. 2018), Lombardy (Fea et al. 2006), Friuli Venezia Giulia (Peruzza et al. 2015), and more recently in Apulia (Cilenti et al. 2017), and Sicily (Deidun et al. 2018). The present study aimed to investigate the distribution and population dynamics of *P. clarkii* in the eastern Po Valley and its Delta inland waters, from where only poor previous information was available (Rossi et al. 2006).

*Procambarus clarkii* was found in 100% of the 12 water bodies sampled, which were representative of an area of  $3,000 \text{ km}^2$ , indicating that red swamp crayfish has now completely colonized the eastern Po Valley. Indeed, *P. clarkii* colonization was predictable, since life history traits (early maturity, rapid growth, large number of offsprings, plastic life cycle) and biological features (tolerance to extreme environments, dispersal, polyphagy, predatory and competitive ability, and behavioral flexibility) predispose this species to spread and to become invasive (Gherardi 2006). It is trivial to foresee that diffusion of such an invasive, large, opportunistic, omnivorous predator will further stress those already stressed aquatic ecosystems. It is well-known that the multilevel impact exerted by *P. clarkii* on the recipient communities and ecosystems includes the competitive superiority over indigenous species, often leading to local extinction (Gherardi and Acquistapace 2007; Gherardi et al. 2008).

Our data were obtained by baited traps, and it is known that this can lead to bias in size and sex (Dorn et al. 2005) and are, therefore, a potential



source of error. Our traps, however, were very similar to those that Paillisson et al. (2011) found to be the most efficient in terms of *P. clarkii* catch probability, i.e. semicylindrical traps made from 5.5 mm mesh wire (ours had a 4 mm mesh). Moreover, since the calculated growth parameters were consistent with published data from other Italian crayfish populations, we are confident that our growth estimates were not macroscopically flawed by sampling bias. The trappable population was composed of crayfish aged at least 1+, which becomes apparent from the growth model we found, where smaller size classes (the 0+ cohort, CL < 10 mm) are largely missing from our length frequency diagrams. The crayfish population structure of the Gramicia canal (but also that of the other canals sampled) was mainly composed of adult *P. clarkii*, and not of juveniles. As a consequence, the length frequency diagrams only partially described the real population structure. The scarcity, if not the absence, of size classes in the first growth lines, where each line represents one year of life, can be a consequence of our method of capture, i.e. the smallest crayfish might not be attracted to the bait, since they have a different diet, or they might be less mobile and abandon their burrows less often, or because they could pass through the mesh of the trap (see Coignet et al. 2012).

The expected mean longevity ( $t_{\max}$ ) of the Gramicia population resulted nearly five years, and was in agreement with other studies (e.g. Frutiger et al. 1999; Huner 2002; Scalici and Gherardi 2007), which posed *P. clarkii* lifespan between four and five years. No appreciable between-sex differences were found in growth patterns of our crayfish population, except for the asymptotic length ( $L_{\infty}$ ), for which females approach higher values. Comparing the Gramicia population with other Italian crayfish populations from Central Italy, the  $L_{\infty}$  computed in this study was among the lowest. This might be a latitudinal effect. Mortality rate ( $Z$ ) was low, and this finding could be indicative of low predation on our crayfish population. In Europe, *P. clarkii* has become a new food source used by fish (eels, catfish, perch), carnivore mammals, as the red fox, and ciconiiform birds, as little egret, grey heron, purple heron and white stork (Correia 2001). Because of the intense human disturbance (the Gramicia canal flows between a ring road, the University sports campus, and residential areas), at the study site the only predators that can be observed during the year are (very rare) grey herons, which most likely exert a low predation pressure. Population density showed peaks in the June to September period, when high temperatures and the availability of food may have favored the activity of crayfish. We caught, however, *P. clarkii* also during the cold months, in contrast to observations made, for example, in lentic habitats of Southern Germany (Chucholl 2011), or central Italy (Dörr et al. 2006; Dörr and Scalici 2013), where no captures were made during winter because crayfish were inhibited by low



temperatures. The lower catches we made in winter, however, may give support to the hypothesis of a reduced activity of *P. clarkii* following lower water temperature.

We found that sex ratio did not significantly differ from 1:1, as also observed by other authors (i.e. Scalici and Gherardi 2007; Dörr and Scalici 2013; Peruzza et al. 2015). A slight dominance of males in spring and early summer could be due to the behavior of females, which are reported to spend the incubation period inside their burrows (Gherardi et al. 2000). Peruzza et al. (2015), analyzing a population living in Friuli Venezia Giulia region (North-eastern Italy), observed that from June to November males were always more abundant than females, whilst the situation was reversed from December to May, splitting the year in two periods where one of the sexes is more active than the other. However, *P. clarkii* sex ratio seems to be very variable geographically, as an increase in the number of females within populations has been observed by Anastácio and Marques (1995) with increasing latitude.

In Southern Mediterranean water bodies, crayfish recruitment exhibited two peaks during the year, in autumn and spring (Alcorlo et al. 2008). Although some juveniles (with CL < 15 mm) were found in our May and November sampling sessions, at our study site the two-yearly peaks described were not so evident. As already mentioned, this fact may depend on the selectivity of our traps, size of the mesh, or by a different behavior of juveniles. However, this observation could also depend on the need of a longer period to attain maturity, thus limiting the number of reproductive events in the northern Mediterranean area. The two-yearly peaks may correspond to two mating phases, as observed by Scalici and Gherardi (2007) in Lake Trasimeno. Huner (2002) reported that water temperature plays an important role in reproduction since it is positively related to sexual maturation. Crayfish populations of the Camargue, which is at the same latitude of the Po Delta, do not exhibit the two-yearly recruitment peaks described in more southern water bodies (Meineri et al. 2014).

Due to its ubiquity in the area, we foresee that it will be impossible to eradicate *P. clarkii* from Po Delta inland waters, where interconnected field irrigation systems have allowed crayfish to spread almost everywhere. This study showed that the red swamp crayfish is abundant in the irrigation canals of our explored area, where salinity does not exceed 2–3 PSU ([www.arpae.it](http://www.arpae.it)), but it has not been found any further, in the terminal stretches of watercourses or in the lagoons. Of great concern is the fact that *P. clarkii* has already been found in brackish wetlands, as the Lagoon of Rossos Aveiro, in Portugal (Fidalgo et al. 2001), and the Palude di Torre Flavia, Central Italy (Scalici et al. 2010). It is therefore probable that the crayfish will be able to invade the estuarine and lagoonal areas of the Po Delta. The lagoons of the Po Delta are fragile ecosystems with high

biodiversity, where shellfish and finfish farming represent the primary revenue in the local economy. Therefore, the spread of the red swamp crayfish will threaten not only the native biodiversity and increase biotic homogenization in a short time, but also threaten local human livelihoods.

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#### Supplementary material

The following supplementary material is available for this article:

**Table S1.** Records of *Procambarus clarkii* in the eastern Po Valley and its Delta.

This material is available as part of online article from:

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