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Original Article

Population structure and dynamics of the invasive *Procambarus clarkii* (Girard, 1852) in a Tiber river Ramsar site, Central Italy

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Abstract: *Procambarus clarkii* is a native species of Central America, but strongly invasive in many regions of the world. An investigation on the red swamp crayfish was carried out to obtain more information about its population dynamics in the Tiber River, in Central Italy. A total of 900 individuals, both males and females, were sampled within two different campaigns (2017 and 2019) aimed at collecting biometric data. A strong fishing effort was deployed (more than 100 nets set), to guarantee a large and randomized number of samples. The crayfish populations were grouped into seven different cohorts, according to Bhattacharya's method. The population showed a balanced sex ratio, the average cephalothorax length was 42.52 mm, with the most represented size class between 40-50 mm. K and L_∞, as well as the growth parameter index (Ø), the mortality rate (Z), and longevity value (t_{max}), were calculated. K and Ø values resulted very high, showing an impressive growth rate in the study area; t_{max} ranged from 4 to 5 years, L_∞ values were lower compared with other studies (58.0-59.0 mm), while Z was very high for this population (4.2-4.5 year). The results revealed that crayfish population dynamics can be complex and vary depending on habitat type, available trophic resource and competition.

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Introduction

The red swamp crayfish, *Procambarus clarkii* Girard, 1852, is a native species of northeastern Mexico and southcentral USA. It is an r-selected species, which shows several invasive characteristics, such as rapid life cycle, strong dispersal capacity and high population densities. Therefore, it is able to colonize and disrupt a great variety of habitats, constituting a serious threat to the natural environment worldwide (Anastácio and Marques, 1997; Rodríguez et al., 2003, Souty-Grosset et al., 2016). *Procambarus clarkii* is considered among the 100 worst invasive species (Daisie, 2011) and it is listed in the "Union list" or "Black list" of invasive alien species (IAS), an important management tool included in the EU regulation 1143/2014 (EU, 2014).

The main introduction agent of freshwater crayfish was farming activity and the hobby stand bait industry (Hänfling et al., 2011). *Procambarus clarkii* has been reported in several continents all over the world, and it represents so far as the second freshwater crayfish species more commercially farmed and more captured globally (Holdich, 1993; FAO 2020). In Europe, crayfish was legally imported from Louisiana for commercial purposes to Seville and Badajoz in 1973 (Habsburgo-Lorena, 1979). Later, the economic success of crayfish sale led to its illegal introductions in several European countries, even after the EU regulation 1143/2014, which banned its farming and sale (Chucholl, 2013). Due to the high dispersal capacity of this species, individuals started a rapid colonization throughout the Mediterranean basin and Central Europe (Anastácio and Marques, 1997; Barbaresi and Gherardi, 2000). Nowadays, *P. clarkii* is widespread and abundant all over Europe, including central and northern European countries

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(Louriero et al., 2015). This species represents a strong competitor and affects native freshwater species via direct predation on eggs, larvae, and juveniles and by competing with them for both resources and habitats (Gherardi, 2006). Moreover, digging activities of crayfish populations were demonstrated to affect river banks stability and to increase water turbidity (Rodriguez, 2005).

Several studies highlighted the significant negative impacts of red swamp crayfish on natural ecosystems, which can be quantified in terms of ecosystem services (ES) loss. In particular, provisioning, regulatory and supporting services could be lost in freshwater habitats as a direct consequence of red swamp crayfish presence (Lodge et al., 2012). For instance, in Italy, where red swamp crayfish spread out in the 1990s, likely as a consequence of accidental release by some aquaculture farms in Piedmont (Del Mastro et al., 1992), *P. clarkii* threatened the native fauna (Barbaresi and Gherardi et al., 2001; Renai and Gherardi, 2004), altering the ecological community structure and reducing the food web complexity (Casellato and Masiero, 2011). These invasive ecological skills make *P. clarkii* an impressive competitor and a habitat destroyer, threatening local biodiversity through a direct impact and a decrease in the environmental quality parameters (Gherardi, 2006; Souty-Grosset, 2016). Crayfish activities affected both habitats used by fish for shelter or spawning and the whole aquatic ecosystem (Lodge et al., 2012; Souty-Grosset, 2016). Plant communities are altered both by direct consumption of macrophytes and by burrowing activities, responsible for shifts from clear water macrophyte-dominated areas to phytoplankton dominated areas (Rodríguez et al., 2003; Geiger et al., 2005; Matsuzaki et al., 2009). *Procambarus clarkii* activities are also believed to induce cyanobacteria blooms (Yamamoto, 2010). Threats and damages caused by red swamp crayfish are not only limited to freshwater communities but also extend to the coastal area, i.e. this species can invade the estuarine and brackish environments of the Adriatic coast as already reported for some Tyrrhenian areas (Scalici et al., 2010). In addition, groundwater native communities may be impaired by the presence of red swamp crayfish in the caves (Mazza et al., 2014).

Here we provide a first study on the population structure of *P. clarkii* in the Regional nature reserve “Nazzano Tevere-Farfa”, an important wetland area of the Tiber river basin as one of Italian protected sites under the International Ramsar Convention (Carp, 1972). The site hosts fragile environments inhabited by species included in both European directives and the IUCN red list (Rondinini et al., 2013). This area needs, therefore, to be highly preserved and it is necessary to identify and characterize possible threats. Hence, the aims of the present study were to: (i) investigate the presence of crayfish in the study area, (ii) gather biological data about the local crayfish population and (iii) preliminary assess the red swamp crayfish population structure and dynamics in an important conservation site. This study will provide a major baseline for the future management and control of this invasive species.

Materials and Methods

This study was carried out along a stretch of the middle course of the Tiber River, within the Nature Regional Reserve of “Nazzano Tevere-Farfa” (Nazzano, Rome). The Reserve is 700 ha wide locating near the confluence of the Farfa Stream, 40 km northern Rome (42°12'N, 12°37'E), at an altitude about 30 m a.s.l. Since 1979, the site is protected according to the Ramsar Convention, the Birds Directive (European commission, 1979) and the Habitat Directive (EU, 1992). The site is also included in a Special Protection Area (SPA) and extends upstream of hydroelectric power station of “Nazzano”, including a section of the Tiber River up to the Poggio Mirteto mountain and a stretch of Farfa River up to Granica bridge. The area is composed by lotic *faces*, associated with the natural flow of the stream, and lentic *faces*, due to a dam construction. The core zone (Fig. 1) is located near the reserve center, within an area of about 15 ha with a maximum wet riverbed length of 420 m. Along the river shores, there are several small coves and islets characterized by reduced hydrodynamism and low

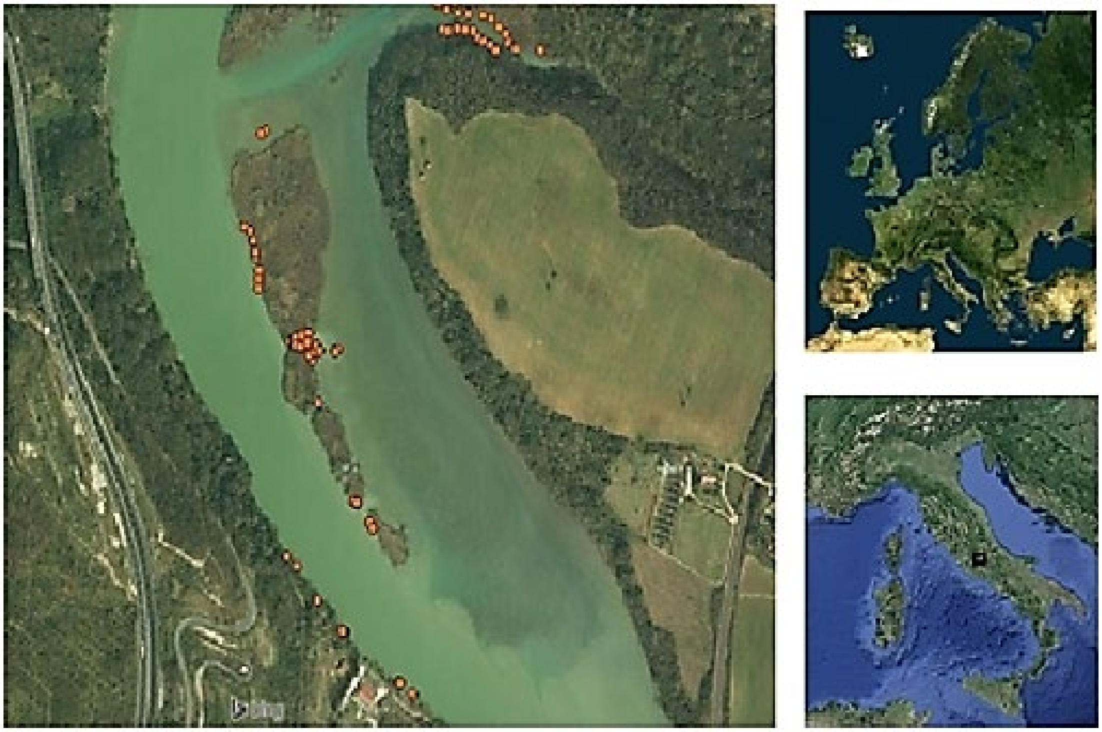

Figure 1. Map of the study area located within Natural Regional Reserve "Nazzano Tevere-Farfa". Each point indicates the exact positions of crayfish traps used during both 2017 and 2019 sampling campaigns.

water depth (<1 m).

Two sampling campaigns (22 samplings) were carried out in 2017 and 2019 (April-October). Sampling frequency was once a week and fixed traps (collapsible cylindrical traps, consisting of nylon threads of 1 cm mesh size with a length of 1 m and a 0.3 m mouth width) were used (Fig. 1). Each trap was baited with pork liver or with "caperlan" boiles. Ten series of fifteen linked-traps were prepared, randomly set in the area, and regularly moved to test the overall catchability in different zones inside the study area. Traps were placed in small coves, characterized by shallow, lentic and turbid water, and in running water, slightly deeper and cleaner, mostly near vegetated banks. Lentic water had a maximum depth of about 50 cm, whereas lotic water ranged from 40 to 100 cm. Water temperature was measured weekly.

A subsample from total catches (about 50-60 individuals) was randomly selected to record biometric data. Cephalothorax length (CL; from the rostrum to the middle margin, to nearest 1 mm), weight (W; to nearest 1 g) and sex (S) were recorded on each specimen. CL was measured using a caliper, and the weight using a precision balance. Gender and female's reproductive status (i.e. without eggs, eggs-carrying, and larvae-carrying) were recorded.

Statistical analysis was performed in the R-Project environment (2.2.0 version), using "TropFishR" (Mildenberger et al., 2017) and "Flife" packages (Kell et al., 2016). Individuals were pooled in groups according to the month of capture, to reach a minimum number of observations (n>150; France et al., 1991) and to apply the Batthacharya method (Bhattacharya, 1967). Fishing selectivity on sex, for each year separately and for the overall fishing period, was determined by chi-squared test. Difference in sex ratio for each sampling group was tested using t-test. Von Bertalanffy equation (Von Bertalanffy, 1938) was used to assess crayfish growth, growth parameter index (Ø) and growth coefficient (k). CL data were used to generate 10 mm frequency distributions, which were analyzed through the Bhattacharya's method, resulting in a yearly plot. The total mortality (Z) was estimated with the Powell-Wetherall Plot equation (Powell et al., 1979; Wetherall et al., 1986) by determination of L_∞ (asymptotic CL) and Z/k,

Table 1. Growth parameters (k, Ø, L∞) and mortality rate (Z) values in different locations.

Site	Sex	Type	Habitat	Origin	Ø	K	L∞	Z
Nazzano Tevere-Farfa Reserve (this study)	M	Lotic	River Creeks	Natural	3.34	0.76	59.00	4.24
	F		Main stream		3.42	0.65	58.00	4.54
River Nile	M	Lotic	River delta	Natural	3.20	-	-	3.65
	F				2.70	-	-	5.60
Delta del Pò	M	Lotic	Pond	Natural	-	0.54	58.80	2.10
	F				-	0.60	63.00	2.48
Qianjiang	M	Lentic	Swamp	Artificial	8.01	0.81	60.93	2.32
	F				7.97	0.86	58.12	1.93
Torre Flavia swamp	M	Lentic	Oligohalin	Natural	-	0.32	68.30	2.99
	F				-	0.33	74.60	4.71
Preola lake Reserve	M	Lentic	lakes	Natural	3.19	0.34	68.25	3.43
	F				3.19	0.35	67.20	3.83
Trasimeno lake	M	Lentic	Shallow Lake	Natural	-	0.59	69.35	5.50
	F				-	0.58	73.71	5.10
Circeo National Park	M	Lentic	Coastal lake	Natural	-	0.66	64.30	3.43
	F					0.70	63.30	4.07

using the length-frequency plot.

The parameter k was also used to estimation of Z and calculated through the Electronic Length Frequency Analysis (Pauly and David, 1981; Taylor, Mildenberger, 2017) using an optimized approach based on simulated annealing (ELEFAN_SA) instead of the classical method based on the k-value scan method. Von Bertalanffy growth curve is described by the equation of $L_t=L_\infty(1-e^{-k(t-t0)})$ (Von Bertalanffy, 1938); where k is the curvature of the function and it is needed to estimate the relative animal growth; t_0 is the time 0, theoretical time at which individuals hatch while t is the time at this moment and L_∞ is the asymptotic CL. In order to have an overview of the crayfish growth, also the growth parameter index (Ø) was estimated through the formula of $Ø=\ln K+2\ln L_\infty$ (Pauly and Munro, 1984). Finally, the value of age at time 0 (when crayfish have CL=0 mm) was assessed by the formula of $\ln(_{-t0})=-0.3922-0.2752\ln L_\infty-1.308\ln K$ (Jin et al., 2019). While the expected longevity (t_{max}), derived from k value and t_0, has been calculated as $t_{max}=k(3+t_0)^{-1}$ (Huang et al., 2012).

Results

During the study period (April-October), water temperature was 21-25°C and water depth of the sampling sites varied 40-180 cm. We analyzed a total of 900 crayfish, 441 males and 452 females, over a 2-year period. In 2017, we collected a total of 421 specimens (158 males and 263 females), and in 2019 472 (283 males and 189 females). Chi-squared test showed for the whole sample a 1:1 sex ratio ($\chi 2$ = 0.05, df=1, P>0.05), while in 2017 the ratio was in favor of females ($\chi 2$=26.188, df=1, P<0.05) compared to 2019, when we observed an opposite trend ($\chi 2$= 20.878, df=1, P<0.05).

The average CL was 42.52 mm for the entire sample (42.52±0.96 mm), whereas in 2017 the mean value was 41.36 mm (41.36±0.93 mm; Fig. 2a) and in 2019, 43.69 mm (43.69±1.00; Fig. 2b). The most represented size class was 40-50 mm CL (n=255) for 2019 (n=154), while for 2017, it was 30-40 mm (n=105). Comparison between sexes of log-transformed CL data per sampling date did not show any significant difference (P>0.05).

According to Bhattacharya's procedure, both sex and years were separately analyzed (Fig. 3) to classify the collected crayfish into several age classes: for all year and sex, we found seven cohorts, except for September 2019 (6 cohorts) and also for females on 2017 (6 cohorts). Comparing the two sampling years, most represented classes showed different patterns: most crayfish of both sexes belonged to 30-40 mm class in July 2017, while in September males and females fell into 40-50 mm class. In April, males' abundance was found in the sixth cohort (50-60 mm) while most of females fit in 40-50 mm length class. Concerning 2019, July and September listed in and, for both sexes, frequency peaks 40-50 mm in each month we recorded.

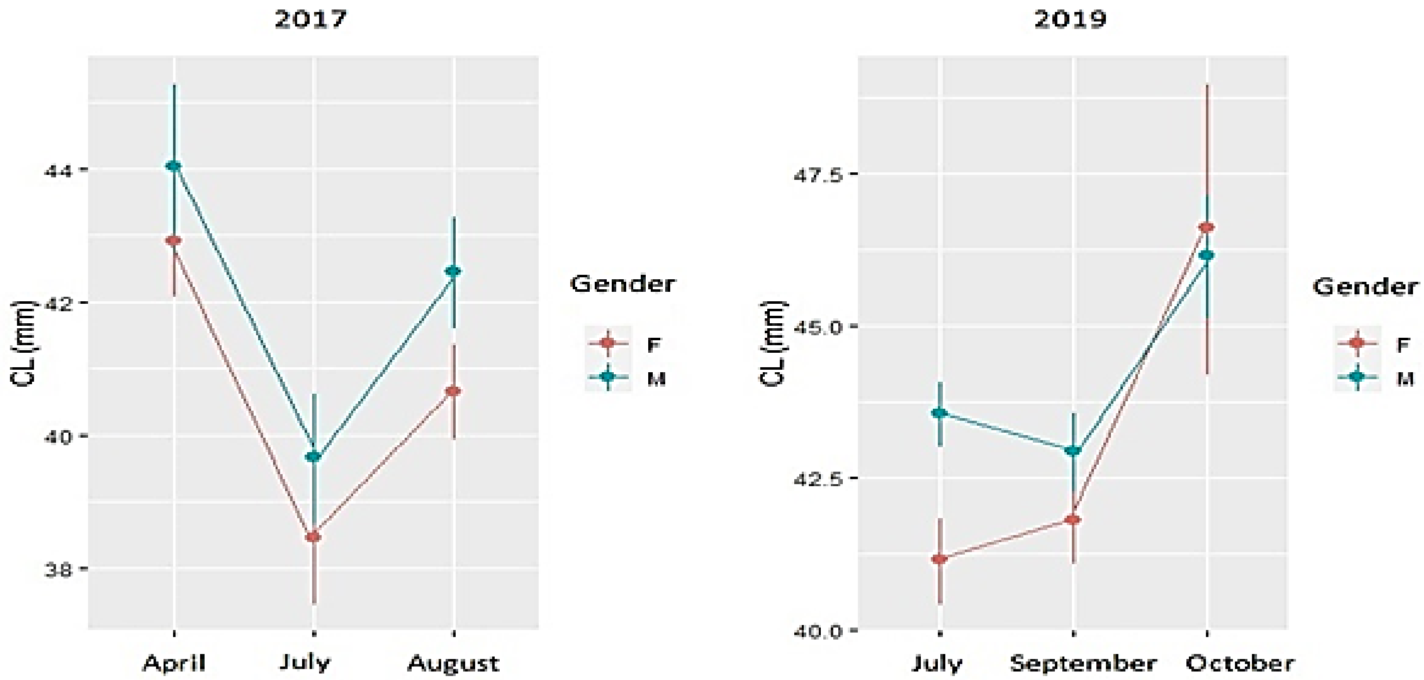

Figure 2. Mean and standard deviation of the cephalothorax length (CL) recorded during field samplings grouped by year (2017, 2019) and gender (females and males).

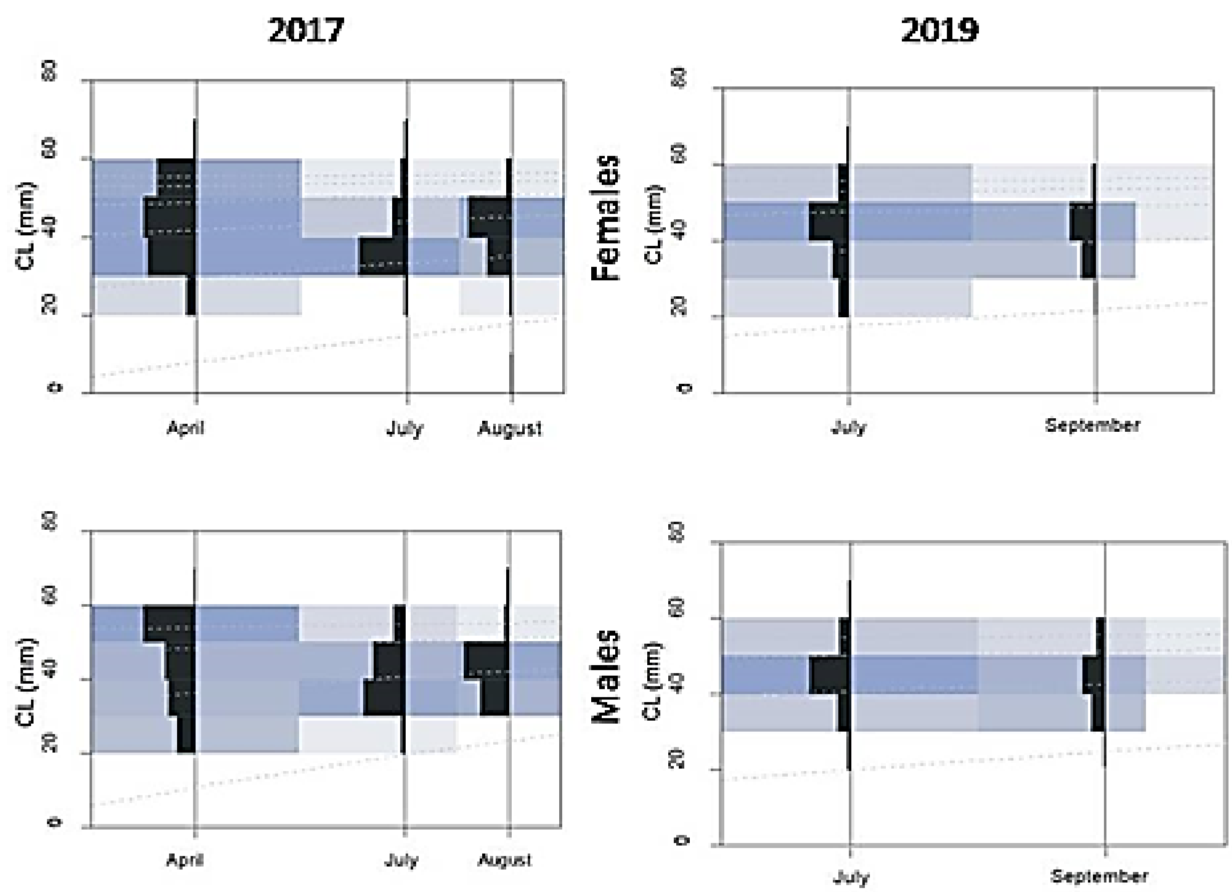

Figure 3. Length-frequency distributions calculated by ELF (Electronic Length Frequency) analysis that uses an optimized approach based on simulated annealing (ELEFAN_SA).

Table 1 reported growth parameters (k and L_∞), growth parameter index (Ø), and mortality rate (Z) for both years. Values in Table 1 were calculated for both sexes and years. Longevity values (t_{max}) ranged from 4 to 5 years: the highest longevity was recorded in 2019 for males (4.9 years), while the lowest value was recorded in 2019 for females (4.3 years old). In 2017, similar values, of about 4.5 years old, were recorded.

Discussions

This study investigated the characteristics of the invasive red swamp crayfish in the Regional Nature Reserve of "Nazzano Tevere-Farfa", an important biodiversity hotspot of Central Italy. First reports of the presence of the red swamp crayfish in the Tiber River in Latium date back to 1997 at the Castel Giubileo dam (Giucca, 1997), about 40 km below the Nature Reserve. This alien species may be very dangerous for the maintenance of ecosystem balances of vulnerable habitats such as freshwater ones. We analyzed a total of 900 specimens in order to gather information about population structure and dynamics, that could be useful for future management actions on *P. clarkii* population of the reserve. The majority of analyzed crayfish were adult (CL>20 mm), likely as a consequence of the fishing gear selectivity (10 mm wide), that only allows the capture of larger individuals.

The population was composed of crayfish aged at least 2+ (20 mm<CL<30 mm) for both years, while smaller size classes (the 0+ cohort, CL < 10 mm) were largely missing from length-frequency diagrams, confirming the poor capabilities of fishing equipment on juveniles. According to other studies (Scalici et al., 2007; Mistri et al., 2019), sex ratio is balanced considering both sampling campaigns. Length-frequency class distribution evidenced the presence of 7 different cohorts: there are no individuals in the first two classes, while for the others, different patterns related to year and sex were found. According to other studies (Maccarone et al. 2016; Jin et al., 2019) a greater number of crayfish belonging to the fifth cohort (40-50 mm) in both July and September can be observed in 2019. During the warm season, crayfish is more active and its catchability is higher, (Reynolds and Souty-Grosset, 2011). In this study, the highest number of individuals belonging to the oldest age classes has been recorded in July as reported in previous works (e.g. Gherardi, 2007b).

The growth coefficient (K) estimated in this study was considerably high, second only to that of the Qianjiang population, that is considered one of the most important production zone of red swamp crayfish in China (Jin et al., 2019). Growth parameter index (Ø) is comparable to several studies shown in Table 1, except for the aforementioned research (Jin et al., 2019). Red swamp crayfish growth rate is influenced by several ecological factors and the most important factors are temperature and food availability (Chucholl, 2011; Dörr and Scalici, 2013). Consequently, the high growth parameters recorded by Jin et al. (2019) are mainly due to their direct feeding by humans and the higher temperature (Jin et al., 2019). Hence, growth coefficient recorded in "Nazzano Tevere-Farfa" Natural Reserve represents the highest ever recorded in natural conditions.

The L_∞ value in the current study was lower respect to other studies. A possible explanation could be the higher population density that leads to a decline in trophic resources availability (Svedäng and Hornborg, 2014). The habitat investigated is characterized by a large amount of debris, particularly consistent near vegetated islets, although limited space availability may result in a strong competition for the resources. The highest L_∞ in previous studies were recorded in typical lentic and very wide habitat, such as the Trasimeno lake (Dörr and Scalici, 2013), characterized by a great amount of trophic and spatial resources.

The estimated mortality rate in this study (Z; >4 years) is one of the highest ever recorded. The population studied is located in the middle stretch of the Tiber River, a lotic environment characterized by slow, shallow and productive waters. Hence, the study area could be ecologically compared to those where crayfish showed high Z values, such as the Nile river (Saadet al., 2015) and Trasimeno lake (Dorr and Scalici, 2013). The river confluence area of the

“Nazzano Tevere-Farfa” Nature Reserve is highly productive due to the large amount of terrigenous contributions and low water flow (Nijboe and Verdonschot, 2004). In addition, the river forms some islets that increase habitat diversity and shelters. This environment was created in the 1950s, immediately after the construction of a hydroelectric dam (unpublished data, 1950). Landscape transformation brought to a deep alteration of physical characteristics, such as oscillation of water level, increase of external inputs and the consequent increase of trophic conditions (Baxter, 1977), influencing biological communities. Local crayfish population found in this area the ideal conditions that allow high density, fast growth rate and longer life span (Momot et al., 1978; Jackson et al., 2017).

Our findings may infer some considerations about crayfish spawning period. Since water temperature plays an important role in crustacean reproduction (Huner, 2002; Alcorlo et al., 2008; Jin et al., 2019), in southern Mediterranean permanent water bodies, spawning may take place even twice a year, in spring and autumn (Scalici and Gherardi, 2007; Alcorlo et al., 2008; Dörr and Scalici, 2013). This phase usually takes place in late summer and presents a variable duration, mostly dependent on water temperature, food resources and habitat (Huner, 2002; Alcorlo et al., 2008; Jin et al. 2019). During samplings, we observed ovigerous females only in the autumnal period (September/October), as already observed in similar studies (Dörr et al., 2006; Jin et al., 2019; Mistri et al., 2019). As aforementioned, the number of spawning crayfish events is mostly related to water temperature and therefore it is linked to local climatic and environmental factors (Jin et al., 2019). Therefore, we can speculate that the peculiar characteristics of the study area allow the crayfish population to have only one autumnal spawning period.

Conclusion

In this study, we found a consistent presence of *Procambarus clarkii*, which colonizes the whole study area. The good health of the studied population, characterized by balanced sex ratio and rapid growth, suggests the need of an effective action plan aimed at rapidly mitigating and remediating the negative impacts of this invasive species.

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