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Habitat use and population structure of the invasive red swamp crayfish *Procambarus clarkii* (Girard, 1852) in a protected area in northern Italy

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Abstract – The red swamp crayfish *Procambarus clarkii* is one of the most invasive alien species in Europe and included in the list of invasive species of Union concern. We describe for the first time some life-history traits of a red swamp crayfish population in the Nature Reserve of the *Lago di Candia* (Italy). We investigated (1) preferences of this species for specific environmental features on the banks of the lake, and (2) differences in size, sex ratio, and condition index between individuals caught in lake and marsh. Moreover, we compared sampling effort and the features of individuals caught in the lake, for two sampling seasons in 2014 and 2015. Findings indicated that the population was well established, and the marsh seemed to have better conditions for growth of individuals than the lake. Accordingly, continuity of riparian vegetation, opportunity to dig burrows, and trophic resource availability seems to facilitate the proliferation of the crayfish in the lake. Our study demonstrated that massive removal efforts over the whole active period of the species and more than one year of trapping are necessary to increase the controlling activities' success. This study could have important implications for further population management projects directed at biodiversity conservation in the area.

Keywords: Invasive alien species / Fulton's Condition Factor / microhabitat / lentic ecosystems / nature reserve / Piedmont

Résumé – **Utilisation de l'habitat et structure des populations d'écrevisse de Louisiane envahissante *Procambarus clarkii* (Girard, 1852) dans une zone protégée du nord de l'Italie.** L'écrevisse de Louisiane *Procambarus clarkii* est l'une des espèces exotiques les plus envahissantes d'Europe et figure sur la liste des espèces envahissantes préoccupantes de l'Union. Nous décrivons pour la première fois quelques traits de l'histoire de vie d'une population de l'écrevisse de Louisiane dans la Réserve Naturelle du *Lago di Candia* (Italie). Nous avons étudié (1) les préférences de cette espèce pour des caractéristiques environnementales particulières sur les rives du lac et (2) les différences de taille, de sex-ratio et d'indice de condition entre les individus capturés dans le lac et le marais. De plus, nous avons comparé l'effort d'échantillonnage et les caractéristiques des individus capturés dans le lac pour deux saisons d'échantillonnage en 2014 et 2015. Les résultats indiquent que la population est bien établie et que le marais semble avoir de meilleures conditions que le lac pour la croissance des individus. Par conséquent, la continuité de la végétation littorale, la possibilité de creuser des terriers et la disponibilité de ressources trophiques semblent faciliter la prolifération des écrevisses dans le lac. Notre étude a démontré que des efforts massifs d'enlèvement sur toute la période active de l'espèce et pendant plus d'un an de piégeage sont nécessaires pour accroître le succès des activités de contrôle. Cette étude pourrait avoir des implications importantes pour d'autres projets de gestion des populations axés sur la conservation de la biodiversité dans la région.

Mots-clés : Espèces exotiques envahissantes / Indice de Condition de Fulton / microhabitat / écosystèmes lentiques / réserve naturelle / Piémont

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1 Introduction

The native range of the red swamp crayfish *Procambarus clarkii* (Girard, 1852) is north-eastern Mexico and south-central USA (Hobbs, 1972). This species has been successfully introduced for aquaculture in many other American states, and also in several other countries in South America, Asia, Africa and Europe (Huner, 2002). It can be now found in all continents except Australia and Antarctica (Huner, 1977; Huner and Avault, 1979). In Europe, *P. clarkii* was first introduced in the Guadalquivir river basin (Spain) in 1974. In only six years the species caused significant damage to the rice fields of the region and it was soon considered an agricultural pest (Adão and Marques, 1993). *Procambarus clarkii* was also introduced in Italy for commercial exploitation and spread in the wild at the beginning of 1990s, probably escaping from two different aquaculture facilities in Piedmont and Tuscany (Mazzoni *et al.*, 2004). *Procambarus clarkii* has the *r*-selected features with rapid growth rates (Scalici and Gherardi, 2007) and highly plastic life cycle (Gutiérrez-Yurrita *et al.*, 1999) typical of an invasive species. Its invasiveness may be increased by its overland spreading potential, thanks to its desiccation resistance (Banha and Anastácio, 2014) and capability to cover long distances out of water, relying on aerial respiration (Gherardi and Barbaresi, 2000; Favaro *et al.*, 2011a).

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 2016/1141) for which effective management measures are required, as it meets the criteria listed in Art. No. 4 of EU Regulation 1143/2014. The species can affect water quality of invaded ecosystems by modifying water properties such as turbidity and dissolved oxygen (Souty-Grosset *et al.*, 2016) as a result of its intense burrowing activity. Indeed, *P. clarkii* dig burrows in banks to cope with hostile environmental conditions like long-term droughts (Kouba *et al.*, 2016), and to create shelters during the sensitive period of their life cycle (Gherardi, 2006), activity that can also damage dams, levees, and irrigation structures (Correia and Ferreira, 1995). Once introduced, *P. clarkii* may also disturb macrophytes and macroinvertebrate communities (Souty-Grosset *et al.*, 2016) and it has negative effects on other animals, such as amphibians (Gherardi *et al.*, 2001; Renai and Gherardi, 2004), fish (Ilhéu *et al.*, 2007) and the native white-clawed crayfish, *Austropotamobius pallipes* (Lereboullet, 1858) (Gherardi and Cioni, 2004; Favaro *et al.*, 2010; Tirelli *et al.*, 2011; Favaro *et al.*, 2011b). Finally, *P. clarkii* can act as a vector of disease, as it may carry the oomycete *Aphanomyces astaci* Schikora, 1906 responsible for the crayfish plague which is lethal to native European crayfish populations (Souty-Grosset *et al.*, 2016).

The first observations of *P. clarkii* in the Nature Reserve of the *Lago di Candia* (north-western Italy) occurred in 2011 (Delmastro, 2017). Since then, the area has been rapidly colonized by this species and specimens of *P. clarkii* can be easily seen walking underwater or overland, both in the lake and in the nearby marsh area. The aims of this study were to describe, for the first time, the habitat use, spatial distribution, size and sex ratio of the *P. clarkii* population established in the Nature Reserve of the *Lago di Candia*, by assessing the population within five years since the first observation and comparing the presence of *P. clarkii* in the lake and the marsh areas in two consecutive years. Results from this study are discussed in the context of new EU regulations and should allow for the implementation of required management actions to limit its spread to potentially suitable nearby habitats.

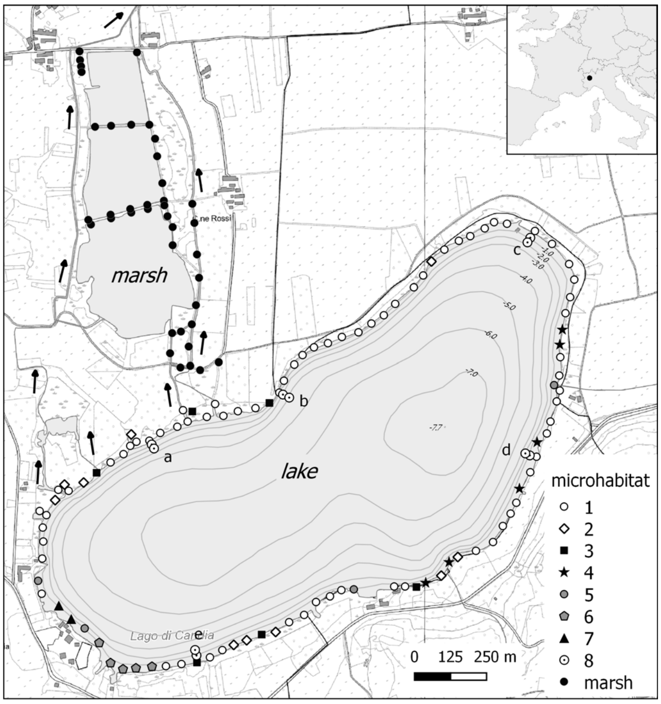

Fig. 1. Location of the study areas (lake and marsh), with sampling points labelled according to microhabitat categories (see Tab. 1). Arrows represent the direction of flow; 'a–e' represent linear transects.

2 Materials and methods

2.1 Study area

The study was conducted in the Nature Reserve of the *Lago di Candia*, north-western Italy (Fig. 1), a protected area included in the European "Natura 2000" Network, which is considered both a Site of Community Importance (SCI, IT1110036) according to the Habitats Directive (92/43/EEC), and a Special Protection Area (SPA) according to the Birds Directive (2009/147/EC). The Reserve has a surface area of 3.35 km^2 and includes two different wetlands, a glacial lake and a marsh, which are connected through a channel system that allows water to flow from the northern bank of the lake to the marsh (Fig. 1). Outgoing water from the marsh is then collected by a ditch that flows into the Dora Baltea river (Po river basin). The lake has an area of 1.52 km^2, a perimeter of 5.5 km and maximum depth of 7.7 m. The banks have continuous cover of riparian vegetation. Water temperature was measured in the lake on all sampling days at 10:00, and it ranged from 22.1 to 27.8 °C (mean 24.8 °C) in July–October 2014 and from 21.6 to 31.7 °C (mean 26.4 °C) in July–September 2015. The marsh has an area of 0.4 km^2, consists of channels and areas with shallow water (less than 0.70 m), and hosts a variety of riparian and aquatic plant species. The *Lago di Candia* is characterized by small bodies of water and

Table 1. Microhabitat categories features and corresponding number of sampling traps.

Microhabitat category	Riparian and aquatic vegetation species	Dominant category of lake bottom	Bank alteration (0–2)	*N* of traps
1	*Phragmites australis, Thelypteris palustris*	muddy	0	70
2	*Phragmites australis, Thelypteris palustris, Alnus glutinosa, Rubus ulmifollius*	muddy	0	10
3	*Alnus glutinosa, Salix caprea*	muddy and pebbly	1	6
4	*Phragmites australis, Nuphar lutea*	muddy	1	7
5	/	pebbly or artificial	2	4
6	*Phragmites australis, Nelumbo nucifera, Salix spp., Alnus glutinosa*	pebbly and sandy	2	5
7	*Trapa natans*	pebbly and sandy	2	2
8	/	muddy	0	10

wetlands created by ancient glacial activities and connected by rivers, small streams, and irrigation ditches.

The most abundant macrophyte species that can be found in the lake are *Najas marina, Nymphoides peltata, Trapa natans, Myriophyllum spicatum.* The site is of great importance for more than 200 bird species, amphibians (*Bufo bufo, Rana dalmatina*), reptiles (*Hierophis viridiflavus, Natrix natrix*), and fish (*Esox lucius*). Moreover, in the protected area, also potential predators on different sizes of *P. clarkii* (Correia, 2001) can be found. These include several introduced fish species, such as *Ictalurus melas, Lepomis gibbosus,* and *Micropterus salmoides.*

2.2 Crayfish sampling

The field activities were carried out during seven weeks from 15th July to 10th October 2014 in both the lake and marsh, and three more times in the marsh until 21st October 2014; and during three weeks from 21st July to 24th September 2015 only in the lake. Volunteer Ecological Guards of the Nature Reserve were trained and involved in sampling activities. All the sampling points were georeferenced with a GPS (Global Positioning System) receiver (GARMIN62stc). In 2014, 104 sampling points, at a distance of 1 m from the bank, were investigated along the entire perimeter of the lake. The total number of captures, sorted by sex, was registered at each sampling point.

Crayfish were sampled using baited, cylindrical traps (90 cm long, mesh size 1 cm, cross-section 30 cm, and two access funnels 10 cm in diameter). The bait consisted of wet cat food (100 g each). During each sampling week, trapping was performed over 4 sequential nights, and traps were checked, emptied and re-baited daily. All crayfish collected were removed from the environment and suppressed by hypothermia. Consequently, crayfish were sexed, measured on cephalothorax length (CTL; from the tip of the rostrum to the cephalothorax posterior portion) by using a 0.1 mm precision calliper, weighted by using a 0.01 g precision digital scale. Finally, the specimens were disposed of in cooperation with the health government body *Istituto Zooprofilattico Sperimentale del Piemonte, Liguria e Valle d'Aosta* (*S.S. Laboratorio Specialistico Ittiopatologia*), and according to the Italian laws.

The Fulton's Condition Factor (FCF; Ricker, 1975) is a condition index calculated for every individual and it was obtained using the following formula: $FCF = weight/(CTL)^3$. All crayfish with missing or regenerating chelae were excluded from the FCF analysis.

Catch per unit effort (CPUE) was calculated for the three sets of samples (lake 2014, marsh 2014 and lake 2015) as the daily number of crayfish collected/number of traps. For each sampling week, CPUE was calculated as the mean of the daily CPUE.

2.3 Microhabitat category identification

To describe habitat use, the following microhabitat parameters were recorded at each sampling point: riparian and aquatic vegetation species, dominant typology of the lake bottom, and level of bank alterations by human activity. For the last parameter, we assigned an alteration value from 0 (absence of alteration) to 2 (intense alteration due to business, for example restaurant, with cut grass). Ten additional sampling points were selected along five linear transects perpendicular to the banks (transects 'a–e', at 25 m and at 50 m) to investigate the presence of *P. clarkii* at greater depths (mean -1.3 ± 0.86 m deep at 25 m from the bank; mean -2.4 ± 0.83 m deep at 50 m from the bank). Consequently, eight different microhabitat categories were identified (from 1 to 8) based on the parameters observed (Tab. 1). Finally, each sampling point was assigned to one of these categories (every category includes, therefore, a different amount of sampling sites). In 2014, sampling sessions at 40 sites in the channels of the marsh area were also carried out. In 2015 in the lake, 26 sampling points were selected from the 104 of the previous year.

2.4 Statistical analyses

Non-parametric statistical tests were carried out because the data did not meet the requirement for normal distribution, even after log-transformation (Sokal and Rohlf, 1995). Because data violated the assumption of homogeneity of variances (Levene's test: $P < 0.001$), but were robust for skewed distributions and large sample sizes (Fagerland, 2012), Welch's ANOVA with post-hoc multiple comparisons was used to evaluate differences in the collected daily biomass (mean crayfish weight collected per trap) in the different microhabitat

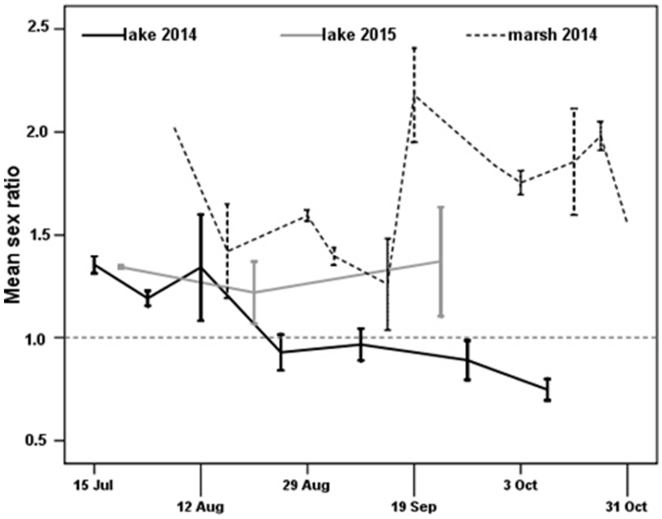

Fig. 2. Mean sex ratio values (males/females) obtained in each sampling week, divided per habitat and per year. Reference dotted line to 1 shows the expected 1:1 balance. Error bars: ±SE.

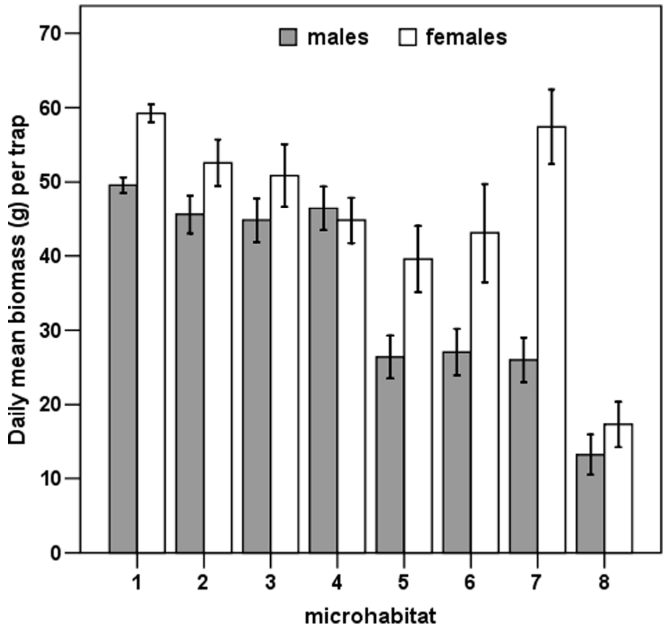

Fig. 3. Daily mean extracted biomass (g) per trap for each microhabitat category (from 1 to 8), divided per sex. Error bars: ±SE.

categories. A Spearman's Rho test and a Chi-squared test (χ^2) were used to investigate the correlation and the presence of the species at different water depths in the lake. A Chi-squared test was also used to assess the sex ratio balance within the three sets of samples (lake 2014, marsh 2014, lake 2015). A Spearman's Rho test allowed us to investigate any correlation between CTL and weight of individuals in both sexes. Finally, Mann-Whitney U tests were used to assess differences in size between individuals caught in the marsh and in the lake in 2014, differences among the calculated Fulton's Condition Factor values of the three sets of samples (lake 2014 *vs.* marsh 2014 and lake 2014 *vs.* lake 2015), and to assess the difference in the total number of captures in the lake between the two years (2014 and 2015). Statistical differences were detected with a level of significance $P < 0.05$ and all statistical analyses were performed using SPSS 22.0 (Statistical Package for the Social Sciences, SPSS Inc.).

3 Results

3.1 Samples features

A total sample of 19,971 individuals (9983 males and 9988 females) was collected in the lake in 2014 and 6073 individuals (3787 males and 2286 females) were collected in the marsh in the same year. In addition, 2634 crayfish (1461 males and 1173 females) were caught in the lake in 2015. These three sets of samples showed significant differences from the expected sex ratio 1:1 within each sampling period (Chi-squared test after contingency table; lake 2014: $\chi^2 = 332.853$, $df = 25$, $P < 0.001$; marsh 2014: $\chi^2 = 51.586$, $df = 18$, $P < 0.001$; lake 2015: $\chi^2 = 28.908$, $df = 11$, $P < 0.005$). In the sample collected in the lake in 2014, males were more abundant than females until the end of August, when a switch of the ratio was recorded and persisted until the end of the sampling activities (first ten days of October). On the other hand, in both the marsh 2014 sample and the lake 2015 sample, more males than females were collected during the entire sampling period (Fig. 2).

3.2 Habitat use

Significant differences were shown in extracted biomass per trap among different microhabitat categories (Welch's ANOVA; $F_{(7;736.761)} = 51.826$, $P < 0.001$), also considering males and females separately (Welch's ANOVA; males: $F_{(7;376.034)} = 35.182$, $P < 0.001$; females: $F_{(7;363.964)} = 25.181$, $P < 0.001$). A clear preference was shown for category 1, characterised by continuous vegetation cover on the bank (*Phragmites australis* and *Thelypteris palustris*), muddy and silty bottom and no physical alteration by human activity. On the contrary, categories 5 and 8, characterized respectively by pebbly and deeper water, were the least frequented; moreover, in both categories, vegetation was absent. Category 7, typified only by the presence of *Trapa natans*, showed a preference peak by females, while it was not much frequented by males (Fig. 3). Consequently, the Dunnett's C post-hoc multiple comparisons pointed out the significant differences among microhabitats for both sexes (Tab. 2).

An inverse relationship between crayfish and depth of the lake was also observed both in terms of biomass (Spearman's Rho test; $\rho = -0.412$, $P < 0.001$) and number of trapped crayfish (Spearman's Rho test; $\rho = -0.412$, $P < 0.001$).

3.3 Catch Per Unit Effort

For each sampling week, CPUE was calculated as a mean value of the daily CPUE (Fig. 4). Overall, CPUE values did not differ significantly between the three sampling periods (Kruskal-Wallis test: $\chi^2 = 1.473$, $df = 2$, $P > 0.05$). Moreover, water temperature did not affect CPUE in the lake (Spearman's Rho test; $\rho = 0.180$, $P > 0.05$).

Table 2. Dunnett's C post hoc multiple comparisons of mean difference between daily biomass (g) per trap for each microhabitat category (from 1 to 8) for females (above the diagonal) and males (below the diagonal). Bold type indicates results that were significant ($P < 0.05$; in brackets, standard error).

Microhabitat	*1*	*2*	*3*	*4*	*5*	*6*	*7*	*8*
1	–	6.7 (3.36)	8.4 (4.39)	**14.4** (3.31)	**19.7** (4.61)	16.1 (6.74)	1.8 (5.16)	**41.9** (3.32)
2	4.0 (2.78)	–	1.7 (5.25)	7.8 (4.39)	13.0 (5.44)	9.5 (7.38)	4.8 (5.91)	**35.3** (4.40)
3	4.8 (3.17)	0.8 (3.93)	–	6.1 (5.22)	11.3 (6.13)	7.8 (7.86)	6.5 (6.55)	**33.6** (5.23)
4	3.2 (3.16)	0.8 (3.93)	1.6 (4.21)	–	5.2 (5.42)	1.7 (7.32)	12.6 (5.89)	**27.5** (4.37)
5	**23.2** (3.07)	**19.2** (3.86)	**18.4** (4.15)	**20.0** (4.14)	–	3.5 (7.99)	17.8 (6.71)	**22.3** (5.42)
6	**22.5** (3.30)	**18.5** (4.05)	**17.8** (4.32)	**19.4** (4.31)	0.7 (4.25)	–	14.3 (8.32)	**25.8** (7.32)
7	**23.6** (3.20)	**19.6** (3.96)	**18.8** (4.24)	**20.4** (4.24)	0.4 (4.17)	1.0 (4.35)	–	**40.1** (5.90)
8	**36.4** (2.91)	**32.4** (3.73)	**31.6** (4.03)	**33.2** (4.02)	**13.2** (3.95)	**13.9** (4.14)	**12.8** (4.05)	–

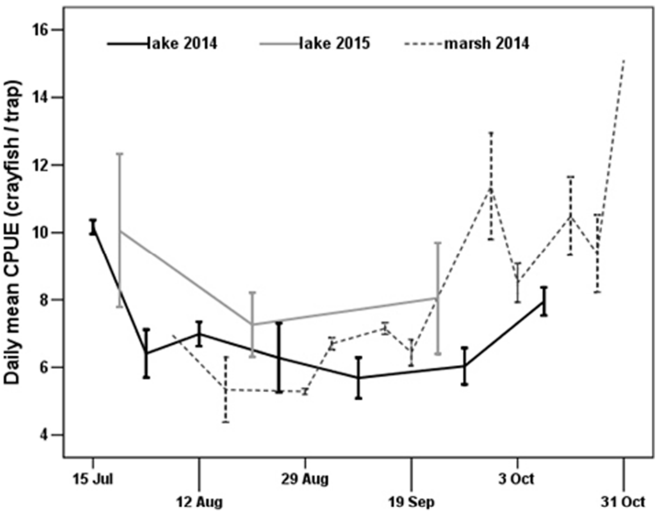

Fig. 4. Daily mean CPUE values, referred to sampling session, in the different habitats (marsh and lake) and years (2014 and 2015). Error bars:±SE.

3.4 Comparison of crayfish size in lake *vs.* marsh habitats

Values of CTL (Fig. 5) and FCF (Tab. 3) were used to carry out comparisons between the set of samples collected in the lake and in the marsh in 2014 in order to identify possible differences in the size and condition of crayfish living in the two habitats. CTL differences between males and females were significant in both habitats (Mann-Whitney test, lake: $U = 43893403.0$, $Z = -39.384$, $P < 0.001$; marsh: $U = 1161867.5$, $Z = -8.298$, $P < 0.001$). Therefore, the test was performed on males and females separately and in both cases significant differences were found between habitats (Mann-Whitney U-test, males: $U = 3635262.5$, $Z = -47.462$, $P < 0.001$; females: $U = 3414629.5$, $Z = -28.011$, $P < 0.001$), with a larger crayfish size in the marsh than in the lake.

Comparisons between different habitats and different years were carried out on values of FCF for males and females separately, because males showed significantly higher FCF values than females (Mann-Whitney U-test, $U = 10617867$, $Z = -39.558$, $P < 0.001$). For both sexes, the statistical analysis did not show any significant difference between crayfish collected in the lake and in the marsh in 2014 (Mann-Whitney U-test, males: $U = 1245977.5$, $Z = -1.580$, $P > 0.05$; females: $U = 771939.5$, $Z = -1.228$, $P > 0.05$). On the contrary, the FCF values in both sexes were significantly different between crayfish caught in the lake in the two years (Mann-Whitney U-test, males: $U = 2145065.5$, $Z = -9.217$, $P < 0.001$; females: $U = 1617983.5$, $Z = -5.039$, $P < 0.001$), with higher FCF values registered in the samples collected in 2015.

3.5 Comparison of captures in the two years

Since the sampling effort was reduced in 2015, only data from corresponding periods and sampling sites in the lake between the consecutive years were compared. The analysis thus involved 1861 individuals (955 males and 906 females) caught in 2014 and all crayfish caught in 2015 (2634 crayfish: 1461 males and 1173 females). Significantly more individuals were collected in 2015 compared to the previous year (after Mann-Whitney U test, $U = 36652.5$, $Z = -5.351$, $P < 0.001$). The difference was also significant considering all three months of activities separately, in each month more crayfish were caught in 2015 than in 2014 (after Mann-Whitney U test, July: $U = 3932.5$, $Z = -3.407$, $P < 0.001$; August: $U = 4220.5$, $Z = -2.744$, $P < 0.01$; September: $U = 4054.0$, $Z = -3.127$, $P < 0.005$).

4 Discussion

The present study describes – for the first time – the habitat use and population biology of the invasive *P. clarkii* in a wetland in north-western Italy. Our findings are supported by a large sample of crayfish collected from the two investigated habitats and over 2 consecutive years.

The sex ratio we observed during the sampling period in all the three sets of samples was different from the expected 1:1 balance usually found for cambarids (Reynolds, 2002). This is probably due to certain factors that can induce a difference in the activity of the two sexes (such as reproduction period, incubation period, and burrowing habit) throughout the year (Gherardi and Barbaresi, 2000; Gherardi *et al.*, 2000; Gherardi *et al.*, 2002). In particular, in 2014, we caught significantly more males than females in the lake, until the second half of August, when we observed a switch in this trend and significantly more females than males were sampled up to

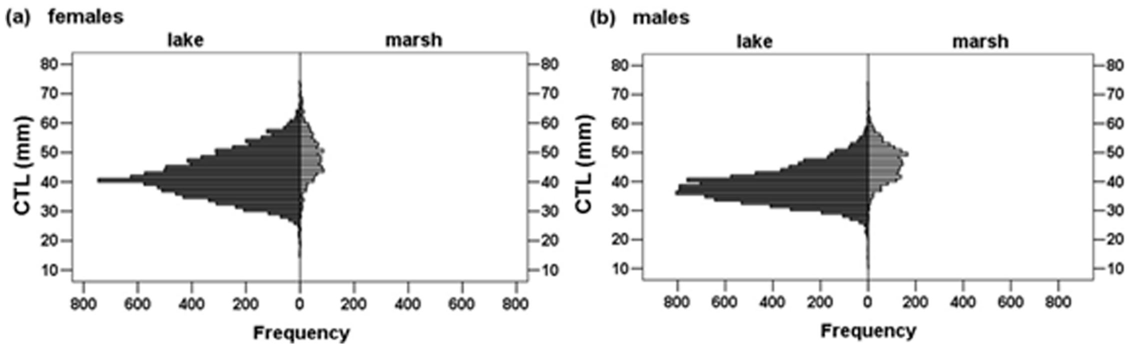

Fig. 5. Cephalothorax length (CTL; mm) frequencies in the two habitats, divided per sex: (a) females, (b) males.

early October. Instead, in the other two sets of samples (marsh 2014 and lake 2015), significantly more males than females were caught over the entire period. Examples of unbalanced sex ratios in different periods of the year have been frequently reported in the Italian populations of *P. clarkii* where either males outnumber females (Chiesa *et al.*, 2006; Ligas, 2008; Dörr and Scalici, 2013; Maccarone *et al.*, 2016) or *vice versa* (Ligas, 2008; Scalici *et al.*, 2010). Moreover, *P. clarkii* females clearly outnumbered males both in Europe and in Africa (*i.e.* Oluoch, 1990; Anastácio and Marques, 1995; Fidalgo *et al.*, 2001). In the original area of distribution (Louisiana), the ratio of females to males calculated throughout the year was approximately equal, even though there was a considerable decrease of females during the warmer months, due to their annual spawning cycle (Penn, 1943; Huner, 1978).

We suggest that the discrepancy in the sex ratio values observed in our study is linked to the reproductive activity of females during the summer months, which tend to remain in their burrows to provide parental care to their offspring (Vogt, 2013). Indeed, in the 2 years in the lake, both egg-bearing and egg-hatched females were rarely trapped and constituted only 1.1% and 1.7% of the females caught in 2014 and 2015, respectively. We therefore presume that removal of 19,971 crayfish from the lake in 2014 could free environmental resources and space, and lead to the immigration of males from the marsh, where they outnumbered females in 2014.

Comparisons performed between the catches in the lake, during the corresponding periods of the 2 years, showed that more crayfish were collected in 2015 than in 2014, despite the remarkable sampling effort of 2014, when almost 20,000 individuals were caught and removed. Two main hypotheses could explain this result. Firstly, the massive catches carried out in 2014 were probably not sufficiently spread out throughout the year, as they affected only 4 consecutive months. Secondly, only 1 year of catches was probably not enough to reduce the abundance of a well-established crayfish population such as that found in the lake. Finally, continuous colonization of the lake by new individuals coming from adjacent areas (fields, irrigation systems, tree plantations) is hypothesised, as also suggested by Savini *et al.* (2008) in a Nature Reserve in northern Italy, and by Cruz and Rebelo (2007) in freshwater habitats of the southwest Iberian Peninsula. Indeed, *P. clarkii* is capable of high mobility and probably has good orientation skills as well (Breithaupt *et al.*, 1995), especially during its "wandering phase" (a spatial strategy opposed to the "stationary phase"). This is a period of short peaks of high speed locomotion, which especially characterizes the behaviour of breeding males, and supports the dispersion of the species. For example, in the rice fields of the Lower Guadalquivir (Spain), crayfish during the "wandering phase" covered up to 17 km in 4 days, and an area of 20 km^2 in the same time span (Gherardi and Barbaresi, 2000). The extent of dispersal and the speed of locomotion seem to differ between individuals, and could depend on the sex of crayfish, as well as environmental parameters and the life cycle period (Barbaresi *et al.*, 2004). Moreover, *P. clarkii* is able to adapt quickly to different conditions due to its ecological plasticity and to spread local parasites and mycoflora on its carapace (Dörr *et al.*, 2011, Dörr et *al.*, 2012a, Dörr *et al.*, 2012b; Chiesa *et al.*, 2014; Bissattini *et al.*, 2015).

Our results revealed significant differences in CTL between sexes in the lake and in the marsh, with females being larger than males in both habitats. In Lake Trasimeno (central Italy), based on total length measures, a previous study also found significant size differences in favour of females (Dörr *et al.*, 2006). However, other studies performed in Italy did not reveal any significant differences between the size of males and females in the collected samples (Scalici and Gherardi, 2007; Scalici *et al.*, 2010). As clutch size (*i.e.* fecundity) significantly increases with body size, females could be subjected to strong selective pressure to quickly reach a large size (Aquiloni and Gherardi, in Scalici and Gherardi, 2007).

Crayfish caught in the marsh were, on average, larger in CTL than those from the lake in the same year; this feature could be attributed to different factors. Crayfish growth rate is influenced by several factors, such as water temperature, light intensity, quantity and quality of nutrients, and population density (Aiken, 1980; Reynolds, 2002). The differences found in size could be related to a difference in the trophic resources present in the different habitats (Paglianti and Gherardi, 2004; Ramalho *et al.*, 2008).

FCF values revealed a significant difference between sexes, with males showing higher values than females. This difference could be the result of a higher average size (and therefore weight) of the chelae in males than in females, confirming what was previously observed by Anastácio and Marques (1998), who calculated FCF by using post orbital carapace length instead of CTL. Lack of significant differences in the FCF values, calculated for individuals caught in the lake and in the marsh in 2014, revealed a homogeneous condition of the two populations for both males and females. However, in

Table 3. Number of crayfish males and females (*N*) collected in the lake and in the marsh in 2014 and 2015 with corresponding CTL and FCF (minimum, maximum, and mean ± standard deviation).

Habitat and year	CTL (mm)						FCF (g/mm^3)					
	Females			Males			Females			Males		
	N	Mean (SD)	Min-Max	*N*	Mean (SD)	Min-Max	*N*	Mean (SD)	Min-Max	*N*	Mean (SD)	Min-Max
Lake 2014	9988	42.2 (7.2)	14.5–74.4	9983	38.8 (5.9)	13.7–74.2	4500	0.0002 (0.0001)	0.0000–0.0047	4787	0.0002 (0.0001)	0.0001–0.0054
Marsh 2014	1320	48.7 (7.6)	17.9–73.2	2116	46.6 (6.1)	10.2–67.1	357	0.0002 (0.0000)	0.0000–0.0006	543	0.0002 (0.0000)	0.0001–0.0004
Lake 2015	1173	41.9 (6.2)	25.0–63.7	1461	37.6 (4.9)	21.0–60.1	809	0.0002 (0.0000)	0.0000–0.0005	1091	0.0002 (0.0000)	0.0000–0.0008

both sexes, crayfish collected from the lake in 2015 showed higher values of FCF than those collected in the previous year, indicating that in 2015 the species probably experienced better environmental conditions related to their ecological needs.

The habitat use study revealed that crayfish tended to frequent shallower waters and remain close to the banks, rather than inhabiting areas at a certain distance from the lake shore. Indeed, greater depths do not provide *P. clarkii* with certain environmental features that they require (*e.g.* food availability or the opportunity to shelter or burrow digging). As shown by other crayfish species like *Procambarus alleni* (Faxon, 1884), population density decreases with water depth and increases with plant biomass (Jordan *et al.*, 1996). Thus, at greater depths, where macrophytes do not occur, crayfish can be consumed by large fish predators; plant biomass, conversely, reduces the danger of predation by birds and fish predators (Heck and Crowder, 1991). In addition, in the *Lago di Candia*, the species shows a minor preference for sites typified by discontinuity of riparian vegetation, pebbly bottom and physical banks altered by human activity that makes burrow digging impossible (features that, to varying degrees, contribute to describe microhabitat categories from 2 to 8). However, the lack of muddy banks does not seem to decrease the survival of *P. clarkii* (Aquiloni *et al.*, 2005). Indeed, crayfish can also find shelter under boulders, and in crevices and vegetated sections of rivers and lakes. Moreover, males more frequently choose to hide under boulders while females, on the contrary, take refuge in complex microhabitats, such as pond sections vegetated by aquatic macrophytes (Aquiloni *et al.*, 2005). Therefore, the habitat use evaluation demonstrated a high degree of suitability of the study area for colonization by crayfish. Indeed, the category 1 microhabitat, which is favoured by the species, covers more than 60% of the investigated sites. Moreover, 50 out of 70 sites belonging to category 1 were located along the northern lake shore, which is the area that links the lake with the marsh, through a web of channels.

In conclusion, we highlighted the presence of an established and reproductively active population in the study area, which presents suitable conditions for *P. clarkii* to proliferate. In addition, our results demonstrated that the marsh showed better conditions than the lake for growth of crayfish individuals within the study area.

5 Impacts and implications for population control

The Nature Reserve of the *Lago di Candia* is a circumscribed area rich in biodiversity and identified both as a Natura 2000 Network SCI and SPA. As suggested by many studies (Gherardi *et al.*, 2011; Nunes *et al.*, 2017) and confirmed by our results, complete eradication of red swamp crayfish is probably impossible to achieve. In any case, it is fundamental to limit the invasion of *P. clarkii,* which is a real threat to the protected area, in order to reduce the negative impacts of the species on the biodiversity of the site. Moreover, reduction in macrophyte biomass and survival has already been recorded in a Dutch peat lake, where *P. clarkii* was expanding rapidly (van der Wal *et al.*, 2013). Surveys carried out in 2011 and in 2015 indicated that some macrophyte species, which were previously present in the lake disappeared over a 4-year period (*e.g. Najas marina* and *Nymphoides peltata*). Some other species showed a dramatic decrease of their occupied surface, *e.g. Myriophyllum spicatum* lost 98.5% and *Trapa natans* lost 87% (CNR and ARPA Piemonte; unpublished data). The influence of *P. clarkii* on macrophyte communities and biomass are well-known and described, not only due to the direct consumption, but also by non-consumptive cutting of the stems (Nyström and Strand, 1996, Gherardi, 2006; Loureiro *et al.*, 2015).

Trapping for mechanical removal is a widespread method for managing *P. clarkii* populations in natural environments. Trapping is considered to work well on huge target populations and to be highly efficient. However, this efficacy is only achieved if trapping activities are conducted for an extended period of time and with regularity, which require intensive manpower and involve high costs. Moreover, the use of net traps shows high selectivity, as it can affect specific classes of individuals in the target population and lacks species-specificity (Gherardi *et al.*, 2011). The results of our study suggest that baited net traps were an efficient way to easily capture and remove large amounts of crayfish individuals from a huge target population. However, traps are affected by the size of individuals due to many factors, the first of which is the mesh size, resulting in higher trappability of larger crayfish. This can have various implications that should be evaluated. Firstly, larger females generally produce more eggs than smaller females; moreover, their eggs have greater weight and volume, factors that result in a greater reproductive output (Gutiérrez-Yurrita and Montes, 1999; Alcorlo *et al.*, 2008). Therefore, removal of larger females could be more beneficial in controlling future generations. However, this kind of selection could also lead to feedback mechanisms with consequent increase of egg production and earlier maturity in females (Holdich *et al.*, 1999). In addition, we suggest that removing large individuals could reallocate the environmental resources to the remaining part of the population and therefore to smaller individuals too. Liberation of resources and space could eventually lead to the arrival of adult individuals from adjacent areas (Gherardi *et al.*, 2011). All these factors could explain the results of our study. Moreover, we argue that the trapping activities carried out in 2014 were efficient, but not adequately extended over time. Some studies show that trapping activities can be highly effective in reducing the size of a population but, in the absence of continuity, populations rapidly return to their former levels (Rogers *et al.*, 1997; Holdich *et al.*, 1999). Therefore, we highlight that massive removals of the species should be performed during the entire period of activity in the study area, and that it is important to consider more than just 1 year of trapping activities, as also suggested by Nunes *et al.* (2017). Continued trapping is thus preferred to intensive short-term trapping (Loureiro *et al.*, 2015). In the case of future massive catches, we think it would be useful to use nets as well as fyke-nets in the connecting area between the lake and the marsh, in order to increase trapping success. Indeed, the water channels that connect the two habitats are probably an important passage for *P. clarkii*, and therefore a strategic position to intercept the crayfish. For the same reason, nets should also be placed in the outgoing streams surrounding the protected area, as these channels could be a pathway for the arrival of new individuals. Reintroduction or restocking of indigenous predators (*Esox lucius* and *Anguilla anguilla*) could also be considered in the perspective of *P. clarkii* population management in the study area. As suggested by Aquiloni *et al.* (2010), the presence of a predator such as the European eel, combined with trapping, may be effective against invasive populations of crayfish. Of course, these actions require accurate feasibility studies to assess their potential for implementation in the area. Finally, we underline the importance of early detection in sites that are potentially suitable for the invasion by *P. clarkii*. To this end, it is important to raise the awareness of local populations, which also aims at reducing the additional spread of the species caused by further liberation of individuals in natural environments. The problems related to the presence of *P. clarkii* are already explained and discussed with visitors and students who frequent the protected area.

Recently *P. clarkii* has been included in the recent "Union List" of invasive alien species of union relevance (implementing regulation EU 2016/1141) by European Union Member States. Therefore, in order to implement an appropriate management plan of these species, the EU Regulation 1143/2014, Article 19 and Article 20, provide guidelines for management measures and restoration of damaged ecosystems. In particular, commercial exploitation of invasive alien species already present can be temporally authorized under management measures for eradication, population control or confinement, but only if strictly justified. Nowadays, the different methods applied to control or reduce populations include mechanical removal; physical methods like drainage of ponds or construction of barriers (Dana *et al.*, 2011); biocontrol, which takes advantage of the natural enemies of the species in question (Aquiloni *et al.*, 2010); pesticides or biocides (Gherardi *et al.*, 2011); and autocidal methods such as sex pheromones or sterile males release technique (SMRT; Aquiloni *et al.*, 2009; Aquiloni and Gherardi, 2010). Moreover, European Member States should engage restoration measures to rebuild ecosystems which have been degraded, damaged or destroyed by invasive species.

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