



An Invasive Species in a Protected Area of Southern Italy: the Structure, Dynamics and Spatial Distribution of the Crayfish *Procambarus Clarkii*

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Abstract

In a WWF reserve of south-western Sicily (Italy), a population of *Procambarus clarkii* has colonized the important protected areas of Preola Lake and the Gorgi Tondi Nature Reserve.

Surveys were conducted in four lakes to investigate the structure and dynamics of the population inhabiting these lakes, and the life history patterns of this crayfish in this characteristic habitat are presented. Significant differences between sex ratio and cephalothorax mean measurements) were not evidenced. The population's parameters were estimated using the Von Bertalanffy growth function and the Bhattacharya method. Similarly to previous work, this study it was found that this species has a lower growth rate and longevity in comparison with other populations living in the Mediterranean region (approximately 4 years old). The spatial analysis evidences a strong heterogeneity and large movement range of the specimens during the study period. The results indicate the high ecological plasticity of *Procambarus clarkii*, suggesting the importance of monitoring this bio-invader to plan appropriate actions for the control of this population in protected areas where it occurs to preserve the local biodiversity.

Keywords: *Procambarus clarkii*, length-frequency distribution, growth parameters, spatial distribution, lake habitat.

Introduction

In recent decades, invasive alien species have been among the most serious threats to the conservation of natural biodiversity; consequently, limitation of their diffusion is a priority for the defence of natural ecosystems (Simberloff and Stiling, 1996; Sousa *et al.*, 2011) as also reported by the EU Regulation on Invasive Alien Species 1143/2014. Several studies have demonstrated the effect of predatory invasive species on trophic conditions (Carpenter *et al.*, 2001). In particular, one of the most-known effects can be manifested both as a decrease in the abundance and distribution of prey species (Correia, 2003; Fraser *et al.*, 1995; Schoener *et al.*, 2001) and as an increase in the same for non-prey species (Correia *et al.*, 2005). *Procambarus clarkii* (Girard, 1852) represents a species that has received great attention as a consequence of intentional or accidental introductions that have led to the establishment of non-native populations (Gherardi and Acquistapace, 2007; Lodge *et al.*, 2000). Moreover, this bioinvaders species could be considered able to drive biodiversity loss (Cardoso and Free, 2008; Sala *et al.*, 2000).

The farming of crayfish for food has been developed in several regions of Europe and some farmed stocks have escaped into the wild, where they may compete with others species altering communities (Holway and Suarez, 1999; Lodge, 1993). In addition, a further threat is represented through the aquarium trade; *P. clarkii* are sold in aquarist centres and owners of such species often discard them into the freshwater environment (Holdich *et al.*, 2009). This practice has interfered with the natural distribution of autochthonous crayfish (Gherardi, 2006). This crayfish is native to the south-eastern United States, but for commercial purposes it has been distributed worldwide (e.g., Adão and Marques, 1993; Gherardi, 2007; Gherardi *et al.*, 2000; Lane and Fujioka, 1998). The introduction of *P. clarkii* has had a significant and negative effect on invaded biotopes, interfering with existing trophic relationships (Ilheu and Bernardo, 1993; Ilheu *et al.*, 2000).

These decapod crustaceans are able to survive in a wide range of biotic and abiotic conditions, are omnivores, and their aggressive behaviours can be directed against several aquatic species, including aquatic plants (Barbaresi and Gherardi, 2000; Hill and

Lodge, 1999), which can result in alterations of the plant density (Rodríguez *et al.*, 2003), other native crayfish populations (Gil-Sanchez and Alba-Tercedor, 2002), endemic invertebrates through predation and competition (Lodge and Hill, 1994; Olsen *et al.*, 1991), and amphibian species (Beja and Alcazar, 2003). Moreover, their habit of burrowing (Barbaresi *et al.*, 2004), which aids the crayfish in tolerating dehydration, can cause bank collapse and increased water turbidity (Rodríguez *et al.*, 2005). Furthermore, based on the results obtained by Águas *et al.* (2014) there is evidence that the passive transport of the recently-hatched offspring of these crustaceans is possible by water birds, which can therefore accelerate the process of invasion.

In general, the environments colonized with the greatest facility are water bodies and wetlands with fresh to moderately brackish water. The biodiversity in terms of richness and trophic complexity of these areas have been noted and widely debated (Sakai *et al.*, 2001), and in recent years several studies have underlined the importance of a non-disturbance period to preserve the natural complexity of these ecosystems (Shea and Chesson, 2002). This disturbance, understood at different levels of impact, tends to interfere in the interrelations of endemic species, and can cause the appearance of new niches for invading species.

The objective of the present study was to understand the spatial distribution, structure and dynamics of the red swamp crayfish population in oligohaline lakes in southern Italy. The study of the dynamics, spatial distribution and principal growth parameters represent useful mean with which to understand the status of a population. This information can provide an estimate of the fitness of a population and its habitat and, moreover, can clarify how demography and population regulation inform the theory of biological control (Jones and Coulson, 2006).

Materials and Methods

Study Area

The present study was performed at the “Riserva Naturale Integrale Lago Preola e Gorgi Tondi”, an area protected by the WWF (World Wildlife Fund for Nature), located in south-western Italy in Sicily.

The study area consists of four oligohaline lakes named Preola Lake, Gorgo Alto, Gorgo Medio and Gorgo Basso (the last three are also referred to collectively as simply “Gorgi Tondi”).

Preola Lake is a medium-sized lake of ca. 30 ha (37°37' N, 12°38' E, 6 m a.s.l.; Figure 1) with an average depth of 1.2 m and a maximum depth of 2 m. Preola Lake has no surface tributary or emissary and is part of a chain of karstic depressions that are separated from the Mediterranean Sea (1.5 km away) by a calcareous ridge ca 30–40 m high (Fig. 1B).

The mean annual temperature is 18.2°C, ranging from 26.3°C in August to 11.4°C in January (source: Osservatorio delle Acque, Regione Siciliana; Bonaccorso *et al.*, 2003). The main physicochemical characteristics of the water were registered over seven sampling sessions in each lake. In particular, surface water temperature, conductivity and pH were measured using a multi-parametric probe (556 MPS, YSI incorporated, OH, USA) at 8 m from the shore and at 1.5 m of depth for each lake.

The climate conditions are typical of Mediterranean areas, with hot, dry summers and mild, moist winters representing a preferred refuge for various endangered species. Most of the landscape surrounding the reserve is managed for agriculture, including vineyards, olive groves, and orchards. Several rare species occur in the aquatic and hygrophilous communities of the reserve, such as *Cladium mariscus*, *Juncellus laevigatus*, *Damasonium bourgaei*, *Galium elongatum* and *Hypericum tetrapterum* (Calò *et al.*, 2012). Halophyte species, including *Arundo donax* L. and *Arthrocnemum macrostachyum* (Moric.), have been observed along the shores of Preola Lake since 1975, suggesting a recent increase in salinity (Calò *et al.*, 2012).

Sampling Period and Measurement

Before the sampling surveys, the WWF Nature Reserve provided authorisations in order to ensure that the lowest disturbance would be caused to migratory species that find refuge in the waters of the lakes by the activities associated with this study.

Five sampling sessions were conducted from May to October 2012 in Preola Lake (1st: 29–31 May; 2nd: 28–30 June; 3rd: 28–30 August; 4th: 28–30 September; 5th: 28–30 October) and five sampling sessions from May to October 2012 in the Gorgi Tondi lakes (1st: 28–30 May; 2nd: 27–29 June; 3rd: 29–31 August; 4th: 27–29 September; 5th: 29–31 October) to assess the spatial distribution, age structure, growth parameters, and the catch per unit effort (CPUE) of the *P. clarkii* population. In total, 116 cylindrical traps in Preola Lake and 39 traps in the Gorgi Tondi lakes (13 traps for each lake) were used. The traps were 600 mm long with 10 mm mesh and two opposing funnels 50 mm in diameter (Scalici and Gibertini, 2005), and were baited with 50 g of liver (Demers and Reynolds, 2002; Hein *et al.*, 2007).

Moreover, to make the sampling more representative, two additional sampling sessions were conducted (11–18 January; 5–12 July 2013). In particular, the samplings were performed using eight traps in Preola Lake and six traps in the Gorgi Tondi lakes (two for each lake). The traps were placed along the edges of the lakes for a week.

Each caught specimen was sexed and the cephalothorax length (CTL) was measured using a vernier calliper from the tip of the rostrum to the medio-dorsal margin of the cephalothorax (Chucholl,

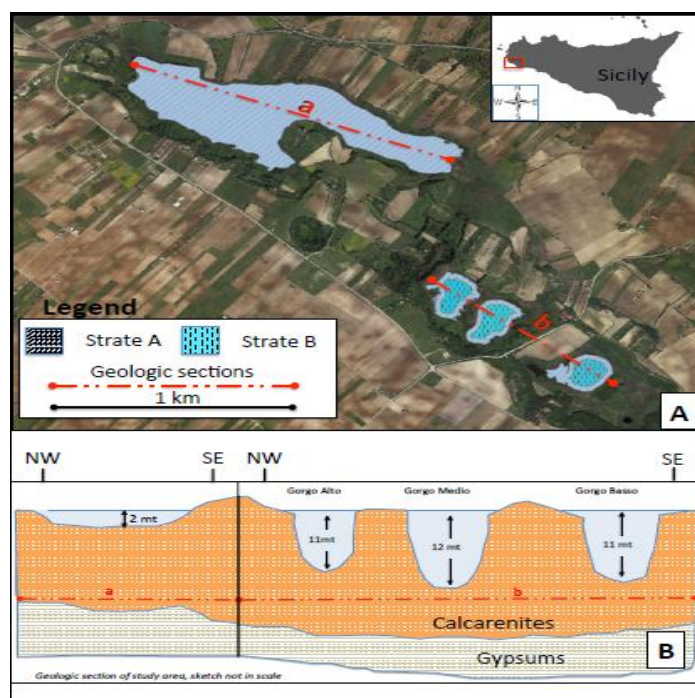


Figure 1. (A) Study area of the Gorghi Tondi WWF Nature Reserve in Mazara del Vallo, Sicily (Italy). (B) Geologic characteristic of study area, figure not to scale, lake depth in metres.

2011; Scalici *et al.*, 2010). All individuals were weighed (Kern KB 650–2NM) to measure the CPUE and the total capture for each sampling point.

When all measurements were finished, the sample was transported to the laboratory for further biochemical and genetic studies.

Differences in body-size and weight among lakes and sexes were assessed using ANOVA. Before running the ANOVA, the assumptions of normality of data and homogeneity of variance were tested using one-way Kolmogorov–Smirnov (K–S) and Levene’s tests, respectively.

When the differences were significant, a Tukey’s HSD test was performed to check which pairs were significantly different.

The sex ratio (males/females), were evaluated by χ^2 tests (with the Yates correction when required) for the entire sample as well as for each sampling session.

All the statistical analyses were carried out using statistical software R (R 3.0.1).

Analysis of CPUE and Spatial Distribution

The CPUE was evaluated as the total biomass captured in each of the four lakes per trap, separated by sex and sampling session. The value of the CPUE rates was expressed in $g \cdot h^{-1}$. Differences in CPUE among the different sites, sampling sessions and sexes were evaluated using the Kruskal–Wallis test.

When the differences were significant, a multiple comparison test was performed to check which pairs were significantly different.

The spatial distributions of the several levels of CPUE during the sampling sessions from May to 2

were evaluated by subdividing the surface area of each lake using a regular grid with 50-m cells on each side (termed the elementary sampling unit, “ESU”). Each ESU was sampled using a trap with a depth-stratified sampling design. The number of traps per stratum was proportional to the stratum surfaces. The depth strata (layers) adopted in each sampling sessions were as follows: layer A from 0 to –2 m and layer B from –2 to –12 m. This division was used to distinguish the areas characterised by the typical vegetation cover of the lakeshores, which is present at depths up to –2 m. In fact, beyond this depth, the lake bottom surfaces are typically muddy and devoid of the aquatic plants described earlier. Preola Lake was subdivided into 120 ESUs (all in layer A); in the Gorghi Tondi lakes, 13 ESUs were identified for each lake (7 in layer A and 6 in layer B). For each lake, the CPUE was calculated as the weight of crayfish caught per trap per 72 h period ($g \text{ of crayfish} \cdot \text{trap} \cdot 72 \text{ h}^{-1}$). Later, each trap was examined to check if cannibalism had occurred. Data were spatially interpolated using an Inverse Distance Weighting (IDW) interpolation method (Hengl, 2009) contained in a geographical information system (Q-GIS 1.4.0, 2014). This interpolation estimates values at unknown points based on nearby known points, weighted by distance.

Structure, Dynamics and Growth Parameters

The cephalothorax length (CTL) was used to assess the population structure. The data were used to generate a 1-mm polymodal frequency distribution (FD), and then the histograms were analysed using the Bhattacharya method (BM) in the software package

FiSAT II (FAO-ICLARM Stock Assessment Tools; (Gayaniilo *et al.*, 1996). The size frequency data were analysed and decomposed into Gaussian components using iterative computations of regression lines until the total decomposition of the overall distributions was achieved (Gayaniilo and Pauly, 1997; Ligas, 2008; Scalici *et al.*, 2010). Each component was identified as cohort. For each Gaussian component, the mean (M) and standard deviation (SD) were calculated, and a separation index (SI) was computed as the difference between two successive means. Values of SI greater than or equal to 2 indicate that the Gaussian component can be considered well-separated.

Parameters of the von Bertalanffy (1938) growth equation were estimated using the ELEFAN I procedures included in FiSAT II (non-parametric scoring; Gayaniilo and Pauly, 1997; Pauly and David, 1981):

$$L(t) = L_{\infty} \{1 - e^{-k(t-t_0)}\}.$$

Longevity (t_{max}) was estimated by $t_{max} = (3/k) + t_0$ and mean (Gayaniilo and Pauly, 1997; Scalici and Gherardi, 2007). The growth performance index (Φ) was obtained applying the Pauly's equation $\Phi = \log(k) + 2\log(L_{\infty})$ (Pauly and Munro, 1984) to assess different stocks' growth performance in terms of length (Pauly and Munro, 1984).

The fishing mortality (F) was calculated by subtracting natural mortality (M) from total mortality (Z) (Pauly, 1980). The total mortality index (Z) was estimated in FiSAT II using the Powell–Wetherall plot equation and M was obtained by solving the equation $\log(M) = -0.0066 - 0.279\log(L_{\infty}) + 0.6543\log(k) - 0.463\log(T)$, where T is the mean environmental temperature during the study period (Pauly, 1980).

Results

During the survey the trap examination did not revealed cannibalism phenomena (parts of carapace, claws or other part attributable at the body of the specimens) among the seven sampling sessions. A total of 1,045 individuals (444 females and 601 males) were captured by trapping, in the study area, during the seven sampling sessions; the sex ratio (Male/Female) deviated significantly from 1 ($\chi^2 = 47.17$, $df = 1$, $P < 0.05$). In particular, the specimens captured in the sampled area were: 717 in Preola Lake (290 females and 427 males) and 328 in the Gorgi Tondi lake (58 females and 48 males in Gorgo Alto; 48 females and 67 males in Gorgo Medio; and 48 females and 59 males in Gorgo Basso).

In the Gorgi Tondi lake, the sex ratio was not significantly different from 1 in Gorgo Alto ($\chi^2 = 1.89$, $p = 0.16$) and Gorgo Basso ($\chi^2 = 2.26$, $p = 0.13$), while the proportion male/female was different from 1 in Gorgo Medio ($\chi^2 = 6.28$, $p = 0.01$). In Preola the proportion male/female was different from 1 ($\chi^2 = 52.35$, $P < 0.001$). The proportions between males and females were the same in January ($\chi^2 = 3.45$, $p = 0.07$), May ($\chi^2 = 0.88$, $p = 0.35$) and in July 2013 ($\chi^2 = 0.33$, $p = 0.56$), while they were different from June to October [June ($\chi^2 = 4.33$, $p = 0.03$); August ($\chi^2 = 24.82$, $P < 0.001$); September ($\chi^2 = 39.69$, $P < 0.001$); October ($\chi^2 = 25.16$, $P < 0.001$)] (Figure 2).

During the seven sampling sessions in the study area the mean CTL was 39.84 ± 7.06 mm in females (min–max: 21.15–64.56 mm) and 40.34 ± 6.03 mm in males (min–max: 21.01–61.06 mm). The average values of the CTL for all samples did not reveal a size difference between males and females ($F_{1,1043} = 0.82$, $p = 0.37$) (Figure 3), nor among the sampling areas

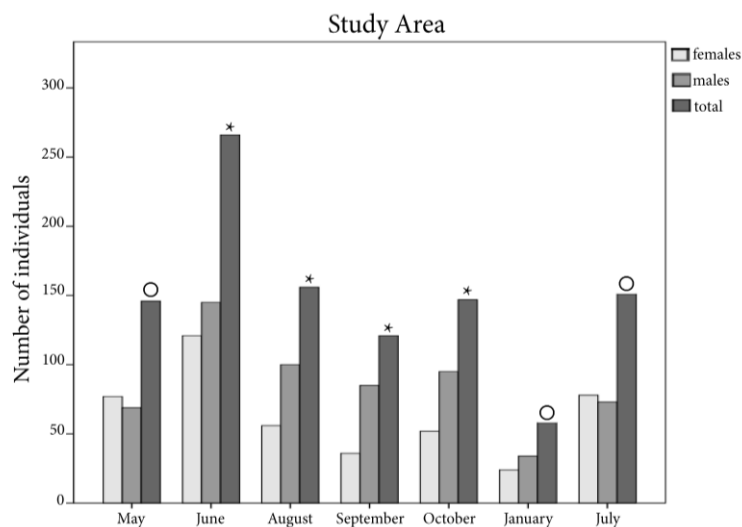


Figure 2. Number of individuals caught in the study area (all lakes) in the seven sampling sessions, separated by sex. Different symbols represent significant differences in sex ratio during the sampling sessions: * samples with a sex ratio significantly different from 1:1 after a chi-squared test; sample with a balanced sex ratio after a chi-squared test; ' difference significance .

($F_{3,1041} = 53.68$, $p > 0.05$). Otherwise, significant differences among the seven sampling session in the mean CTL value were found between males ($F_{6, 594} = 7.16$; $p < 0.05$) and females ($F_{6, 437} = 7.27$; $P < 0.05$) captured (Figure 4).

CPUE and Spatial Distributions

The mean CPUE value in males increased from May to June, when they reached the maximum ($0.29 \text{ g} \cdot \text{h}^{-1}$), whereas the lowest value ($0.06 \text{ g} \cdot \text{h}^{-1}$) was observed in January. The CPUE trend was similar in females; the maximum values were registered in September ($0.31 \text{ g} \cdot \text{h}^{-1}$), whereas the minimum values were recorded in January ($0.07 \text{ g} \cdot \text{h}^{-1}$). No specimens were captured in the five sampling sessions considered in layer B. CPUE values seem to have a similar distribution in females and males (Figure 5).

Kruskal-Wallis test showed that the CPUE captures were not significant different among lakes in the same layers ($KW = 2.45$, $p = 0.48$). CPUE captures

were significant different between sexes ($KW = 12.86$; $P < 0.001$) and were dissimilar among sampling periods ($KW = 202.69$, $P < 0.001$), showing differences between each pair of sampling period except June-September.

A graphical representation of the abundances of *P. clarkii* in the four lakes during five sample sessions and between sexes was made using a geographical information system (GIS) (Figures 6 A, B).

Structure, Dynamics and Growth Parameters

All CTL measurements were used to generate size frequency distributions for the seven sampling periods (Table 1). The CTL data for the captured specimens were grouped into two different datasets for males and females in order to apply the Bhattacharya method. The frequency distribution distinguished by sexes with information on the reproductive biology of the species (Chiesa et al., 2006; Gherardi et al., 1999) allowed to classify the

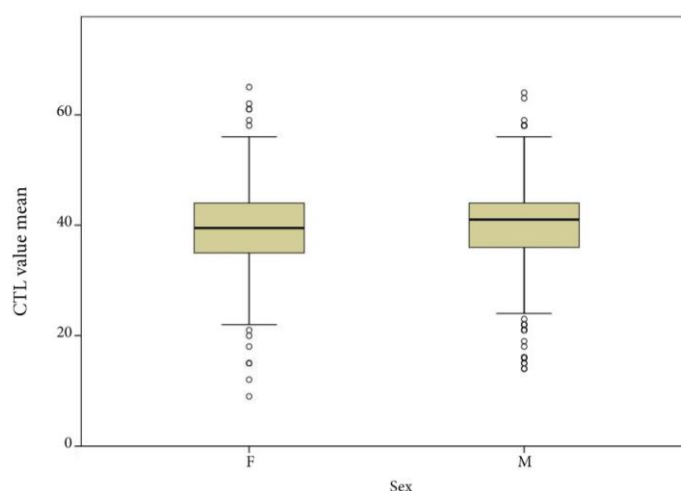


Figure 3. Values of cephalothorax length (CTL) (Median \pm 25th–75th percentiles; whiskers \pm 1st–99th percentiles) of specimens captured in the study area during the seven sampling sessions.

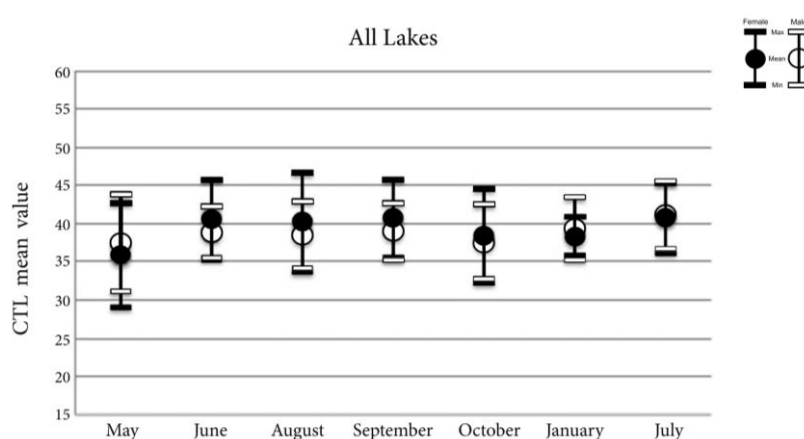


Figure 4. Mean and standard deviation of the cephalothorax length (CTL) for the seven sampling session, separated by sex.

crayfish collected into 3–5 age classes or cohorts for females and into 2–5 for males (Table 1). In particular, five Gaussian components were found for both females and males in all sampling sessions, except the January session, when two components were observed in males and three in females. All outputs from the Bhattacharya method analysis were not significant (after χ^2 test, $P > 0.05$ always).

Table 2 reports the estimates of the growth parameters ($k = 0.340$ and 0.350 , $L_\infty = 68.25$ and 67.20 , $t_0 = -0.110$ and -0.260 for male and female respectively), the growth performance index ($\Phi = 3,19$ for both sexes), total mortality ($Z = 3.43$ and 3.83 for male and female respectively), natural mortality ($M = 1,14$ and $1,16$ for male and female respectively), fishing mortality ($F = 2.29$ and 2.67 for male and

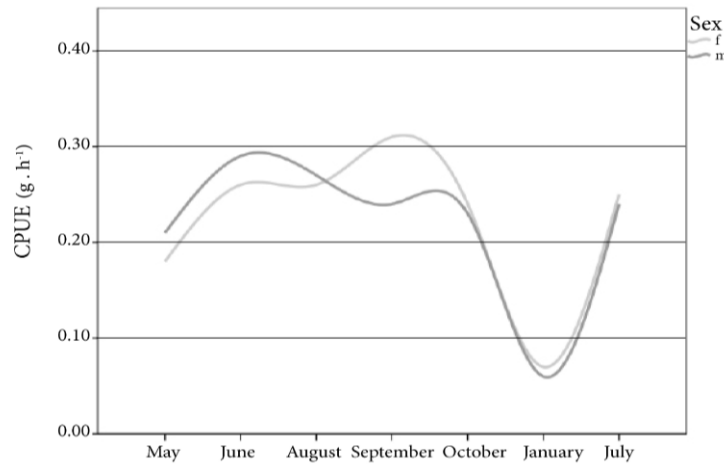


Figure 5. Mean value of the CPUE for the seven sampling sessions, separated by sex.

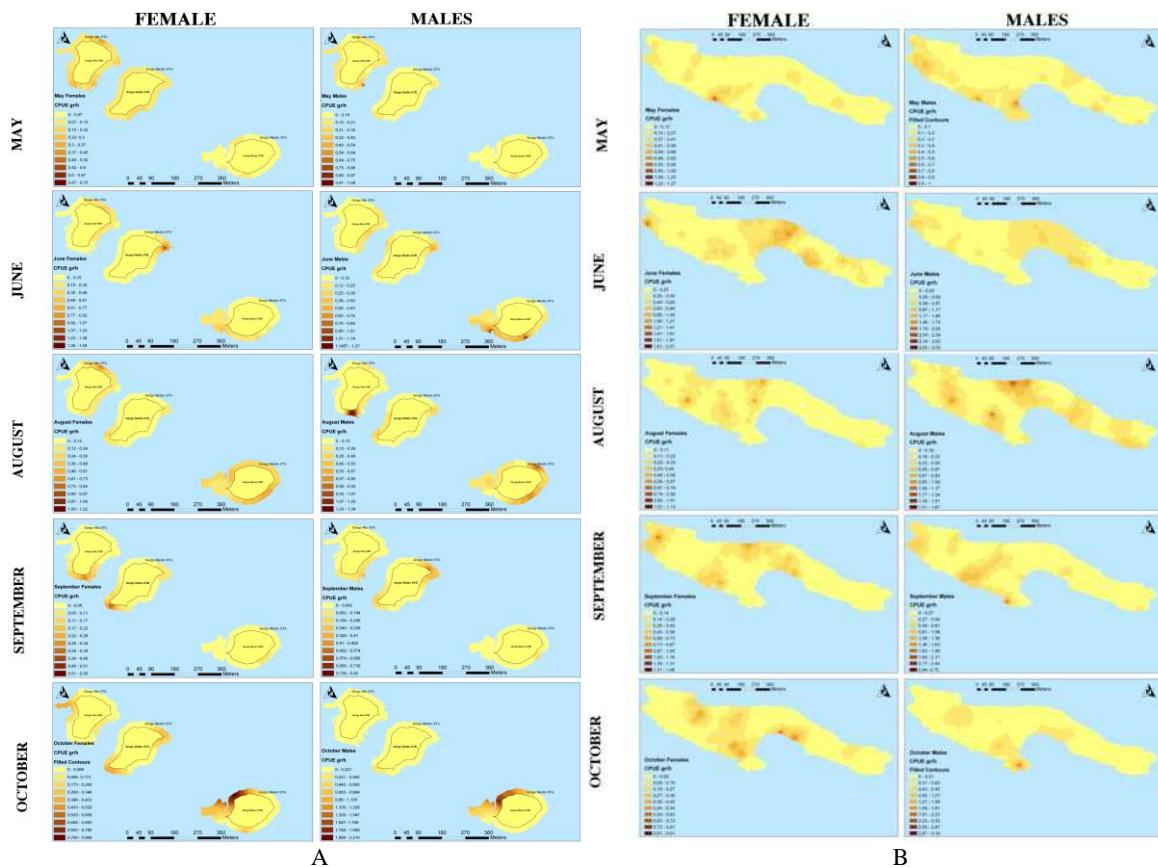


Figure 6. Spatial distribution during the sampling session expressed as a CPUE ($gr^{*}h^{-1}$) in the Gorgho Tondi lakes (A) and Preola Lake (B). Values of CPUE expressed in the map of interpolation shows the rates of captures per sex in each lake for each sampling session.

Table 1. Values of chemico-physical parameters recorded in Preola and Gorgi Tondi lakes during each sampling session

Month	Lake	Hours of sampling	Temperature	Sallinty ‰	DO %	DO mg/l	Ph
May 2012	Preola	08.25 a.m.	22.42	2.28	72.60	6.21	7.97
	Gorgo Alto	09.32 a.m.	21.21	1.93	65.68	5.78	8.13
	Gorgo Medio	10.18 a.m.	21.13	1.59	89.60	7.89	7.81
	Gorgo Basso	14.01 p.m.	24.47	1.60	89.40	7.39	8.05
June 2012	Preola	09.15 a.m.	28.54	2.25	96.50	7.41	8.20
	Gorgo Alto	10.22 a.m.	27.37	2.47	65.01	8.83	8.50
	Gorgo Medio	11.40 a.m.	26.93	2.39	67.90	5.35	8.35
	Gorgo Basso	14.05 p.m.	27.27	2.47	79.80	6.24	8.37
August 2012	Preola	08.43 a.m.	27.80	1.98	96.50	8.91	8.49
	Gorgo Alto	10.06 a.m.	25.77	1.60	30.90	2.48	8.24
	Gorgo Medio	11.45 a.m.	25.26	1.75	18.40	1.50	8.19
	Gorgo Basso	14.30 p.m.	28.48	1.60	89.40	6.84	8.16
September 2012	Preola	09.03 a.m.	27.52	2.49	98.20	7.62	8.68
	Gorgo Alto	09.55 a.m.	26.68	2.54	95.90	7.58	8.53
	Gorgo Medio	10.36 a.m.	26.99	2.51	88.58	7.82	8.35
	Gorgo Basso	12.30 p.m.	26.15	2.57	93.70	7.47	8.38
October 2012	Preola	08.15 a.m.	24.81	2.14	70.30	5.70	8.07
	Gorgo Alto	09.59 a.m.	23.47	2.58	76.20	7.00	7.00
	Gorgo Medio	11.16 a.m.	22.96	2.51	77.90	6.90	8.10
	Gorgo Basso	12.45 p.m.	21.86	2.30	73.40	6.23	8.40
January 2013	Preola	09.16 a.m.	13.10	1.88	12.01	1.99	7.41
	Gorgo Alto	10.17 a.m.	14.00	1.61	11.00	1.12	7.35
	Gorgo Medio	13.44 p.m.	14.13	1.42	10.40	1.50	7.36
	Gorgo Basso	12.15 p.m.	13.09	1.50	9.90	1.05	7.42
July 2013	Preola	13.00 p.m.	29.26	2.80	89.50	6.78	8.18
	Gorgo Alto	10.24 a.m.	28.48	2.51	89.40	6.84	8.43
	Gorgo Medio	09.19 a.m.	27.27	2.47	79.80	6.24	8.37
	Gorgo Basso	11.10 a.m.	26.93	2.47	67.90	5.35	8.35

female respectively), longevity ($t_{max} = 8.71$ and 8.31 for male and female respectively), and mean lifetime ($t_{1/2} = 4.36$ and 4.16 for male and female respectively). Figure 7 describes the growth model obtained with ELEFAN.

Discussion

The present study aimed to improve the knowledge of the population of *P. clarkii* in the Preola Lake and Gorgi Tondi WWF Nature Reserve by adopting two types of sampling design.

Highest crayfish captures were concentrated in summer and autumn, in line with what has been observed in other similar studies (Anastácio and Marques, 1995; Chucholl, 2011; Fidalgo et al., 2001; Scalici and Gherardi, 2007; Scalici et al., 2010). Adult mostly composed the captured specimens, which was demonstrable by the minimum CTL length of the crayfish in females (21.15 mm) and in males (21.01 mm) as affirmed in the studies of Huner (2002) and Scalici et al. (2010).

At the end of the study, considering the seven sampling sessions jointly, the sex ratio result was not balanced, contrary to what was observed in Portugal, Kenya and Louisiana (Fidalgo et al., 2001; Huner, 2002). This result of sex ratio alterations has been widely documented (Anastácio and Marques, 1995; Costa et al., 1996; Dörr and Scalici, 2013; Fidalgo et

al., 2001; Frutiger et al., 1999; Ligas, 2008; Ouloch, 1990; Pérez-Bote et al., 2000), but it is difficult to link it to a specific cause because the environmental conditions of the habitat, the type and availability of food, and the reproduction period can vary across the considered populations of *P. clarkii* (Dörr and Scalici, 2013; Ligas, 2008; Scalici and Gherardi, 2007; Scalici et al., 2010). Nevertheless, it could be explained by taking into account the natural behaviour of some species of decapod crustaceans, i.e. individual females in a premoult state occupy temporary burrows, presumably to accumulate reserves sufficient for producing a clutch of eggs (Christy and Salmon, 1984; Turner et al., 2003), creating muscle growth (Medici et al., 2006), and providing parental care (Thiel, 2000). Fig. 2 shows that these hypotheses can be linked to our results; in fact, the discrepancy in the sex ratio appears within the months in which the females are in full reproductive activity (Anastácio and Marquez, 1995; Scalici et al., 2010). In addition, the role played by the bait should be considered; in fact, the feeding preferences of *P. clarkii* can be very different in function of several factors: typology of bait, habitat, seasonality, sex, and size. These variables could influence both the sex ratio and the capture capacity of the traps (Alcorlo et al., 2004; Smart et al., 2002).

The movements of crayfish inhabiting streams, ditches and ponds have been described by several authors (Fast and Momot, 1973; Flint, 1977; Gherardi

Table 2 Analysis of cephalothorax length (CTL) frequencies after the application of Bhattacharya's method in the study area. Means (M) and standard deviation (s.d.) of the cephalothorax length, S.I denote the separation index and N is the theoretical number of individuals

	Females					Males			
	CG	M	s.d.	S.I	N	M	s.d.	S.I	N
May	1	30.05	1.70		23	24.50	1.20		7
May	2	34.54	1.47	3.46	18	28.80	1.00	3.91	11
May	3	39.87	1.20	3.34	14	33.94	1.84	3.62	27
May	4	43.13	0.99	3.09	15	41.56	1.38	4.73	16
May	5	45.50	0.79	2.66	6	46.69	1.47	3.60	6
June	1	31.17	1.20		8	33.00	0.67		5
June	2	39.23	1.81	5.36	45	36.72	1.46	3.49	22
June	3	44.24	1.97	2.65	38	42.13	1.39	3.80	51
June	4	50.70	1.29	3.96	11	45.49	1.49	2.33	31
June	5	54.64	0.97	3.49	3	51.37	1.16	4.40	10
August	1	36.90	1.81		17	33.50	1.57		6
August	2	41.22	0.91	3.18	6	38.52	2.32	2.58	36
August	3	44.38	0.93	3.43	11	44.86	2.11	2.86	47
August	4	49.45	1.28	4.59	18	49.04	1.45	2.35	8
September	1	31.17	1.70		6	28.81	1.36		7
September	2	36.71	1.69	3.27	5	36.38	1.49	5.31	13
September	3	42.15	1.70	3.21	14	39.97	0.89	3.02	13
September	4	49.00	0.67	5.78	5	43.03	1.51	2.55	36
September	5	56.50	1.20	8.20	7	46.90	1.71	2.40	28
October	1	32.89	1.03		14	30.17	0.69		7
October	2	35.94	0.72	3.49	6	34.00	0.67	5.63	5
October	3	40.50	1.20	4.75	7	37.52	1.04	4.12	7
October	4	43.00	0.70	2.63	5	43.02	1.19	4.93	38
October	5	50.50	0.85	9.68	10	47.90	2.17	2.30	18
January	1	39.50	2.950		5	20.19	1.86		16
January	2	41.50	0.960	2.08	4	25.50	0.85	3.99	5
January	3	47.23	0.820	2.19	7				
July	1	27.50	1.20		7	32.00	0.85		9
July	2	34.56	1.70	4.8	13	35.02	0.66	4.00	15
July	3	37.77	0.90	2.4	28	38.20	0.81	4.33	24
July	4	42.17	1.59	3.5	23	41.50	0.91	3.84	14
July	5	47.05	1.92	2.7	18	46.50	1.20	4.74	7

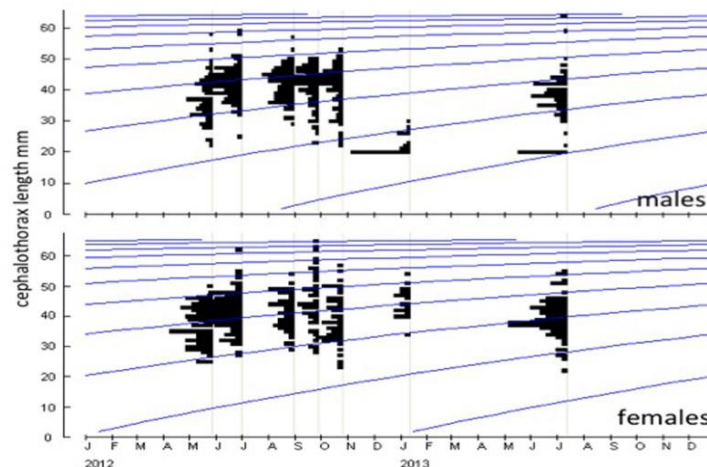


Figure 7. Growth model obtained by electronic length frequency analysis (ELEFAN), distinguished by sex, the histograms represent the distribution of the lengths of the crayfish samples; the curve represent the VBGF model for each size class.

et al., 1998; Guan and Wiles, 1997; Hazlett *et al.*, 1974, 1979), who have indicated how a number of factors, such as density pressure, availability of burrows, reproductive conditions, sediment types,

water level, and other physical parameters (e.g. light) are responsible for the distribution of crayfish in their habitat. In the present work, within the entire study area the results for the CPUE underlined the strong

seasonal trend in the capture rates, as already observed in other studies (Anastácio *et al.*, 2009; Chucholl, 2011; Dörr *et al.*, 2012; Fidalgo *et al.*, 2001; Gherardi *et al.*, 2000; Scalici *et al.*, 2010). In Preola Lake, the IDW-GIS analysis evidenced heterogeneous catch rates between areas near and far from the shore and between sexes and sampling sessions (Fig. 6A). On the other hand, in the Gorgi Tondi lakes the captures always occurred along the shore (Fig. 6B), and no specimens were found in layer B (more than 2 m in depth). This difference could be related to the characteristics of the lakes' bed, as evidenced in other cases (Paillisson *et al.*, 2011), as well as to the movement patterns of the species (Gherardi *et al.*, 2000, 2002, 2007).

P. clarkii has a very plastic life cycle and is capable of surviving in tropical, subtropical and temperate

climates worldwide (Chucholl, 2011). Within its natural subtropical-temperate range, this species breeds year round, with distinct recruitment peaks from the late summer to early winter and again in the spring (Chucholl, 2011). In southern Spain, *P. clarkii* was shown to reproduce from April to late October, with ovigerous females found to be continuously present from May to October (Gutiérrez-Yurrita *et al.*, 1999). The present study suggests the possibility of two recruitment phases, once in late summer and again in winter, as described by Anastácio and Marques (1995) about a *P. clarkii* population in central Portugal and by Gherardi *et al.* (1999) about a population in central Italy. Our hypothesis has been confirmed by the characteristics of the samples collected in January and June (Fig. 7), where the number of young specimens is more consistent compared with the crayfish from the other sampling sessions, which is confirmed by others studies in the same latitudes (Dörr and Scalici, 2010; Dörr *et al.*, 2006; Scalici *et al.*, 2010). Nevertheless, in some populations, up to three recruitment events were found per year (Anastácio and Marquez, 1995; Huner, 2002).

To define age classes in the study population the Bhattacharya method was applied to size frequency. This is instrumental in compensating for the obvious absence of more reliable data from skeletal structures, such as scales, vertebrae, and otoliths, which are not reliable for determining the age of individuals (Scalici and Gibertini, 2009). In the present study the values obtained for t_{max} were the result of the frequency/length relation. In fact, the population structure, analysed with the Elefan method, showed a maximum of five cohorts for both females and males (Table 1). However, considerations on the trap typology and its selectivity in terms of captured individuals' size should be noted (Paillisson *et al.*, 2011). The first regards the study areas: these are natural sites characterised by the presence of a large number of protected species, among which is *Emys trinacris*, an endemic turtle species. For this reason,

and to minimise the risk of bycatches, the traps were designed to minimise these accidents – indeed, no bycatches occurred during the entire sampling period. Furthermore, the choice to use small portions of bait (50 g of liver) was opted to minimise the disturbance of fishing surveys in this protected ecosystem. The second regards the sampling design: indeed the traps were selected for specimens with CTL > 21 mm; in fact, beyond this size class, the captured animals began to be more numerous. However, it is possible to hypothesise that this selectivity also affected the largest specimens, preventing the access, and then capture, of individuals with CTL > 64 mm. This hypothesis can be confirmed by the results of other studies that used traps with a different and similar gear selectivity (Dörr and Scalici, 2013; Dörr *et al.*, 2006; Scalici *et al.*, 2010). In these cases, specimens were found with a CTL > 65 mm from populations of *P. clarkii* (Dörr *et al.*, 2006; Scalici *et al.*, 2010). Observing the Bhattacharya computed plot (Fig. 7), it is possible to note that the t_{max} results in eight growth lines, equivalent to about eight cohorts (Table 2). In this area and for this population, two recruitment phases per year were hypothesised (late summer and winter) (Sommer, 1984), as shown by other authors (Chiesa *et al.*, 2008; Dörr *et al.*, 2006; Scalici *et al.*, 2007, 2010). For these reasons it seems appropriate to give an approximate lifespan of 4 years, as also suggested by other authors (Chiesa *et al.*, 2006; Huner, 2002; Ligas, 2008), whereas other studies proposed that the lifespan of *P. clarkii* can be much longer, at 5–6 years (Chucholl, 2011; Dörr *et al.*, 2013).

Conclusions

This report describes the first study conducted in a southern Sicily reserve area concerning the assessment of the spatial distribution, population structure and growth parameters of *P. clarkii*. The study area is a very important site from the perspective of conservation; in fact, it was included in the NATURA 2000 network (SIC ITA010005; ZPS ITA010031).

The study conducted on the population dynamics has confirmed the arguments of other authors. In particular, the growth parameters were similar to other studies conducted in the Mediterranean region. This can be attributed to several contingency factors, including the geographical, thermal and biotic characteristics of the habitat. In fact, the population was more active during the warm months (from June to September). In contrast, in the autumn and winter months, the lower activity of the species was evidenced by lower catch rates.

Moreover, our results highlight no difference in CTL between males and females and CPUE showed high values in males from May to August, probably due to ovigerous female activity (Scalici and Gherardi, 2007) linked to the burrowing habit of

berried females (Huner, 2002), while the opposite evidence was found from September to October.

The capture maps evidenced a heterogeneous use of the lakes' surface; in fact, during the five sampling sessions a preferred zone of population distribution inside the lakes' surface areas was not found. This could be attributed to three factors: homogeneous characteristics of vegetation cover and geological structure along the shores; the limited extension of the colonised areas; a large movement range of the specimens (Gherardi *et al.*, 2000).

The presence of bioinvader species in the Natura 2000 network could threaten many valuable species and habitats. This study underlines the importance of knowledge of the population dynamics of alien species and their use of the invaded habitat to ensure that future management is ecologically and economically sustainable.

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