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-RESEARCH ARTICLE-

First Scientific Records of the Invasive Red Swamp Crayfish, *Procambarus clarkii* (Girard, 1852) (Crustacea: Cambaridae) in Malta, a Threat to Fragile Freshwater Habitats.

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Abstract

This study reports the first records of the invasive fresh water red swamp crayfish, *Procambarus clarkii* in Malta, first spotted in the wild in summer 2016. In spring 2017, 26 specimens of *P. clarkii* were collected, sexed, measured and identified both morphologically and genetically. The distribution and density of the species along the same valley were investigated at Fiddien Valley and Chadwick Lakes. As these two locations are associated with one of the major valley systems that cross the island, the presence of this prolific, aggressive and opportunistic feeding crayfish species increases the vulnerability of native freshwater species.

Keywords:

Procambarus clarkii; freshwater crayfish; invasive; new record; Malta

Article history:

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Introduction

Procambarus clarkii (Girard, 1852), the red swamp crayfish, is a freshwater species native to North-Eastern Mexico and South-Central USA (Hobbs, 1989). It is a generalist opportunistic omnivore (Alcorlo et al., 2004) and has behavioural plasticity that allows it to cope with changing environmental and biological conditions (Ilheu et al., 2003; Dörr et al., 2006; Grey and Jackson, 2012). *Procambarus clarkii* has an ability to tolerate pollution, acting as a bioaccumulator

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threatening higher trophic levels (Gherardi, 2006; Suárez-Serrano et al., 2010), while having a lower susceptibility to *Aphanomyces astaci*, the pathogen responsible for the crayfish plague, giving *P. clarkii* an advantage over other crayfish species (Schrimpf et al., 2012). Additionally, this species has a high ability to proliferate as it reaches sexual maturity within a few months, has high fecundity ranging between 100 to 500 eggs (GISD, 2017) and in areas with hydroperiods longer than 6 months it is able to undergo a second reproductive period (Dörr et al., 2006). These various attributes compounded by the intentional and accidental releases from the aquarium and aquaculture industry (GISD, 2017; Nunes et al., 2015) have a synergistic effect on the invasiveness of *P. clarkii* outside their natural range, making it the most widely distributed freshwater crayfish worldwide (Gherardi, 2006; Holdich et al., 2009). This has lowered native species richness in the areas it invades while causing the largest heterogeneity of impacts (Garcia et al., 2015). *Procambarus clarkii* is not the only introduced non-indigenous crayfish species (NICS), as the increasing influx of NICS is becoming a major concern in Europe (Savini et al., 2010), with almost every country having records of NICS (Nunes et al., 2015).

The invasion of *P. clarkii* in Europe has started a couple of decades ago, with the first records in Spain dating back to 1973 (Holdich et al., 2009). Since then several successive records of this species have been recorded in various freshwater bodies in most of the Central and Southern European countries (Holdich et al., 2009; Cilenti et al., 2017), and other Mediterranean localities including Israel (Wizen et al., 2008), and islands (Holdich et al., 2009) such as Sicily (D'Angelo and Lo Valvo, 2003; Di Leo et al., 2014). In Europe, the occurrence of this NICS is considered as a major threat, causing localized extinctions of native species such as such as, *Astacus leptodactylus* and *Austropotamobius pallipes*, leading to large annual economic losses (Holdich et al., 2009; Sundseth, 2014; Souty-Grosset et al., 2016). In 2016, the EU commission through EU regulation 2016/1141 has enlisted both *P. clarkii* and *P. fallax* f. *virginalis* as species that fall within the legal framework of EU regulation 1143/2014, as invasive species of concern to European biodiversity (EUR-Lex, 2017). Thus at EU level, there is a clear objective to prevent the introduction, establishment and spread of these species. The establishment of early detection systems and rapid response measures would lead to better effective management measures (EUR-Lex, 2017).

Material and methods

The first specimens of *P. clarkii* were spotted at Fiddien Valley (35°53'21.85"N 14°22'50.51"E) in September, 2016 by Denis Magro (Magro 2016; *pers. comm*. 2017). Scientific field surveys at the area and further down the valley to Chadwick Lakes (35°53'24.94"N 14°23'3.43"E) were conducted in Spring 2017 (Figure 1 and 2). During the latter surveys, estimates of the density of the specimens in the drying pools of the areas were measured. 26 specimens were also collected, 13 from Fiddien Valley and 13 from Chadwick Lakes. Once captured, the specimens were photographed (Figure 3) and morphologically identified according to Morehouse and Tobler, (2013) in the lab. They were sexed and their body size (length and weight) measured to the nearest 0.1 mm and 0.1 g respectively. Ten specimens (five from each sampling site) were used to collect tissue samples and were preserved in 100% ethanol. The rest of the specimens were stored at -20°C for further studies at the Conservation Biology Research Group laboratory.

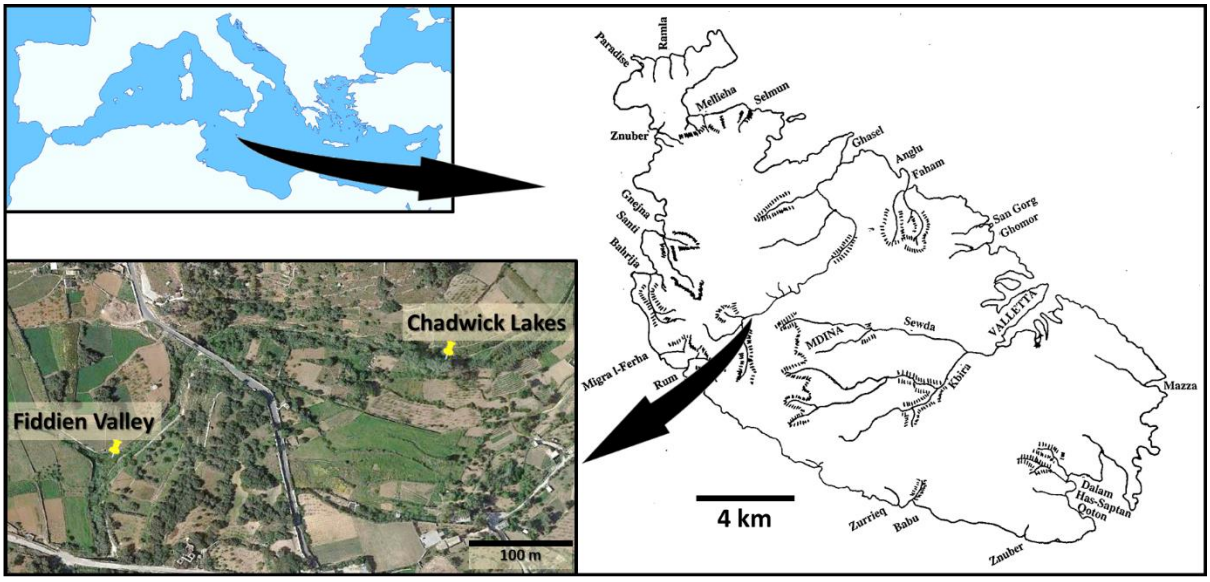

Figure 1. A map showing the locations from where *Procambarus clarkii* was collected (Map of Malta: Haslam and Borg, 1998; Aerial photo of the areas studied: Google Earth, 2013).

Figure 2. Photos showing *Procambarus clarkii* individuals recorded on the sides of clay bottom freshwater courses towards the end of the wet season in Malta (Photo on left: Fiddien Valley - characterized by sub-adults and adult individuals; Photos on right: Chadwick Lakes - characterized by juveniles and sub-adults).

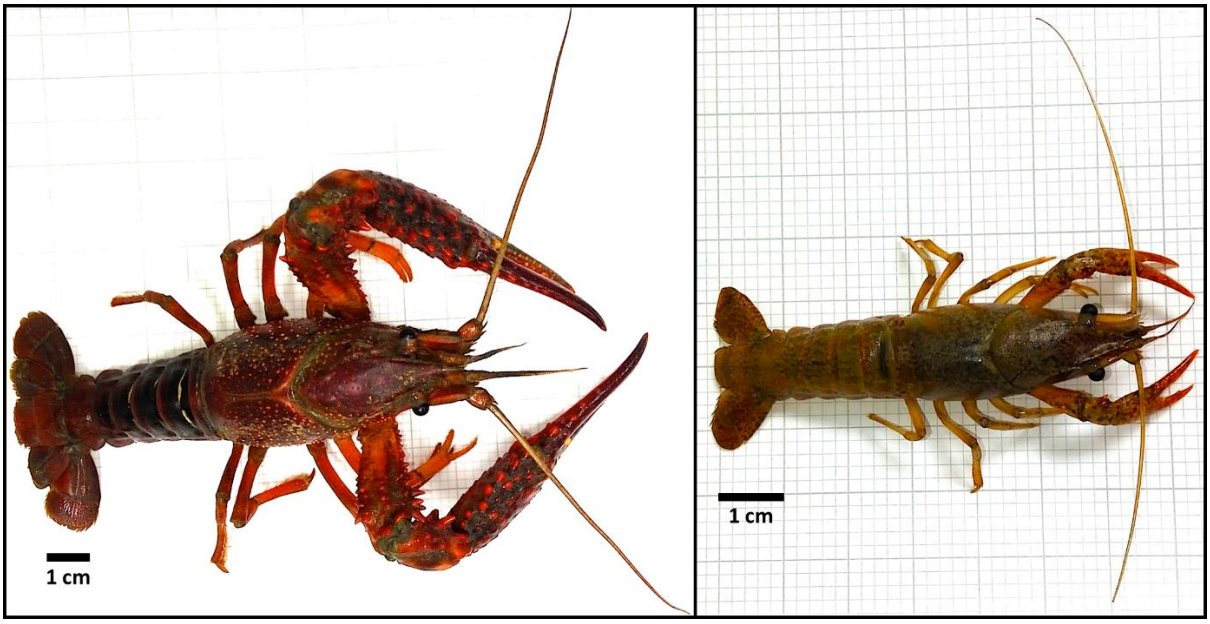

Figure 3. Photos showing two of *Procambarus clarkii* specimens collected (Left: specimen collected from Fiddien Valley; Right: specimen collected from Chadwick Lakes).

The genomic DNA was extracted from the preserved tissue samples using AccuPrep Genomic DNA Extraction Kit (Bioneer Inc.). PCR amplification of the cytochrome c oxidase I gene (COI) was carried using the primers HCO1490 and LCO2198 (Folmer et al., 1994), following the amplification protocol described in Kambhampati and Smith, (1995). PCR products were purified and sequenced via ABI3730XL sequencer (Applied Biosystems) using both the forward and reverse primers. Sequences were checked for consistency using Geneious v10 (Kearse et al., 2012) and were compared to other sequences available in genomic databases using BOLD and BLASTn (Ratnasingham and Hebert, 2007; NCBI, 2017). The genetic data generated was deposited in GenBank, under accession numbers MF170527-36.

Discussion

These results provide scientific and conservation considerations on the occurrence of *P. clarkii* in one of Malta's major valley freshwater courses. The ability of this species to adapt to periods of drought (Ilheu et al., 2003; McClain, 2013) together with its ability to survive outside aquatic environment makes it capable of moving to more favourable environmental conditions especially during the dry season when the water availability and aquatic food sources diminish. The latter allows this species to find refuge in artificial freshwater reservoirs, such as those used for agriculture or the freshwater systems including underground waters as noted in Italy (Mazza et al., 2014). Since its detection in September 2016, the species has expanded its population size and range, covering at least 400m between October 2016 and March 2017, consistent with similar studies conducted in Italy (Cilenti et al., 2017). This spreading rate is of conservation concern, given that the area studied marks the beginning of one of the longest water systems on the island (Figure 1), and does not exclude the possibility that this species could spread further from the main valley system expanding its range through smaller water courses in the area, possibly effecting other valleys.

Also of concern is how such an invasive species was artificially introduced in this vulnerable freshwater habitat in the first place by human actions, pointing toward the pressing need to monitor these habitats to prevent further introductions and spreading of such alien species. It has been found that in Malta species of the genus *Procambarus*, including *P. clarkii* and *P. fallax*, are imported as part of the aquarium industry (Malta Aquarist Society, 2011), with the latter species having a parthenogenetic form (Martin et al., 2010) that is already invading parts of Europe (Holdich et al., 2009; Bohman et al., 2013; Vojkovská et al., 2014). Reckless release of these species in the wild together with the lack of local awareness and lack of local enforcement on the trading of NICS can lead to adverse effects to the local freshwater habitats and biodiversity.

The decline in native species observed at the studied Fiddien Valley pond, indicates that *P. clarkii*'s prolonged presence led to food web impairment as its opportunistic feeding behaviour on macrophytes and macrofauna (Alcorlo et al., 2004; van der Wal et al., 2013; Garcia et al., 2015) depleted most of the aquatic resources in its surrounding environment. The predatory behaviour towards tadpoles recorded in Chadwick Lakes, has caused the disappearance of this frog species in the Fiddien Valley pond too. As an invasive species, it grows becoming the dominant species and disrupting the native community, while also changing the structure of the habitat. The occurrence of juvenile crayfish in the Fiddien Valley area was very low and claw remains of crayfish were also observed. These observations support the cannibalistic behaviour of larger adults on sub-adults and juveniles as densities increase (Marcal Correia, 2003; Alcorlo et al., 2004; Mueller, 2007). The latter allowing some control on the population growth of this invasive species when other prey resources are depleted.

The Fiddien Valley is part of a large water system (Figure 1) and is in close proximity to other freshwater courses some of which host the protected endemic Maltese Freshwater Crab, *Potamon fluviatile lanfrancoi* (Capolongo and Cilia, 1990). The *P. clarkii*'s aggressiveness, feeding behaviour and habitat modification abilities are a potential threat to this highly vulnerable species. Moreover, it cannot be excluded that these crayfish might be carriers of exotic microorganisms, such as *Aphanomyces astaci* (Dieguez-Uribeondo et al., 1995; Aquiloni et al., 2011), spreading it to other crustaceans (Svoboda et al., 2014). In other freshwater systems throughout Europe this species has been found to drastically reduce the biomass and survival of establishing macrophytes (van der Wal et al., 2013) and macroinvertebrates (Garcia et al., 2015). While other recent research in Italy has found that some microfungal flora species living in its gut are potential phytopathogenic, making *P. clarkii* a potential vector of plant diseases (Garzoli et al., 2014), further increasing the risk to local biodiversity especially in an ecologically important Tree Protected Area, such as at Fiddien Valley (Government Notice 473, 2011).

In Malta, freshwater bodies are very restricted in range and their presence is highly influenced by the wet season making them susceptible to climate change and to other increasing anthropogenic activities, including the introduction of invasive alien species. *Procambarus clarkii*'s range expanding ability linked with its ecological plasticity, omnivorous feeding habits, negative impacts on native fauna and ecosystem organisation and function is not to be underestimated. Therefore, this study calls for immediate management measures to control and eradicate this invasive species preferably prior to the beginning of the following wet season, so as to minimize the possibility of further spreading to other freshwater systems. Subsequently, regular monitoring and awareness campaigns have to be conducted to ensure that this or any other potentially invasive species would not be allowed to establish itself in this fragile freshwater natural environment.

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