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From Fresh to Salt Waters: First Data about the Invasiveness of the Red Swamp Crayfish *Procambarus clarkii* (Girard, 1852) in Mediterranean Marine Waters

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Abstract: Background: The red swamp crayfish *Procambarus clarkii* (Girard, 1852), native to the United States and northern Mexico, has been introduced in many countries worldwide. The species has a remarkable invasiveness, due to its high reproductive rate and to its ability to tolerate broad ranges of water parameters like salinity. Nevertheless, no consistent observations of *P. clarkii* have been reported in the marine environment until now. **Methods**: Through the observations of a personal acquaintance and of two volunteers who published photos of the species on Facebook marine biology and fishing groups, we collected data showing the presence of the crayfish within marine ecosystems. **Results**: *P. clarkii* has been spotted in three different localities, in two of which observations are recurrent. The species can live miles from the nearest freshwater stream, and can colonise the depths up to 20 meters. **Conclusions**: The multiple observations of *P. clarkii* collected in our study uncover a persistent presence of the species within some marine areas, rather than its mere sporadic occurrence. As resistance to increasing salinities is influenced by multiple genes, genetic admixture between genetically-differentiated individuals coming from distinct geographical origins could have brought together different salinity-resistance genes, leading to the formation of these resilient phenotypes.

Keywords: crayfish; *Procambarus clarkii*; alien species; invasive species; alien species adaptation; salinity tolerance

1. Introduction

The processes of biological invasions have gained significant attention in the field of ecology and environmental sciences, due to their marked and detrimental impacts on both native species and ecosystem functioning [1]. In an increasingly interconnected world, biological invasions have become more frequent and widespread. Furthermore, factors like trade and climate change have facilitated the spread of non-indigenous species to new areas far from their native distribution range [2]. The introduction of invasive species poses a major threat to ecosystems structure and integrity, leading to species extinction and biodiversity loss worldwide [3,4]. In fact, albeit the magnitude of impacts can strongly vary across different taxa [5], invasive species have been implicated in the extinction of enormous numbers of animal and plant species [6]. In addition, invasive alien species (IAS) can have a negative impact on the economy and even on human health [7].

Biological invasions involve a multi-step process. Initially, introduced species must successfully arrive, survive, and establish themselves in a new area. Subsequently, these alien species can become invasive, spreading and impacting native environments and species [8,9]. Throughout these stages, complex ecological processes unfold in the invaded habitats, including changes in the biological community, characteristics of the invaders, abiotic environment, and ecosystem component interactions. The temporal dimension is a critical factor when analyzing the impacts of IAS [10]. Research focusing on later invasions phases often reveals extensively altered communities, whereas studying the early stages provides insights into how ongoing processes modify the invaded communities. Within the freshwater environments the emerging scenario poses critical challenges. In fact, due to intense human modifications and to the numerous routes of introduction, freshwater environments are particularly subject to biological invasions [11]. Together with the numerous other anthropogenic stressors (e.g. pollution, climate change, hydropower, and habitat degradation), the introduction of alien species jeopardizes the biodiversity conservation, that in freshwater ecosystems is much more vulnerable than elsewhere [12]. For example, freshwater environments are thought to be more sensitive to the introduction of predators than their marine or terrestrial counterparts. This phenomenon is potentially attributable to the high heterogeneity in predation regimes between the different freshwater systems, that promotes naiveté in native preys [13]. In this context, the worst scenario is represented by extinction of native species by invasive ones [14]. For this reason, an early assessment is fundamental to monitoring and predict the impacts of IAS from damaging environmental integrity and native species [9].

In Europe, alien crayfishes now outnumber native crayfish species, and represent the first threat for their conservation. In fact, at least 9 alien crayfish species have formed established populations in European freshwater environments, coming from both the Americas and Australia. For example, in North America, non-indigenous crayfishes have displaced indigenous ones through direct competition [15]. In Europe, a similar situation has occurred, but the presence of the crayfish plague has exacerbated the devastation among the limited number of native species to a far greater extent. The success of non-indigenous crayfishes in Europe can be attributed primarily to their resilience, rapid individual and population growth, high reproductive capacity, and invasive characteristics, as noted by Lindqvist and Huner [16]. Tricarico et al. [17] introduced the "Freshwater Invertebrate Invasiveness Scoring Kit (FI-ISK)" to evaluate the invasive potential of various crayfish species, including those from the Astacidae, Cambaridae, and Parastacidae families, in the Italian freshwater ecosystem. Their findings attributed high risk scores for the red swamp crayfish *Procambarus clarkii* (Girard, 1852), in contrast to other species that were classified as having a moderate risk. This assessment highlights the utility of FI-ISK as a valuable tool for aiding decision-makers and policymakers in categorizing freshwater invertebrates based on their potential invasiveness. Importantly, this information is relevant to compliance with the 2007 European Union regulation (Council Regulation No. 708/2007) concerning the introduction of alien species for aquaculture and related activities.

The genus *Procambarus* encompasses several IAS in Europe, and even other species often subject to aquarium trade [18]. Among them, *P. clarkii*, native to the United States and northern Mexico, has been introduced in many countries across the globe for commercial purposes [19]. This species has displayed a remarkable capacity to adapt to and thrive in diverse natural and artificial habitats, leading to significant consequences for local ecosystems and their prey-predator dynamics [19,20]. Among its various ecological impacts, *P. clarkii* has been observed to significantly reduce the abundance, diversity, or biomass of many native species, feeding on amphibians [20,21], insects [14,20], snails [22], and macrophites [22,23]. Moreover, this species serves as a vector for the oomycete *Aphanomyces astaci* Schikora, which is responsible for the crayfish plague, a disease to which the European crayfish species are highly susceptible [18]. These combined factors underscore the considerable ecological and economic challenges posed by the widespread introduction ad proliferation of *P. clarkii* in various regions. In Italy, *P. clarkii* was observed for the first time in the wild in the Turin province (northern Italy) in 1989 [24]. However, albeit the presence of the species in the country was previously considered nearly limited to the northern and central regions, with just few records occurring in the south [25], over recent decades, it rapidly spread across the nation [26]. In fact, recent studies suggest that *P. clarkii* is constantly increasing its presence in southern regions, as well as in the two main Italian islands (i.e. Sicily and Sardinia) [27]. This poses a serious threat to the native crayfish communities, which are particularly rich and complex in Italy [18]. Its invasiveness is attributed to a confluence of many different factors. First of all, the species is r-selected, exhibiting high early maturity, rapid growth rates and large numbers of offspring production [19]. Its burrowing behaviour further enhances its adaptability, enabling *P. clarkii* to also live in temporary streams [18,19,28]. Besides, it has great dispersal abilities and a generalist diet that can change among the different habitats, further enhancing its invasiveness [19]. It can also tolerate highly polluted environments. Lastly, *P. clarkii* can withstand broad ranges of water quality parameters: for example, it tolerates a wide range of salinity. In fact, it has been proposed that the species could be able to descend some rivers up to the sea, and thus migrate from coastal waters to new, upstream, freshwater environments [29]. A similar invasion route has also been proposed for other freshwater species, like the topmouth gudgeon *Pseudorasbora parva*, a small cyprinid native to East Asia, apparently able to withstand coastal salinities in the proximity of streams [30]. Due to its remarkable biological adaptability, behavioral flexibility, and dispersal capabilities, *P. clarkii* has the capacity to establish itself in a broad spectrum of environments, including lagoons and brackish ecosystems [29]. For all these reasons, this species is ranked by the European Union in the top places among the 100 most harmful IAS (Regulation (EU) 1143/2014, see [31]). Evolutionary histories in harsh environments with changing environmental conditions can lead to the development of phenotypic plasticity [32]. For this reason, species originating from brackish and transitional waters are often predisposed to invasion, as they often show broad plasticity in terms of resistance to varying levels of salinity and other environmental variables [33]. This makes salinity tolerance one of the most studied "predictors of invasion". Moreover, given euryhaline species are often able to colonize multiple environments, they can also take advantage of multiple invasion routes. For example, it has been proposed that increased salinity tolerance can promote invaders' ability to survive in ships ballast waters [34]. Despite the broad salinity tolerance of *P. clarkii*, however, no consistent observations of the species in the marine environment have been reported until now, nor have interactions with marine fishing activities been documented. In this study, on the basis of recent records coming from citizen scientists, we provide the first evidence of the species presence within marine ecosystems, and its interaction with artisanal fishery.

2. Materials and Methods

2.1. Data collection

The data for this research was obtained through the kind collaboration of three volunteer citizens. The first source was a professional fisherman who published on a Facebook fishing group some images depicting captured specimens of *P. clarkii* among marine fishes. The second contributor was a personal acquaintance of one of the authors (FT) who once spotted the species in the marine environment while snorkelling. The last collaborator published a photo of the crayfish in an Italian Facebook marine biology group, asking for help to identify the species. All these "citizen scientists" have been all interviewed to gather all the relevant data regarding their observations or captures.

2.2. Fisherman report

The fisherman was privately contacted to gather more information about the captures. Additional details regarding the catched individuals (i.e. the locality, the proximity to freshwater streams, the depth, tool and frequency of capture) were collected to thoroughly understand the context of the observations. Additionally, explicit consent was obtained from the fisherman to utilize the photos shared on the social network for the purpose of this study.

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2.3. Second report

The personal acquaintance was asked some information about the observation (i.e. locality, proximity to freshwater streams, depth, and substrate) as well. Furthermore, he was asked to inquiry about the presence of the species at the local beach resort. In particular, he asked some more information to the resort staff to better understand the context and extent of the species occurrence in the area. Explicit consent to utilize the provided photos was obtained from this collaborator as well.

2.4. Third report

Similarly to the fisherman's record, the volunteer was privately contacted to gather some data about the spotted specimen (i.e. precise coordinates, date, proximity to freshwater streams and habitat of the specimen). Even here, the volunteer provided us with explicit consent to utilize the published pictures for this work.

3. Results

3.1. Fisherman report

The alien *P. clarkii* was captured alive several times (Figure 1) between the years 2022 and 2023 by the fisherman collaborator in Metaponto (Basilicata, Italy; 40.357 N, 16.844 E). Specimens were captured on many dates, including, for example, 17th October 2022, 29th April 2023, and 20th June 2023. These captures were carried through the usage of a trammel net with an approximate mesh size of 20 mm. The net was employed to catch the striped prawn *Penaeus kerathurus* (Forskål, 1775) at a depth ranging from 15 to 20 meters. The fishing locality was geographically distant from the nearest freshwater streams, namely the Basento River and the Bradano River, both located more than 3 Km away.

Figure 1. Specimen of *P. clarkii* captured by the fisherman collaborator. Photo by Vincenzo Celico.

3.2. Second report

The personal acquaintance observed a single viable specimen of *P. clarkii* (shown in Figure 2) in Cassano All'Ionio (Calabria, Italy; 39.762 N, 16.489 E) on the 3rd of August, 2023. The individual was spotted at a depth of 1.5 meters on a mixed bottom of sand and gravel. Notably, it was stationing in close proximity to a buoy anchorage. The observer also reports that observations of alive specimens from the local beach resort staff are very recurrent, even outside the water on the shoreline. According to Google Maps, the nearest freshwater stream is approximately 500 meters south from the point of observation; the second is the Raganello stream, located approximately 1.4 km north.

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Figure 2. Specimen of *P. clarkii* spotted by the second collaborator. Photo by Leonardo Pugliese.

3.3. Third report

The collaborator spotted a single viable specimen of *P. clarkii* (shown in Figure 3) on the 23rd of July 2021 in the waters of Terracina (Lazio, Italy; 41.284 N, 13.241 E). The specimen stationed on floating freshwater vegetation on the shoreline. According to the collaborator, the individual came from Porto Badino (3.3 Km west), where the Portatore River joins the sea. Watching the zone on Google Maps, we recognise the presence of a further canal of the Portatore River that joins the nearby port, located at approximately 1.7 Km east of the observation coordinates. The collaborator also reports that the species inhabits every canal, stream, river, and lake (even brackish) in the nearby areas.

Figure 3. Specimen of *P. clarkii* spotted by the third collaborator. Photo by Antonio Di Lello.

4. Discussion

The invasiveness of a species is considered to be bound by the genetic diversity within its invading propagules, albeit many other factors can intervene. The adaptations of alien species to the habitats of introduction indeed encompass a wide array of processes, including genetic, epigenetic and ecological aspects [35].

Our results underscore the potential of *P. clarkii* to adapt to very high salinity levels, as previously proposed by other authors who carried experimental manipulations to test the salinity resistance of the species [29]. However, a consistent presence of the species in marine environments has never been reported within its native range, where the genetic variability of the populations should be expected to be higher (but see [19]). One explanation could be related to the occurrence of genetic admixture between genetically-differentiated individuals in the new range. In fact, in the new range, invading populations coming from distinct geographical origins (and thus with different genetic backgrounds, adapted to the local environment) can often come in contact and interbreed, enhancing gene flow [36–39]. Given that resistance to increasing salinities is influenced by diverse molecular factors, and thus multiple genes [29], admixture between genetically-differentiated individuals could have brought together different salinity-resistance genes, leading to the formation of resilient phenotypes. This phenomenon could allow, in some specific populations, the

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development of the necessary adaptations to live in the marine environment. This hypothesis is consistent with the invasive evolutionary history of *P. clarkii*, that is believed to have been introduced in Italy and other European countries by multiple areas of origin, leading to a higher genetic diversity than expected by a single founder event [19]. Due to the high mobility of the species (breeding males can move up to 17 Km in 4 days, see [40]), and to its high life expectancy (up to 4 years, see [41]), it is possible that reported individuals might derive from a recent migration from the closest freshwater streams. This seems particularly likely for the third report, likely transported by the floating freshwater vegetation. However, the recurrent presence of alive individuals within some marine environments arises the question of whether the species could also be able to reproduce in them, at least in the proximity of the river mouth. Overall, this possibility should be concretely considered: in fact, in Italy, the reproduction of the species has already been reported in brackish waters that reach salinity values of 29.6 ppt [42]. The species also inhabits terrestrial natural or semi-natural wetlands and terrestrial humid zones, such as meadows or areas managed as rice fields. Notably, this invasive crayfish was discovered in European caves, specifically in Portugal and Italy. The presence of *P. clarkii* in caves is indeed significant, posing a fresh threat to groundwater ecosystems due to its potential adverse effects on native communities [43]. Indeed, it can prey on a various cave endemic species, as its diet is adaptable and dependent on the availability of different food sources. Furthermore, *P. clarkii* can be viewed as an ecosystem engineer, as it thoroughly alters the environments it invades. Through the utilization of burrows, this species not only copes with environmental challenges, such as high temperature and dehydration, but also safeguards itself from predators during critical stages of its life cycle [19].

The possible impacts of the species in marine environments are difficult to predict. However, considering the its dietary flexibility and its wide ranges of preys in invaded freshwater environments [14,20–23], it is highly likely that it would be able to feed on many invertebrates, including molluscs, crustaceans and polychaetes. Besides, considering the plasticity of its diet in the freshwater ecosystems, the prospect of *P. clarkii* to broaden its diet to other taxa, characteristic of the marine environment (e.g. bryozoans or echinoderms), should not be excluded. Besides, even small fish species and their eggs wouldn't be safe from this predator. Indeed, compared to indigenous crayfish species, *P. clarkii* is generally more active and aggressive, tends to exist in higher population densities, and exhibits increased interactions with fishes, as noted by Reynolds [44]. Red swamp crayfish are known to prey on the eggs of *Micropterus salmoides* (Lacepède, 1802), likely also consuming the larger, vegetation adhering eggs of *Esox lucius* Linnaeus, 1758. Invasive *P. clarkii* also play a significant role as fish predators in temporary Mediterranean streams, according to Ilhéu et al. [45]. In this context, in addition to the ecological harm resulting from the invasion of the marine environment by this species, there could also be an impact on fishing activities, similar to what is happening with the bearded fireworm, *Hermodice carunculata* (Pallas, 1766), a highly invasive and thermophilic expanding species, that is causing serious direct and indirect economic damage to artisanal fishery in the Ionian Sea [46]. In fact, *P. clarkii* might find it easier to prey on organisms already trapped in fishing nets rather than those free from them, often ending up entangled in the nets itself. So this species could also have a negative impact on economic activities at sea, such as fishing, and not just, as is already happening, for example, on rice production [31].

Climate change and invasive alien species, often referred to as the "double trouble" [47] can work in synergy to pose a significant threat to native species and ecosystems. Notably, climate changes can ease the introduction, establishment, and successful reproduction of invasive species, enabling their long-term survival and expansion, especially in the case of warm-adapted IAS like *P. clarkii* [48]. Maintaining a constant update on the status of this invasive species is crucial, and modern communication tools like smartphones and social networks offer effective means for this purpose. Attitudes towards the introduction and eradication of this species can vary and depend on a combination of factors, including stakeholder interests, individual socio-demographic characteristics, environmental behavior, and personal experiences [48]. For instance, in Doñana National Park in Spain, people are informed about the red swamp crayfish and its ecological impacts [31]. However, fishermen may be less inclined to support eradication efforts due to the economic benefits they derive from its sale.

Finally, based on the information discussed so far, the importance of citizen science in monitoring and early detection of alien species becomes evident. It is noteworthy that all three significant records presented in this work have been contributed by citizen scientists, underscoring their pivotal role in scientific research. Citizen science greatly extends the geographical coverage and public engagement in data collection, allowing for more comprehensive and widespread monitoring of alien species [49]. This approach not only facilitates data gathering on a larger spatial scale, but also involves citizens in rising awareness and understanding of issues related to IAS [50]. Engaging citizens in monitoring invaders can serve as a valuable tool for timely identification and addressing of ecological threats. Furthermore, it promotes community involvement in advocating for the conservation of native species and the management of natural resources. In summary, citizen science is an essential component in the battle against IAS, fostering active collaboration between researchers and the public, which contributes to a better understanding and management of such ecological challenges.

5. Conclusions

The invasiveness of a species is intricately linked to the genetic diversity within its invading propagules, alongside various other contributing factors. The adaptation of alien species to their introduced habitat encompasses a wide array of processes, including genetic, epigenetic, and ecological aspects [35]. Our study contributes new insights into the adaptability and invasive potential of the crayfish *P. clarkii*. In fact, the species appears to be able to thrive in conditions of elevated salinity, as previously reported with laboratory experiments by other authors. However, a consistent presence of the species in marine environments has never been reported within its native range, where higher genetic variability in populations would be expected. This great adaptability in the invaded range is the result of the evolutionary history of the species; however, its specific drivers remain elusive. To unravel them, genetic and epigenetic approaches should be carried out. Since all individuals here reported were alive at the time of observation, these sightings uncover a persistent presence of the species within some marine environments, rather than its mere sporadic occurrence. The prospect of *P. clarkii* being able to reproduce in marine environments underscores the need for careful monitoring of the species' population dynamics in the proximity of river mouths. This possibility should be considered, as in Italy, reproduction of the species has already been reported in brackish waters with salinity values reaching 29.6 ppt [42]. The potential impacts of the species in marine environments are challenging to predict. However, considering the opportunistic feeding behaviour of the species [14,20–23], it is highly likely that it could feed on various marine invertebrates and even small fishes and their demersal eggs. In this context, aside from the ecological harm caused by the invasion of the marine environment by the species, there could also be impacts on fishing activities. Finally, our findings highlight the importance of citizen science in the monitoring and early detection of alien species.

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