

ORIGINAL ARTICLE

Can marginal aquatic habitats serve as corridors for *Procambarus clarkii* expansion towards the lagoon and sea waters?

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Abstract: This study investigated the distribution, abundance, and population structure of the invasive *Procambarus clarkii* in understudied small marginal water bodies along the Venice lagoon. These water bodies comprise expanses of brackish water, shallow freshwater canals, and small ponds, previously constituting part of a fishing valley. The aim was to fill the knowledge gap about the species colonization of these specific environments, analyse its relationships with the aquatic fauna and evaluate whether it could also inhabit coastal environments with increasing salinities. Sampling was conducted using fishing pots in 10 stations, each characterized by distinct environmental characteristics such as salinity, nutrient concentrations, water body width, water flow and floristic cover. The results showed that *P. clarkii* successfully invaded most of these marginal habitats. Although poor water quality and high nutrient levels do not preclude the presence of this species, they seem to influence its abundance. In contrast, salinity resulted to be a primary factor determining the presence or absence of the species, acting as a physiological barrier, limiting *P. clarkii* expansion toward more sea-influenced parts of the lagoon coastal environments. Moreover, pH, while not affecting the presence of the species, resulted in affecting the growth rates of individuals. In terms of community dynamics, the native nektonic fauna appears largely replaced by more adaptable non-native species, with *P. clarkii* likely contributing to this displacement, alongside other impacts. Moreover, although strong direct associations with specific aquatic fauna are limited, evidence suggests reciprocal predation between crayfish and fish during different life stages.

Keywords: red swamp crayfish; invasive species; water characteristics; salinity; nektonic fauna

Sažetak: MOGU LI MARGINALNA VODENA STANIŠTA SLUŽITI KAO KORIDORI ZA ŠIRENJE VRSTE *PROCAMBARUS CLARKII* PREMA LAGUNSKIM I MORSKIM VODAMA? U ovom istraživanju analizira se rasprostranjenost, brojnost i populacijska struktura invazivne vrste *Procambarus clarkii* u slabo istraženim malim marginalnim vodenim staništima uz Venecijansku lagunu. Ta vodena staništa obuhvaćaju područja bočate vode, plitke slatkovodne kanale i male bare, koje su nekada bile dio ribolovne doline. Cilj istraživanja bio je proširiti znanje o kolonizaciji vrste u ovim specifičnim staništima, analizirati njezine odnose s vodenom faunom te procijeniti može li nastanjivati i obalna područja s povećanim salinitetom. Uzorkovanje je provedeno pomoću ribarskih vrša na 10 postaja, od kojih je svaka bila obilježena različitim okolišnim karakteristikama, uključujući salinitet, koncentracije nutrijenata, širinu vodenog tijela, protok vode i floristički pokrov. Rezultati su pokazali da je *P. clarkii* uspješno kolonizirao većinu tih marginalnih staništa. Iako loša kvaliteta vode i visoke razine nutrijenata ne sprječavaju prisutnost ove vrste, čini se da utječu na njezinu brojnost. Nasuprot tome, salinitet se pokazao kao glavni čimbenik koji određuje prisutnost ili odsutnost vrste, djelujući kao fiziološka barijera koja ograničava širenje vrste *P. clarkii* prema dijelovima lagunskog obalnog okoliša pod jačim utjecajem mora. Osim toga, utvrđeno je da pH vrijednost, iako ne utječe na samu prisutnost vrste, ipak utječe na stope rasta jedinki. U kontekstu dinamike zajednice, čini se da je autohtona nektonska fauna u velikoj mjeri zamijenjena prilagodljivijim alohtonim vrstama, pri čemu *P. clarkii*, zajedno s drugim čimbenicima, vjerojatno doprinosi tom istiskivanju. Nadalje, iako su izravne povezanosti s određenim skupinama vodene faune ograničene, postoje dokazi o uzajamnoj predaciji između rakova i riba tijekom različitih životnih stadija.

Ključne riječi: crveni močvarni rak; invazivna vrsta; karakteristike vode; salinitet; nektonska fauna

INTRODUCTION

The red swamp crayfish *Procambarus clarkii* (Girard, 1852) is a freshwater crayfish native to North America (Hobbs, 1972), but it has been introduced in all

geographic regions except Antarctica and Oceania (Gherardi, 2006; Kouba *et al.*, 2014; Loureiro *et al.*, 2015) due to the aquarium trade (Loureiro *et al.*, 2015; Souty-Grosset *et al.*, 2016), aquaculture, and its value as a food source (Huner and Linqvist, 1995; Souty-Grosset *et al.*,

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2016). This species has been spreading after escaping or being released into freshwater bodies in several locations owing to its strong invasive character and adaptability (Gherardi, 2006; Souty-Grosset *et al.*, 2016) and has entered the list of the 100 worst invasive species in Europe owing to its negative impact on colonised environments and native species (Roy *et al.*, 2020). In Italy, the red swamp crayfish was first introduced and likely escaped from aquaculture facilities. The first observation of this species in the wild was recorded in the Turin Province in 1989 (Delmastro, 1992). It has been spreading since then, mainly in the North and Centre of Italy, and more recently in the South and the islands of Sicily and Sardinia (Morpurgo *et al.*, 2010; Cilenti *et al.*, 2017; Lo Parrino *et al.*, 2020). By 2020, the presence of this species had been confirmed in 86 of the 107 Italian provinces (Lo Parrino *et al.*, 2020). This successful colonisation and establishment are facilitated by both the r-selected characteristics of *P. clarkii*, as well as by secondary introductions. Rapid growth rates (Paglianti and Gherardi, 2004; Scalici and Gherardi, 2007) and a highly plastic life cycle (Gutiérrez-Yurrita and Montes, 1999) have indeed allowed it to invade and survive in a wide variety of habitats, causing damage to various environments (Gherardi, 2006; Scalici *et al.*, 2010; Siesa *et al.*, 2014). Moreover, *P. clarkii* may also rely on long-distance passive transportation for its dispersal, mediated by humans through e.g., moving vehicles that could carry crayfish individuals also for large-scale translocations, but also mediated by other vectors such as birds (Acevedo-Limón *et al.*, 2020; Scalici and Gallitelli, 2025).

Procambarus clarkii exhibits high tolerance to a wide range of critical environmental conditions, including temperature fluctuations, prolonged droughts, extreme variations in oxygen levels, acidity, and high concentrations of nutrients and toxic compounds (Huner and Lindqvist, 1995; Barbaresi and Gherardi, 2000; Maceda-Veiga *et al.*, 2013; Loureiro *et al.*, 2015; Sun *et al.*, 2023). Its strong desiccation resistance, relying on aerial respiration (Favaro *et al.*, 2011; Donato *et al.*, 2018), allows it to survive out of water for over 10 hours and facilitates overland dispersal across watersheds for several kilometres at speeds up to 90 m/h (Ramalho, 2012; Banha and Anastácio, 2014; Souty-Grosset *et al.*, 2016). Moreover, the use of burrows allows this species to withstand environmental extremes, such as high temperatures and dehydration, and protects the species from predators during vulnerable life stages (Huner and Barr, 1991; Gherardi, 2006), such as during egg laying (Huner and Barr, 1991). The high tolerance of crayfish to hypoxia is due to their ability to move to the air-water interface and switch their energy metabolism from aerobic to anaerobic respiration, enabling them to thrive where other species cannot (Reiber and McMahon, 1998; McMahon, 2001; Bonvillain *et al.*, 2012, 2015). Additionally, high nutrient levels can indirectly promote crayfish growth by stimulating the food sources (phytoplankton

and zooplankton) on which they rely (Mischke and Zimba, 2004; Sun *et al.*, 2023).

However, this species exhibits relatively low physiological tolerance to salinity (Huner and Barr, 1991; Kang and King, 2012), which affects both its distribution and reproduction (Meineri *et al.*, 2014). Salinity reduces the probability of capturing *P. clarkii* year-round, with adults typically found only in waters with salinity up to 10, although reproduction is limited to waters below 5 (Huner and Barr, 1991; Meineri *et al.*, 2014). It has also been hypothesised that this species may be able to invade brackish waters, such as estuaries and lagoons, using these environments as ecological corridors to descend from the rivers to the sea and subsequently migrate upstream to new freshwater environments (Dörr *et al.*, 2020; Nota *et al.*, 2023; Scalici and Gallitelli, 2025). Therefore, marginal aquatic habitats between freshwater and lagoon systems that serve as interfaces between these two types of environments may represent a potential gateway for invasive species to different habitats. Therefore, owing to their mixed and variable characteristics, these marginal aquatic habitats are critical for investigating the colonisation of red swamp crayfish. Moreover, because these environments are often managed for aquaculture activities or naturalistic purposes, they can be regularly monitored, providing the opportunity to intervene and limit the spread of the species. The aim of this study was firstly to evaluate the distribution, abundance, and population structure of *P. clarkii* across a mosaic of understudied small marginal water bodies adjacent to the Venice lagoon (Northern Italy). Secondly, we analysed the relationships between population dynamics, environmental and hydromorphological characteristics, and aquatic faunal composition, dominated by alien fish species.

MATERIAL AND METHODS

Study area

Valle Averte is an ancient, dammed, traditional fishing valley located in the southern part of the Venice lagoon. The area comprises expanses of brackish water interspersed with salt marshes, embankments, reedbed areas, numerous shallow freshwater canals, and small ponds. These canals and ponds receive freshwater supplies via a hydraulic system built in the 18th century, which is still functional (Bioprogram, 2006; Curiel *et al.*, 2008). The entire area covers approximately 524 hectares, of which 78 hectares constitute a WWF Oasis. The Oasis is characterised by a wide variety of environments of naturalistic interest for its flora and fauna, representative of the upper Adriatic wetlands, and its diverse avifauna (LIFE ForestAll, 2019). In this study, we focused on the marginal water bodies located within the inner part of the oasis. Here, a wide network of canals flows through small ponds, remnants of the former traditional fishing valley systems, providing a direct connec-

tion between the freshwater habitats and the lagoon. The exact year of the introduction of *Procambarus clarkii* into Valle Averte is unknown, but its presence has been observed since at least 2012 (S. Borella, personal communication), with the first collected data dating back to 2014 (Zanardo, 2015).

Sampling and laboratory analysis

Sampling campaigns were conducted at 10 stations (Fig. 1) located within the WWF Oasis. These stations were selected after an initial field inspection to represent the diverse environmental and morphological conditions present in the oasis. The characteristics of the sampling stations were strongly influenced by the complex water circulation within the study area, which is regulated by sluice gates positioned between the channels, while tidal variations do not influence the water level within the channel network (Bioprogram, 2008). Station V18 is located at the only inlet of a freshwater stream into the oasis (Canale Novissimo). From there, the water flows through canals and small ponds along various paths. Stations V7, V9, V12, and V14 were located along the canals, whereas V8 and V10 were located within small ponds. Station V27 is located at the intersection of several canals, whereas station V31 is located along the canal that discharges water from the oasis to the lagoon. Station V2 was located in a small pond at the end of a

canal, completely cut off from the main water network within the study area. This makes it the only station located in a water body isolated from the others. Moreover, the water bodies where the stations were located exhibited additional diverse characteristics, including varying water flow, width and depth, reeds and riparian vegetation densities. Specifically, water depth ranged from 0.4 to 1.2 m (Bioprogram, 2008), while all the other main characteristics of each sampling station are summarized in the Supplement Material Table S1.

Three field sampling campaigns were conducted in June, July, and September 2022. In our study area, this period of the year coincides with the peak of red swamp crayfish activity (late spring/early summer) and the start of the activity drop (late summer/early autumn) (Scalici *et al.*, 2010). Moreover, summer represents a critical period of the year for water stream conditions due to decreased flow because of low precipitation, high evapotranspiration, and high temperatures, which might cause thermal stress on aquatic organisms, a decrease in dissolved oxygen that could cause anoxic crises, and also a possible increase of salinity (Gasith and Resh, 1999; Bioprogram, 2008; Bonada and Resh, 2013). These challenges that could affect small marginal water bodies make it relevant to focus the study on this critical season of the year.

During each field campaign, aquatic fauna, environmental variables, chlorophyll *a*, and nutrient (ammonia,

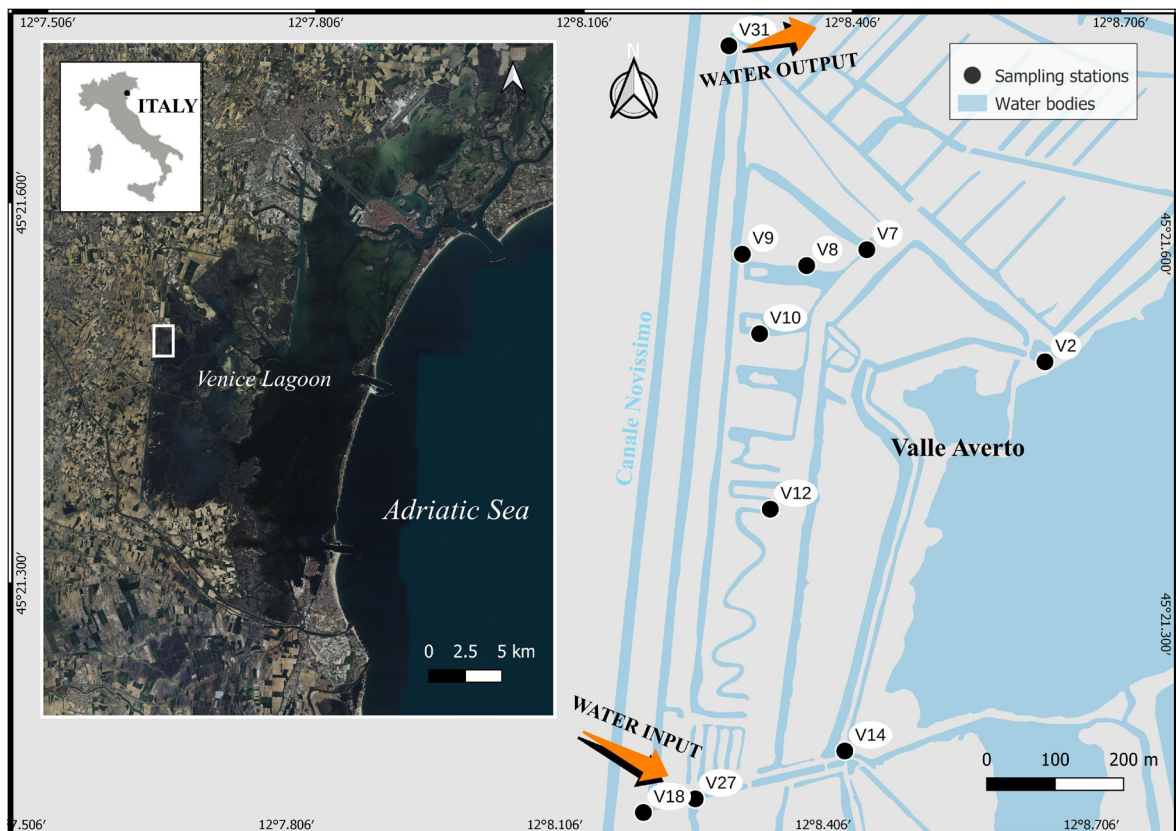


Fig. 1. Map of the ten sampling stations studied within the WWF Oasis, located in Valle Averte, Venice lagoon (Northern Italy).

nitrate, and phosphate) concentrations were monitored. Samplings and measurements of environmental variables were conducted at the same time each month to ensure consistency. The width of the water body along which sampling stations were located, and the presence of vegetation, were also recorded. Sampling was not performed in V14 in September because of the drought at the station. Sampling of aquatic fauna was carried out using two different types of fishing pots (traps): a fine mesh fishing pot, rectangular in shape with dimensions of 25 x 25 x 40 cm, mesh size of 0.4 cm, and a 7 cm diameter opening on the two sides; a larger mesh cylindrical fishing pot with a length of 60 cm, a diameter of 30 cm, a mesh of 1.0 cm, and a 13 cm diameter opening on the two sides. Four traps were deployed a few meters apart from each other in all sampling stations: two of each type, to sample species and specimens of different sizes, even though a bias was still present as smaller and larger animals were not captured. Traps were deployed in the late afternoon and retrieved the following morning so that they remained in the water for approximately 16 hours, in order to sample both diurnal and nocturnal species. Pots were baited with a mixture of bread, pellets, and fish (sardines or anchovies) to attract species with different feeding habits. Pots were positioned to ensure that one corner always protruded beyond the surface of the water to allow specimens of the European pond turtle *Emys orbicularis* (Linnaeus, 1758) caught to reach the water surface to breathe.

Samples of nektonic fauna (fish and decapod crustaceans) were instead euthanized through ice immersion following ethical standards and regulations regarding invasive species (EU Regulation 1143/2014, European Union, 2014; D.Lgs. 230/2017, Italian Ministry of the Environment, 2017), and then brought to the laboratory for identification at species level using literature and scientific iconography with the aid of a stereomicroscope (Nikon SMZ 745, 6.7x-50x) for the observation of distinctive characters. Each *P. clarkii* specimen was analysed for sex determination, weighed (wet weight, W) using a technical precision balance (± 0.001 g), and measured using a calliper (± 0.01 mm). The measurements included body length (TL), carapace length (CL), and carapace width (CW), as proposed by Yi et al. (2020). Each fish specimen was weighed (wet weight, W) using a technical precision balance (± 0.001 g) and measured (Standard Length, SL) using a calliper (± 0.01 mm). Specimens of *E. orbicularis* were instead immediately released and only their carapace length was measured (± 1 mm) in the field.

During trap retrieval, the following environmental parameters were recorded with a multi-parametric probe (Hanna Instrument 9829) at each sampling station: temperature (± 0.01 °C), pH (± 0.01), salinity (± 0.01), dissolved oxygen (± 0.1 saturation ± 0.01 mg/l), and turbidity (± 0.1 fnu). To determine chlorophyll *a* concentrations ($\mu\text{g/l}$), water was collected with a bucket, taking care not to touch the streambed to reduce the resuspension

of sediments and not alter the natural composition of the water. 200 mL of water was filtered on a Whatman GF/F filter (diameter: 47 mm and porosity: 0.7 μm). The filter was stored in a freezer until it was analysed. Chlorophyll *a* concentrations were determined using the fluorometric method proposed by Lorenzen (1966), using Trilogy Laboratory Fluorometer (Turner Designs) for acetone extraction analysis. To determine nutrient concentrations (ammonia, nitrate and phosphate; mg/l), the filtered water collected after chlorophyll *a* sampling was stored in a 250 mL jar and kept refrigerated until laboratory determination using a Hach DR1900 portable spectrophotometer.

Vegetation was distinguished between reeds (*Phragmites australis* (Cav.) Trin. ex Steud., 1841), and riparian trees (*Ulmus minor* Mill., 1768 and *Fraxinus angustifolia* Vahl 1804): for each type of vegetation, values between 0 and 2 were assigned based on their absence (0), presence (1), or abundant presence (2). These scores reflected both the density and total cover of reeds and riparian trees (data on vegetation are reported in Supplement Material Table S1).

Data processing

The water environmental characteristics of each sampling station are reported using boxplots to ensure a transparent visualization of the data and a clear comparison across the different sampling stations. Furthermore, we used the LIMeco water quality index (DM 260/2010; Italian Ministry of the Environment, 2010) to assess the differences between sampling stations (Azzellino et al., 2015). This tool is used to evaluate the quality of Italian water streams under the Water Framework Directive 2000/60/EC (European Union, 2000), and the variables used for its calculation are nitrate (mg/l), ammonia (mg/l), total phosphorus (mg/l), and dissolved oxygen (saturation %). At each station, a score from 1 to 0 was assigned for each variable according to its concentration; the average score of the four variables was used to allocate each station on a 5-level scale of water quality: excellent, good, sufficient, poor, and bad (the detailed method to calculate LIMeco Index is reported in Italian Law DM 2060/10). In the present study, the LIMeco index was used as an indicative tool to obtain a better view of the complex system of small marginal water bodies, as our intent was not to evaluate the quality of the water streams. The obtained results are thus not meant to classify the sampling stations, as i) the quality of the streams should be evaluated in a three years long time interval, ii) we used phosphate instead of total phosphorus, with a potential slight overestimate of the index, and iii) our study area might not represent the typical environment this index was implemented for, but probably still the most suitable. The relative abundance of red swamp crayfish was expressed as Catch Per Unit Effort (CPUE) with raw capture data from each 16-hour soaking period standardized to 24-hour and therefore calculated as

the number of individuals *per trap per day*, and reported using histograms, while sizes were reported using box-plots. To account for the potential non-independence of samples collected from the same locations across different months, we employed Linear Mixed-Effects Models (LMM). In these models, ‘Month’ was treated as a fixed effect, while ‘Sampling Station’ was included as a random effect. This approach allowed us to partition the variance associated with specific sites and formally account for the intrinsic correlation between repeated measures at the same station. Furthermore, the potential depletion effect caused by the removal of specimens was assessed by testing the correlation between mean CPUE and cumulative removals across the study period. A Pearson correlation analysis showed no significant relationship ($r = -0.92$, $df = 1$, $P = 0.247$), indicating that the physical removal of individuals did not significantly bias the capture probability in subsequent sessions. The sex ratio was calculated as males/females proportion, and size classes were set at intervals of 5 mm (total length; TL) to compare the abundance by size class between sampling stations and months using the non-parametric Kruskal-Wallis test (significant differences *per* $P < 0.05$) as the data do not follow a normal distribution. The monthly average CPUE of all sampled nektonic species was reported, furthermore, red swamp crayfish CPUE data were divided and reported for three size classes based on total length (TL): small (≤ 7.5 cm), medium ($7.5 \geq 9.5$ cm), and large (> 9.5 cm), to explore possible relations between different-sized specimens and the other variables. Spearman’s rank correlation coefficients between each couple of variables (including water environmental variables, water bodies characteristics, and aquatic fauna biomass) were then explored (significant values *per* $P < 0.05$) to test whether as one variable increases, the other tends to increase (positive correlation) or decrease (negative correlation). Moreover, a redundancy analysis (RDA) was performed to explore the overall distribution of the species in relation to the water characteristics and to the hydromorphology and floristic composition of the sampling stations. Biotic inputs included the CPUE data of all the species whose CPUE represented at least the 0.5% of the total CPUE, to avoid statistical noise by including species only occasionally present, with red swamp crayfish CPUE analysed separately for the size classes (small, medium, and large). Multicollinearity among the 13 initial environmental variables was assessed using the Variance Inflation Factor (VIF). An iterative process was applied, and chlorophyll *a* was excluded from the final model as it exhibited a $VIF > 10$, indicating high redundancy with other environmental predictors. The species abundance matrix was Hellinger-transformed to account for the ‘double-zero’ problem, while environmental variables were centered and scaled to ensure comparability across different units of measurement. The significance of the RDA and the unique contribution of each predictor were assessed using marginal permutation tests (999 permuta-

tions). To account for the non-independence of repeated seasonal measures, permutations were stratified by sampling station (strata = Station). The overall model fit was evaluated *via* the adjusted R^2 . All the statistical analyses were performed using R (v.4.2.3; R Core Team, 2023), including the *vegan*, *lme4* and *lmerTest* packages (Bates *et al.*, 2015; Oksanen *et al.*, 2015; Kuznetsova *et al.*, 2017).

RESULTS

Environmental characterisation of the study area

The water environmental variables (salinity, turbidity, pH, temperature, dissolved oxygen, chlorophyll *a*, nitrate, ammonia, and phosphate) are shown in Fig. 2. Salinity was low (< 1) at all stations except V2, where it ranged from 8.96 to 10.43. The highest values of salinity, turbidity, and chlorophyll *a* were recorded in V2, whereas pH reached the highest values in V14. Nutrient concentrations were also high, mostly in V2 and V14, but also in V18. The lowest chlorophyll *a* and pH values were recorded in V18, whereas the minimum oxygen values were detected in V2 and V12. The LIMeco Index results presented in Table 1 show that some stations (V7, V8, V10, and V27) were always in excellent status during our samplings. Instead, stations such as V14 and V18 always ranged from poor to sufficient status during all sampling months. Other stations showed more variable results, especially V2, which was in a poor and bad status in June and July, respectively, but in a good status in September.

Red swamp crayfish population

A total of 885 specimens of *Procambarus clarkii* were sampled (380 in June, 345 in July, and 160 in September, and they were found in 9 out of 10 sampling stations (Fig. 3), with a marked seasonal trend. The mean CPUE remained stable between June (14.3 ± 8.4 individuals *per trap per day*) and July (12.9 ± 5.5), with no statistically significant differences ($P = 0.534$, LMM). However, a sharp and significant reduction was recorded in September (6.3 ± 4.4 ; $P < 0.05$), coinciding with the decrease in water temperature. According to Spearman’s rank correlation, captures were indeed directly related to temperature. The mean CPUE *per station* varied from a minimum of 0.0 (in V2) to a maximum of 17.1 (in V27). The highest CPUE recorded during a single sampling event was 27.4 at V9 in June.

The overall sex ratio of sampled crayfish recorded was 1.25, with a total of 492 males and 393 females captured throughout the study. Sex ratio remained skewed toward males during each month of sampling and at all sampling stations, with the sole exception of V31. The size-structure of captures did not differ significantly between sexes, whether analysed monthly or overall (Kruskal-Wallis, $P > 0.05$ for TL). The highest mode of

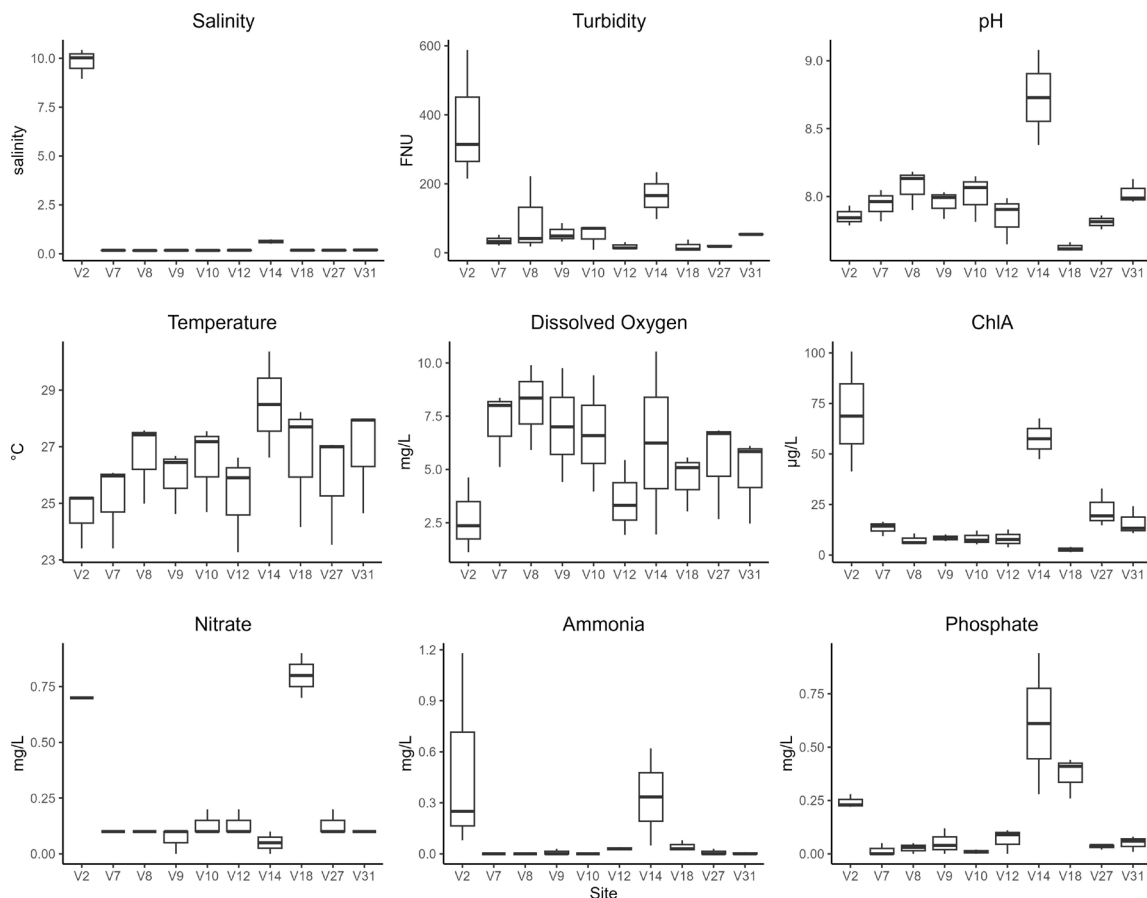


Fig. 2. Boxplot of detected environmental variables of water (salinity, turbidity, pH, temperature, dissolved oxygen, chlorophyll *a*, nitrate, ammonia and phosphate). Maximum, minimum and median values are shown for each sampling station; boxes represent first and third quartiles.

both male and female specimens was consistently observed between 7.5 and 8 cm TL. However, significant differences in size-structure were detected among sampling stations ($P < 0.05$). Stations V14 and V18 represented the extremes: the former exhibited a size-structure

skewed toward the smaller individuals (median TL = 7.54), while the latter was skewed toward larger individuals (median TL = 8.52). Size differences among stations are presented in Fig. 4 and were more pronounced in June, when specimens sampled at some stations, such

Table 1. Results of the LIMeco water quality Index in each sampling station per month during 2022. From best to worst quality class: Excellent, Good, Sufficient, Poor, Bad (DM 260/2010, Italian Ministry of the Environment, 2010).

LIMeco Index	June	July	September
V2	Poor	Bad	Good
V7	Excellent	Excellent	Excellent
V8	Excellent	Excellent	Excellent
V9	Excellent	Sufficient	Excellent
V10	Excellent	Excellent	Excellent
V12	Good	Good	Good
V14	Sufficient	Poor	n/a
V18	Poor	Sufficient	Sufficient
V27	Excellent	Excellent	Excellent
V31	Excellent	Good	Excellent

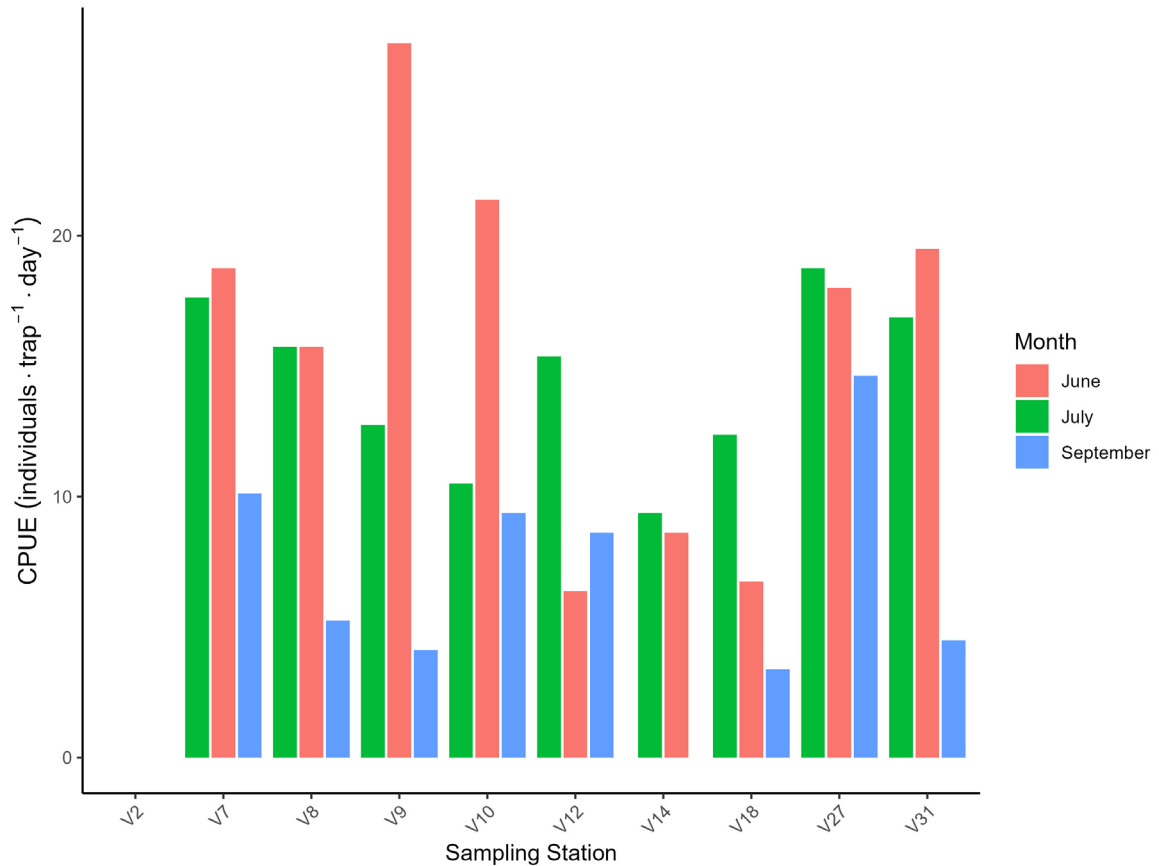


Fig. 3. Total of *Procamburus clarkii* specimens caught per station during each sampling campaign.

as V12 (median TL = 9.57) and V27 (median TL = 9.14), were significantly larger ($P < 0.05$) than those at other stations, such as V14 (median TL = 7.70). An overall decrease in size over time was detected ($P < 0.05$), with median TL decreasing from 8.38 cm in June to 8.03 cm in July, and finally to 7.82 cm in September.

Red swamp crayfish relations with fish fauna and environmental variables

In addition to *Procamburus clarkii*, the other species caught were the invasive fishes *Gambusia holbrooki* Girard, 1859, *Pseudorasbora parva* (Temminck & Schlegel, 1846), *Ameiurus melas* (Rafinesque, 1820), *Cyprinus carpio* Linnaeus, 1758, *Lepomis gibbosus* (Linnaeus, 1758), the only native fish *Alburnus alborella* (Bonaparte, 1841), the native grass shrimp *Palaemonetes antennarius* H. Milne-Edwards, 1837, and the native European pond turtle *Emys orbicularis* (Linnaeus, 1758). Monthly average CPUE for all sampled species is presented in Table 2 for each station, with *P. clarkii* data further categorized into three size classes. *P. clarkii* exhibited the highest CPUE at all stations, with the exceptions of V2 (where it was absent), V14, and V18; with its maximum CPUE was recorded at V27. Among the three size classes of *P. clarkii*, the medium size class consist-

ently exhibited the highest CPUE. *Pseudorasbora parva* was the species with the highest CPUE at V14 and V18, while *P. antennarius* was the species with the highest CPUE at V2. The three most abundant fish species (*A. melas*, *P. parva*, and *G. holbrooki*) were detected at all stations except V2; specifically, *A. melas* reached its maximum CPUE at V18 and V31, while *P. parva* peaked at V14 and V31, and *G. holbrooki* at V14 and V27. Other species were sampled less frequently: *C. carpio* was detected at 6 out of 10 stations, with its maximum CPUE at V31 and V14, while *L. gibbosus* was detected at only two stations, with its peak CPUE at V18. *Alburnus alborella* was recorded at only one station (V18). No fish species were found at V2, where *P. antennarius* and *E. orbicularis* were the only sampled species. *Palaemonetes antennarius* was present at seven stations, but its occurrence was sporadic at most sites, except for V2, where it recorded the highest CPUE. *Emys orbicularis* was present at all sampling stations except for V27 and V31, reaching its highest CPUE at V12 and V14.

The redundancy analysis (RDA; Fig. 5) significantly explained the variation in the aquatic community ($R^2_{adj} = 0.61$, $F = 4.69$, $P = 0.001$), with seven environmental variables identified as significant through the marginal permutation test: water temperature ($P = 0.005$), ammonia concentration ($P = 0.002$), and flowing waters ($P = 0.008$)

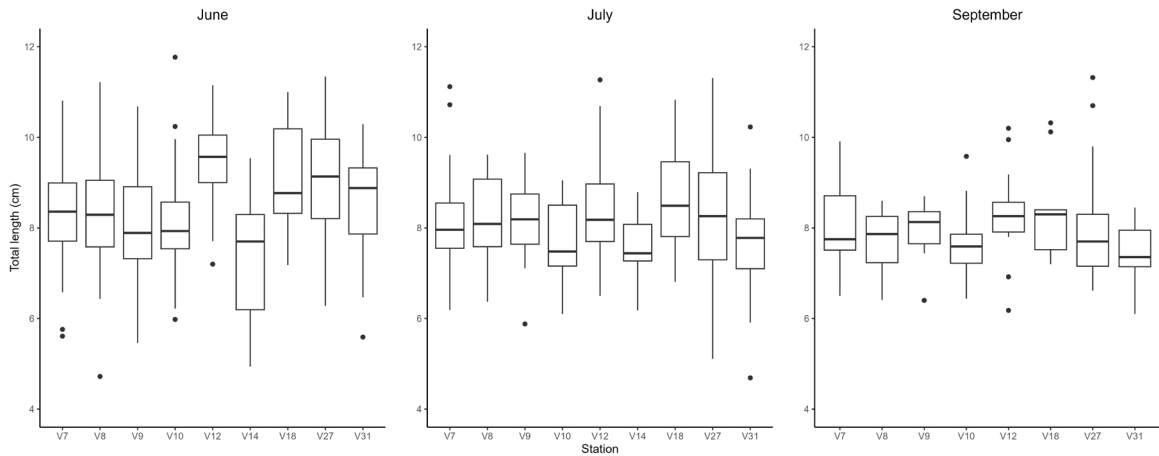


Fig. 4. Boxplot showing the sizes of the caught individuals of *P. clarkii* in each sampling station in June, July and September of 2022. First quartile, median and third quartile are shown in the graphic, dots represent outliers.

emerged as the primary drivers, followed by salinity ($P = 0.020$), riparian tree cover ($P = 0.026$), pH ($P = 0.029$), and water body width ($P = 0.033$). The first two axes accounted for 56.27% of the total variance (RDA1: 29.84%; RDA2: 26.43%), capturing effectively the main gradients of the species distribution. Overall, two primary gradients were observed: i) factors such as salinity, turbidity, nutrients concentrations (nitrate, ammonia and phosphate) and reeds cover were negatively correlated with flowing water and riparian tree cover. These variables primarily influenced the distribution of *P. clarkii* (small and medium sizes), as well as *P. antennarius*; ii) dissolved oxygen and pH appeared to be the main drivers for *E. orbicularis*, *P. parva*, and *G. holbrooki*, while large individuals of *P. clarkii* were strongly correlated with temperature.

Specifically, a positive association was detected between *P. clarkii* and temperature, with this relation-

ship being more pronounced for large size. In contrast, negative associations were observed between *P. clarkii* and nutrient concentrations (nitrate, ammonia, and phosphate), turbidity, salinity and water body width, with higher crayfish abundance detected in narrower canals. In contrast, riparian trees cover and flowing waters were correlated with small and medium size crayfish, with dissolved oxygen also being related to the smaller size class but losing its influence with increasing size of *P. clarkii*. Indeed, the three sizes of *P. clarkii* followed a clear gradient: strong associations were observed between small and medium-sized individuals, as well as between medium and large-sized individuals, whereas only a weaker correlation was detected between the small and large size classes. Regarding the relationships between *P. clarkii* and other species, small-sized crayfish showed a positive association with part of the fish fauna,

Table 2. Average monthly CPUE (individuals • trap-1 • day-1) of each sampled species: *Procambarus clarkii* (total and by size class), *Gambusia holbrooki*, *Pseudorasbora parva*, *Ameiurus melas*, *Cyprinus carpio*, *Alburnus alborella*, *Lepomis gibbosus*, *Palemonetes antennarius*, *Emys orbicularis* per sampling station.

	V2	V7	V8	V9	V10	V12	V14	V18	V27	V31
<i>P. clarkii</i>	0.0	15.5	12.3	14.8	13.8	10.1	9.0	7.5	17.1	13.6
TL ≤ 7.5 cm	0.0	3.5	3.1	4.4	5.0	1.4	4.3	1.0	4.5	3.6
7.5 < TL ≤ 9.5 cm	0.0	10.6	8.1	9.1	8.1	6.6	45.	4.4	8.8	8.5
TL > 9.5 cm	0.0	1.4	1.0	1.3	0.6	2.1	0.2	2.1	3.9	1.5
<i>G. holbrooki</i>	0.0	8.4	5.1	8.6	11.4	8.0	12.0	3.1	13.5	1.1
<i>P. parva</i>	0.0	4.5	0.8	3.1	2.6	0.0	39.0	7.8	0.8	8.3
<i>A. melas</i>	0.0	1.8	1.1	1.9	1.1	1.9	1.5	2.8	1.4	2.0
<i>C. carpio</i>	0.0	0.0	0.5	0.1	0.5	0.0	1.7	0.0	0.3	2.1
<i>A. alborella</i>	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.5	0.0	0.0
<i>L. gibbosus</i>	0.0	0.0	0.0	0.1	0.0	0.0	0.0	0.3	0.0	0.0
<i>P. antennarius</i>	24.9	0.1	0.0	0.4	0.0	0.4	0.0	0.4	0.1	0.1
<i>E. orbicularis</i>	0.4	0.3	0.3	0.1	0.1	0.3	0.6	0.3	0.0	0.0

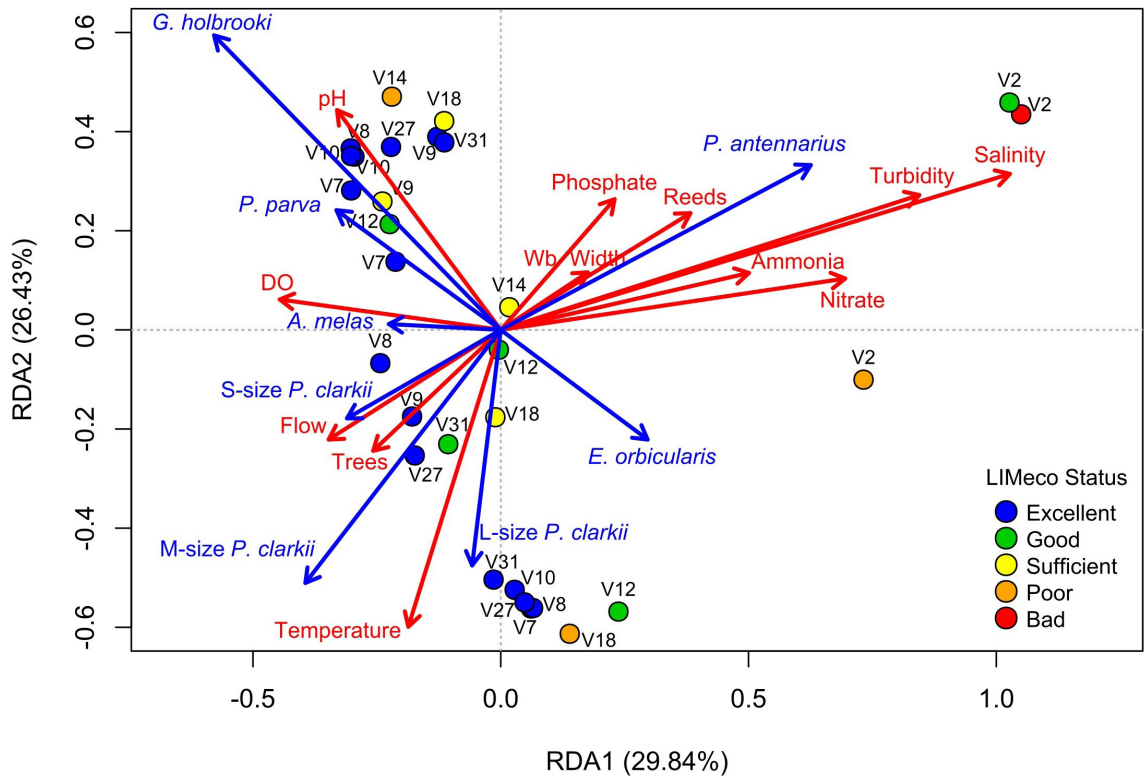


Fig. 5. Redundance analysis with environmental variables (red arrows) and biotic variables (CPUE; blue arrows) by sampling month (June, July, September 2022). Environmental variables include temperature, salinity, pH, dissolved oxygen ("DO"), nitrate concentrations, ammonia concentrations, phosphate concentrations, riparian trees cover ("Trees"), reeds cover, water body width ("Wb width"), and water flow ("flow"). The dots' colour represents the LIMeco Index status (Blue = Excellent, Green = Good, Yellow = Sufficient, Orange = Poor, Red = Bad). Explained variance by axes I) 29.84%; II) 26.43%.

primarily with *A. melas*, while large-sized crayfish were more associated with *E. orbicularis*. In contrast, a negative association was detected between large-sized crayfish and *G. holbrooki*, as well as between all sizes of *P. clarkii* and *P. antennarius*. Furthermore, *E. orbicularis* was not related to most of the environmental variables but exhibited a negative correlation with most of the fish species, particularly *G. holbrooki* and *P. parva*. The fish species were all positively associated with one another, as well as variables such as oxygen and pH. All primary associations identified by RDA were corroborated by Spearman's rank correlation coefficients (Supplement Material Fig. S1), although not all of them reached statistical significance.

DISCUSSION

Environmental characterisation of the study area

The aim of this work was to evaluate the distribution, abundance, and population structure of *Procambarus clarkii* in understudied small marginal water bodies. Additionally, it sought to analyse the population dynamics of the species in relation to other aquatic species inhabiting these small marginal coastal environments, as well

as hydromorphological characteristics and water chemistry, although acknowledging the potential influence from natural events, such as high precipitation or heatwaves occurring in the days prior to sampling. This research shows that the Valle Averno Oasis represents a notable mosaic of aquatic marginal coastal habitats, with water flowing through a complex net of wider or narrower canals or stagnating in small ponds and isolated canals. Water chemistry characteristics were relatively homogeneous among some stations (V7, V9, V8, and V10), where spatial proximity probably outweighed morphology differences. Conversely, strong variability was observed across other stations, particularly for pH, salinity, nutrients, and chlorophyll *a* concentrations. This variability resulted in considerable variation in the water LIMeco Index, which ranged from Excellent to Bad quality class. This variance could be explained by a mix of factors, including distance, isolation, reeds and riparian vegetation, morphology, and hydrological connectivity (Mitsch and Gosselink, 2000; Lindenschmidt *et al.*, 2005). The water input canal entering from the surrounding countryside (V18) exhibited high concentrations of nutrients, which could be attributed to run off from the cultivated fields (Coppola *et al.*, 2024). Canals partially isolated (V14) or cut out from the main hydrological network (V2), ex-

hibited critical water chemistry, including low dissolved oxygen, high nutrients concentrations, and considerably higher salinity compared with canals with flowing water, reflecting these characteristics into the worst water quality index and different aquatic fauna composition (Amoros and Bornette, 2002). On the contrary, stations located towards the end of the oasis hydrological network exhibited better water quality, presumably benefiting from the buffering and depuration capacity of the riparian vegetation (Tamburini *et al.*, 2020).

Red swamp crayfish population and relations with environmental variables

Despite the variability among sampling stations, the results of the study showed that the red swamp crayfish has successfully invaded most of these marginal habitats, although with different densities, sex ratio and mean carapace size. Although *P. clarkii* could exhibit great tolerance and adaptability to a wide range of critical environmental conditions, such as low oxygen, high nutrient concentrations, and high turbidity (Huner and Lindqvist, 1995; Maceda-Veiga *et al.*, 2013), it was completely absent at station V2, representing the only area where the species was never sampled among the ten investigated throughout the entire season. Results suggest that the main factor limiting the presence of the species could be salinity, suggesting that brackish waters with a consistent salinity around 10 could not enable a stable colonization (Huner and Barr, 1991; Meineri *et al.*, 2014). The poor water quality detected in June and July represents a possible contribution to the absence of the species, but probably not the main reason, as similar conditions were also detected at other stations, such as V14 and V18, during the same period. Moreover, water characteristics at V14 and V2 were overall quite similar, except for salinity, that remained below 1 at all sampling stations, including V14, with the only exception of V2, due to the station's isolation from freshwater input. Additionally, considering *P. clarkii*'s ability to move overland (Bernardo *et al.*, 2011; Souty-Grosset *et al.*, 2016), and the proximity of other canals inhabited by the species, if low water quality had represented a limitation, the species could have easily reached V2 when better water quality permitted its survival, e.g., in September. The presence of species with strong salinity tolerance, such as *Palaemonetes antennarius* and *Emys orbicularis* (Ungherese *et al.*, 2008; Agha *et al.*, 2018) at V2 further supports the idea that water quality did not represent a limitation alone, strengthening the hypothesis of salinity as the primary determinant.

However, the absence of *P. clarkii* at V2 could also be due to a combination of several stressors (e.g., potential anoxic crises and high concentration of nutrients), which would require an excessive physiological cost for the crayfish to sustain simultaneously with higher salinity. Indeed, while not preventing the species' presence alone, low water quality likely affects its abundance, as significant negative associations between *P. clarkii* and

nutrients concentration was recorded. The lowest biomass of the red swamp crayfish was found at low water quality stations (V14 and V18), where long exposure to extreme conditions might not be optimal for the species. Such exposure may ultimately damage the immune and antioxidant system, subsequently leading to disease, especially in juvenile individuals, who exhibit weaker resistance to bacterial infection (Zeng *et al.*, 2024). Despite variable abundances, the species was ubiquitous at all the other stations, showing a slight preference for narrower water bodies, which may be due to reduced predation pressure from large fish (Elvira *et al.*, 1996; Gavioli *et al.*, 2018). Moreover, the species was abundant at both stations with slower flow, as the red swamp crayfish preferably inhabit lentic warmer waters, where they could find favourable conditions for burrowing activity (Donato *et al.*, 2018), but also at flowing water stations, where especially large sized individuals were sampled, likely due to their stronger resistance to water flow (Maude and Williams, 1983). *Procambarus clarkii* was confirmed to be more abundant during warmer months, benefiting from its temperature optimum (Anastácio *et al.*, 1999; Scalici *et al.*, 2010). The sex ratio, which was skewed toward males, could be explained by seasonal variations, as during summer, females in their reproductive phase generally remain in their burrows (Gherardi *et al.*, 2000; Vogt, 2013; Donato *et al.*, 2018; Mistri *et al.*, 2019). Moreover, crayfish sizes varied among stations and were negatively associated with pH. The extremes were detected at V14 (highest pH, minimum size) and V18 (lowest pH, maximum size), supporting the role of pH in affecting growth rates, with maximum growth occurring around pH 7.8 and decreases as pH increases (Yue *et al.*, 2009), whenever also additional factors such as crayfish densities or high nutrients concentrations may also contributed to this result.

Red swamp crayfish relations with aquatic fauna

Procambarus clarkii is a generalist species, and no strong associations with fish species were detected, suggesting either minimal reciprocal influence or that a relevant spatial segregation is occurring. However, a trend was observed in which larger red swamp crayfish were more closely associated with fish species like *G. holbrooki* and positively related to *E. orbicularis*. This complex relationship, which includes reciprocal predation (crayfish predating fish eggs; fish predating crayfish juveniles) (Ilhéu *et al.*, 2007; Anastácio *et al.*, 2011; Souty-Grosset *et al.*, 2016), could explain the lack of strong overall correlation. The low diversity of the native nektonic community likely reflects the negative impact of the invasive crayfish and the history of invasion of other alien fish species, including large carps and catfish. Our sampling method did not permit the capture of larger species, such as *C. carpio* and *Silurus glanis* Linnaeus, 1758, which are widely distributed in the oasis (LIFE ForestAll, 2019; S. Borella, personal communication).

Nevertheless, the fish fauna was dominated by non-native species (*G. holbrooki*, *P. parva*, *Ameiurus melas*); native species (except for the rarely recorded *A. alborella*) have largely been replaced due to the high adaptability of these non-native generalists. The wide distribution and impacts of *P. clarkii* are considered a crucial factor contributing to the drastic change in the nektonic community (Gherardi, 2006; Scalici *et al.*, 2010). However, the crayfish also serves as a food source for native predators like *E. orbicularis* and various avifauna, exerting also a positive impact on the invaded area (Correia, 2001; Ottonello, 2005; Tablado *et al.*, 2010; Delmastro, 2015). In conclusion, the red swamp crayfish has found a favourable environment in the Valle Averno Oasis, successfully colonizing most of the marginal water bodies and likely contributing to their modification. However, the brackish nature of certain habitats appears to limit its expansion, with the species failing to stably colonize water bodies with higher salinity.

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REFERENCES

- Acevedo-Limón, L., Oficialdegui, F.J., Sánchez, M.I., Clavero, M. 2020. Historical, human, and environmental drivers of genetic diversity in the red swamp crayfish (*Procambarus clarkii*) invading the Iberian Peninsula. *Freshwater Biology*, 65(8), 1460-1474. <https://doi.org/10.1111/fwb.13513>
- Agha, M., Ennen, J.R., Bower, D.S., Nowakowski, A.J., Sweat, S.C., Todd, B.D. 2018. Salinity tolerances and use of saline environments by freshwater turtles: Implications of sea level rise. *Biological Reviews*, 93(3), 1634-1648. <https://doi.org/10.1111/brv.12410>
- Amoros, C., Bornette, G., 2002. Connectivity and biocomplexity in waterbodies of riverine floodplains. *Freshwater Biology*, 47(4), 761-776. <https://doi.org/10.1046/j.1365-2427.2002.00905.x>
- Anastácio, P.M., Nielsen, S.N., Marques, J.C. 1999. CRISP (crayfish and rice integrated system of production): 2. Modelling crayfish (*Procambarus clarkii*) population dynamics. *Ecological Modelling*, 123(1), 5-16. [https://doi.org/10.1016/S0304-3800\(99\)00164-7](https://doi.org/10.1016/S0304-3800(99)00164-7)
- Anastácio, P.M., Leite, E.P., Ferreira, M., Vicente, L., Correia, A.M. 2011. Ontogenic shifts in predatory roles involving two invasive species. *Limnologia*, 41(3), 228-234. <https://doi.org/10.1016/j.limno.2010.12.002>
- Azzellino, A., Canobbio, S., Çervigen, S., Marchesi, V., Piana, A. 2015. Disentangling the multiple stressors acting on stream ecosystems to support restoration priorities. *Water Science and Technology*, 72(2), 293-302. <https://doi.org/10.2166/wst.2015.177>
- Banha, F., Anastácio, P.M. 2014. Desiccation survival capacities of two invasive crayfish species. *Knowledge and Management of Aquatic Ecosystems*, 413, 1. <https://doi.org/10.1051/kmae/2013084>
- Barbaresi, S., Gherardi, F. 2000. The invasion of the alien crayfish *Procambarus clarkii* in Europe, with particular reference to Italy. *Biological Invasions*, 2, 259-264. <https://doi.org/10.1023/A:1010009701606>
- Bates, D.M., Mächler, M., Bolker, B.M., Walker, S.C. 2015. Fitting linear mixed-effects models using lme4. *Journal of Statistical Software*, 67(1), 1-48. <https://doi.org/10.18637/jss.v067.i01>
- Bernardo, J.M., Costa, A.M., Bruxelas, S., Teixeira, A. 2011. Dispersal and coexistence of two non-native crayfish species (*Pacifastacus leniusculus* and *Procambarus clarkii*) in NE Portugal over a 10-year period. *Knowledge and Management of Aquatic Ecosystems*, 401, 28. <https://doi.org/10.1051/kmae/2011047>
- Bioprogram. 2006. Area protetta di Valle Averno - Aggiornamento delle conoscenze sull'erpetofauna della Riserva Naturale dello Stato di Valle Averno (Protected area of Valle Averno - Updated knowledge on the herpetofauna of the Valle Averno State Nature Reserve). Bioprogramm s.c.r.l., 17 pp.
- Bioprogram. 2008. Progetto preliminare della sistemazione idraulica di Valle Averno e relativi rilievi tecnici conoscitivi (Preliminary design for the hydraulic reclamation of Valle Averno and related technical fact-finding surveys). Bioprogramm s.c.r.l., 118 pp.
- Bonada, N., Resh, V.H. 2013. Mediterranean-climate streams and rivers: Geographically separated but ecologically comparable freshwater systems. *Hydrobiologia*, 719(1), 1-29. <https://doi.org/10.1007/s10750-013-1634-2>
- Bonvillain, C.P., Rutherford, D.A., Kelso, W.E., Green, C.C. 2012. Physiological biomarkers of hypoxic stress in red swamp crayfish *Procambarus clarkii* from field and laboratory experiments. *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology*, 163(1), 15-21. <https://doi.org/10.1016/j.cbpa.2012.04.015>
- Bonvillain, C.P., Rutherford, D.A., Kelso, W.E. 2015. Effects of environmental hypoxia on population characteristics of red swamp crayfish *Procambarus clarkii* in the Atchafalaya River Basin, Louisiana. *Hydrobiologia*, 743, 309-319. <https://doi.org/10.1007/s10750-014-2049-4>
- Cilenti, L., Alfonso, G., Gargiulo, M., Chetta, F.S., Liparoto, A., D'Adamo, R., Mancinelli, G. 2017. First records of the crayfish *Procambarus clarkii* (Girard, 1852) (Decapoda, Cambaridae) in Lake Varano and in the Salento Peninsula (Puglia region, SE Italy), with review of the current status in southern Italy. *BioInvasions Records*, 6(2), 153-158. <https://doi.org/10.3391/bir.2017.6.2.11>
- Coppola, A., Zucaro, R., Baralla, S., Satta, M., Ruberto, M., Comegna, A., Dragonetti, G., *et al.* 2024. Monitoring and modelling fluxes of water and nutrients to surface drainage network from irrigated agricultural fields in a hydraulically reclaimed coastal area. *Ecohydrology*, 17(8), e2723. <https://doi.org/10.1002/eco.2723>
- Correia, A.M. 2001. Seasonal and interspecific evaluation of predation by mammals and birds on the introduced red swamp crayfish *Procambarus clarkii* (Crustacea, Cambaridae) in a freshwater marsh (Portugal). *Journal of Zoology*, 255(4), 533-541. <https://doi.org/10.1017/S0952836901001625>

- Curiel, D., Boscolo, N., Marzocchi, M. 2008. Il macrofitobenthos delle valli da pesca della Laguna di Venezia (The macrophytobenthos of the fishing ponds in the Venice Lagoon). *Lavori Società Veneziana Scienze Naturali*, 33, 59-70.
- Delmastro, G.B. 1992. Sull'acclimatazione del gambero della Louisiana *Procambarus clarkii* (Girard, 1852) nelle acque dolci italiane (Crustacea: Decapoda: Cambaridae) (in Italian). *Pianura*, 4, 5-10.
- Delmastro, G.B. 2015. Il gambero della Louisiana (*Procambarus clarkii*): Una specie invasiva alla conquista del Piemonte (The Louisiana crayfish (*Procambarus clarkii*): An invasive species conquering Piedmont). IV convegno regionale "La Fauna del Piemonte", Città di Cherasco, 16 pp.
- Donato, R., Rollandin, M., Favaro, L., Ferrarese, A., Pessani, D., Ghia, D. 2018. Habitat use and population structure of the invasive red swamp crayfish *Procambarus clarkii* (Girard, 1852) in a protected area in northern Italy. *Knowledge and Management of Aquatic Ecosystems*, 419, 12. <https://doi.org/10.1051/kmae/2018002>
- Dörr, A.J.M., Scalici, M., Caldaroni, B., Magara, G., Scoparo, M., Goretti, E., Elia, A.C. 2020. Salinity tolerance of the invasive red swamp crayfish *Procambarus clarkii* (Girard, 1852). *Hydrobiologia*, 847(9), 2065-2081. <https://doi.org/10.1007/s10750-020-04231-z>
- Elvira, B., Gnicola, G., Almodovar, A. 1996. Pike and red swamp crayfish: A new case on predator-prey relationship between aliens in central Spain. *Journal of Fish Biology*, 48(3), 437-446. <https://doi.org/10.1111/j.1095-8649.1996.tb01438.x>
- European Union. 2000. Directive 2000/60/EC of the European Parliament and of the Council of 23 October 2000 establishing a framework for Community action in the field of water policy. *Official Journal of the European Communities*, L 327, 1-73.
- European Union. 2014. Regulation (EU) No 1143/2014 of the European Parliament and of the Council of 22 October 2014 on the prevention and management of the introduction and spread of invasive alien species. *Official Journal of the European Union*, L 317, 35-55.
- Favaro, L., Tirelli, T., Gamba, M., Pessani, D. 2011. Sound production in the red swamp crayfish *Procambarus clarkii* (Decapoda: Cambaridae). *Zoologischer Anzeiger*, 250(2), 143-150. <https://doi.org/10.1016/j.jcz.2011.01.002>
- Gasith, A., Resh, V.H. 1999. Streams in Mediterranean climate regions: Abiotic influences and biotic responses to predictable seasonal events. *Annual Review of Ecology, Evolution and Systematics*, 30(1), 51-81. <https://doi.org/10.1146/annurev.ecolsys.30.1.51>
- Gavioli, A., Milardi, M., Lanzoni, M., Mantovani, S., Aschonitis, V., Soana, E., Fanno, E.A., et al. 2018. Managing the environment in a pinch: Red swamp crayfish tells a cautionary tale of ecosystem based management in northeastern Italy. *Ecological Engineering*, 120, 546-553. <https://doi.org/10.1016/j.ecoleng.2018.07.013>
- Gherardi, F. 2006. Crayfish invading Europe: The case study of *Procambarus clarkii*. *Marine and Freshwater Behaviour and Physiology*, 39(3), 175-191. <https://doi.org/10.1080/10236240600869702>
- Gherardi, F., Barbaresi, S., Salvi, G. 2000. Spatial and temporal patterns in the movement of *Procambarus clarkii*, an invasive crayfish. *Aquatic Sciences*, 62(2), 179-193. <https://doi.org/10.1007/PL00001330>
- Gutiérrez-Yurrita, P.J., Montes, C. 1999. Bioenergetics and phenology of reproduction of the introduced red swamp crayfish, *Procambarus clarkii*, in Doñana National Park, Spain, and implications for species management. *Freshwater Biology*, 42(3), 561-574. <https://doi.org/10.1046/j.1365-2427.1999.00484.x>
- Hobbs, H.H. 1972. Biota of freshwater ecosystems, identification manual 9: Crayfishes (Astacidae) of North and Middle America. US Environmental Protection Agency, Washington DC, 173 pp.
- Huner, J.V., Barr, J.E. 1991. Red swamp crawfish: Biology and exploitation. Louisiana Sea Grant College Program, 136 pp.
- Huner, J.V., Lindqvist, O.V. 1995. Physiological adaptations of freshwater crayfishes that permit successful aquacultural enterprises. *American Zoologist*, 35(1), 12-19. <https://doi.org/10.1093/icb/35.1.12>
- Ilhéu, M., Bernardo, J.M., Fernandes, S. 2007. Predation of invasive crayfish on aquatic vertebrates: The effect of *Procambarus clarkii* on fish assemblages in Mediterranean temporary streams. In *Biological invaders in inland waters: Profiles, distribution, and threats* (ed. F. Gherardi). Springer, Dordrecht. pp. 543-558. https://doi.org/10.1007/978-1-4020-6029-8_29
- Italian Ministry of the Environment and Protection of Land and Sea. 2010. Decreto 8 novembre 2010, n. 260. Regolamento recante i criteri tecnici per la classificazione dello stato dei corpi idrici superficiali (Decree No. 260 of 8 November 2010. Regulation on technical criteria for the classification of the status of surface water bodies). *Official Gazette of the Italian Republic, General Series No. 30 (Suppl. Ord. No. 29)*.
- Italian Ministry of the Environment and Protection of Land and Sea. 2017. Decreto Legislativo 15 dicembre 2017, n. 230. Adeguamento della normativa nazionale alle disposizioni del regolamento (UE) n. 1143/2014 (Legislative Decree No. 230 of 15 December 2017. Adjustment of national legislation to the provisions of Regulation (EU) No. 1143/2014). *Official Gazette of the Italian Republic, General Series No. 24*.
- Kang, S.-R., King, S. L. 2012. Influence of salinity and prey presence on the survival of aquatic macroinvertebrates of a freshwater marsh. *Aquatic Ecology*, 46(4), 411-420. <https://doi.org/10.1007/s10452-012-9410-3>
- Kouba, A., Petrusek, A., Kozák, P. 2014. Continental-wide distribution of crayfish species in Europe: Update and maps. *Knowledge and Management of Aquatic Ecosystems*, 413, 5. <https://doi.org/10.1051/kmae/2014007>
- Kuznetsova, A., Brockhoff, P.B., Christensen, R.H.B. 2017. lmerTest package: Tests in linear mixed effects models. *Journal of Statistical Software*, 82(13), 1-26. <https://doi.org/10.18637/jss.v082.i13>
- LIFE ForestAll. 2019. LIFE18 NAT/IT/001020. <https://lifeforestall.eu/> (accessed 19 February 2025).
- Lindenschmidt, K.E., Poser, K., Rode, M. 2005. Impact of morphological parameters on water quality variables of a regulated lowland river. *Water Science & Technology*, 52(6), 187-193. <https://doi.org/10.2166/wst.2005.0167>
- Lo Parrino, E., Ficetola, G.F., Manenti, R., Falaschi, M. 2020. Thirty years of invasion: The distribution of the invasive crayfish *Procambarus clarkii* in Italy. *Biogeographia - The Journal of Integrative Biogeography*, 35, 43-50. <https://doi.org/10.21426/B635047157>
- Lorenzen, C.J. 1966. A method for the continuous measurement of *in vivo* chlorophyll 117 concentration. *Deep-Sea Research*, 13, 223-227.

- Loureiro, T.G., Anastácio, P.M.S.G., Araujo, P.B., Souty-Grosset, C., Almerão, M.P. 2015. Red swamp crayfish: Biology, ecology and invasion - An overview. *Nauplius*, 23(1), 1-19. <https://doi.org/10.1590/S0104-64972014002214>
- Maceda-Veiga, A., De Sostoa, A., Sánchez-Espada, S. 2013. Factors affecting the establishment of the invasive crayfish *Procambarus clarkii* (Crustacea, Decapoda) in the Mediterranean rivers of the northeastern Iberian Peninsula. *Hydrobiologia*, 703(1), 33-45. <https://doi.org/10.1007/s10750-012-1335-2>
- Maude, S.H., Williams, D.D. 1983. Behavior of crayfish in water currents: Hydrodynamics of eight species with reference to their distribution patterns in southern Ontario. *Canadian Journal of Fisheries and Aquatic Sciences*, 40, 1. <https://doi.org/10.1139/f83-010>
- McMahon, B.R. 2001. Respiratory and circulatory compensation to hypoxia in crustaceans. *Respiration Physiology*, 128(3), 349-364. [https://doi.org/10.1016/S0034-5687\(01\)00311-5](https://doi.org/10.1016/S0034-5687(01)00311-5)
- Meineri, E., Rodriguez-Perez, H., Hilaire, S., Mesleard, F. 2014. Distribution and reproduction of *Procambarus clarkii* in relation to water management, salinity and habitat type in the Camargue. *Aquatic Conservation: Marine and Freshwater Ecosystems*, 24(3), 312-323. <https://doi.org/10.1002/aqc.2410>
- Mischke, C.C., Zimba, P.V. 2004. Plankton community responses in earthen channel catfish nursery ponds under various fertilization regimes. *Aquaculture*, 233(1-4), 219-235. <https://doi.org/10.1016/j.aquaculture.2003.09.044>
- Mistri, M., Sfriso, A., Sfriso, A.A., Munari, C. 2019. Distribution and population structure and dynamics of the red swamp crayfish *Procambarus clarkii* (Girard, 1852) in the eastern Po valley and its delta (northeastern Italy). *BioInvasions Records*, 8(1), 142-153. <https://doi.org/10.3391/bir.2019.8.1.16>
- Mitsch, W.J., Gosselink, J.G. 2000. *Wetlands*. Wiley, 936 pp.
- Morpurgo, M., Aquiloni, L., Bertocchi, S., Brusconi, S., Tricarico, E., Gherardi, F. 2010. Distribuzione dei gamberi d'acqua dolce in Italia (Distribution of freshwater crayfish in Italy). *Studi Trentini di Scienze Naturali*, 87, 125-132.
- Nota, A., Santovito, A., Gattelli, R., Tiralongo, F. 2023. From fresh to salt waters: First reports of the red swamp crayfish *Procambarus clarkii* (Girard, 1852) in Mediterranean marine waters. *Hydrobiology*, 3(1), 1-10. <https://doi.org/10.3390/hydrobiology3010001>
- Oksanen, J., Blanchet, F.G., Kindt, R., Legendre, P., Minchin, P., O'Hara, B., Simpson, G.L., et al. 2015. *Vegan: Community ecology package*. R Package Version 2.2-1. 2. 1-2.
- Ottonello, D., Salvidio, S., Rosocchi, E. 2005. Feeding habits of the European pond terrapin *Emys orbicularis* in Camargue (Rhône delta, Southern France). *Amphibia Reptilia*, 26(4), 562-565. <https://doi.org/10.1163/156853805774806241>
- Paglianti, A., Gherardi, F. 2004. Combined effects of temperature and diet on growth and survival of young-of-year crayfish: A comparison between indigenous and invasive species. *Journal of Crustacean Biology*, 24(1), 140-148. <https://doi.org/10.1651/C-2374>
- R Core Team. 2023. *R: A language and environment for statistical computing*. R Foundation for Statistical Computing, Vienna, Austria. <https://www.R-project.org/>
- Ramalho, R.M.O. 2012. Dispersal and population regulation of the red swamp crayfish (*Procambarus clarkii*). PhD thesis, University of Évora, 182 pp.
- Reiber, C.L., McMahon, B.R. 1998. The effects of progressive hypoxia on the crustacean cardiovascular system: A comparison of the freshwater crayfish, (*Procambarus clarkii*), and the lobster (*Homarus americanus*). *Journal of Comparative Physiology B*, 168, 168-176. <https://doi.org/10.1007/s003600050133>
- Roy, D., Alderman, D., Anastasiu, P., Arianoutsou, M., Augustin, S., Bacher, S., Başnou, C., et al. 2020. DAISIE - Inventory of alien invasive species in Europe. Version 1.7. Research Institute for Nature and Forest (INBO). Checklist dataset. <https://doi.org/10.15468/ybwd3x> (accessed 19 April 2024).
- Scalici, M., Chiesa, S., Scuderi, S., Celauro, D., Gibertini, G. 2010. Population structure and dynamics of *Procambarus clarkii* (Girard, 1852) in a Mediterranean brackish wetland (Central Italy). *Biological Invasions*, 12(5), 1415-1425. <https://doi.org/10.1007/s10530-009-9557-6>
- Scalici, M., Gherardi, F. 2007. Structure and dynamics of an invasive population of the red swamp crayfish (*Procambarus clarkii*) in a Mediterranean wetland. *Hydrobiologia*, 583(1), 309-319. <https://doi.org/10.1007/s10750-007-0615-8>
- Scalici, M., Gallitelli, L. 2025. Invasive crayfish stepping as a potential threat for coastal waters. *Water*, 17(10), 1519. <https://doi.org/10.3390/w17101519>
- Siesa, M.E., Padoa-Schioppa, E., Ott, J., De Bernardi, F., Ficeola, G.F. 2014. Assessing the consequences of biological invasions on species with complex life cycles: Impact of the alien crayfish *Procambarus clarkii* on Odonata. *Ecological Indicators*, 46, 70-77. <https://doi.org/10.1016/j.ecolind.2014.05.036>
- Souty-Grosset, C., Anastácio, P.M., Aquiloni, L., Banha, F., Choquer, J., Chucholl, C., Tricarico, E. 2016. The red swamp crayfish *Procambarus clarkii* in Europe: Impacts on aquatic ecosystems and human well-being. *Limnologia*, 58, 78-93. <https://doi.org/10.1016/j.limno.2016.03.003>
- Sun, X., Wang, H., Wang, F., Zhao, Y., Wang, H., Zhu, J., Wei, S., et al. 2023. Effects of different fertilization patterns on the dietary composition of *Procambarus clarkii* in a rice-crayfish coculture system. *Aquaculture Reports*, 33, 101801. <https://doi.org/10.1016/j.aqrep.2023.101801>
- Tablado, Z., Tella, J.L., Sánchez-Zapata, J.A., Hiraldo, F. 2010. The paradox of the long-term positive effects of a North American crayfish on a European community of predators. *Conservation Biology*, 24(5), 1230-1238. <https://doi.org/10.1111/j.1523-1739.2010.01483.x>
- Tamburini, E., Soana, E., Monti, M., Fano, E. A., Castaldelli, G. 2020. Introducing life cycle assessment in costs and benefits analysis of vegetation management in drainage canals of lowland agricultural landscapes. *Water*, 12(8), 2236. <https://doi.org/10.3390/w12082236>
- Ungherese, G., Boddi, V., Ugolini, A. 2008. Eco-physiology of *Palaemonetes antennarius* (Crustacea, Decapoda): The influence of temperature and salinity on cardiac frequency. *Physiological Entomology*, 33(2), 155-161. <https://doi.org/10.1111/j.1365-3032.2008.00620.x>
- Vogt, G. 2013. Abbreviation of larval development and extension of brood care as key features of the evolution of freshwater Decapoda. *Biological Reviews*, 88(1), 81-116. <https://doi.org/10.1111/j.1469-185X.2012.00241.x>
- Yi, S., Zhang, L., Li, Y., Shi, L., Chen, J., Wang, W., She, L., et al. 2020. Genetic diversity and phenotypic variation of the red swamp crayfish, *Procambarus clarkii* (Girard, 1852)

- (Decapoda: Astacidea: Astacidae), in China. *The Journal of Crustacean Biology*, 40(5), 574-583.
<https://doi.org/10.1093/jcobiol/ruaa055>
- Yue, C.F., Wang, T.T., Wang, Y.F., Peng, Y. 2009. Effect of combined photoperiod, water calcium concentration and pH on survival, growth, and moulting of juvenile crayfish (*Procambarus clarkii*) cultured under laboratory conditions. *Aquaculture Research*, 40(11), 1243-1250.
<https://doi.org/10.1111/j.1365-2109.2009.02216.x>
- Zanardo, E. 2015. Il popolamento nectonico in Valle Averte (laguna sud): Implicazioni gestionali e conservazionistiche (in Italian). MSc thesis, Ca' Foscari University, 54 pp.
- Zeng, Q., Luo, M., Qin, L., Guo, C., Liu, J., Zhang, T., Feng, G., *et al.* 2024. Effects of hypoxia stress on survival, antioxidant and anaerobic metabolic enzymes, and related gene expression of red swamp crayfish *Procambarus clarkii*. *Biology*, 13(1), 33.
<https://doi.org/10.3390/biology13010033>