Received: 14 June 2023 | Accepted: 19 March 2024

DOI: 10.1002/2688-8319.12325

Ecological Solutions and Evidence | BRITISH ECOLOGICAL SOCIETY

RESEARCH ARTICLE

Mechanical excavation of wetland habitat failed to eradicate invasive American red swamp crayfish (*Procambarus clarkii*) in Malta

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Funding information
Endeavour Scholarship Scheme (Malta); European Union—European Social Fund (ESF)

Handling Editor: Insu Jo

Abstract

1. Invasive crayfish are an important ecological concern in many freshwater ecosystems. Many efforts have been made to eradicate them, but there is very little documentation of the effectiveness of these efforts. Between 2019 and 2020, a restoration project funded by the European Regional Development Fund tried to eradicate invasive American red swamp crayfish *Procambarus clarkii* from the Fiddien valley system in Malta by mechanically excavating the valley's stream bed and exporting contaminated debris securely off-site to a dry quarry.
2. Three years post-intervention, we systematically surveyed the valley system to explore the distribution and relative abundance of the invasive crayfish population. We placed traps in a stratified random sample of stream segments (both those that were included in the original restoration project and those that were not) and recorded catch per unit effort (crayfish caught per trap night) and the size/frequency distribution of crayfish caught.
3. The invasive crayfish were still abundant in the upper reaches of the valley system, and, despite the excavation effort, crayfish were present at the highest relative abundance (4.3–14.8 CPUE, median = 12.3) within the restored area.
4. Despite substantial effort and spending of more than 700,000 €, mechanical excavation did not eradicate invasive crayfish populations. We urge caution for future projects planning to attempt crayfish eradication using this approach and call for much greater impact evaluation, and at the very least post-project monitoring, to ensure lessons can be learnt from such failures in future.

KEYWORDS
eradication, invasive, mechanical excavation, red swamp crayfish, stream restoration

1 | INTRODUCTION

Global freshwater biodiversity is in crisis and declining even faster than in terrestrial and marine ecosystems due to pressures including habitat degradation, exploitation, climate change, diseases, pollution and invasive species (Reid et al., 2019; WWF, 2022). Invasive species reduce the abundance and diversity of aquatic communities due to direct (predation, competition and grazing) and indirect (habitat alteration) interactions (Gallardo et al., 2016). Invasive species are notoriously difficult to control, and while there are a range of interventions aimed at controlling invasive species in freshwater ecosystems (Thomaz et al., 2015), information on the effectiveness of these interventions is limited (Havel et al., 2015).

The American red swamp crayfish *Procambarus clarkii*, originally from southern U.S.A. and north-eastern Mexico (Hobbs, 1989), has become established in 40 countries across the globe, following intentional introduction for food, as well as unintentional releases through the pet trade (Oficialdegui et al., 2020). This invasive crayfish species is of conservation concern because it eats native species and so impacts freshwater food webs (Twardochleb et al., 2013), and because it is an important vector for the crayfish plague *Aphanomyces astaci* (Souty-Grosset et al., 2016), which has decimated native crayfish populations across Europe (Jussila et al., 2014; Theissinger et al., 2021). There is also recent evidence that the species can transmit the chytrid fungus *Batrachochytrium dendrobatidis*, a disease linked to the decline of 501 amphibian species and 90 presumed extinctions globally (Oficialdegui et al., 2019; Scheele et al., 2019).

Numerous efforts to eradicate invasive crayfish species have been attempted with mixed success (Gherardi et al., 2011). The Conservation Evidence database (Conservation Evidence, 2023) has classified these actions and summarized available evidence of effectiveness. This highlights how limited the available evidence base is. Trapping combined with the encouragement of predators is classified as 'likely to be beneficial' based on two studies (Aquiloni et al., 2010; Neveu, 2001). Trapping and removal are also considered 'likely to be beneficial' but this is based on a single study that shows that crayfish can be effectively trapped, but does not demonstrate effectiveness at eradication at the landscape scale (Aquiloni & Gherardi, 2010). Sterilization of males has been tested in laboratory conditions only (Aquiloni et al., 2009). Similarly, while the chemical pyrethrum has been shown to kill invasive crayfish, and that it can be used locally (e.g. by injecting into individual burrows), there is no evidence that it could be used to eradicate crayfish at a larger scale (Cecchinelli et al., 2012). One study shows that building carefully designed small dams can prevent the spread of invasive crayfish (Dana et al., 2011). Mechanical excavation, the act of using mechanical equipment to capture and extract crayfish from natural sites, is not documented within Conservation Evidence or the review of invasive crayfish control by Gherardi et al. (2011). We know of a single case study using this approach. Peay (2001) reports on an attempt to eradicate invasive signal crayfish, *Pacifastacus leniusculus*, from a small pond at the headwater of River Vyrnwy in Wales through mechanical excavation. The authors concluded that the eradication was unsuccessful.

Red swamp crayfish were first documented within the Maltese Islands in 2016 (Deidun et al., 2018; Vella et al., 2017) across eight valley systems, including the Fiddien valley system. The Fiddien valley is classified as a special area of conservation (SAC) of National Importance because it hosts populations of the only amphibian species in Malta, the painted frog, *Discoglossus pictus*. It also provides important habitat for species of conservation concern, such as the threatened endemic freshwater crustacean sub-species, the Maltese freshwater crab *Potamon fluviatile* ssp. *Lanfrancoi* (Environmental Resource Authority, 2023), the endemic land snail *Trochoidea spratti* and the white garden snail *Theba pisana*. The valley system contains an ephemeral stream that dries during the summer month.

There are records of other invasive species of crayfish in Malta, in particular, the Australian red claw crayfish *Cherax quadricarinatus*, the marbled crayfish *Procambarus virginalis* (in a nearby water reservoir) and the signal crayfish *Pacifastacus leniusculus* (Deidun et al., 2018), but their presence in the Fiddien valley system is unknown.

The European Union funded an ecological restoration project under the European Regional Development Fund (ERDF) to restore the Fiddien valley's biodiversity, improve the valley's accessibility for tourism purposes and enhance its water storage capacity (European Commission, 2023). The project included an effort to eradicate the crayfish by excavating and dredging the silt in the valley bed during the summer months, with all debris transported off-site to a dry, secure location to cull any crayfish caught (ADI Associates, 2017a; Gherardi et al., 2011). We evaluate this intervention's efficacy by assessing the invasive crayfish's population distribution and relative abundance within this valley system 3-year post-restoration. While this 'after-only' design is clearly far from an ideal approach to impact evaluation, the lack of data on the magnitude and distribution of the crayfish population before the eradication makes it the only approach possible. It is certainly sufficient for demonstrating the lack of effectiveness of the intervention: if crayfish are abundant after the eradication programme, the eradication failed. Learning from failure is important for the effectiveness of conservation interventions to be improved (Catalano et al., 2019; Dickson et al., 2023). Making even data from such simple study designs valuable.

2 | MATERIALS AND METHODS

2.1 | The intervention

The ERDF-funded restoration project was conducted during the summer months (May–September) of 2019 and 2020 within the Fiddien valley system (110–130 m a.s.l.; 35°53′30.9″ N, 14°23′23.5″ E, Figure 1) while the stream is dry. It included three main measures: (i) *Eradication of invasive species from the valley* via *excavation* (specifically the American red swamp crayfish *Procambarus clarkii* and the Giant reed *Arundo donax*); (ii) *restoration of the degraded area by planting saplings of native tree and shrub species*; and (iii) *installation of bioengineering works (gabion dams & live crib-wall systems) to create a mosaic of microhabitats* (ADI Associates, 2017b). Our study focused on the effectiveness of the effort to eradicate the invasive crayfish.

Excavation was conducted with heavy machinery (Model: Caterpillar 312E Excavator) by a private company. The stream bed was excavated to a depth of 0.5–3 m. The depth varied because of the varying depth of the bedrock and other practical constraints, such as the need to avoid the roots of protected tree species such as White Poplar *Populus alba* present in the stream banks (Calleja, 2018)

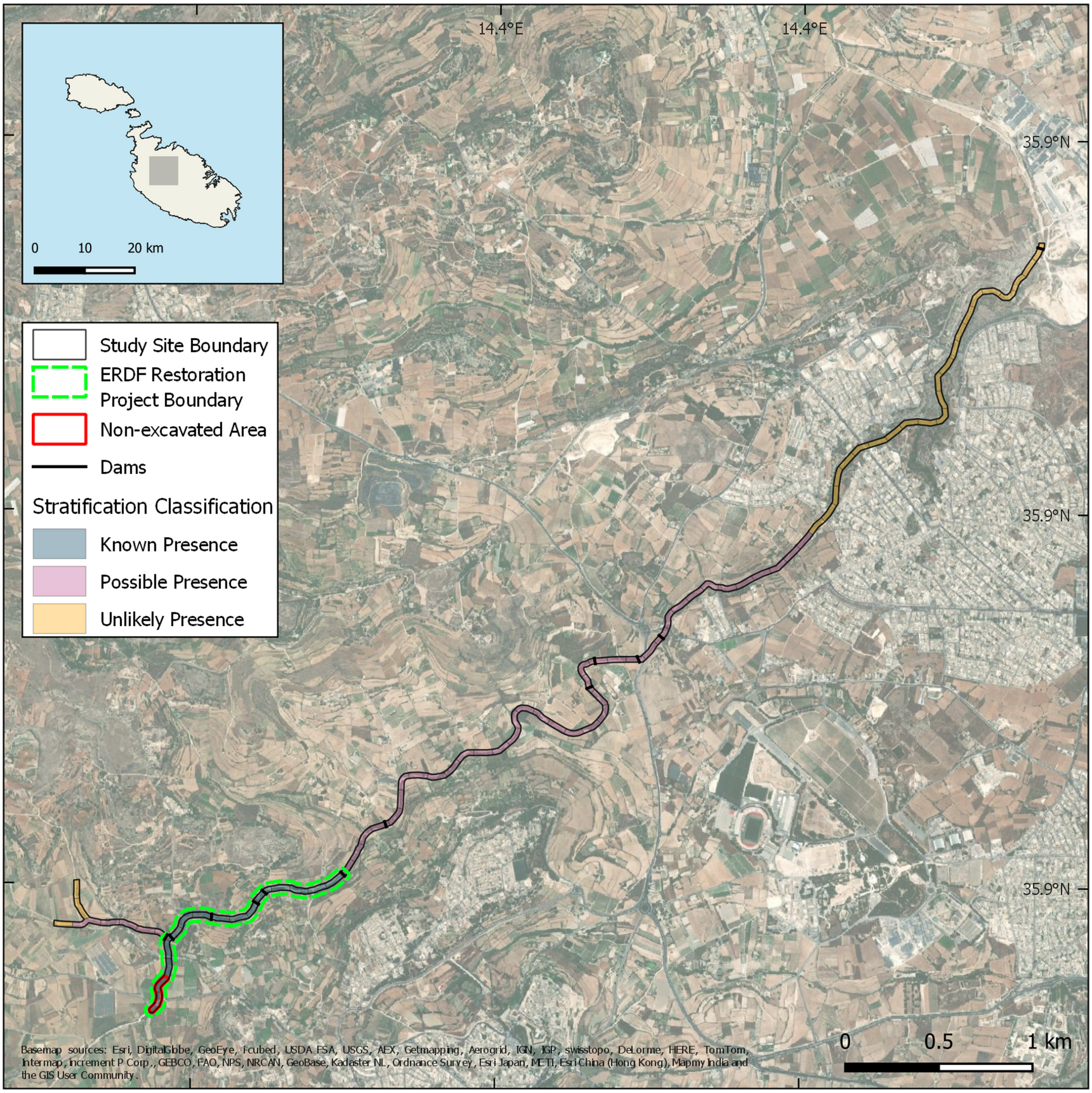

FIGURE 1 Fiddien valley system boundary and stratification used to allocate crayfish trapping effort (Table 1).

(Figure 2) or where the stream bed was inaccessible (Figure 3). Within some zones, the valley width was also widened by approximately 1–3 m to increase the overall area cover of riparian habitat and increase the overall water capacity (ADI Associates, 2017a). All the silt and sediment excavated from the valley bed was transported securely in industrial dump trucks to a nearby abandoned quarry, so that any crayfish caught would be killed.

The overall budget of the ERDF-funded restoration project was 4,237,000 € (European Commission, 2023). The cost of the excavation work aimed at eradicating the invasive crayfish conducted totalled 728,000 € (A. Cutajar, personal communication, 22 October 2022).

2.2 | Evaluation

Unfortunately, no data are available from before the restoration project as to the distribution and abundance of the invasive crayfish. Therefore, an 'after-only' evaluation is all that is possible.

In March and April 2023, we carried out a survey of the crayfish's distribution and relative abundance throughout the Fiddien valley system (including the restored area). We split the study site into 100 m stream segments based on stream data (Parks Malta, 2020) via a geographical information system and stratified our sampling according to three strata: (i) *known presence of invasive crayfish*; (ii)

FIGURE 2 Pictures to demonstrate the constraints on efforts to ensure no refuges were left for crayfish during mechanical excavation. (a) Red arrows mark a handful of protected White Poplar *Populus alba* trees, which meant the bank could not be excavated (note the blue arrow shows where bedrock was struck during excavation). (b) Shows the traditional rubble wall alongside the valley system. A 0.5 m buffer was left along such walls to retain their structural integrity. Crayfish could have survived in burrows alongside these buffers.

possible presence; and (iii) *unlikely presence* based on information from previous studies as well as satellite imagery for water presence or indicator species (Figure 1). Sampling was focused on the stream segments considered most suitable for hosting red swamp crayfish to determine the current distribution of the species (Table 1).

Commercially available nylon crayfish traps (brand: Fishkit Ltd., 60 cm in length, 30 cm in width with a 12 mm mesh) were placed approximately 15–30 m apart systematically in the watercourse in each sampled stream segment. The traps were baited with approximately 50 g of fresh mackerel and were deployed in the morning (07:00–09:00 h) and collected after 24 h. The carapace length (±0.1 mm), sex and species of each trapped crayfish were recorded.

A preliminary test was conducted within the first 4 days of the fieldwork to determine the trapping protocol's efficiency at capturing crayfish where they are known to be present. Our initial tests (using six traps per 24 h) found that 47 of the 48 traps deployed had caught at least one crayfish (98% catch rate), and thus, at all subsequent sites, we used just three traps per night per site to increase the coverage of the study.

2.3 | Crayfish identification

We identified the species by comparing the identifying features of crayfish caught with pictures in the published literature. We verified our identification by sending selected pictures to an expert (Z. Faulkes, McMaster University, 2023).

2.4 | Environmental data

We collected additional environmental parameters to provide further context for the habitats present within each sampled stream segment. The *stream depth* was manually calculated via a rope attached to a metal weight, the *stream width* was calculated via a 100 m rule (or satellite imagery where access was not possible), *aquatic vegetation cover* was measured through a 50 cm quadrant with 10 cm × 10 cm guidelines, *stream substrate* was classified by feel and *riparian vegetation height* was measured with a measuring tape.

2.5 | Ethics

Due to the highly invasive nature of this species, all captured crayfish were culled on-site using a humane method. Crayfish were initially stunned by placing them within an ice slurry for 30 min (Weineck et al., 2018) and then culled by splitting with a knife (Conte et al., 2021). This work was approved by both Bangor University's Ethics Committee (COESE2023AC01A) and Malta's Environmental Resource Authority under *Environmental Permit 1040/23/1H/1J*.

2.6 | Statistical analysis

We calculated catch per unit effort (CPUE) in terms of crayfish numbers per trap night and used it as an indicator of crayfish abundance throughout the valley system. CPUE was calculated via Microsoft Excel and visualized through Quantum GIS (QGIS) in a bubble heat map.

We recorded the carapace length of all crayfish caught and present a box and whisker plot of carapace length as an indicator of age and using CPUE as an indicator of population density, categorized by whether the crayfish were caught within or outside the restoration area. Then we analysed the data through a Welch *t*-test as the populations were independent and there was unequal variance in the data between crayfish caught within and outside the restoration area.

The environmental data collected was also analysed alongside crayfish CPUE in the form of scatter plots and bar plots. We carried out linear regression to explore the relationship between environmental variables and crayfish CPUE. All statistical analyses and plots were generated through R Studio (version 2023.03.0).

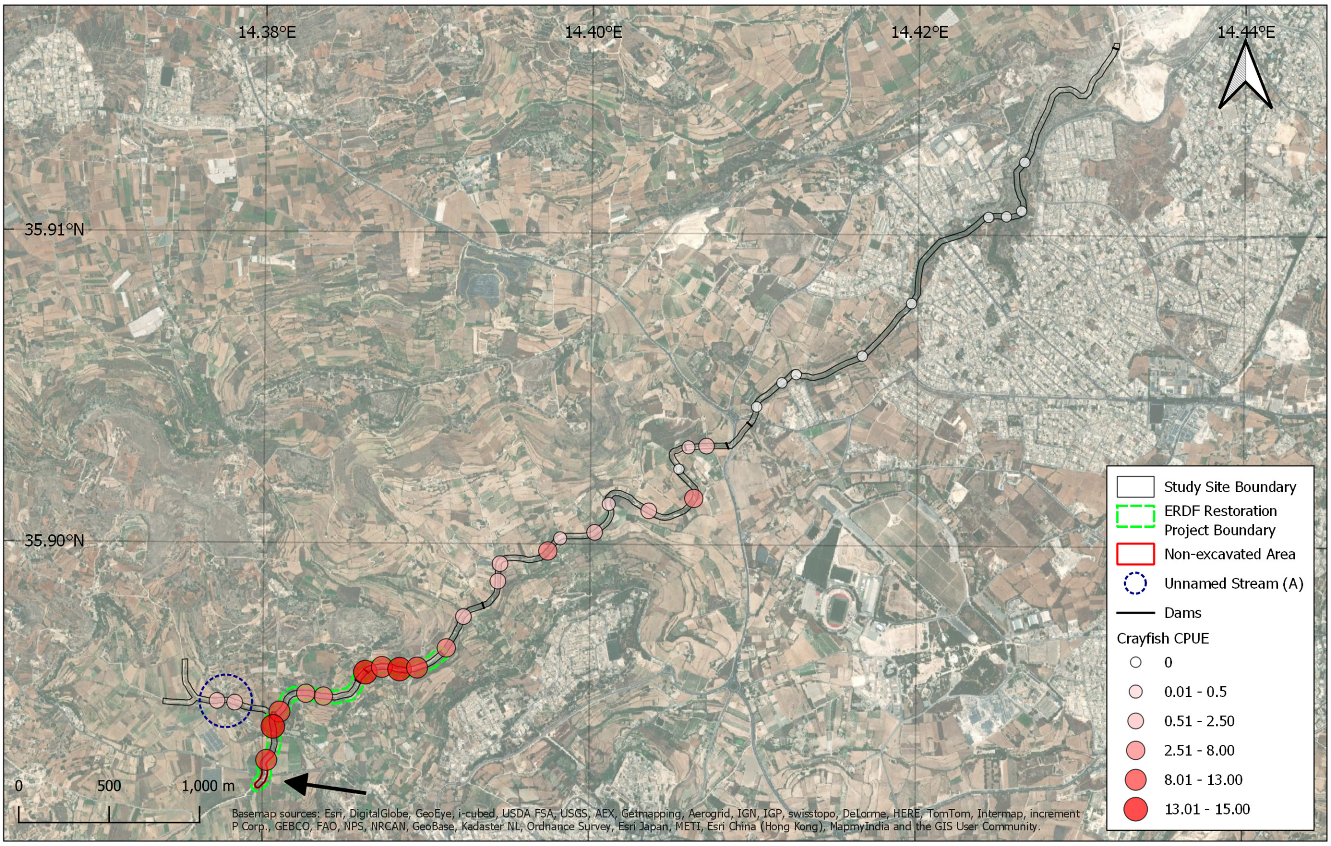

FIGURE 3 Invasive crayfish distribution and relative abundance (CPUE) across the Fiddien valley system recorded in 2023. The circles indicate trapping locations, with the size of the circle and colour indicating the catch per unit effort (crayfish per trap night). The arrow indicates a small area of the restoration site that was inaccessible and where mechanical excavation did not occur. Raw data are available to download (Caruana, 2024).

TABLE 1 Stratified random sampling stratum classification and information on the area of each stratum, the weighting and the resulting sampling effort (trapping in a number of 100 m stream segments) dedicated to each stratum.

Stratum name	Stratum description	Area (m^2)	Stream segments	Sampling weighting	Sampled stream segments
Known presence	*Procambarus clarkii* has been previously documented by to be present within this area	45,540	15	0.27	9
Possible presence	The habitat found within this area is potentially suitable to host a population of *Procambarus clarkii*, as streams and water sources are known to be temporarily present during the wet season. The greater reed (*Arundo donax*) was also used as an indicator species for water presence	133,000	44	0.58	19
Unlikely presence	The habitat found within this area is unlikely to host a population of *Procambarus clarkii* as there is very limited or no water presence for the majority of the year	78,690	26	0.15	5
Total		257,230	85	1	33

3 | RESULTS

We caught 656 crayfish, all of which were *Procambarus clarkii* (Data S1). Raw data are available online (Caruana, 2024) alongside a layman report (Caruana & Farrugia, 2024). Crayfish were found at high CPUE throughout the excavated area (4.3–14.8 CPUE, median = 12.3, Figure 3). Outside the restored area, relative abundance was lower (0–9.0 CPUE with a median of 0.3, Figure 4). In the lower reaches of the Fiddien valley (more than 4 km of excavated area), no crayfish were found in any of the nine sampling sites (Figure 3).

Carapace length of crayfish caught within the excavated area (50.0 ± 9.2 mm) was significantly higher than that found in

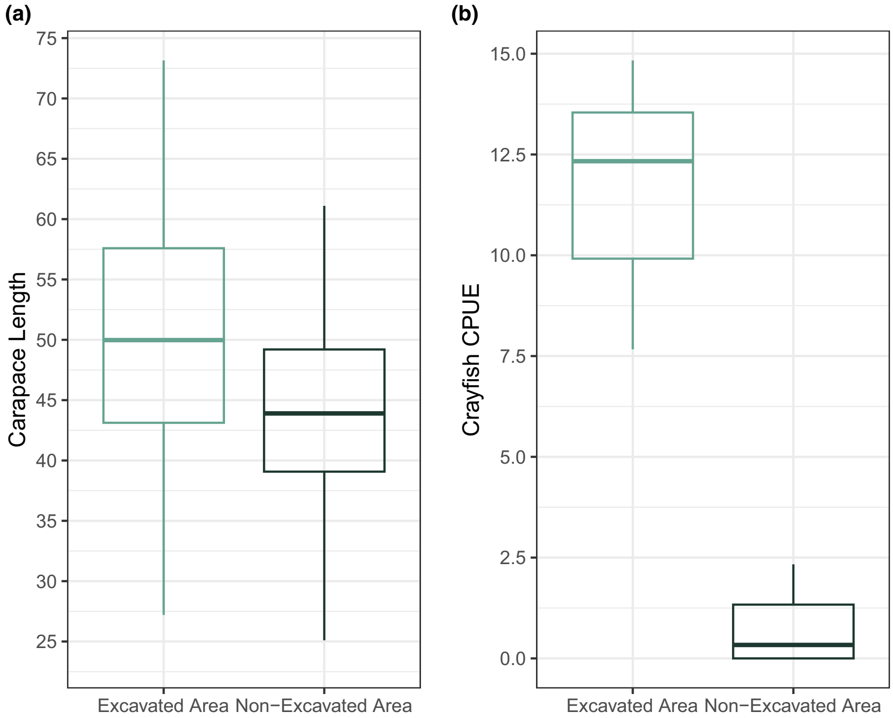

FIGURE 4 Box and whisker plots showing the differences between the carapace length and overall CPUE between the excavated and non-excavated areas in the valley system. The carapace length (a) of crayfish found within the excavated area ($n=536$) was significantly higher than that in the non-excavated area ($n=120$) (a). Crayfish CPUE (b) was higher in excavated areas (4.3–14.8, median = 12.3) than non-excavated areas (0–9.0, median = 0.3).

non-excavated areas (43.4 ± 7.6 mm); $t(206.34)=8.3031$, $p<0.001$ (Welch two sample t-test with a 95% confidence interval, Figure 4). A histogram of the crayfish carapace's length categorized by sex alongside the population's sex distribution can be found within Data S2.

There is a weak relationship between stream width, depth and riparian vegetation height in the sampled stream segments in excavated and non-excavated areas with CPUE. Furthermore, the aquatic vegetation cover appeared to have no relationship with crayfish relative abundance. However, crayfish were more abundant on sand and silt substrates, rather than cobble or bedrock (Figure 5).

4 | DISCUSSION

4.1 | Possible reasons why eradication failed

While the lack of pre-project data on the size and distribution of the invasive crayfish population is not ideal from the perspective of robust impact evaluation (Baylis et al., 2016), an after-only analysis is sufficient to conclude that the intervention failed as invasive crayfish remained present within the restored area. Even with the limitations of the study design, we can conclude that mechanically excavating the valley bed inhabited by invasive crayfish failed to eradicate invasive crayfish from the Fiddien valley system in Malta. We can conclude that, despite 728,000 € being spent, this project failed to eradicate this species. Given the paucity of published evidence about the effectiveness of many conservation interventions, case studies such as this, which report the clear failure of an intervention, can be helpful (White et al., 2022). There are two possible explanations why mechanical excavation failed.

First, while extensive excavation did occur within the restoration site, it is very possible that not all crayfish burrows and individuals were successfully removed. While the average crayfish burrows ranged from 0.28 to 0.58 m in depth in Portugal, some burrows were documented as deep as 4.2 m (Correia & Ferreira, 1995), thus the excavation depth of 0.5–3 m could have been insufficient. Furthermore, some sections of the stream bank could not be excavated due to protected tree species being present and the need to avoid undercutting roads and paths. Since red swamp crayfish reside within burrows during environmental extremes (e.g. drought), it is highly likely that some crayfish may have survived within these unexcavated stream banks (Gherardi, 2006). Additionally, individuals were hidden in structures such as rubble walls alongside the valley system, and a small section of the restoration project was not accessible by the mechanical excavator, which meant this section was not excavated. These could have provided the source for the re-invasion of the entire restoration area.

Second, crayfish could have reinvaded even if eradication was successful within the project site. No information is available concerning the distribution of the crayfish population pre-intervention. It is possible that crayfish were already present outside the area marked for restoration by the time of the restoration project. For example, a stream shown in the bottom-left of Figure 3 outside the excavation area and positioned 10–20 m a.s.l. higher than the valley system harboured a population of red swamp crayfish. This could have acted as a reservoir from which the crayfish reinvaded the project area, and continued to spread downstream, following the restoration project. The re-invasion could also have come from crayfish lower down the Fiddien valley (crayfish were found up to 4 km from the downstream boundary). However, while red swamp crayfish can spread distances upwards of 1.13–3.10 km per year in fast-flowing rivers (Bernardo et al., 2011), individuals in lakes, ponds and marshes

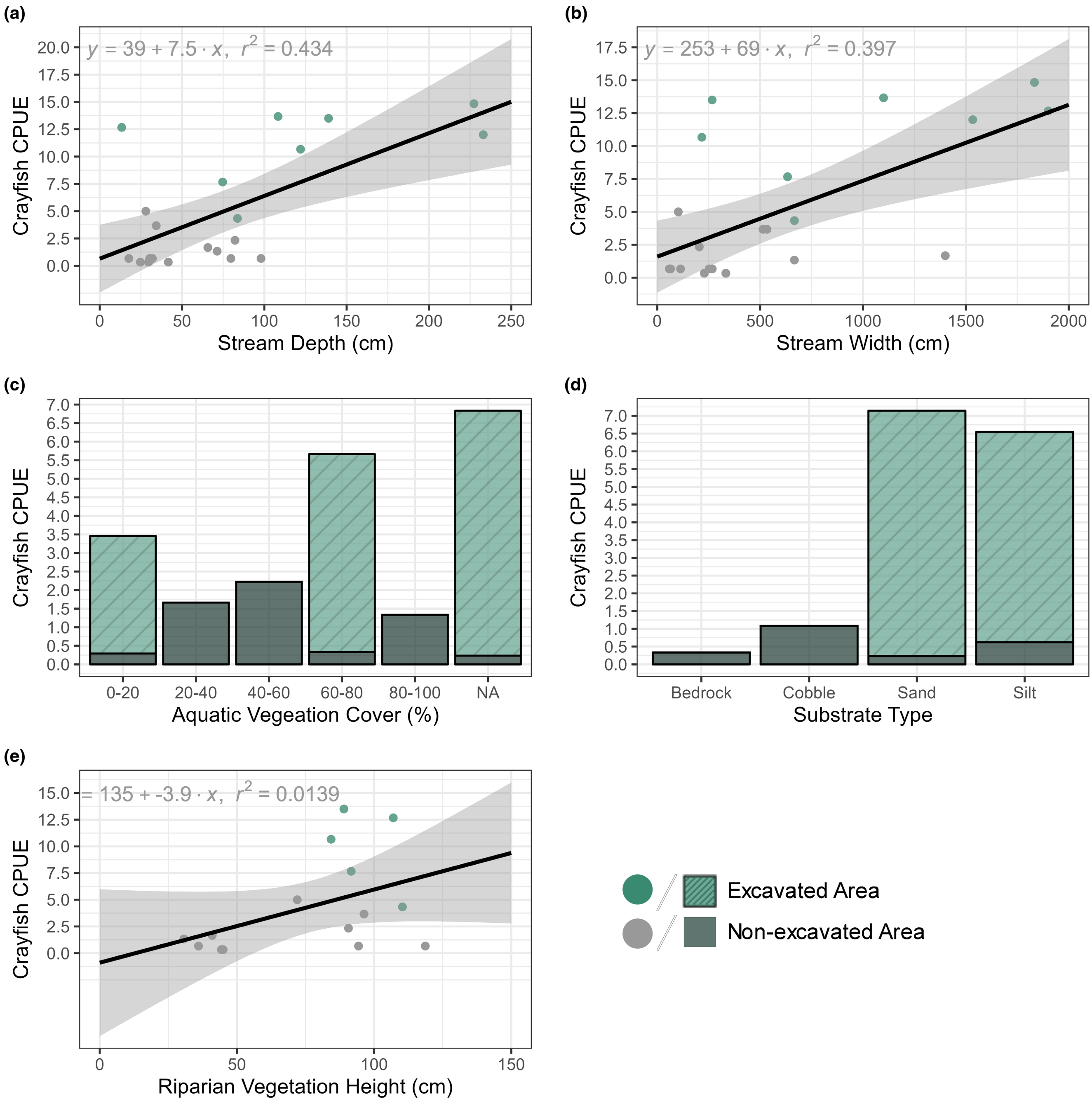

FIGURE 5 The relationship between crayfish relative abundance (CPUE) and stream depth (a), stream width (b) and riparian vegetation height (e). (c) and (d) illustrate that the majority of crayfish were caught in areas with a predominant silt and sand substrate and that aquatic vegetation cover is not correlated with relative abundance.

generally opt to remain within their home range ($3\,m^2$) (Barbaresi et al., 2004) and have an average annual movement range of 44m (Gherardi et al., 2000). Given the limited movement range, the lower abundance and smaller median carapace length of crayfish in the lower reaches (Figure 4), we believe the crayfish within the lower reaches might have relatively recently colonized this area from the upper reaches of the stream (the target of the restoration project), where the crayfish have been known to exist since at least 2016 (Deidun et al., 2018; Vella et al., 2017).

A single berried female remaining post eradication would potentially have been sufficient for the eradication to fail. While we do not have data on the crayfish's reproductive period in Malta, within its native habitat, recruitment often occurs between the end of summer and the beginning of autumn (Gherardi, 2006), and similar reproductive periods have been documented in Portugal (Carvalho et al., 2001). Buried females may well therefore have been present when excavation works ended in the beginning of September.

4.2 | Lessons for future red swamp crayfish eradication attempts

In contrast to this case study, many efforts to eradicate invasive species often apply multiple methods (Stebbing, 2016), making it worth considering whether, had mechanical excavation been combined with other methods, eradication may have been more effective. Biocontrol through predation by native fish such as the European Eel (*Anguilla anguilla*) has been proposed as an approach to reduce the density of red swamp crayfish (Aquiloni et al., 2010). However, the stream dries up in the summer, and eels are not natively present. Given that this species is critically endangered (Pike et al., 2020), manipulating populations in these circumstances with the aim of controlling invasive crayfish seems unlikely to be ethical or practical. Pyrethrum has also been shown to be an effective biocide in crayfish eradication; however, its impacts on amphibians and benthic communities remain unknown and require further research (Peay et al., 2019). Systematic trapping has been shown to be a relatively effective method of suppressing crayfish populations (Conservation Evidence, 2023; Gherardi et al., 2011), and perhaps, alongside mechanical excavation, it could have suppressed the population enough to be functionally eradicated (Green & Grosholz, 2021); however, it would still require management post-excavation to ensure populations remain low. This EU-funded eradication attempt collected no information on effectiveness (our study was completely independent of the funded project). Certainly, future attempts at eradication should build in effective monitoring so lessons can be learned.

4.3 | Habitat associations

This is the first study of red swamp crayfish in Malta. Therefore, we report the information we collected on habitat associations. Crayfish were more abundant on sand and silt substrates rather than cobble or bedrock and were more abundant in wider, deeper streams and in those with higher riparian vegetation. However, these characteristics are those more associated with the upper reaches of the Fiddien valley system (the area where crayfish have been longer established and the target of the restoration project). Therefore, this information does not necessarily provide information on the habitat preferences of the crayfish.

5 | CONCLUSIONS

Due to fast growth rates and high fecundity (Gherardi & Paglianti, 2004), crayfish can recolonize quickly from even a very small population. To have any chance of success, an eradication project therefore needs to ensure it has identified the full extent of the crayfish population before work starts, needs to access all parts of the population and needs to be able to remove all individuals. Given the experience of this project, we do not suggest mechanical excavation to be used as an eradication protocol for invasive crayfish. The costs are high, with specialized machinery and personnel being required, and there is no evidence of the excavation having any impact on the crayfish's population. Ultimately, complete eradication of red swamp crayfish is difficult, and possibly impossible, once populations become established.

The ecological restoration project, which included this effort to eradicate invasive crayfish, received substantial funding from the EU's ERDF programme. Unfortunately, no post project monitoring was carried out. Without this study, there would be no information in the public domain concerning the effectiveness of this intervention. If conservation efforts are to become more effective, then more information is needed about the outcomes of conservation interventions (whether successful or failures).

AUTHOR CONTRIBUTIONS

Alex Caruana and Julia P. G. Jones conceived the research; Alex Caruana, Benjamin Camilleri and Luke Farrugia collected the data; Alex Caruana analysed the data; and Alex Caruana, Benjamin Camilleri and Julia P. G. Jones wrote the manuscript. All authors contributed critically to the drafts and gave final approval for publication.

ACKNOWLEDGEMENTS

We thank Z. Faulkes for his assistance in verifying the crayfish species found within our study. The research included in this publication is funded by the Endeavour Scholarship Scheme (Malta). Project part-financed by the European Union—European Social Fund (ESF) under Operational Programme II—Cohesion Policy 2014–2020, '*Investing in human capital 295 to create more opportunities and promote the well-being of society*'.

CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

PEER REVIEW

The peer review history for this article is available at https://www.webofscience.com/api/gateway/wos/peer-review/10.1002/2688-8319.12325.

DATA AVAILABILITY STATEMENT

Data and analysis code are available in the Zenodo repository, https://doi.org/10.5281/zenodo.10868594 (Caruana, 2024).

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

Data S1: Invasive crayfish species identification.

Data S2: Crayfish population histogram plot and sex distribution.

How to cite this article: Caruana, A., Camilleri, B., Farrugia, L., & Jones, J. P. G. (2024). Mechanical excavation of wetland habitat failed to eradicate invasive American red swamp crayfish (*Procambarus clarkii*) in Malta. *Ecological Solutions and Evidence*, *5*, e12325. https://doi.org/10.1002/2688-8319.12325