



## No traces of emerging and priority organic pollutants in the muscles of *Procambarus clarkii* suggest the feasibility of its regulated and sustainable control from uncontaminated environments

Dario Savoca<sup>a,b,\*</sup>, Vincenzo Arizza<sup>a,b</sup>, Gaetano Cammilleri<sup>c</sup>, Leonardo Cerasino<sup>d</sup>, Antonella Maccotta<sup>a,b</sup>, Federico Marrone<sup>a</sup>, Licia Pantano<sup>c</sup>, Nico Salmaso<sup>b,d</sup>, Francesco Paolo Faraone<sup>a</sup>

<sup>a</sup> Department of Biological, Chemical and Pharmaceutical Sciences and Technologies (STEBICEF), University of Palermo, Palermo 90123, Italy

<sup>b</sup> NBFCC, National Biodiversity Future Center, Palermo 90133, Italy

<sup>c</sup> Istituto Zooprofilattico Sperimentale della Sicilia, Palermo 90129, Italy

<sup>d</sup> Research and Innovation Centre, Fondazione Edmund Mach San Michele all'Adige 38098, Italy

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### ABSTRACT

A monitoring of organic contaminants in the muscles of the *Procambarus clarkii* and environmental samples of water and sediment was conducted in three Sicilian wetlands (Italy). The substances investigated in the biological samples were per- and polyfluoroalkyl substances (PFAS), phthalic acid esters (PAEs), pesticides, antibiotics, and microcystins (MCs), all of which were below the detection limit. Given that the Louisiana red swamp crayfish is considered a bioaccumulator, the results of this study indicate that these environments are not significantly contaminated by the selected pollutants. Furthermore, the study suggests the potential uses of the edible portions of this alien species. Despite *P. clarkii* cannot be marketed in several countries, including Italy, it is possible that the animal biomass obtained in the frame of the monitoring, control and eradication activities carried out by local authorities, could be exploited for various purposes, such as food, feed and biotechnology. This would reduce the costs associated with disposal and make these activities more sustainable in the long term, thereby contributing to the preservation of ecosystems that are currently threatened by this invasive species.

### 1. Introduction

The presence of contaminants of emerging concern (CECs), including pharmaceuticals and personal care products (PPCPs), cyanotoxins, pesticides and perfluoroalkyl substances (PFAS), in freshwater samples has been increasingly documented, and their detection poses a risk to the health of environments and biota (Kazakova et al., 2018; Manjarrés-López et al., 2023; Ríos et al. 2013; Savoca et al., 2023a).

PPCPs encompass a diverse array of chemical compounds, including antibiotics and plasticisers such as phthalates (Rehman et al., 2024). The escalating demand for antibiotics has led to a significant increase in their utilisation, both by humans and in various other fields (Gambino et al., 2022). The high solubility in water and the presence of polar functional groups facilitate the dispersion of antibiotics and their metabolites in surface water, which is also facilitated by the inefficient removal in

water treatment plants and sewage systems (Mahmud et al., 2024). They pose a serious threat to aquatic ecosystems due to their pseudo-persistence, their bioactivity and bioaccumulability (Branchet et al., 2021). Among the main risks associated with antibiotic exposure are the development of antibiotic resistance, toxicity, mutagenicity, and carcinogenicity (Rehman et al., 2024).

Phthalates are easily found in the aquatic environments due to the absence of covalent bonding with the matrices in which they are mixed (Savoca et al., 2023b). The slow degradation of plastics leads to the dispersion of micro- and nanoparticles, which in turn make phthalates bioavailable due to their persistence and lipophilicity (Savoca et al., 2023b). The ubiquity of phthalates is further aggravated by their natural origin, as they can be biosynthesised by various organisms (Pace et al., 2024). As endocrine disrupting chemicals (EDCs) phthalates causes a range of effects, such as the onset of reproductive, growth and

\* Corresponding author at: Department of Biological, Chemical and Pharmaceutical Sciences and Technologies (STEBICEF), University of Palermo, Palermo 90123, Italy.

E-mail address: [dario.savoca@unipa.it](mailto:dario.savoca@unipa.it) (D. Savoca).

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developmental disorders (Savoca et al., 2022).

Despite the restrictions on the use of phthalates (Commission Regulation (EU) 2018/2005), environmental and food contamination continues to persist (Pace et al., 2024). PFAS are other persistent organic pollutants classified as EDCs. They have been identified in a variety of aquatic environments globally and are known as 'forever chemicals' due to their exceptional chemical and thermal stability (Mojiri et al., 2023). Recently, the EU and the US have adopted several regulatory measures for their management (Reinikainen et al., 2024).

PFAS have been used for a variety of applications, including the formulation of pesticides. The latter have long been used in private gardens, farmland and other public areas (Mojiri et al., 2020; Vaglica et al., 2024) to eliminate unwanted organisms.

Although pesticides are subject to varying degrees of regulation, their use in wetlands is frequent with consequent risk to the ecosystem (Barreca et al., 2021).

In addition to PFAS and PAEs, a variety of pesticides have been identified as EDCs, with evidence indicating their association with a multitude of adverse health outcomes. These include systemic toxicity and multifactorial diseases such as metabolic syndromes, teratogenicity, genetic aberrations, and even carcinogenesis (Mojiri et al., 2020; Savoca and Pace, 2021; Savoca et al., 2022; Yang et al., 2024).

The occurrence of cyanobacterial blooms in water bodies has been identified as a global concern (Chorus and Welker, 2021; Jablonska et al., 2024). The massive development of cyanobacteria, which is often sustained by excessive nutrients input, may lead to high levels of toxic metabolites (cyanotoxins) in the water. The most frequent cyanotoxins are microcystins, anatoxins, cylindrospermopsins, and nodularins). In particular, microcystins (MCs) are of concern due to their long-term toxicological effects, their high prevalence in freshwaters, and their remarkable chemical stability, which makes them prone to accumulate in aquatic biota and food crops (Chorus and Welker, 2021). These factors pose a significant health hazard to humans and other animals through the food chain. The potential repercussions of MCs accumulation encompass organ impairment, with the liver, intestines, brain, kidneys, lungs, heart and reproductive system exhibiting heightened vulnerability (Massey et al., 2018). The toxicity, ranging from acute to chronic, is characterised by the formation of a permanent covalent bond between MCs and protein phosphatases, particularly in hepatocytes' cytosol (Massey et al., 2018). This process is associated with an increase in reactive oxygen species (ROS), resulting in morphological and functional alterations in hepatocytes, potentially causing damage to cellular structures and a number of harmful effects, including oxidative stress, lipid peroxidation, apoptosis, cytoskeleton rupture, potential carcinogenesis and autophagy (Ríos et al., 2013; Massey et al., 2018). They can be produced by several common cyanobacterial genera, including *Microcystis*, *Planktothrix*, *Nostoc*, and *Fischerella* (Rastogi et al., 2014; Kurmayer et al., 2017).

Routes of expositions for humans to MCs can be i) direct: through exposition to contaminated water (drinking, inhalation, skin contact); and ii) indirect: through consumption of contaminated aquatic organisms, fish included (Ríos et al., 2013).

The analysis of CECs in aquatic biota could provide a more detailed insight into pollution relationships than would be possible through analysis of contamination only in environmental matrices (Manjarrés-López et al., 2023).

Among the bioindicators most informative about the state of environmental pollution, there are bioaccumulative alien species (Goretti et al., 2016; Spyra et al., 2019).

The processes of globalization, industrialization, the deliberate introduction of exotic species for food production, and climate change facilitate the spread of species beyond their natural habitats (Faraone et al., 2008, 2019; Mori et al., 2022). This phenomenon is exemplified by the Louisiana crayfish (*Procambarus clarkii*), one of the most widespread freshwater crayfish in the world which was introduced for commercial purposes and has become an invasive species in Mediterranean

wetlands, including in Sicily (Italy) (Faraone et al., 2017; Vecchioni et al., 2022). To date, it is known that *P. clarkii* contain a considerable quantity of proteins, amino acids, unsaturated fatty acids, carotenoids, and chitin (Conde and Domínguez, 2015). It can be reasonably deduced that these decapods have the potential to be utilised as functional additives in the food industry, industrial feed and fertilisers in agriculture (Azelee et al., 2023). The economic benefits of its introduction encouraged its exploitation in areas with similar habitats.

Its robust physiology, which allows it to withstand low oxygen levels, high temperatures, and high-water pollution confers it a pivotal role in the food chain, facilitating the transfer of energy and pollutants between trophic levels, rendering it an optimal species for use as a bioindicator (Mistri et al., 2020; Manjarrés-López et al., 2023).

Although *P. clarkii* has been introduced in several countries, it is primarily exploited industrially in the USA, China and Spain (Souty-Grosset et al., 2016). In most European countries, several laws protect native biota by banning the importation of non-indigenous crayfish and/or regulating their use due to their potential enormous economic and ecosystemic impact (Souty-Grosset et al., 2016).

Most EU countries now prohibit the importation of live crayfish, nevertheless, a unified and strengthened legislative framework should be established at the European level to ensure a total ban on the import, trade and holding of live *P. clarkii* (Souty-Grosset et al., 2016).

In this context, there is a need to monitor, control and mitigate the impact of this species through its capture and disposal. Concurrently, there is a necessity to identify alternative and sustainable food or biotechnological products with the objective of addressing population growth and the depletion of natural resources, not only at the European level but also on a global scale. It is therefore necessary to find a sustainable solution to this impact from a circular economy perspective.

In view of the impact of the Louisiana crayfish on Sicilian biota (Marrone and Naselli-Flores, 2015) and its possible use as a resource, it is of primary importance to carry out accurate monitoring the status of the species in regional inland waters, and the assessment of the possible role of the species as a vector of toxic substances or pathogens in Sicily (Italy) as one of the European regions experiencing the deterioration in water quality (Zuccarello et al., 2021; Tricarico and Zanetti, 2023). This information would allow the drafting of "best practices" plans for the management, control and, where possible, eradication of the species. This would be in line with Article 22 of Legislative Decree 230/17, according to which it is possible to authorise the commercial use of specimens of invasive alien species of Union or national importance as part of the management measures aimed at their eradication, numerical control or containment (Reg. EU 1143/14) (Tricarico and Zanetti, 2023).

The high commercial value of this species suggests that the disposal of carcasses at an additional cost could be avoided.

However, as a species capable of accumulating pollutants, it is essential to ensure the safety of the product through chemical analysis.

The aim of this study is to investigate for the first time the occurrence of various CECs in edible portions of *Procambarus clarkii* and in selected environmental matrices collected from three representative areas of Sicily. Furthermore, this study seeks to:

- Compare the results of analyses of different classes of organic pollutants, in particular antibiotics, pesticides, PFAS, PAEs and MCs, for the same sample in order to identify potential differences in contamination profile.
- Compare the results of this study with similar research conducted on the same species.
- Evaluate the feasibility of sustainable and regulated exploitation of this species, based on the results obtained.

## 2. Material and methods

### 2.1. Samples origin and samples preparation

The choice of study areas was made with the aim of testing the studied parameters under quite different environmental conditions. With this approach the following three areas were selected for sampling. Cuccumella reservoir, province of Siracusa (37°21'36"N, 14°54'48"E), is an agricultural pond in the context of organic rice crops. Gorgo Basso, province of Trapani (37°36'30"N, 12°39'19"E), is a natural pond in the context of the nature reserve "Lago di Preola e Gorgi Tondi", surrounded by vineyards and orchards where high levels of lanthanides (D'Angelo, 2013) and *Microcystis* sp. (Naselli-Flores et al., 2007) have been previously detected. San Leonardo river, province of Palermo (37°54'20"N, 13°36'35"E), is a river section upstream of a large reservoir ("Rosamarina"), mainly surrounded by pastures and uncultivated lands.

In July 2023, a total 500 individuals of *Procamburus clarkii* from Cuccumella Reservoir, 159 from Gorgo Basso and 266 from San Leonardo River were caught using baited hoop traps, as described in Vecchioni et al. (2020). Once collected, the specimens of *P. clarkii* were immediately transported to the laboratory in refrigerated containers and then stored at -20°C. Of these 925 individuals, only the amount needed to form sample pools for each site was randomly selected. The pools were constituted with the objective of enhancing the homogeneity and representativeness of the measurements, as well as facilitating comparisons between the various investigated analytes, which were analysed from the same pool.

In detail, for the analyses of PAEs, PFAS, pesticides and antibiotics, a total of 463 specimens were dissected: 183 from the Cuccumella Reservoir, 64 individuals from the San Leonardo River, 118 from the Gorgo Basso. For each site, *P. clarkii* samples were divided into five pools and dissected to obtain only the edible part, without intestine for a total of 15 samples. The biometric data of these individuals are present in Table S1 of supplementary material.

Instead, the specific case of microcystins analyses, a total of 98 specimens were dissected: 48 from the Cuccumella Reservoir, 24 individuals from the San Leonardo River, 26 from the Gorgo Basso. For each site, *P. clarkii* samples were divided into six pools and dissected to obtain samples of muscle with intestine and samples of muscle without intestine for a total of 36 samples. The biometric data of these individuals are recorded (see Table S2 of supplementary material).

All the samples were weighed, homogenised, and stored in sterile polypropylene tubes and frozen at 20 °C until freeze drying.

At the same date, from each site, three sampling points located at 50 m from each other were chosen along a transect; water samples (500 mL each) and sediment samples (500 g each) were collected in each sampling point, and then transported in the laboratory in refrigerated polypropylene (or glass for PAEs analyses) containers previously washed with water collected in situ and stored at 4 °C.

This samples of water were analysed for determination PAEs, PFAS, pesticides and antibiotics while sediment samples were analysed for determination PAEs, PFAS, and pesticides.

Both during sampling and sample preparation, the utmost care was spent to avoid cross contamination or contact contamination, using new or thoroughly washed ceramics and stainless steel after each operation, and implementing solvent cycles of acetone and water liquid chromatography - mass spectrometry (LC-MS) grade.

### 2.2. Pollutants analysis

The class of pollutants investigated were antibiotics, microcystins, pesticides, PFAS, and PAEs.

All extraction and analysis, procedures included the procedural blanks, were performed in duplicate and adapted to the type of matrix

and analyte.

The extraction and analyses of pesticides, PFAS and PAEs were conducted by Chimica Applicata Depurazione Acque s.n.c. di Filippo Giglio e C. (CADA). The methods/protocols used for muscle, sediment and water samples are presented in the [supplementary material](#), together with the full list of all these analytes and their detection limits (Table S3 in the [supplementary material](#)).

The extraction of the toxins was conducted in accordance with the methodology described by Adamovský and Bláha (2016), with minor modifications (see [Supplementary Material](#)). For the LC-MS/MS analysis, the procedures outlined by Cerasino and Salmaso (2020) were employed (see [Supplementary Material](#)).

The detection of macrolides, tetracyclines, sulphonamides, quinolones was carried out by a LC-HRMS method. The method was validated according to the Decision EC 657/2002. The complete extraction and analysis procedures in accordance with Cammilleri et al. (2019) are presented in the [Supplementary Materials](#).

## 3. Results and discussion

Most research on the bioaccumulation in the natural environment of pollutants in *P. clarkii* focuses on trace elements and heavy metals (Ariano et al., 2021; El-Aziz et al., 2022; Mo et al., 2022, Selvaggi et al., 2023; Savoca et al., 2024 and references therein) while, in proportion, few are the research concerning organic pollutants (Song et al., 2018; Yang et al., 2019; Manjarrés-López et al., 2023, Stecconi et al., 2023 and references therein).

In the present study, all biological and environmental samples were below the detection limits for each class of pollutant investigated, except for two water samples, which exhibited the presence of a phthalate.

### 3.1. Phthalates

Benzil butyl phthalate was identified in a water sample from Gorgo Basso ( $0.029 \pm 0.01 \text{ mg L}^{-1}$ ) and in a water sample from the San Leonardo River ( $0.079 \pm 0.028 \text{ mg L}^{-1}$ ); while in all other samples (water, sediment and muscles of *P. clarkii*), the concentration of phthalates was below the level of detection (please refer to Table S3 in the [supplementary material](#) for the complete list of phthalates analysed).

Phthalates contaminations are common in freshwater aquatic environments, both in biota and environmental samples such as water and sediment (Dong et al., 2022; Chaudhary et al., 2023; Liu et al., 2024).

The results indicate the presence of a punctiform contamination at a specific sampling point.

It is well established that these PAEs can be derived from both anthropogenic (for example, the dispersion of plastic material) and natural factors including the biosynthesis of certain phthalates by plant, algae, fungi, and bacteria (Pace et al., 2024). This suggests that it cannot be excluded that there is moderate contamination of natural origin of BBP.

To the best of our knowledge, no studies have been conducted on the presence of phthalates in *P. clarkii*. Consequently, comparisons with other works in the literature are not possible.

### 3.2. Pesticides

In the present work all pesticides analysed were below the detection limits (please refer to Table S3 in the [supplementary material](#) for the complete list of pesticides analysed). A comparable outcome was observed by Song et al. (2018) in China, who reported that the levels of 60 pesticides in the meat of *P. clarkii* were below the quantification limit. Similarly, in another study, pendimethalin residues in *P. clarkii* were below the detection limit, in contrast to the presence of these residues in water and sediment (Yang et al., 2019). In contrast, *P. clarkii* individuals sampled from eight southern states of the USA and analysed for 34 pesticides including organochlorine, organophosphate and pyrethroid

compounds exhibited positive results (Santerre, et al., 2000). The average total DDT in *P. clarkii* was  $0.047 \text{ mg kg}^{-1}$ , in particular only p, p'-DDE was detected in the 8 % of individuals while other pesticides were below the detection limit (Santerre, et al., 2000).

In a more recent study, Manjarrés-López et al. (2023) examined the presence of 54 pesticides in the soft tissue of three freshwater invasive species, including 17 individuals of *P. clarkii* collected from the Albufera Natural Park of Valencia (Spain). In the latter work, the pesticides detected were dichlofenthion, DMA, fenthion (mean value:  $255 \text{ ng g}^{-1}$  in estimated in dry weight considering an 80 % water contribution), imidacloprid, methiocarb, molinate, spinosyn A, and terbutryn (Manjarrés-López et al., 2023). However, with the exception of phention ( $< \text{LOD}: 5 \text{ ng g}^{-1}$ ), none of the 95 pesticides investigated in our study on the muscle of *P. clarkii* was in common with those detected by Manjarrés-López et al. (2023) therefore it is not possible to make comparisons.

### 3.3. Antibiotics

Target antibiotics investigate in this work were not detected (please refer to Table S4 in the supplementary material for the complete list of antibiotics analysed). Our result suggests that there is no significant contamination from antibiotics compounds or that it is not sufficient to be detected in the matrices analysed, probably due to the absence of potential sources of contamination in the surrounding environment considering their pseudo-persistence nature (Harrower et al., 2021). In fact, the persistence of pharmaceuticals in the aquatic environment is contingent upon a number of factors, including biodegradation, sunlight photolysis, and other abiotic transformations, such as hydrolysis. Moreover, the half-life of antibiotics in the aquatic environment is subject to variation depending on the prevailing environmental conditions. This can range from a few hours to several days (Yamamoto et al., 2009; Felis et al., 2020, Harrower et al., 2021). These degradation factors or combinations of them may have led to the degradation of antibiotics in the environment before bioaccumulation in *P. clarkii*, as such degradation processes would be more effective on a small or intermittent intake of antibiotics.

Differently a constant and consistent inflow of these pollutants was observed by Kazakova et al., (2018), with the source identified as persistent sources of landfills, such as livestock farming activities.

In the latter study, samples of *P. clarkii* were collected from six surrounding areas of the Doñana National Park (Spain). Among the 23 different pharmaceutical active ingredients, ciprofloxacin, ibuprofen, salicylic acid, flumequine and carbamazepine were detected at some of the selected sampling points. Furthermore, flumequine and carbamazepine have been identified in samples of *Procambarus clarkii* at concentrations of approximately  $30 \text{ ng g}^{-1}$  and  $14 \text{ ng g}^{-1}$ , respectively. Data from captured samples of *P. clarkii* indicate that this organism may serve as a useful bioindicator, despite the absence of any selected drug in the water samples at certain sampling points. Similarly in Spain, the investigation of 87 pharmaceutically active compounds showed median values above the limit of quantification for 20 of them (Manjarrés-López et al., 2023).

### 3.4. PFAS

Although PFAS are commonly found in aquatic environments, our analysis did not detect their presence in the various environmental and biological matrices (please refer to Table S3 in the supplementary material for the complete list of PFAS analysed). Specifically, some studies have identified the presence of PFAS contamination in the muscle tissues of *P. clarkii* (Manjarrés-López et al., 2023; Stecconi et al., 2024) and in particular the maximum values recorded were for PFOS ( $60 \text{ ng g}^{-1}$ ), PFODA ( $74 \text{ ng g}^{-1}$ ) in Manjarrés-López et al. (2023), and PFDoDA ( $240 \text{ ng g}^{-1}$ ) and PFTrDA ( $200 \text{ ng g}^{-1}$ ) in Stecconi et al. (2023). The results obtained by Manjarrés-López et al. (2023) and Stecconi et al.

(2023) for these PFASs were higher than those obtained in the present work, with values below the detection limits ( $< 2 \text{ ng g}^{-1}$ ). Although Stecconi et al. (2023) identified PFAS values that were higher than those observed in our study, their research indicated that the concentration of total PFAS in muscle followed a descending order among the species analysed, with European eel exhibiting the highest levels, followed by red fish, European perch and red swamp crayfish. In contrast, liver levels were relatively similar across species. These findings suggest that *P. clarkii* may not be the most polluting accumulator. Similarly, as observed in China by Zhou et al. (2022), the maximum value of the sum of 11 PFAS recorded in muscle of *P. clarkii* was  $6 \text{ ng g}^{-1}$  (estimated in dry weight considering an 80 % water contribution). This tissue is typically less contaminated with PFAS than other organs showed values on average about four times higher (exoskeleton of the cephalic region) or even about 14 times higher (hepatopancreas) (Zhou et al., 2022). Similar results were also found in in Chinese commercially available red swamp crayfish by Bian et al., (2024) who found the total concentration of PFAS in the hepatopancreas (median:  $160 \text{ ng g}^{-1}$ ) significantly higher than that in muscle tissue ( $5.95 \text{ ng g}^{-1}$ ), as well as the concentration of each individual substance.

Wang et al. (2024) obtained higher concentration levels of PFAS in China, with average values of approximately  $14 \text{ ng g}^{-1}$  (estimated in dry weight considering an 80 % water contribution) for the sum of 14 PFAS. The highest concentration levels were observed for PFOA, with an average concentration of  $6.5 \text{ ng g}^{-1}$  (estimated in dry weight considering an 80 % water contribution).

Regarding environmental samples, the mean concentration found in water samples by Zhou et al. 2022, was  $49 \text{ ng g}$  for the sum of 11 PFAS while, in our work the sum of 20 PFAS was below the detection limit ( $< 10 \text{ ng g}^{-1}$ ).

### 3.5. Microcystins

The MCs investigated in the present work in muscles (with and without intestine) of *P. clarkii* were below the limit of quantification (LOD range:  $10\text{--}15 \text{ ng toxin/g dry biomass}$ ) (please refer to microcystins extraction and analysis section in supplementary material).

The literature also reports that the *P. clarkii* is able to accumulate MCs, as has been demonstrated in Portuguese estuaries (Vasconcelos et al., 2001) and Chinese waters (Chen and Xie, 2005). In these cases, the concentrations are mainly high in the less edible parts of the animal, such as the gut or hepatopancreas.

A study of the presence of toxins in 15 water bodies in Sicily revealed that the only toxins detected were microcystins. The highest values were recorded in summer, (Zuccarello et al., 2021) according to cyanobacterial blooms that usually occur in this season (Simoni et al., 2004).

Furthermore, Rios et al. (2013) conducted a study to determine the presence of MCs in edible parts of *P. clarkii* and water collected from three ponds in Extremadura (Spain). In the latter work, it was demonstrated that *P. clarkii* accumulated MCs in their tissues in summer period. MC-LR ( $2.3\text{--}18.1 \text{ } \mu\text{g MCLR/g body weight}$ ) was identified as the predominant MC variant in all the crayfish samples. MC-RR was quantified in 50 % of the samples analysed, with concentrations ranging between  $1.4$  and  $7.8 \text{ } \mu\text{g MC-RR/g body weight}$ . No MC-YR was detected. The results indicated that crayfish could accumulate free MCs by toxic cyanobacteria species and emphasised the need for regular monitoring if the health risks associated with their consumption are to be avoided.

Furthermore, a study has demonstrated that *P. clarkii* collected in Massaciucoli Lake (90 km from Florence, Italy) accumulates microcystins in its organs and tissues, particularly in the intestine. The latter tissues have a higher concentration of toxins than the edible part (muscle) and cannot be detoxified after a short period, as observed for the edible parts (Tricarico et al., 2008).

The absence of MCs detection in this study suggests that individuals were not exposed to these toxins during the monitoring period. However, given the seasonal fluctuations in toxin production associated with

algal blooms and the impact of environmental factors such as water temperature and nutrient enrichment (which enhance their production) (Filatova et al., 2020), continuous long-term monitoring is essential to guarantee the safe utilisation of *P. clarkii* from this contamination.

### 3.6. Future development trend and direction

Although *P. clarkii* is usually considered a pollutant bioaccumulator (Souty-Grosset et al., 2016) this must be contextualised with the level of pollution in the environment in which it lives. In fact, if the environments are not heavily polluted because they are far from the sources of pollution, it is possible that the biota present will be slightly affected, regardless of its capacity for double accumulation. In this context, the eradication, monitoring and commercial exploitation of alien species represent an opportunity with several positive aspects:

- improving the quality of ecosystems by helping to restore their natural biotic composition and promoting the conservation of endemic species and existing biodiversity;
- reducing the environmental impact of disposal by exploiting all parts of individuals, including non-edible parts that could be used for high added-value products such as biotechnology, nutraceuticals, cosmetics, etc.;
- To promote the local economy, scientific progress and social awareness of the impact of the spread of these alien species;
- To support management and numerical control and monitoring actions in a sustainable and cost-effective manner of the revenues related to products from resource exploitation;
- To monitor the environment from both an ecological and chemical point of view.

In this context, the European Commission promotes policies and regulations that aim to support environmental sustainability (Reg. EU 1143/14) (Tricarico and Zanetti, 2023).

The eradication and re-use of *P. clarkii* would be perfectly in line with this and would be particularly important for the most threatened ecosystems.

Given the lack of research in this area, monitoring studies should be extended to cover areas where this species is present. In particular, chemical analyses should be systematically carried out both for the purpose of environmental biomonitoring and to assess the contamination of *P. clarkii* meat before use as food or feed. In this context it is desirable to utilise and/or develop existing multi-residue analytical methods for the most common pollutants as well as to carry out specific analyses for those pollutants which may be more easily found in the area under investigation through information on the type of local pollution.

Comparing the results obtained in this study with other studies, it was found that contamination levels for common pollutants in our samples were similar or lower.

These encouraging results should be confirmed and provide a starting point for future research and applications.

## 4. Conclusions

In general, the results of this study indicate that at the sites monitored on the dates of the study, there is no particular concern about the impact of PFAS, pesticides, phthalates, antibiotics and microcystins.

The organic pollutants investigated in the muscles of *P. clarkii* were found to be below the detection limit, which is likely attributable to the lack of significant pollution in the wetland, thereby preventing the pollutants bioaccumulation. This finding is encouraging from both the perspective of environmental quality and for the potential utilization of *P. clarkii*'s specimens collected in the frame of monitoring or control activities. This would reduce the management and environmental costs associated with eradication and related carcass disposal of this invasive species, while simultaneously facilitating the development of

sustainable products. Further research is required to ascertain whether red swamp crayfish meat can be used for food or feed purposes. It would also be beneficial to conduct the same environmental and biological analysis on other sites to gain a better understanding of the contamination levels in the muscles of Sicilian *P. clarkii* populations. Nevertheless, it is of paramount importance to pursue long-term biomonitoring for these and other pollutants at the sites under investigation in this study, as well as at all locations where cases of the species have been documented, in order to corroborate these findings, which may be subject to variation due to potential seasonal fluctuations in pollutant levels. This would help to determine if the muscles of Sicilian *P. clarkii* can be routinely exploited as a foodstuff or for other applications.

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## CRediT authorship contribution statement

**Licia Pantano:** Software, Resources, Formal analysis, Data curation. **Federico Marrone:** Writing – original draft, Visualization, Supervision, Project administration, Investigation, Funding acquisition. **Francesco Paolo Faraone:** Writing – review & editing, Visualization, Supervision, Software, Methodology, Investigation, Data curation, Conceptualization. **Nico Salmasso:** Writing – review & editing, Visualization, Supervision, Software, Resources, Methodology, Investigation, Formal analysis, Data curation. **Vincenzo Arizza:** Visualization, Supervision, Resources, Investigation. **Dario Savoca:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Software, Resources, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Leonardo Cerasino:** Writing – review & editing, Visualization, Supervision, Software, Resources, Methodology, Investigation, Formal analysis. **Gaetano Cammilleri:** Software, Resources, Formal analysis, Data curation. **Antonella Maccotta:** Writing – review & editing, Visualization, Supervision, Methodology, Investigation, Conceptualization.

## Declaration of Competing Interest

The underlisted authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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## Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at [doi:10.1016/j.hazl.2024.100140](https://doi.org/10.1016/j.hazl.2024.100140).

## Data availability

Data will be made available on request.

## References

- El-Aziz, M.A.E.A., Hassan, A.M., El-Naggar, H.A., Abbas, M.M.M., Bashar, M.A.E., 2022. Potential carcinogenic and non-carcinogenic health risks of heavy metals ingestion from consumption of the crayfish, *Procambarus clarkii* in El-Rahawy Drain and El-Kanater in the River Nile, Egypt. *Egypt. J. Aquat. Biol. Fish.* 26, 667–686. <https://doi.org/10.21608/ejabf.2022.244364>.
- Adamovský, O., and Bláha, L., 2016. Extraction of Microcystins from Animal Tissues. Handbook of Cyanobacterial Monitoring and Cyanotoxin Analysis, 358–361. Eds {C} J. Meriluoto, L. Spoof and G.A. Codd{C}. <https://doi.org/10.1002/9781119068761.ch40>.
- Ariano, A., Scivicco, M., D'Ambola, M., Velotto, S., Andreini, R., Bertini, S., Zaccaroni, A., Severino, L., 2021. Heavy metals in the muscle and hepatopancreas of red swamp crayfish (*Procambarus clarkii*) in Campania (Italy). *Animals* 11, 1933. <https://doi.org/10.3390/ani11071933>.
- Azelee, N.I.W., Digvijay, D., Ayothiraman, S., Noor, N.M., Rased, Z.I.A., Ramli, A.N.M., Ravindran, B., Iwuchukwu, F.U., Selvasembian, R., 2023. Sustainable valorization approaches on crustacean wastes for the extraction of chitin, bioactive compounds and their applications-A review. *Int. J. Biol. Macromol.*, 126492 <https://doi.org/10.1016/j.ijbiomac.2023.126492>.
- Barreca, S., Busetto, M., Forni, C., Colzani, L., Clerici, L., Daverio, D., Balzamo, S., Calabretta, E., Peleggi, M., Dellavedova, P., 2021. Determination of antibiotics, pesticides, herbicides, fungicides and hormones in water bodies in Italy in occurrence with European Watch List mechanism by using an UHPLC-MS/MS system: method validation, quantification and evaluations. *Pollutants* 1 (4), 207–216. <https://doi.org/10.3390/pollutants1040017>.
- Bian, J., Xu, J., Guo, Z., Li, X., Ge, Y., Tang, X., Lu, B., Chen, X., Lu, S., 2024. Per-and polyfluoroalkyl substances in Chinese commercially available red swamp crayfish (*Procambarus clarkii*): Implications for human exposure and health risk assessment. *Environ. Poll.*, 124369 <https://doi.org/10.1016/j.envpol.2024.124369>.
- Branchet, P., Arpin-Pont, L., Piram, A., Boissery, P., Wong-Wah-Chung, P., Doumenq, P., 2021. Pharmaceuticals in the marine environment: What are the present challenges in their monitoring? *Sci. Tot. Environ.* 766, 142644. <https://doi.org/10.1016/j.scitotenv.2020.142644>.
- Cammilleri, G., Pulvirenti, A., Vella, A., Macaluso, A., Lo Dico, G.M., Giaccone, V., Giordano, V., Vinciguerra, M., Cicero, N., Cicero, A., Giangrosso, G., Vullo, S., Ferrantelli, V., 2019. Tetracycline residues in bovine muscle and liver samples from Sicily (southern Italy) by LC-MS/MS method: A Six-Year Study. *Molecules* 24 (4), 695. <https://doi.org/10.3390/molecules24040695>.
- Cerasino, L., Salmaso, N., 2020. Co-occurrence of anatoxin-a and microcystins in Lake Garda and other deep perialpine lakes. *Adv. Oceanogr. Limnol.* 11 (1), 11–21. <https://doi.org/10.4081/aol.2020.8677>.
- Chaudhary, G., Jasrotia, A., Raj, P., Kaur, R., Kumari, A., Rajput, V.D., Minkina, T., Mandzhieva, S., Kaur, R., 2023. Contamination of water and sediments of Harike Wetland with phthalate esters and associated risk assessment. *Water* 15 (6), 1009. <https://doi.org/10.3390/w15061009>.
- Chen, J., Xie, P., 2005. Tissue distributions and seasonal dynamics of the hepatotoxic microcystins-LR and-RR in two freshwater shrimps, *Palaemon modestus* and *Macrobrachium nipponensis*, from a large shallow, eutrophic lake of the subtropical China. *Toxicol* 45 (5), 615–625. <https://doi.org/10.1016/j.toxicol.2005.01.003>.
- Chorus, I., and Welker, M., 2021. Toxic cyanobacteria in water: a guide to their public health consequences, monitoring and management (p. 858). Taylor & Francis. London, UK. <https://doi.org/10.1201/9781003081449>.
- Conde, A., Domínguez, J., 2015. A proposal for the feasible exploitation of the red swamp crayfish *Procambarus clarkii* in introduced regions. *Conserv. Lett.* 8 (6), 440–448. <https://doi.org/10.1111/conl.12164>.
- D'Angelo, S., 2013. Studio della biologia, morfologia ed ecologia di tre popolazioni di testuggine palustre siciliana *Emys trinacris* (Fritz et al., 2005): valutazione della presenza di lantanidi e caratterizzazione genetica. Università Ca' Foscari Venezia, Phd thesis.
- Dong, L., Lin, L., Pan, X., Zhang, S., Lv, Z., Mi, C., 2022. Distribution dynamics of phthalate esters in surface water and sediment of the middle-lower Hanjiang River, China. *Int. J. Environ. Res. Public Health* 19 (5), 2702. <https://doi.org/10.3390/ijerph19052702>.
- Faraone, F.P., Barraco, L., Giacalone, G., Muscarella, C., Schifani, E., Vecchioni, E., 2019. First records of the Brahminy blind snake, *Indotyphlops braminus* (Daudin, 1803) (Squamata: Typhlopidae), in Italy. *Herpetol* 12, 1225–1229.
- Faraone, F.P., Giacalone, G., Canale, D.E., D'Angelo, S., Favaccio, G., Garozzo, V., Giancontieri, G.L., Isgro, C., Melfi, R., Morello, B., Navarra, F., Russo, G., Tinnirello, V., Torre, A., Torre, D., Torre, G., Urso, G., Vinci, P., Zizzo, M.G., Marrone, F., 2017. Tracking the invasion of the red swamp crayfish *Procambarus clarkii* (Girard, 1852) (Decapoda Cambaridae) in Sicily: a “citizen science” approach. *Biogeogr. J. Integr. Biogeogr.* 32, 25–29. <https://doi.org/10.21426/B632135512>.
- Faraone, F.P., Lillo, F., Giacalone, G., Lo Valvo, M., 2008. The large invasive population of *Xenopus laevis* in Sicily, Italy. *Amphib. -Reptil.* 29, 405–412. <https://doi.org/10.1163/156853808785112075>.
- Felis, E., Kalka, J., Sochacki, A., Kowalska, K., Bajkacz, S., Harnisz, M., Korzeniewska, E., 2020. Antimicrobial pharmaceuticals in the aquatic environment-occurrence and environmental implications. *Eur. J. Pharmacol.* 866, 172813. <https://doi.org/10.1016/j.ejphar.2019.172813>.
- Filatova, D., Picardo, M., Núñez, O., Farré, M., 2020. Analysis, levels and seasonal variation of cyanotoxins in freshwater ecosystems. *Trends Environ. Anal. Chem.* 26, e00091. <https://doi.org/10.1016/j.teac.2020.e00091>.
- Gambino, D., Savoca, D., Sucato, A., Gargano, V., Gentile, A., Pantano, L., Vicari, D., Alduina, R., 2022. Occurrence of Antibiotic Resistance in the Mediterranean Sea. *Antibiotics* 11 (3), 332. <https://doi.org/10.3390/antibiotics11030332>.
- Goretti, E., Pallottini, M., Ricciarini, M.I., Selvaggi, R., Cappelletti, D., 2016. Heavy metals bioaccumulation in selected tissues of red swamp crayfish: an easy tool for monitoring environmental contamination levels. *Sci. Total Environ.* 559, 339–346. <https://doi.org/10.1016/j.scitotenv.2016.03.169>.
- Harrower, J., McNaughtan, M., Hunter, C., Hough, R., Zhang, Z., Helwig, K., 2021. Chemical fate and partitioning behavior of antibiotics in the aquatic environment—a review. *Environ. Toxicol. Chem.* 40 (12), 3275–3298. <https://doi.org/10.1002/etc.5191>.
- Jablonska, M., Cerasino, L., Boscaini, A., Capelli, C., Greco, C., Klemenčič, A.K., Mischke, U., Salmaso, N., Kurmayer, R., 2024. Distribution of toxigenic cyanobacteria in Alpine lakes and rivers as revealed by molecular screening. *Water Res* 258, 121783. <https://doi.org/10.1016/j.watres.2024.121783>.
- Kazakova, J., Fernández-Torres, R., Ramos-Payán, M., Bello-López, M.Á., 2018. Multiresidue determination of 21 pharmaceuticals in crayfish (*Procambarus clarkii*) using enzymatic microwave-assisted liquid extraction and ultrahigh-performance liquid chromatography-triple quadrupole mass spectrometry analysis. *J. Pharm. Biomed. Anal.* 160, 144–151. <https://doi.org/10.1016/j.jpba.2018.07.057>.
- Kurmayer, R., Sivonen, K., Wilmotte, A., Salmaso, N., 2017. Molecular Tools for the Detection and Quantification of Toxigenic Cyanobacteria. John Wiley & Sons, Ltd., West Sussex, UK <https://doi.org/10.1002/9781119332169.ch1>.
- Liu, B., Lv, L., Ding, L., Gao, L., Li, J., Ma, X., Yu, Y., 2024. Comparison of phthalate esters (PAEs) in freshwater and marine food webs: Occurrence, bioaccumulation, and trophodynamics. *J. Hazard. Mater.* 466, 133534. <https://doi.org/10.1016/j.jhazmat.2024.133534>.
- Mahmud, F., Banhi, T.S., Roy, H., Dihan, M.R., Islam, S., Cai, Y., Asiri, A.M., Rahman, M. M., Hasan, M.M., Shenashen, M.A., Islam, A., SheikhM, C., Awual, R., 2024. Antibiotic-contaminated wastewater treatment and remediation by electro-advanced oxidation processes (EAOPs). *Groundw. Sustain. Dev.*, 101181 <https://doi.org/10.1016/j.gsd.2024.101181>.
- Manjarrés-López, D.P., Vitale, D., Callejas-Martos, S., Usuriaga, M., Picó, Y., Pérez, S., Montemurro, N., 2023. An effective method for the simultaneous extraction of 173 contaminants of emerging concern in freshwater invasive species and its application. *Anal. Bioanal. Chem.* 415 (29), 7085–7101. <https://doi.org/10.1007/s00216-023-04974-3>.
- Marrone, F., Naselli-Flores, L., 2015. A review on the animal xenodiversity in Sicilian inland waters (Italy). *Adv. Oceanogr. Limnol.* 6 (1-2). <https://doi.org/10.4081/aol.2015.5451>.
- Massey, I.Y., Yang, F., Ding, Z., Yang, S., Guo, J., Al-Osman, M., Kamegni, R.B., Zeng, W., 2018. Exposure routes and health effects of microcystins on animals and humans: A mini-review. *Toxicol* 151, 156–162. <https://doi.org/10.1016/j.toxicol.2018.07.010>.
- Mistri, M., Munari, C., Pagnoni, A., Chenet, T., Pasti, L., Cavazzini, A., 2020. Accumulation of trace metals in crayfish tissues: is *Procambarus clarkii* a vector of pollutants in Po Delta inland waters? *Eur. Zool. J.* 87 (1), 46–57. <https://doi.org/10.1080/24750263.2020.1717653>.
- Mo, A., Huang, Y., Gu, Z., Liu, C., Wang, J., Yuan, Y., 2022. Health risk assessment and bioaccumulation of heavy metals in *Procambarus clarkii* from six provinces of China. *Environ. Sci. Pollut. Res.* 29 (2), 2539–2546. <https://doi.org/10.1007/s11356-021-15855-6>.
- Mojiri, A., Zhou, J.L., Ozaki, N., KarimiDermani, B., Razmi, E., Kasmuri, N., 2023. Occurrence of per-and polyfluoroalkyl substances in aquatic environments and their removal by advanced oxidation processes. *Chemosphere*, 138666. <https://doi.org/10.1016/j.chemosphere.2023.138666>.
- Mori, E., Andreone, F., Viviano, A., Faraone, F.P., Di Nicola, M.R., Borri, B., Bruni, G., Mazza, G., Banchi, R., Zaccaroni, M., Mezzadri, S., Baratti, M., 2022. Aliens Coming by Ships: Distribution and Origins of the Ocellated Skink Populations in Peninsular Italy. *Animals* 12, 1709. <https://doi.org/10.3390/ani12131709>.
- Naselli-Flores, L., Barone, R., Marrone, F., D'Angelo, S., 2007. 100 milioni di *Microcystis spp.* + 5 *Procambarus clarkii* = 0 *Emys trinacris*; ovvero tossine, invasori ed estinzione nei Gorgi Tondi, laghi salmastrici della Sicilia sud-occidentale. *Ecologia, Limnologia e Oceanografia: Quale futuro per l'ambiente?* Società Italiana di Ecologia. Parma, pp. 76–77.
- Pace, A., Vaglica, A., Maccotta, A., Savoca, D., 2024. The Origin of Phthalates in Algae: Biosynthesis and Environmental Bioaccumulation. *Environments* 11 (4), 78. <https://doi.org/10.3390/environments11040078>.
- Rastogi, R.P., Sinha, R.P., Incharoensakdi, A., 2014. The cyanotoxin-microcystins: current overview. *Rev. Environ. Sci. Biotechnol.* 13, 215–249. <https://doi.org/10.1016/j.watres.2020.116543>.

- Rehman, M.U., Nisar, B., Yattoo, A.M., Sehar, N., Tomar, R., Tariq, Ali, S., Ali, A., L., Rashid, S.M., Ahmad, S.B., Aldossari, R.M., 2024. After effects of Pharmaceuticals and Personal Care Products (PPCPs) on the biosphere and their counteractive ways. *Sep. Purif. Technol.*, 126921 <https://doi.org/10.1016/j.seppur.2024.126921>.
- Reinikainen, J., Bouhoule, E., Sorvari, J., 2024. Inconsistencies in the EU regulatory risk assessment of PFAS call for readjustment. *Environ. Int.*, 108614 <https://doi.org/10.1016/j.envint.2024.108614>.
- Ríos, V., Moreno, I., Prieto, A.I., Puerto, M., Gutiérrez-Praena, D., Soria-Díaz, M.E., Cameán, A.M., 2013. Analysis of MC-LR and MC-RR in tissue from freshwater fish (*Tinca tinca*) and crayfish (*Procambarus clarkii*) in tench ponds (Cáceres, Spain) by liquid chromatography–mass spectrometry (LC–MS). *Food Chem. Toxicol.* 57, 170–178. <https://doi.org/10.1016/j.fct.2013.03.025>.
- Santerre, C.R., Ingram, R., Lewis, G.W., Davis, J.T., Lane, L.G., Grodner, R.M., Wei, C.I., Bush, P.B., Xu, D.H., Shelton, J., Alley, E.G., Hinshaw, J.M., 2000. Organochlorines, organophosphates, and pyrethroids in channel catfish, rainbow trout, and red swamp crayfish from aquaculture facilities. *J. Food Sci.* 65 (2), 231–235. <https://doi.org/10.1111/j.1365-2621.2000.tb15985.x>.
- Savoca, D., Pace, A., 2021. Bioaccumulation, biodistribution, toxicology and biomonitoring of organofluorine compounds in aquatic organisms. *Int. J. Mol. Sci.* 22 (12), 6276. <https://doi.org/10.3390/ijms22126276>.
- Savoca, D., Barreca, S., Lo Coco, R., Punginelli, D., Orecchio, S., Maccotta, A., 2023b. Environmental Aspect Concerning Phthalates Contamination: Analytical Approaches and Assessment of Biomonitoring in the Aquatic Environment. *Environments* 10 (6), 99. <https://doi.org/10.3390/environments10060099>.
- Savoca, D., Lo Coco, R., Melfi, R., Pace, A., 2022. Uptake and photoinduced degradation of phthalic acid esters (PAEs) in *Ulva lactuca* highlight its potential application in environmental bioremediation. *Environ. Sci. Pollut. Res. Int.* 29 (60), 90887–90897. <https://doi.org/10.1007/s11356-022-22142-5>.
- Savoca, D., Pace, A., Arizza, V., Arculeo, M., Melfi, R., 2023a. Controlled uptake of PFOA in adult specimens of *Paracentrotus lividus* and evaluation of gene expression in their gonads and embryos. *Environ. Sci. Pollut. Res. Int.* 30 (10), 26094–26106. <https://doi.org/10.1007/s11356-022-23940-7>.
- Savoca, D., Vazzana, M., Arizza, V., Maccotta, A., Orecchio, S., Longo, F., Giudice, V., D'Oca, G., Messina, S., Marrone, F., Mauro, M., 2024. Contamination Profiles of Selected Pollutants in *Procambarus clarkii* Non-Edible Portions Highlight Their Potential Exploitation Applications. *J. Xenobiot.* 14 (3), 893–906. <https://doi.org/10.3390/jox14030049>.
- Selvaggi, R., Pallottini, M., Caldaroni, B., Dörr, A.J.M., Magara, G., Gravina, P., Grispoli, L., Cenci-Goga, B., Goretti, E., La Porta, G., Elia, A.C., Cappelletti, D., 2023. Sex and seasonal differences in metal accumulation of selected tissues in red swamp crayfish from Lake Trasimeno (Umbria, Italy). *Environ. Sci. Pollut. Res.* 30, 6234–6244. <https://doi.org/10.1007/s11356-022-22582-z>.
- Song, S., Zhu, K., Han, L., Sapozhnikova, Y., Zhang, Z., Yao, W., 2018. Residue analysis of 60 pesticides in red swamp crayfish using QuEChERS with high-performance liquid chromatography–tandem mass spectrometry. *J. Agric. Food Chem.* 66 (20), 5031–5038. <https://doi.org/10.1021/acs.jafc.7b05339>.
- 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>.
- Spyra, A., Ciepłok, A., Strzelec, M., Babczyńska, A., 2019. Freshwater alien species *Physella acuta* (Draparnaud, 1805)—A possible model for bioaccumulation of heavy metals. *Ecotoxicol. Environ. Saf.* 185, 109703. <https://doi.org/10.1016/j.ecoenv.2019.109703>.
- Stecconi, T., Stramenga, A., Tavoloni, T., Bacchiocchi, S., Ciriaci, M., Griffoni, F., Palombo, P., Sagratini, G., Siracusa, M., Piersanti, A., 2024. Exploring Perfluoroalkyl Substances (PFASs) in Aquatic Fauna of Lake Trasimeno (Italy): Insights from a Low-Anthropized Area. *Toxics* 12 (3), 196. <https://doi.org/10.3390/toxics12030196>.
- Tricarico, E., and Zanetti, M., 2023. Piano di gestione nazionale del gambero rosso della Louisiana (*Procambarus clarkii*) Available online: 687 ([https://www.mase.gov.it/sites/default/files/archivio/allegati/biodiversita/piano\\_gestione\\_gambero\\_louisiana.pdf](https://www.mase.gov.it/sites/default/files/archivio/allegati/biodiversita/piano_gestione_gambero_louisiana.pdf)) (accessed on 15 January 2024).
- Tricarico, E., Bertocchi, S., Brusconi, S., Casalone, E., Gherardi, F., Giorgi, G., Mastromei, G., Parisi, G., 2008. Depuration of microcystin-LR from the red swamp crayfish *Procambarus clarkii* with assessment of its food quality. *Aquaculture* 285 (1–4), 90–95. <https://doi.org/10.1016/j.aquaculture.2008.08.003>.
- Vaglica, A., Cerulli, A., Piacente, S., Bruno, M., Badalamenti, N., Pavela, R., Maggi, F., 2024. Phytochemical investigation and evaluation of acaricidal activity of *Ammi crinitum* Guss. against the two-spotted spider mite *Tetranychus urticae* Koch. *Crop Prot.*, 106791 <https://doi.org/10.1016/j.cropro.2024.106791>.
- Vasconcelos, V., Oliveira, S., Teles, F.O., 2001. Impact of a toxic and a non-toxic strain of *Microcystis aeruginosa* on the crayfish *Procambarus clarkii*. *Toxicon* 39 (10), 1461–1470. [https://doi.org/10.1016/S0041-0101\(01\)00105-2](https://doi.org/10.1016/S0041-0101(01)00105-2).
- Vecchioni, L., Faraone, F.P., Stoch, F., Arculeo, M., Marrone, F., 2022. Diversity and Distribution of the Inland Water Decapods in Sicily (Crustacea, Malacostraca). *Diversity* 14 (4), 246. <https://doi.org/10.3390/d14040246>.
- Vecchioni, L., Marrone, F., Arculeo, M., Fritz, U., Vamberger, M., 2020. Stand out from the crowd: Small-scale genetic structuring in the endemic Sicilian pond turtle. *Diversity* 12 (9), 343. <https://doi.org/10.3390/d12090343>.
- Wang, X., Rao, Q., Zhang, Q., Liu, C., Li, Y., Wang, D., Huang, D., Li, Y., Yao, C., Song, W., 2024. Tissue distribution and exposure risk assessment of dioxins, dioxin-like polychlorinated biphenyls and perfluoroalkyl substances in red swamp crayfish. *Food Control* 166, 110747. <https://doi.org/10.1016/j.foodcont.2024.110747>.
- Yamamoto, H., Nakamura, Y., Moriguchi, S., Nakamura, Y., Honda, Y., Tamura, I., Hirata, Y., Hayashi, A., Sekizawa, J., 2009. Persistence and partitioning of eight selected pharmaceuticals in the aquatic environment: laboratory photolysis, biodegradation, and sorption experiments. *Water Res.* 43 (2), 351–362. <https://doi.org/10.1016/j.watres.2008.10.039>.
- Yang, M., Wang, Y., Yang, G., Wang, Y., Liu, F., Chen, C., 2024. A review of cumulative risk assessment of multiple pesticide residues in food: Current status, approaches and future perspectives. *Trends Food Sci. Technol.*, 104340 <https://doi.org/10.1016/j.tifs.2024.104340>.
- Yang, Q., Ai, X., Li, S., Liu, H., Liu, Y., 2019. Determination of pendimethalin in water, sediment, and *Procambarus clarkii* by high performance liquid chromatography–triple quadrupole mass spectrometry. *Environ. Monit. Assess.* 191, 1–9. <https://doi.org/10.1007/s10661-019-7794-4>.
- Zhou, M., Zhao, F., Chen, M., Yu, Q., Liu, P., Wu, K., Wang, H., Liu, Y., Wang, Q., Liu, X., Wu, Y., Gong, Z., 2022. Exposure and health risk assessment of per-and polyfluoroalkyl substances in crayfish from the middle and lower reaches of the Yangtze River. *J. Agric. Food Chem.* 71 (1), 825–835. <https://doi.org/10.1021/acs.jafc.2c06365>.
- Zuccarello, P., Manganelli, M., Conti, G.O., Copat, C., Grasso, A., Cristaldi, A., De Angelis, G., Testai, E., Stefanelli, M., Vichi, S., Fiore, M., Ferrante, M., 2021. Water quality and human health: A simple monitoring model of toxic cyanobacteria growth in highly variable Mediterranean hot dry environments. *Environ. Res.* 192, 110291. <https://doi.org/10.1016/j.envres.2020.110291>.