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Research Article

Differential elemental accumulation of the signal crayfish (*Pacifastacus leniusculus*) along an invasion gradient

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Abstract

The non-native signal crayfish (*Pacifastacus leniusculus*) can influence elemental cycling in aquatic ecosystems through bioaccumulation and transfer of chemical elements, with potential spatial variation along invasion gradients. In this study, we investigate the differences in elemental exposure in signal crayfish in the Rabaçal River, Portugal. We focus on potential intra-population differences along a well-defined invasion gradient, comparing individuals from the invasion core (upstream) and the downstream expanding front (n = 30 each). We examined 57 elements in the muscle of the signal crayfish, including essential elements (EEs): Mn, Co, Ni, Cu, Zn, Mg, Ca, Fe, Se, V, S; non-essential and potentially toxic elements (PTEs): Cd, Hg, Pb, U, As, Sr, Ba, Cr, Zr, Cs, Tl; and technology-critical elements (TCEs): Ti, Rb, La, Ce, Pr, Gd, Dy, Ho, Er, Yb, Ga, Ge, Hf, Ta, In, Re, Te, Pt. We explored the relationship between element concentrations and signal crayfish trophic ecology, inferred through stable isotope analysis ($\delta^{13}C$ and $\delta^{15}N$), behaviour, and epibiotic associate load – factors known to shape invasion success through their effects on resource acquisition, competition, and physiological stress. Significant differences in element concentrations were found between individuals from the core and front. Individuals from the front showed higher levels (mean µg/kg, dry weight) of Co: (476 vs. 297), V: (390 vs. 262), Mn: (2.6×10^4 vs. 1.0×10^4), Hg: (2526 vs.1658), and Ta: (21 vs. 11). These patterns suggest that front individuals, with higher $\delta^{15}N$ values and more exploratory behaviour, feed at higher trophic levels (e.g., macroinvertebrates), which may explain the elevated concentrations of biomagnifying elements such as Hg and Ta. On the other hand, individuals from the core had higher levels of Pb: (361 vs. 234), and Sr: (39772 vs. 20018), likely due to a diet based on basal resources, as supported by the strong negative correlation between Pb and $\delta^{13}C$, indicating reliance on benthic sources more prone to lead accumulation. This study increases our understanding of contaminant accumulation along an invasion gradient, offering insights for management practices – such as targeted removal of highly contaminated individuals, improved monitoring of metal levels in invaded areas, and public awareness campaigns – to minimise ecological impacts on higher trophic levels.

Key words: Behaviour, diet, freshwater ecosystems, invasion gradient, non-native species, parasitic load, potentially toxic elements, stable isotopes, technology-critical elements

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Introduction

Freshwater ecosystems are crucial biodiversity hotspots, providing essential ecosystem services such as water purification and nutrient cycling (Dudgeon 2019). Despite their ecological significance, these systems are among the most threatened globally, facing biodiversity declines at a rate surpassing terrestrial and marine ecosystems (WWF 2024; Sayer et al. 2025). For example, the abundance of monitored freshwater vertebrate populations has decreased by 85% since 1970 (WWF 2024), many times driven by the introduction and establishment of non-native species, which can disrupt ecological balances, modify habitats, and alter trophic dynamics (Gurevitch and Padilla 2004; Simberloff 2010; Vörösmarty et al. 2010; Gutiérrez et al. 2014; Dudgeon 2019; Li et al. 2023).

The signal crayfish (*Pacifastacus leniusculus*), native to North America, is an example of a highly invasive species, as defined by Soto et al. (2024), given its ability to establish self-sustaining populations, spread rapidly, and cause ecological, economic, and social disruptions in invaded ecosystems. Its ecological plasticity, rapid growth, early maturity, and high reproductive output further facilitate its spread (Capurro et al 2015; Vedia 2018). This species was first detected in Portugal in 1997 in the Maçãs River (Bernardo et al. 2011), having invaded adjacent river basins, such as the Rabaçal River, in 2013 (Sousa et al. 2015, 2019; Carvalho et al. 2022). Beyond its ecological impacts, the signal crayfish may also accumulate macroelements and trace elements in aquatic ecosystems (Nędzarek et al. 2020). This accumulation may be particularly relevant because crayfish can act as bioindicators of environmental contamination, reflecting pollution levels in the sediments and the water column (Nędzarek et al. 2020). Additionally, their ability to accumulate both essential and non-essential elements raises concerns about potential trophic transfer, where contaminants may biomagnify along the food chain, affecting native predators such as fish, birds, and mammals, and posing risks to human consumers (Nędzarek et al. 2020; Li et al. 2023). In this context, pollution is not only an environmental concern but also a compounding factor that can exacerbate the ecological impacts of biological invasions (Li et al. 2023).

Trace elements play dual roles in ecosystems, with essential elements (EEs) such as Zn, Cu, and Fe being vital for physiological functions at low concentrations but also toxic at elevated levels (Islam et al. 2023). Non-essential and potentially toxic elements (PTEs), such as Pb, Cd, and As, pose additional risks to organisms even at low concentrations (Saad et al. 2014; Zoroddu et al. 2019). Technology-critical elements (TCEs) are increasingly generating environmental concern due to their growing use, especially in technological industry, and are considered emerging contaminants (Gwenzi et al. 2018; Balaram 2019). The elevated accumulation of these compounds in nature results in negative environmental impacts, especially for aquatic organisms, which absorb and bioaccumulate them, compromising the normal functioning of organs and reproduction (Picone et al. 2022; Espejo et al. 2023). These elements remain largely understudied, so their increasing use makes them an uncertain environmental threat (Adeel et al. 2019; Malhotra et al. 2020). The ability of *P. leniusculus* to bioaccumulate these elements, particularly in the hepatopancreas and exoskeleton, highlights its role in both ecosystem and human health (Nędzarek et al. 2020). This duality underscores the need to monitor non-native species not only for their ecological impact but also for human health since humans can consume them (Nędzarek et al. 2020; Dobrzycka-Krahel et al. 2024).

Invasion gradients offer a unique opportunity to investigate how environmental pressures shape the traits of non-native species (Sousa et al. 2024). Theoretically, individuals at the invasion front face distinct selective pressures, such as distinct exposure to predators, enhanced resource availability, and reduced intraspecific competition, compared to those in the invasion core (Sousa et al. 2024; Alves et al. 2025). These differences can drive phenotypic and behavioural adaptations, such as heightened exploratory behaviour, aggression, and risk-taking, which may enhance the success of individuals in colonising new habitats (Phillips et al. 2010; Gruber et al. 2018; Yagound et al. 2022). Conversely, individuals in the core often experience higher intraspecific competition, and potentially more significant loads of associated organisms, such as parasites which can influence their behaviour and contaminant accumulation patterns (Rebrina et al. 2015; Herse et al. 2018; Sousa et al. 2024).

Despite advances in understanding the ecological impacts of non-native species, intra-population differences along invasion gradients remain poorly explored, particularly regarding trace element accumulation and its interactions with diet, behaviour, and parasitism (Sousa et al. 2024). While previous studies have focused on species-level impacts (Nędzarek et al. 2020; Li et al. 2023), few have delved into population-level dynamics, which are critical for elucidating the mechanisms underlying invasion success and for better understanding environmental contamination at meaningful spatial scales (Morales 2004). Few studies analysed elements such as rare earths, with the majority focusing on studying elements such as arsenic (As), mercury (Hg), and lead (Pb) (Suárez-Serrano et al. 2010; Bellante et al. 2015; Gedik et al. 2017; Zhang et al. 2023). This gap is critical, as intra-population variation can reveal how local environmental pressures, diet, behaviour, and ecological interactions shape contaminant bioaccumulation and subsequent ecological impacts (Herse et al. 2018). For example, previous research on invasive red swamp crayfish *Procambarus clarkii* has demonstrated population-level differences in trophic interactions (Li et al. 2023). However, these studies often overlook behavioural traits, such as risk-taking and aggression, which may drive differential exposure to contaminants along invasion gradients. Therefore, understanding these accumulation patterns and their ecological interactions is essential for developing targeted strategies to manage non-native species (Alves et al. 2025). By considering population-level dynamics, including behavioural traits and trophic differences, mitigation approaches such as selective removal programs and long-term contaminant monitoring can be more effectively designed to reduce ecological and trophic transfer risks (Sousa et al. 2024).

This study contributes to investigating this gap by analysing elemental bioaccumulation, the influence of diet through stable isotope analysis ($\delta^{15}N$ and $\delta^{13}C$), and assessing differences in behaviour and parasitic load in *P. leniusculus* along an invasion gradient in the Rabaçal River, Portugal. We hypothesize that individuals at the invasion front will exhibit higher exploratory behaviour and risk-taking, correlating with increased accumulation of elements due to higher metabolic rates associated with dispersal and differences in foraging strategies. Understanding these dynamics provides critical insights into how invasive species interact with contaminants, adapt to ecological pressures, and influence ecosystem health.

Material and methods

Study area and sampling

This study was conducted on the Rabaçal River (Fig. 1), located in northeastern Portugal, with a length of 88 km and its source in Spain (Sousa et al. 2018). The river experiences low anthropogenic pressure, and part of it lies within the Montesinho Natural Park, one of Portugal's protected areas, established in 1979 to preserve the rich biodiversity across an area of 748 km^2 (Pereira et al. 2007; Castro et al. 2010).

On the Rabaçal River, eight sampling sites (R1–R8) were selected to delineate the invasion gradient of the signal crayfish. R1, located in Edroso (41.92053, -7.1224), represents the invasion core, while R7, in Candedo (41.86436, -7.11961), marks the current invasion front. The selection of these sites allowed us to determine the extent of the invasion and establish a reference framework. However, we focused on individuals from the invasion core and front, as they best represent different invasion stages. All signal crayfish individuals were captured using coarse-mesh trap nets during the summer of 2023 for elemental, diet, behavioural, and epibiotic associate analyses. The traps were submerged for 24-hour periods and subsequently removed from the river. All individuals were measured (carapace length in cm), and their sex was recorded.

Environmental characterisation

At each sampling site, we measured key abiotic parameters – temperature, conductivity, dissolved oxygen, and pH – using a YSI EXO 2 multi-parameter probe. To assess suspended matter, 1 L of water was filtered through GFC filters. Filters were dried at 60 °C for 48 hours and then combusted at 550 °C for 8 hours in a muffle

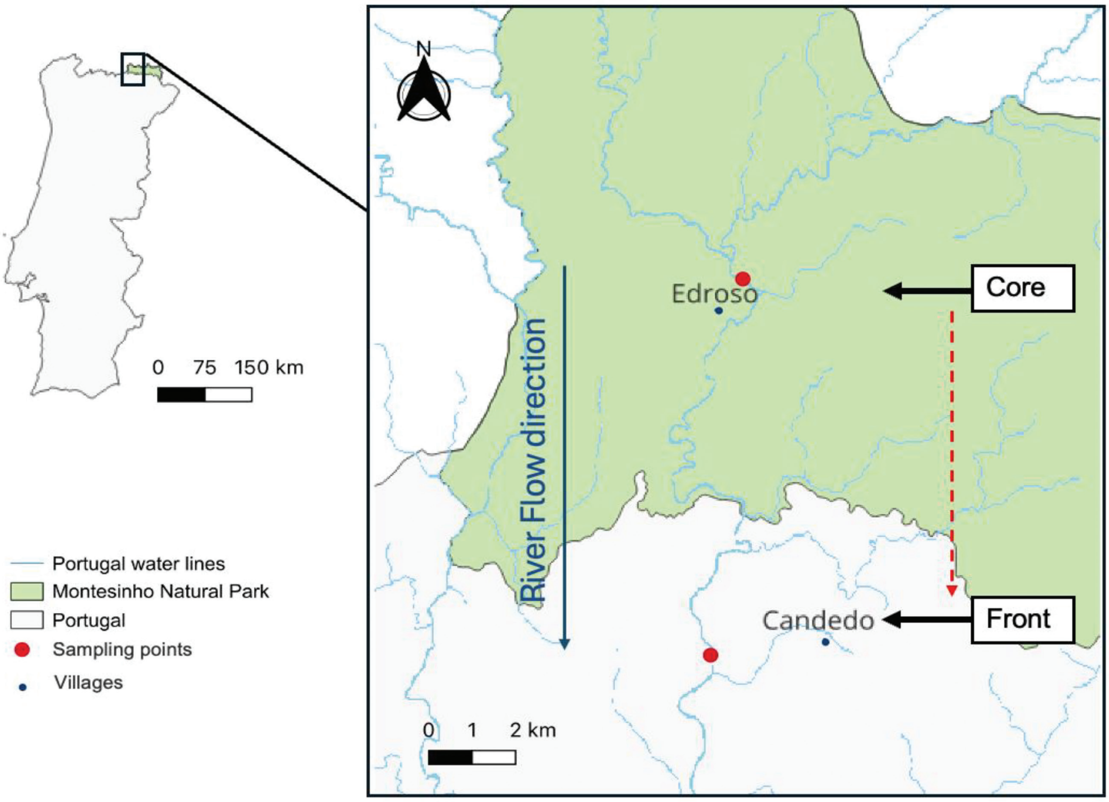


Figure 1. Core and front sampled sites along the Rabaçal River. The core site is within Montesinho Natural Park.

furnace, allowing us to calculate total (TSS) and organic suspended solids (OSS) based on mass differences, following Zieritz et al. (2018). These procedures were conducted near the midstream riverbed, during late morning periods.

To characterise sediment, samples were collected with a 10 cm-diameter core sampler placed about 2 m from the river's left margin. Granulometry and organic matter were analysed using the method described by Sousa et al. (2007). In addition, both sites were surveyed using the River Habitat Survey (RHS) protocol to assess habitat complexity, riparian vegetation structure, aquatic plant types, and human-induced alterations along the river channel. Surveys were conducted along 500-meter reaches in the core and front sampling sites and collected information was used to calculate the Habitat Modification Score (HMS) and Habitat Quality Assessment (HQA) indices (Raven et al. 1998), providing a comparative assessment of anthropogenic influence at the sampling sites.

Trace element analysis

The trace elements are divided into essential elements (EEs), potentially toxic elements (PTEs), and technology-critical elements (TCEs) (Gwenzi et al. 2018; Balaram 2019; Zoroddu et al. 2019). Sample preparation began with the freezing sacrifice of 15 males and 15 females randomly collected from each site (core vs. front of invasion). Specimens were stored at -20 °C until analysis at IB-S (Institute of Science and Innovation for Bio-Sustainability, Braga, Portugal). The total lengths of the individuals, measured from the rostrum to the telson (following Sousa et al. 2013), from the core, ranged from 6.5 cm to 10.5 cm, while those from the front ranged from 7.0 cm to 11.8 cm. Muscle samples were dried at 40 °C for 24 hours in an Infors HT, Multitron Pro, ground in a mortar to achieve a homogeneous powder consistency, and subsequently sent to the Department of Analytical Chemistry in Poznań, Poland. Approximately 0.80 ± 0.02 g of each sample was digested with 3 mL 65% HNO_3 using a microwave oven (Mars 6, CEM Corporation, Austria). The samples were then made up to 10 mL with Milli-Q water (Direct-Q system, Millipore, Germany). Immediately before the elemental analysis, the samples were diluted 20 times with HNO_3 1%. A total of 57 elements – including EEs: Mn, Co, Ni, Cu, Zn, Mg, Ca, Fe, Se, V, S, Al, Na, P, K, B, Si, PTEs: Cd, Hg, Pb, U, As, Sr, Ba, Cr, Zr, Cs, Tl, Be, Nb, Ru, Pd, W, Os, Au and TCEs: Ti, Rb, La, Ce, Pr, Gd, Dy, Ho, Er, Yb, Ga, Ge, Hf, Ta, In, Re, Te, Pt, Nd, Sm, Eu, Lu – were analysed using inductively coupled plasma mass spectrometry (ICP MS, PlasmaQuant® MS, Analytik Jena, Germany).

For quality control, analytical blanks (n = 4) and certified reference materials – DORM 5: (fish protein; n = 3; National Research Council, Canada) and BCR 668 (mussel tissue; n = 4; Institute for Reference Materials and Measurements, Belgium) were submitted to the same procedures as the samples. Detection limits ranged from 0.00017 µg/kg for In to 1203 µg/kg for S (Suppl. material 1: table S2). The recovery rates for certified materials were according to international QA/QC criteria being between 80 and 120%. For non-certified elements, accuracy was monitored using a standard solution. To ensure optimal precision and accuracy in the analyses, a solution containing – Bi, Ir, Li, Rh, Sc, and Y – was used as internal standards. For multi-element determination, the following conditions were standardised: nebuliser gas flow at 1.02 L/min, auxiliary gas flow at 1.5 L/min, plasma gas flow at 9.0 L/min, and Radio Frequency (RF) power set to 1.20 kW. Signal measurements were conducted over 20 scans in 5 replicates, using three sequential modes: without gas addition, and with the integrated Collision Reaction Cell (iCRC), utilising hydrogen as the reaction gas and helium as the collision gas to minimise mass interference.

Stable isotope analysis of crayfish

Stable isotope analysis was performed on the abdominal tissue of the same crayfish individuals used for trace element analysis, to assess their trophic position and potential dietary sources. The sample preparation procedure was the same as for analysing trace elements. The elemental and isotopic composition analyses were carried out on a Thermo Scientific Flash 2000 organic elemental analyser (EA), Organic Elemental Analyser, connected to an isotope ratio mass spectrometer (IRMS) Delta V Advantage via Conflo IV, at Ciimar (Centro Interdisciplinar de Investigação Marinha e Ambiental) in Porto, Portugal. Stable carbon and nitrogen isotope values were calculated using the following formula:

$$\delta X = (Rsample/Rstandard - 1) \times 1000,$$

where X is the isotope of carbon or nitrogen, and R is the ratio between the heavy and light isotopes of carbon or nitrogen.

The $\delta^{15}N$ and $\delta^{13}C$ values of the samples and standards were normalised with reference materials for each element (IAEA-N-1, IAEA-N-2 and IAEA-NO3 for nitrogen, International Atomic Energy Agency, Austria; USGS-24 and USGS-40 for carbon, United States Geological Survey, USA) with a margin of analytical error of approximately 0.1‰. Using an internal standard from the sea bass *Dicentrarchus labrax* (read after 12 analyses to have analytical control), the isotopic composition of the samples present in the extracted abdominal tissue was accurately determined. Corrections were also made to the $\delta^{15}N$ and $\delta^{13}C$ values according to a calibration curve derived from the delta values of N and C, based on the mass amplitudes of a caffeine standard. The elemental composition was also determined using the Chlorella K factor, and the samples were analysed twice to keep the coefficient of variation below 10%.

Behavioural tests

Three behavioural tests were conducted using individuals collected from both the core and front sampling sites of the invasion gradient. Before testing, crayfish underwent an acclimation period of three days in arenas maintained at 18 °C, without food. For the behavioural tests, the individuals were placed in a 60-litre arena (46 × 63 × 32 cm) made of polyethylene at a controlled laboratory room of 18 °C.

We performed the Risk-taking and Neophilia Tests. Neophilia, or novelty-seeking behaviour, is the tendency of individuals to explore and interact with novel objects or environments, which may influence their risk-taking behaviour and adaptability in new habitats. In two tests, 16 individuals (8 females and 8 males, ranging from 8 to 11 cm in total length) were randomly selected from both the core and the front of the invasion gradient. These individuals were placed inside a shelter (17 × 20 × 16 cm, polyethene, rectangular shape) within the main experimental arena and given 5 minutes for acclimatisation before testing (following Brown et al. 2007; Suppl. material 1: fig. S1) for 15 minutes. The objectives were to assess the individuals' risk-taking ability (measuring the time, in seconds, to leave the shelter) and neophilia (measuring the time, in seconds, to leave the shelter, approach the object, and touch it). For the neophilia test, a fish bait shaped like an adult beetle was used as a visual stimulus without any associated odour. The object was placed in motion to encourage interaction.

Aggression tests were conducted to assess dominance interactions in signal crayfish. Four combat groups were established: (1) core female vs. front female, (2) core male vs. front male, (3) core female vs. front male, and (4) core male vs. front female. Each combat group was replicated eight times (n = 8 per combat group), totalling 32 fights, with each individual participating in only one fight to avoid repeated exposure effects. In each trial, two individuals of similar size (carapace length within ± 0.5 cm, Suppl. material 1: table S3) and without visible morphological abnormalities (e.g., missing or regenerating chelae or walking legs) were randomly selected. A statistical evaluation (ANOVA, $p > 0.05$) confirmed that size differences between groups were not significant. The aggression tests were conducted in the same 60 L experimental arena (46 × 63 × 32 cm) previously used for the risk-taking and neophilia tests.

For the aggression trials, two individuals were placed on opposite sides of the experimental arena without any additional acclimatization period inside the test arena. These individuals were distinct from those used in the risk-taking and neophilia tests to prevent potential carryover effects from previous trials. Additionally, only individuals without visible ectobionts (e.g., *Branchiobdella astaci*) were selected to eliminate potential confounding effects of parasite load on aggression.

Two types of resources were contested: food (i.e., a standard piece of cat food, positioned centrally between the two individuals) and territory (i.e., a defined space within the arena). Each trial lasted 15 minutes, during which we recorded aggressive interactions related to food and space competition. The latency to the first attack, the number of aggressive interactions (including antennal contact, chelae displays, and strikes), and the overall fight duration were all recorded. Victory was determined by two criteria: (1) the individual who maintained exclusive control of the food for at least 30 seconds without being displaced, and (2) the individual who controlled the designated space at the end of the 15 minutes. If no clear dominance was established within the observation window, the contest was considered unresolved.

Prior to all these tests, signal crayfish underwent a three-day acclimatisation period in individual containers filled with aerated water and maintained at 18 °C. This period aimed to reduce prior social hierarchies and standardise hunger levels, as recommended by previous studies on crayfish aggression (Breithaupt and Eger 2002; Aquiloni and Gherardi 2010).

Epibiotic associate analysis

The abundance of external epibiotic associate (*Branchiobdella astaci*) was analysed *in situ* in the core and front sampling sites based on visual assessment. The individuals were assessed for an epibiotic associate in the claws and classified on a 4-value qualitative scale (0- absence; 1- few; 2- some; 3- many). Representative images of each infestation category are provided in Suppl. material 1: fig. S1.

Data analysis

All data analysis was carried out in the R Studio software (R Core Team 2022), using various packages such as "*readxl*" (Wickham and Bryan 2023), "*ggplot2*" (Wickham 2016), "devtools" (Wickham et al. 2022), "*dplyr*" (Wickham et al. 2023), "rjags" (Plummer 2024), "SIBER" (Jackson and Parnell 2023), and "multcomp" (Hothorn et al. 2008). The results for the 57 trace elements were filtered to select the elements that could be analysed, removing those with value below detection limits. A total of 49 elements were analysed, and the mean and standard deviation were calculated for each group (core female, core male, front female, and front male). The statistical analysis consisted of a two-way ANOVA, to check for possible differences between the four groups (core female, core male, front female, and front male) and a Tukey test. Boxplots were made for all the elements whose results were significant in both analyses. A Pearson correlation analysis was conducted between the concentrations of 49 trace elements and individual weight to evaluate potential relationships.

For the Pearson correlation analysis between individual weight and element concentrations, data from both sexes and invasion locations (core and front) were combined to increase statistical power and provide a comprehensive assessment of elemental accumulation trends. A Pearson correlation was also made between the 49 trace elements analysed and the stable isotopes $\delta^{15}N$ and $\delta^{13}C$ to identify the possible influence of diet on the concentration of the trace elements analysed. We consider a weak relationship when the correlation is up to 0.3, moderate from 0.3–0.7 and strong from 0.7–1 (following Schober et al. 2018).

For the behavioural tests, the Shapiro-Wilk test indicated that the data did not follow a normal distribution; therefore, they were log10-transformed to improve normality before statistical analyses of the risk-taking and neophilia tests. For these tests, ANOVAs were used to determine the significance of the invasion site and sex. For the aggressiveness test, chi-squared tests were performed to assess whether individuals at the front were more aggressive and to evaluate differences in behaviour between sexes. This test is appropriate for categorical data identifying which individual reached the food first or claimed the space. Fisher's exact tests were also conducted due to the small sample size.

To analyze epibiotic associate data, a chi-square test was carried out to evaluate the influence of location and sex on parasitism. Pairwise comparisons were also explored to assess differences between groups.

Results

Environmental characterisation

The sampled sites, located 15 km apart, exhibit highly similar environmental conditions (Suppl. material 1: table S1). The studied river experiences minimal human disturbance, and the RHS survey results are nearly identical for both sampling sites (Suppl. material 1: table S1). Therefore, we consider that the observed variations between core and front sampling sites are unlikely to be driven by environmental differences.

Table 1. Mean values ± standard deviation obtained for the trace elements under study (μg/kg, dry weight) and stable isotopes for the signal crayfish (*Pacifastacus leniusculus*).

Local	**Front**		**Core**	
Sex	**Female**	**Male**	**Female**	**Male**
n	14	17	13	15
Al	104902 ± 73604	129438 ± 55905	51298 ± 69884	42411 ± 28330
V	401 ± 298	380 ± 166	268 ± 163	256 ± 102
Mn	17893 ± 11502	35156 ± 44898	7238 ± 5150	13911 ± 12406
Co	489 ± 459	463 ± 373	293 ± 162	301 ± 157
Ni	453 ± 571	397 ± 466	397 ± 300	1162 ± 1989
Cu	78762 ± 65501	63478 ± 28898	64864 ± 28095	81726 ± 43015
Zn	108026 ± 74333	89012 ± 39519	76690 ± 16325	110156 ± 80689
Cd	211 ± 163	171 ± 100	150 ± 72	192 ±129
La	53 ± 108	50 ± 62	28 ± 29	153 ± 273
Ce	115 ± 200	125 ± 122	75 ± 59	123 ± 248
Pr	16 ± 26	13 ± 15	8.62 ± 8.49	14 ± 36
Nd	62 ± 95	63 ± 58	41 ± 25	68 ± 128
Sm	22 ± 22	26 ± 17	19 ± 8.06	28 ± 24
Eu	1.8 ± 1.3	2.35 ± 2.49	2.75 ± 3.29	2.61 ± 1.66
Gd	11 ± 13	15 ± 11	10 ± 5.02	14 ± 12
Dy	7 ± 6.92	9.32 ± 7.53	7.56 ± 3.82	8.10 ± 3.19
Ho	3.33 ± 3.67	4.16 ± 3.35	2.80 ± 2.56	3.53 ± 3. 86
Er	3.67 ± 3.74	5.43 ± 4.27	4.49 ± 3.25	4.38 ± 1.77
Hg	2858 ± 1956	2195 ± 918	1647 ± 942	1670 ± 964
Na	13173834 ± 9124346	9809414 ± 4025267	10650455 ± 4306602	10689730 ± 6460304
Mg	1815545 ± 1243487	1289553 ± 496082	1750486 ±534711	1761397 ± 694511
P	13367983 ± 9453734	11581499 ± 5068599	7737853 ± 1327441	9614962 ± 4709918
K	22432189 ± 1653791	18189038 ± 8103566	1430104 ± 3945718	20983848 ± 18855258
Ca	6942514 ± 5696204	3432673 ± 1734778	6934692 ± 3239298	11854283 ± 7933547
Fe	157276 ± 106304	156705 ± 88189	153796 ± 172209	133133 ± 52397
B	29865 ± 26558	31606 ± 11562	14768 ± 10544	38502 ± 13350
Rb	58228 ± 42622	41813 ± 17649	43008 ± 17426	81837 ± 119259
Sr	26046 ± 20521	13990 ± 6519	33194 ± 13393	46351 ± 27108
Ba	3839 ± 3028	3087 ± 1842	4948 ± 2480	7320 ± 4001
Pb	411 ± 612	58 ± 52	547 ± 593	176 ± 56
U	25 ± 33	16 ± 11	15 ± 11	15 ± 10
Cr	902 ± 908	814 ± 695	437 ± 201	795 ± 744
As	1190 ± 1174	773 ± 325	1202 ± 1112	814 ± 538
Se	907 ± 638	686 ± 289	670 ± 244	542 ± 436
S	15811652 ± 10396139	12121667 ± 4554600	12197292 ± 2917404	12678303 ± 5839739
Ti	4893 ± 6529	3635 ± 3917	2134 ± 2553	2084 ± 1308
Ga	158 ± 130	138 ± 78	219 ± 111	311 ± 171
Ge	44 ± 40	58 ± 32	46 ± 17	62 ± 25
Yb	5.33 ± 5.51	7.11 ± 5.19	6.04 ± 3.39	6.75 ± 3.22
Hf	9.16 ± 7.61	9.35 ± 4.70	6.74 ± 2.05	29 ± 68
Ta	25 ± 22	16 ± 7.24	9.55 ± 2.98	13 ± 4.18
Be	10 ± 7.99	14 ± 7.17	14 ± 11	18 ± 8.25
Zr	73 ± 68	49 ± 33	26 ± 10	550 ± 1811
Te	32 ± 22	34 ± 11	29 ± 5.87	51 ± 29
Cs	2361 ± 1586	1852 ± 684	2148 ± 877	3592 ± 4983
W	27 ± 24	22 ± 17	8.25 ± 6.79	62 ± 90
Pt	10 ± 7.94	7.37 ± 2.71	1.42 ± 1.01	4.55 ± 1.79
Tl	34 ± 26	26 ± 12	24 ± 10	30 ± 29
Si	91945 ± 56399	97099 ± 67142	71535 ± 54459	72719 ± 50208
δ^{15}N	8.58 ± 0.55	8.56 ± 0.38	8.09 ± 0.32	8.31 ± 0.39
δ^{13}C	-23 ± 066	-23 ± 066	-23 ± 0.67	-23 ± 0.55

Elemental analysis

The concentration values (mean ± standard deviation) for 49 elements analysed plus the stable isotopes of carbon and nitrogen are shown in Table 1, in µg/kg (dry weight). The statistical tests did not consider eight elements (lutetium, niobium, ruthenium, palladium, indium, rhenium, osmium and gold) because they were below the detection limit.

For the EEs Ca, B, Mn, and Sr ($p < 0.001$; $p = 0.002$; $p = 0.03$; $p < 0.001$, respectively, Fig. 2), significant differences between the sexes for each location were detected. For calcium, males from the core presented higher concentrations than males from the front. In the case of boron, core females exhibited the lowest concentrations, whereas males, both from the core and the front, had significantly higher values. Manganese concentrations were higher in males from the front compared to females from the core. For strontium, males from the core had the highest concentrations overall, followed by females from the front. Conversely, males from the front and core females exhibited lower values.

For PTEs and TCEs, significant differences were found for aluminium, tellurium, platinum, lead, gallium, tantalum, and tungsten ($p < 0.001$; $p = 0.02$; $p = 0.001$; $p = 0.005$; $p = 0.003$; $p = 0.01$; $p = 0.03$, respectively). Aluminium levels were significantly higher in front males compared to core females and males. For lead and gallium, core males exhibited higher concentrations than front males and females. Platinum showed significantly higher values in front of females compared to core females and males. Tantalum concentrations were higher in front females

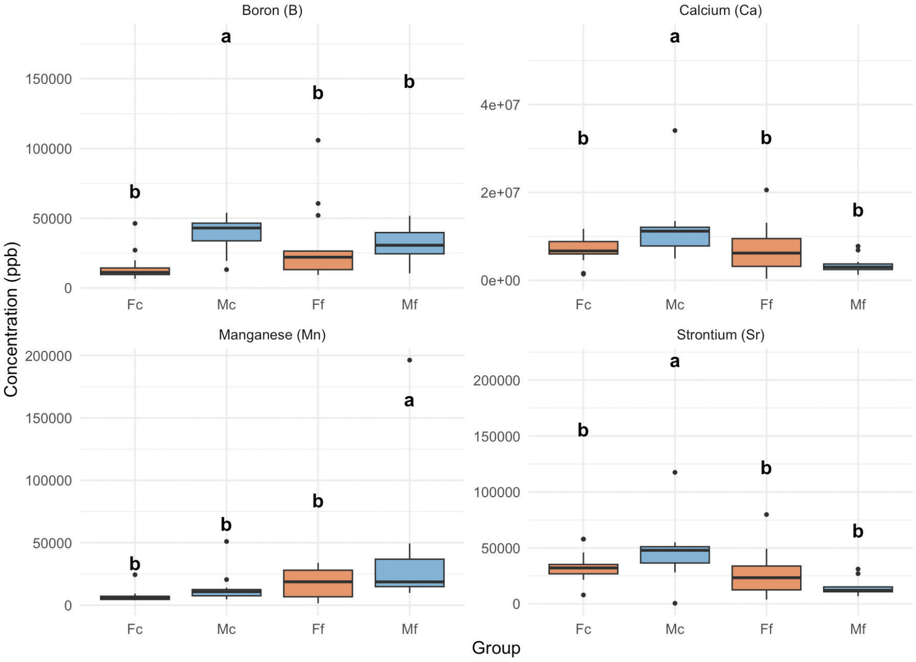


Figure 2. The concentration of calcium, boron, manganese, and strontium in core female (Cf), core male (Cm), front female (Ff), and front male (Fm) of signal crayfish (*Pacifastacus leniusculus*). Each box plot shows the median (horizontal line inside the box), interquartile range (box limits), and minimum and maximum values excluding outliers (whiskers). Dots represent outliers. Different letters indicate statistically significant differences between groups ($p < 0.05$, Tukey's HSD test). The assignment of letters does not correspond to a ranking of concentration values but rather to statistically distinct groups.

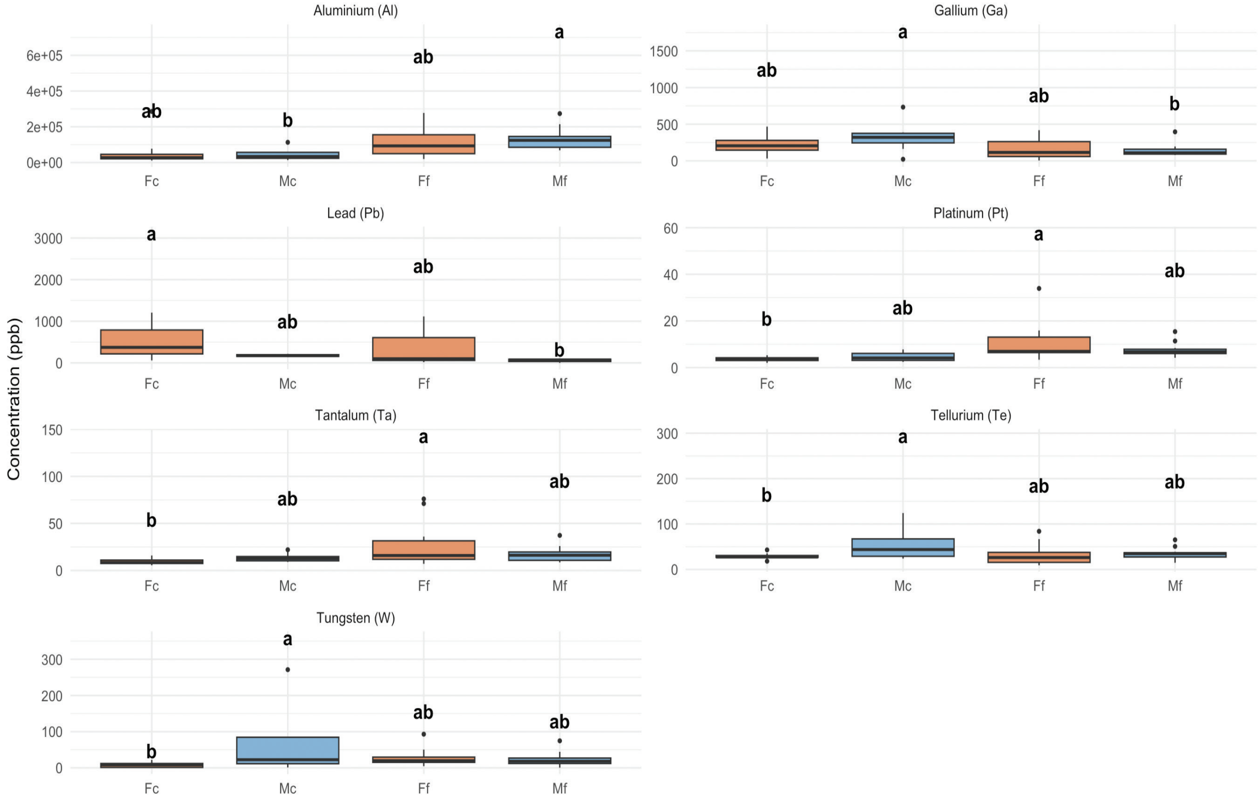


Figure 3. Potential toxic elements and technology-critical elements (TCEs) for core female (Cf), core male (Cm), front female (Ff), and front male (Fm): aluminium, tellurium, platinum, lead, gallium, tantalum, and tungsten for signal crayfish (*Pacifastacus leniusculus*). Each boxplot shows the median (horizontal line inside the box), interquartile range (box limits), and minimum and maximum values excluding outliers (whiskers). Dots represent outliers. Different letters indicate statistically significant differences between groups ($p < 0.05$, Tukey's HSD test). The assignment of letters does not correspond to a ranking of concentration values but rather to statistically distinct groups.

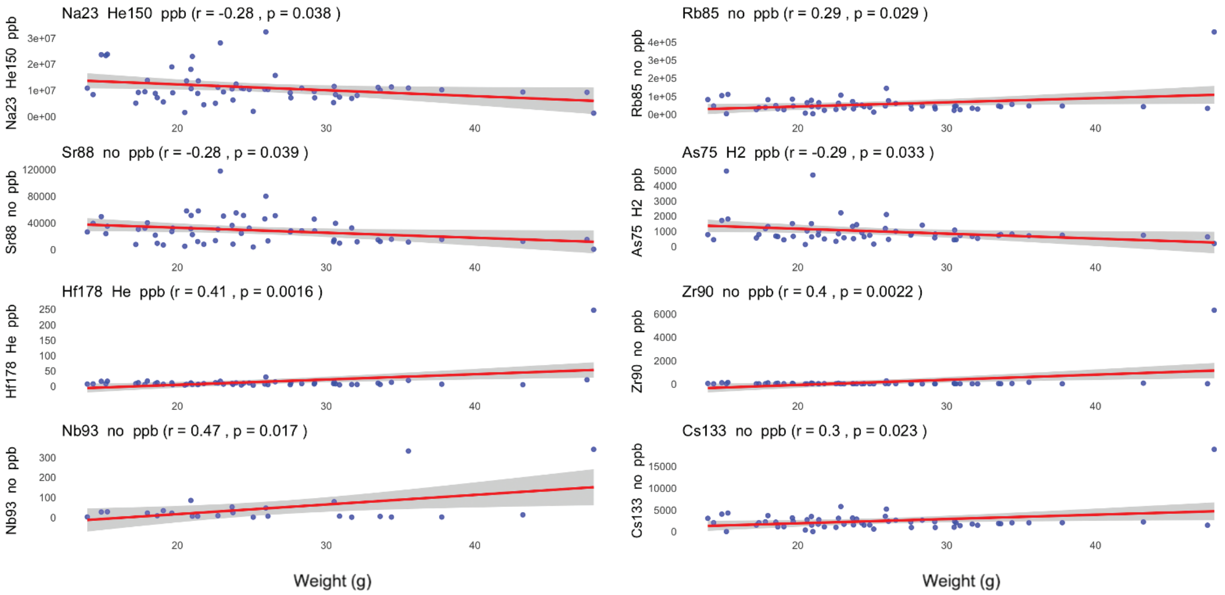


Figure 4. Scatterplots showing the relationship between individual weight and the concentrations of significant metals (Na, Rb, Sr, As, Hf, Zr, Nb, and Cs). Each plot includes a fitted linear regression line (red) with a 95% confidence interval (grey shading). Negative correlations were observed for Na, Sr, and As, while positive correlations were found for Rb, Hf, Zr, Nb, and Cs.

compared to core females. Finally, differences in tungsten and tellurium were observed between core females and core males, with core females generally exhibiting lower concentrations of these elements (Fig. 3).

The Pearson correlation analysis between individual weight and element concentrations revealed several significant relationships (Fig. 4). Sodium exhibited a negative correlation with weight ($r = -0.278$, $p = 0.038$), as strontium ($r = -0.277$, $p = 0.039$) and arsenic ($r = -0.286$, $p = 0.033$). Conversely, rubidium ($r = 0.292$, $p = 0.029$), hafnium ($r = 0.411$, $p = 0.0016$), zirconium ($r = 0.401$, $p = 0.0022$), niobium ($r = 0.475$, $p = 0.016$), and cesium ($r = 0.302$, $p = 0.023$) exhibited positive correlations. Among them, hafnium and zirconium displayed the strongest positive correlations, whereas arsenic and strontium had the most pronounced negative associations. For all the other elements no correlation was found (data not shown).

Stable isotope analysis of signal crayfish

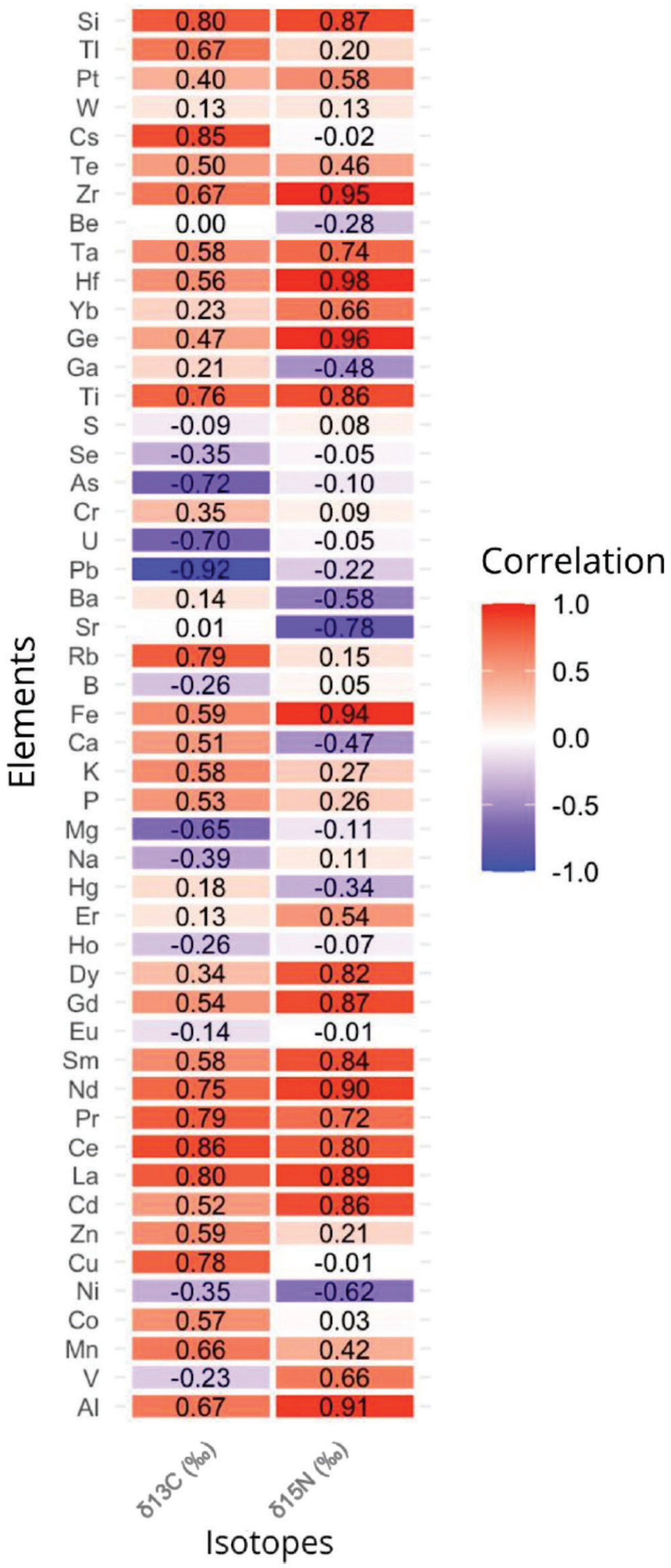
The ANOVA results for stable isotope values revealed a significant difference between the core and front groups ($F(1, 58) = 11.27$, $p = 0.001$). Post hoc analysis using the Tukey HSD test confirmed this finding, with $\delta^{15}N$ values being significantly lower in the individuals from the core compared to the front sampling site (95% CI: -0.58 to -0.15, $p = 0.001$).

To assess potential relationships between resource use and trophic position to trace elements, a Pearson correlation was carried out between the 49 elements and the stable isotopes $\delta^{13}C$ and $\delta^{15}N$ (Fig. 5). There is a positive and strong correlation between the $\delta^{13}C$ isotope and the elements silicon, caesium, titanium, rubidium, neodymium, praseodymium, cerium, lanthanum, copper, and strongly negative with arsenic, uranium and lead. There was also a strong positive correlation between $\delta^{15}N$ and the elements silicon, zirconium, tantalum, hafnium, germanium, titanium, strontium, iron, dysprosium, gadolinium, samarium, neodymium, praseodymium, cerium, lanthanum, cadmium and aluminium, and strongly negative with strontium.

Behavioural tests

The results of the behavioural tests (Suppl. material 1: fig. S3) showed no significant differences in the time it took for signal crayfish to leave the shelter during the risk-taking test (Suppl. material 1: fig. S3) between invasion sites ($p = 0.25$) or between sexes ($p = 0.49$). Similarly, no significant differences were found for the time to leave the shelter (S2B), spent near the object in the neophilia test (S2C), or for the time taken to touch the object (S2D), with no variation between core and front sampling sites ($p = 0.32$, $p = 0.84$, respectively) or between sexes ($p = 0.54$, $p = 0.79$, respectively).

Significant differences were found in the percentage of victories for reaching the food first in the core male vs. front female fight ($p = 0.01$), while no significant differences were observed in the core male vs. front male, core female vs. front female, and core female vs. front male fights ($p = 0.62$; $p = 0.13$; $p = 0.13$, respectively) (Fig. 6A). Regarding space dominance, significant differences were detected in the core male vs. front male and core female vs. front female fights ($p < 0.01$; $p = 0.03$, respectively), whereas no significant differences were observed in the female vs. male in core and female in front vs. male in front fights ($p = 0.28$; $p = 0.28$, respectively) (Fig. 6B).



Elements	δ13C (‰)	δ15N (‰)
Si	0.80	0.87
Tl	0.67	0.20
Pt	0.40	0.58
W	0.13	0.13
Cs	0.85	-0.02
Te	0.50	0.46
Zr	0.67	0.95
Be	0.00	-0.28
Ta	0.58	0.74
Hf	0.56	0.98
Yb	0.23	0.66
Ge	0.47	0.96
Ga	0.21	-0.48
Ti	0.76	0.86
S	-0.09	0.08
Se	-0.35	-0.05
As	-0.72	-0.10
Cr	0.35	0.09
U	-0.70	-0.05
Pb	-0.92	-0.22
Ba	0.14	-0.58
Sr	0.01	-0.78
Rb	0.79	0.15
B	-0.26	0.05
Fe	0.59	0.94
Ca	0.51	-0.47
K	0.58	0.27
P	0.53	0.26
Mg	-0.65	-0.11
Na	-0.39	0.11
Hg	0.18	-0.34
Er	0.13	0.54
Ho	-0.26	-0.07
Dy	0.34	0.82
Gd	0.54	0.87
Eu	-0.14	-0.01
Sm	0.58	0.84
Nd	0.75	0.90
Pr	0.79	0.72
Ce	0.86	0.80
La	0.80	0.89
Cd	0.52	0.86
Zn	0.59	0.21
Cu	0.78	-0.01
Ni	-0.35	-0.62
Co	0.57	0.03
Mn	0.66	0.42
V	-0.23	0.66
Al	0.67	0.91

Figure 5. Pearson's correlation between 49 trace elements and the stable isotopes of δ^{15}N and δ^{13}C for signal crayfish (*Pacifastacus leniusculus*). 0–0.3 is a weak correlation, 0.3–0.7 is a medium correlation, and 0.7–1 is a strong correlation. A reddish colour indicates a positive correlation and a bluish colour a negative correlation.

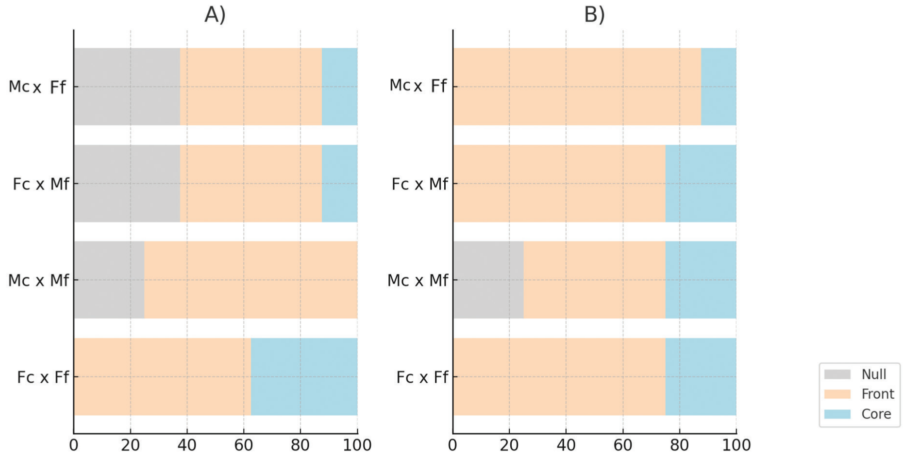


Figure 6. **A** The proportion of victories for space in the aggression trials, comparing tested groups of signal crayfish (*Pacifastacus leniusculus*) **B** the proportion of victories for access to food in the aggression trials, considering four fight pairings: core male (Mc) vs. front female (Ff), core female (Fc) vs. front male (Mf), core male (Mc) vs. front male (Mf), and core female (Fc) vs. front female (Ff).

Epibiotic associate analysis

The results of the classification of external epibiotic associated by location and sex are shown in Fig. 7. The chi-square tests revealed a highly significant difference between location and score categories (p-value < 0.001), suggesting that the distribution of scores differs strongly between the core and front sampling sites. Additionally, when analyzing the relationship between sex and score categories within each sampling site, no significant associations were observed for either the core (p-value = 0.57) or the front (p-value = 0.29) groups.

Discussion

This study investigates differences in element bioaccumulation in the signal crayfish along an invasion gradient and how these differences may be linked to factors such as trophic position and resource use, behaviour, and epibiotic associated load. We assume, based on the environmental characterisation and on recent studies (Sousa et al. 2015, 2018, 2019, 2020; Nogueira et al. 2021a, Nogueira et al. 2021b; Alves et al. 2025; Carvalho et al. 2025), that the environmental conditions in the two sites are very similar. Hence, their contribution to the possible differences is minimal. We observed that individuals at the invasion front exhibit higher concentrations of certain potentially toxic, technology-critical, and essential elements, which may be associated with their higher trophic position, more exploratory behaviour, and lower epibiotic associate load. Conversely, individuals from the invasion core tend to accumulate elements such as lead and strontium. This pattern is likely influenced by their longer residence time in the core area, where they have been established for a prolonged period, leading to increased exposure through both dietary intake and sediment contact. Additionally, crayfish abundance is significantly higher in the core compared to the invasion front, resulting in greater intraspecific competition (Alves et al. 2025). This competition can constrain resource availability, forcing individuals to rely more on benthic food sources such as plant detritus. Consequently, these ecological pressures may drive the observed differences in

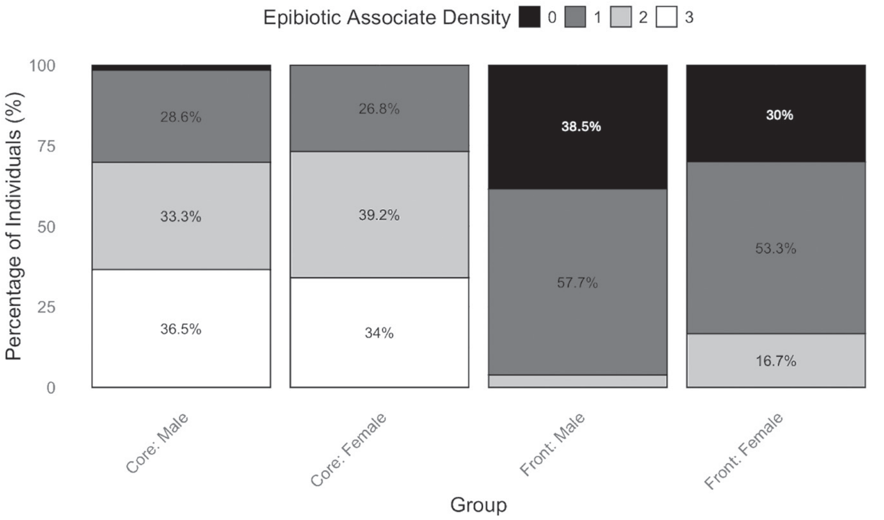


Figure 7. Percentage distribution of epibiotic associate categories by location (Core and Front) and sex (Female and Male) of signal crayfish (*Pacifastacus leniusculus*). The bars represent the percentage of individuals in each epibiotic associate category (0, 1, 2, and 3).

elemental accumulation between the core and the front organisms; findings are particularly relevant for understanding how non-native species influence contaminant transfer within ecosystems.

Elemental accumulation

Crustaceans can accumulate elements through bioaccumulation via water absorption, sediment uptake, and food ingestion (Nędzarek et al. 2020; Li et al. 2023). Due to their omnivorous diet, the signal crayfish is particularly susceptible to accumulating toxic elements, which can affect growth and development (Li et al. 2023). Elemental analysis indicates a higher tendency for crayfish at the invasion front to accumulate more elevated concentrations of essential elements such as cobalt, phosphorus, vanadium, and manganese, as well as non-essential elements including aluminium, mercury, platinum, and tantalum. The higher nitrogen values observed in individuals at the invasion front may suggest a biomagnification process, indicating that signal crayfish act as vectors for increased exposure to potentially harmful elements, such as mercury and tantalum, for their predators in invaded sites, such as the Eurasian otter *Lutra lutra* and brown trout *Salmo trutta* (Espejo et al. 2018; de Almeida Rodrigues et al. 2019). Thus, further studies comparing element concentrations between signal crayfish and native species occupying similar trophic niches are necessary to assess whether this transfer poses a greater ecological risk than under natural conditions.

Conversely, calcium (an essential element for crustaceans) exhibited higher concentrations in core individuals, which may be partially explained by the higher abundance and prolonged residency in the core. These conditions lead to increased competition and potential cannibalistic interactions, which have been reported to influence calcium accumulation in crayfish (Guan and Wiles 1997). Additionally, lead, strontium, and gallium (non-essential elements) also exhibited higher concentrations in core individuals. These differences in elemental concentrations in the muscle tissue of individuals from the core and the front of the invasion gradient can likely be attributed to variations in diet between the two sites (see further discussion below). Some elements, such as TCEs group, pose a threat due to their high toxicity, long environmental persistence, and potential for bioaccumulation and biomagnification in the food chain (Mistri et al. 2020). TCEs, including tantalum and platinum, showed higher concentrations in individuals from the invasion front. These elements are increasingly used in advanced technological applications, such as electronics, solar panels, and medical devices, raising concerns about their potential negative ecological impacts (Gwenzi et al. 2018; Balaram 2019). Tantalum, for example, may be linked to biomagnification processes, as this element has been reported to accumulate in aquatic food webs (Espejo et al. 2018). Platinum, commonly associated with catalytic converters and industrial emissions, suggests possible contamination from atmospheric deposition or local runoff sources (Picone et al. 2022). The observed patterns reinforce the idea that non-native species can serve as bioindicators of emerging contaminants, highlighting the need for further research on the ecological and toxicological implications of TCE accumulation in freshwater ecosystems.

When compared with other studies (Suppl. material 1: table S4), the concentrations of lead, mercury, and arsenic appear to fall within values found in the literature, while the concentration of copper is higher than that obtained in other studies with crayfish. The concentration of cadmium is similar to that obtained in the study by Rowe et al. (2001) but higher than the values obtained in the other studies.

There appear to be some sex-related differences in some of the elements studied, such as B and Sr. For example, in the study by Nędzarek (2020), for the elements Se, Ni, Cd, and Pb there were significant differences in the concentrations present in females and males. A possible explanation is that egg-laying in female crayfish could serve as a means of excreting certain elements, leading to different accumulation patterns compared to males (Güner 2010). However, this remains speculative, and only further studies can shed some light on this topic.

A positive correlation of the elements with $\delta^{15}N$ indicates that there is an increase in the concentrations of the element with increasing position in the trophic chain, which may suggest biomagnification processes, as higher trophic levels accumulate more of these elements through dietary exposure (Dung et al. 2023). Conversely, a negative correlation indicates a higher concentration of the element as it moves down the trophic chain, which could be indicative of biodilution, where lower trophic levels accumulate more of the element from the environment (Dung et al. 2023). The results showed a strong positive correlation with the elements Si, Zr, Ta, Ti, Hf, Ge, Sr, Fe, Nd, Pr, Ce, La, Dy, Gd, Sm, Cd and Al, which means that along the trophic chain, species that are higher up the chain, such as in the case of the Eurasian otter or brown trout may be more exposed to a higher concentration than species lower down the food chain (Esposito et al. 2020). On the other hand, species at the top of the chain will accumulate a lower concentration of Sr, as this element was negatively correlated. In this case, the process of biodilution occurs (Dung et al. 2023).

Possible mechanisms explaining different elemental accumulation along the invasion gradient

The signal crayfish's omnivorous diet reflects high dietary plasticity, enabling them to consume a wide variety of food sources such as fish, invertebrates, aquatic plants, and detritus (Olsson et al. 2009; Jackson and Britton 2014). Food availability influences their trophic position, and as Alves et al. (2025) observed using the

SIBER model, individuals at the invasion front in the Rabaçal River occupy a different trophic niche, benefiting from the higher availability of higher trophic-level prey, such as macroinvertebrates, compared to core individuals who rely more on plants due to increased competition and resource depletion given their higher abundance in this site (Galib et al. 2022). It is known that individuals feeding on resources at the bottom of the water column (e.g. algae, organic matter such as leaf litter) may have higher C^{13} values when compared to individuals feeding on organisms in the water column (e.g. macroinvertebrates and fish) (Pacioglu et al. 2019). These results corroborate our findings, indicating that individuals in the core rely more on benthic food sources compared to those in the front. Previous studies have demonstrated that trophic niche differentiation occurs between core and front populations, with front individuals exhibiting a diet richer in higher trophic-level prey, such as macroinvertebrates, while core individuals rely more on plant detritus and basal resources due to higher competition and abundance in the core (Pacioglu et al. 2019). These dietary differences may contribute to variations in element accumulation between groups.

Many organisms rapidly adapt their behavioural traits to expand their range and make risk-related decisions, driven by environmental challenges (Biro and Stamps 2008; Yagound et al. 2022). In signal crayfish, the behavioural adaptations observed at the invasion front, including higher aggression and risk-taking, likely interact with trophic ecology and elemental accumulation. Studies by Groen et al. (2012) and Myles-Gonzalez et al. (2015) support this, showing that front individuals take more risks and move faster, as observed in our trials where they were the first to reach the food and dominate the space. This greater exploration and aggression, combined with their elevated $\delta^{15}N$ values likely contribute to higher bioaccumulation of elements prone to biomagnification, such as cobalt, mercury, and manganese, in front of individuals (Kouba et al. 2010; Johnson et al. 2014). To ensure that observed behavioural differences were not influenced by potential symbiont effects (Skelton et al. 2013), only individuals without visible epibionts were selected for behavioural tests. Thus, the variations in aggression and risk-taking behaviours are attributed to invasion-related ecological factors rather than potential interactions with branchiobdellid worms. The reduced aggression observed in core individuals during behavioural tests further supports the hypothesis that competition may constrain their ability to exploit higher trophic levels, influencing elemental accumulation (Galib et al. 2022). However, some studies show the opposite. For example, Hudina et al. (2015) observed that the signal crayfish in a Croatian population had higher rates of aggression in the core, even though they had better physical conditions. One possible explanation is that aggression in the core may not necessarily provide an immediate dispersal advantage. Still, it could instead help maintain dominance in established populations where competition is intense. These variations highlight the importance of studying behavioural traits at the population level rather than assuming uniform patterns across the species (Sousa et al. 2024). Understanding these behavioural differences is essential for predicting invasion success and developing targeted management strategies (see below).

Interestingly, individuals from the invasion core exhibited higher loads of epibiotic associates, possibly due to greater crayfish density in this area, which may facilitate their transmission (DeWitt et al. 2013). Although branchiobdellids are often considered commensals or mutualists – by cleaning gill surfaces and consuming detritus – their effects are context-dependent. High loads, especially in gill chambers, have been associated with increased physiological stress and reduced growth in crayfish (Lee et al. 2009; Rosewarne et al. 2012). While we did not assess gill colonisation directly, the greater abundance of branchiobdellids in core individuals may indirectly influence metabolic demands and feeding behaviour, potentially affecting elemental accumulation patterns. These interactions highlight the importance of considering symbiotic loads when evaluating contaminant dynamics along invasion gradients.

Importantly, the presence of *Branchiobdella astaci* in signal crayfish populations in Portugal raises biogeographic questions. Since the only native European crayfish known to host this symbiont, *Austropotamobius pallipes*, does not occur in Portugal, it is likely that *B. astaci* was co-introduced with the signal crayfish from populations already carrying the symbiont, possibly from Spain where both occur (Let et al. 2023). This highlights the potential for invasive species to act as vectors not only for contaminants but also for symbionts, affecting native symbiont communities. For instance, Let et al. (2023) found that *B. parasita*, a native symbiont species, quickly disappears following signal crayfish invasion, likely due to predation during grooming or lack of compatible hosts. Therefore, while in our study the presence of *B. astaci* provides a useful proxy for host condition, in other systems, the introduction of signal crayfish may lead to overlooked biodiversity losses among symbionts. These contrasting outcomes underscore the need for further research on the dynamics of symbiont communities in invasion contexts and their ecological consequences.

Overall, the interplay between elemental accumulation and ecological traits such as diet (trophic position), behaviour, and parasitism highlights the complexity of invasion dynamics. Reduced parasitism at the invasion front may confer an advantage, allowing individuals to allocate more energy to behaviours that enhance dispersal and resource acquisition. In contrast, higher parasitic loads at the core may constrain ecological flexibility, reinforcing the observed differences in diet and element accumulation between sampling sites (Lee et al. 2009; Skelton et al. 2013). Future studies should further explore these relationships to better understand how parasitism interacts with elemental accumulation in invasive species. This information may be crucial for better understanding the dynamics of invasive species and possible impacts on the bioaccumulation and biomagnification, or biodilution of contaminants and should be considered when designing management strategies.

Management implications

Management strategies should primarily focus on preventing and limiting contamination at its source, reducing the overall environmental burden of toxic elements before they enter aquatic food webs. This includes stricter regulations on industrial and agricultural emissions, improving wastewater treatment facilities, and monitoring contamination hotspots to mitigate bioaccumulation risks in freshwater ecosystems (Nędzarek et al. 2020). In addition, targeted removal programs could be considered in areas where signal crayfish act as bioaccumulators of hazardous contaminants such as mercury, potentially reducing their availability to predators like the Eurasian otter (*Lutra lutra*) and brown trout (*Salmo trutta*) (Ficetola et al. 2012; Johnson et al. 2014). However, we acknowledge that the dynamic nature of invasion fronts, characterised by low densities and continuous spatial expansion, presents logistical challenges that may limit the effectiveness of large-scale removals. Therefore, these programs should be integrated into broader management plans, such as localised trapping efforts in areas where crayfish densities are high enough to make removal feasible or in regions where they pose a direct threat to native species and human activities. Complementary strategies may involve the selective removal of aggressive individuals at the invasion front, habitat modifications to reduce refuges, reinforcement of native predators, and the use of biological control agents to limit crayfish success. These efforts should be integrated with public awareness campaigns and citizen science initiatives to enhance community engagement and support long-term management goals (Gherardi et al. 2011; Alves et al. 2025).

Recent studies highlight the human health risks associated with consuming signal crayfish due to their ability to bioaccumulate toxic metals. Nędzarek et al. (2020) demonstrated that signal crayfish from the Wieprza River (southern Baltic) accumulate elements such as lead, cadmium, and arsenic, which can exceed acceptable dietary intake limits in certain body parts. While the abdominal meat of crayfish can be a source of essential elements such as calcium, potassium, and zinc, consumption of hepatopancreatic tissues poses a potential health risk due to the high concentration of toxic elements. This highlights the need for risk assessment before promoting crayfish harvesting for human consumption.

To make these strategies more effective, it is crucial to incorporate an understanding of individual and population-level traits, such as trophic ecology, behaviour, and parasitic load, as these factors can influence contaminant accumulation and dispersal dynamics (Sousa et al. 2024). These aspects are often neglected in management studies, yet they may play a key role in determining the success of mitigation strategies (Haubrock et al. 2024; Sousa et al. 2024). Finally, long-term contaminant monitoring is essential to track bioaccumulation trends across different invasion stages. However, to develop truly effective and adaptive management strategies, it is first necessary to characterise the contamination profile of each site – including both absolute concentrations and relative differences across locations – to better understand the environmental context and the potential risks associated with trace element exposure (Nędzarek et al. 2020).

Conclusion

This study provides insight into differential element accumulation in the signal crayfish along an invasion gradient. Our findings reveal significant intra-population variations in elemental concentrations, which appear to be influenced by trophic positioning, behavioural traits, and host-parasite dynamics.

Signal crayfish from the invasion front exhibited higher concentrations of certain essential and potentially toxic elements, such as cobalt, vanadium, manganese, mercury, and tantalum. These differences are likely associated with distinct trophic positions, as front individuals displayed higher δ^{15}N values, suggesting a diet richer in macroinvertebrates and higher metabolic demands. Conversely, individuals from the core accumulated higher levels of elements like lead and strontium. Rather than solely reflecting longer exposure, this pattern may be influenced by higher intraspecific competition and reliance on more benthic food sources, such as plant detritus. However, we acknowledge that muscle tissue is not the primary organ for metal accumulation, and future studies should explore element deposition in other tissues such as the hepatopancreas and gills (Balzani et al. 2021, 2022).

Behavioural tests confirmed that individuals at the invasion front exhibited greater aggression, traits that may facilitate spread and resource acquisition.

Additionally, the reduced parasitic load observed in the front of the invasion gradient underscores the potential adaptive benefits of lower host densities and novel environments, leading to higher consumption and potentially higher accumulation of certain elements.

Overall, these findings underscore the complex interactions between invasion dynamics, environmental contaminants, and ecological traits. By combining elemental analysis, stable isotope data, and behavioural observations, this study contributes to a better understanding of the ecology of a highly invasive species in freshwater ecosystems.

Additional information

Conflict of interest

The authors have declared that no competing interests exist.

Ethical statement

No ethical statement was reported.

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Author contributions

Conceptualization: JAGP. Data curation: JAGP, DG. Formal analysis: DG, JAGP. Funding acquisition: RS, PN. Investigation: JSK, RS, DG. Methodology: JSK, PN, JAGP. Project administration: RS. Resources: PN, JSK, JAGP. Supervision: JAGP, RS. Validation: RS. Writing – original draft: JAGP, DG. Writing – review and editing: JAGP, DG, RS, JSK, PN.

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Data availability

All of the data that support the findings of this study are available in the main text or Supplementary Information.

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Supplementary material 1

Supplementary figures and tables

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