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Stuck in the mangrove mud: The risk of trace element exposure to shore crabs in restored urban mangroves

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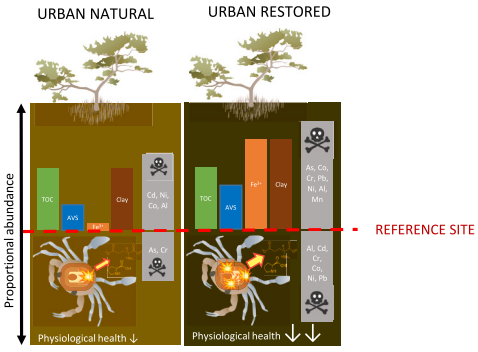
HIGHLIGHTS

- Urban contaminants threaten the success of coastal restoration
- Sediment and crabs from an urban restored site were the most contaminated
- Crabs from urban sites had distinct metabolome profiles
- Contaminant burden should be a key metric when monitoring restoration outcomes
- Linking metabolome profiles to contaminant levels is challenging in complex ecosystems

GRAPHICAL ABSTRACT



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ABSTRACT

The restoration of mangroves in urban environments can increase the risk of contaminant exposure and subsequent health effects to resident biota, yet this risk is rarely considered in mangrove restoration programs. Here we assessed the influence of sediment chemistry on contaminant bioaccumulation in shore crabs from restored and natural mangroves in urban environments compared to a reference site. The concentrations of some trace elements were several-fold higher in the sediment and crab tissues of the urban restored site compared to the natural reference site (Cd = 6×, Co = 7×, Cr = 4×, Mn = 30×, and Ni = 18× greater in sediments, while Cd = 4×, Co = 2×, Cr = 2×, Mn = 6×, and Ni = 3× greater in crab tissues). NMR-based metabolomics on crabs revealed higher abundances of proline and glutamate at urban sites, which may be indicative of physiological stress from trace element contamination. Choice experiments were used to test habitat selectivity by crabs from each population, and showed that crabs avoided sediments from the contaminated urban sites. Our results suggest that restoring mangroves in contaminated environments could create ecological sinks, where animals take residence in the new habitat but are exposed to sediment-based contaminants, with potential implications for organism and population health.

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1. Introduction

Coastal ecosystems, such as mangrove forests, contribute substantially to the provision of ecosystem services. At the intersection of land and sea, mangroves mitigate climate change by storing carbon, mediate water quality by sequestering pollutants and nutrients, and protect the shoreline from extreme weather and erosion (Friess et al., 2019; Lovelock et al., 2014). Rapid urban and industrial development of the world's coastlines has, however, led to the loss and degradation of mangroves and the services they provide (Adame et al., 2021; Goldberg et al., 2022). Improved understanding of the environmental and economic benefits of restoring mangroves has resulted in major global investment in their restoration in recent years (Waltham et al., 2020). Mangrove restoration often takes place in, or adjacent to, urban environments, as this is where the greatest historical losses have occurred (Goldberg et al., 2020). Restoration sites are therefore often downstream of wastewater discharges or industrial emissions, or in areas receiving non-point source run-off from stormwater, agriculture, and mining (Hanfi et al., 2020). While mangrove plants tend to survive in such nutrient rich environments (Reef et al., 2010), quantifying restoration success solely by vegetation growth and persistence may be misleading, since it fails to consider the broader ecosystem (Lee et al., 2019). Ideally, 'success' should encompass habitat condition, animal health, and the restoration of ecosystem services, all of which can be affected by contaminant exposure (Basconi et al., 2020; Sievers et al., 2022). Yet, of the studies incorporating sediment chemistry in restoration outcome assessment (Feng et al., 2017; Sulochanan et al., 2022), few go on to investigate the impacts of sediment contaminants on recruited fauna.

Contaminants deposited in mangrove sediments include trace elements (metals and metalloids; Lewis et al., 2011). Trace elements are ubiquitous in nature, and their fundamental properties render them either essential or non-essential in biological systems (Walker et al., 2012). Many essential elements serve a crucial role in biochemistry, and an imbalance of trace element concentrations can have serious detrimental effects on biota (Bjerregaard et al., 2022; Roe et al., 2020). Environmental pollution results when the concentrations of contaminants are elevated by anthropogenic activity to the extent that they cause ecological damage (Sun et al., 2019). Such damage has repeatedly been documented in animals inhabiting polluted coastal ecosystems, including cytotoxicity, organ damage, developmental impairment and reproductive failure (Hill et al., 2022; Lavery et al., 2009; Lu et al., 2020; Melvin et al., 2018). As such, restoration projects - particularly those in areas with known contamination - should consider, at least, the potential impacts on fauna.

Measuring contaminant concentrations in exposed organisms provides an indication of the potential risks of contaminant bioaccumulation, but additional measures are needed to characterise biological effects. One of the most sensitive techniques to evaluate physiological or metabolic change in an organism is metabolomic analysis (Zhang et al., 2021). Metabolome assessment provides a snapshot of the phenotypic outcome of contaminant stress, identifying biochemical changes at the cellular level which are useful for assessing resilient estuarine species that are well adapted to extreme environments (Roessner and Bowne, 2009). Assessment can provide insights regarding the toxicity of contaminants prior to the occurrence of irreversible effects such as tumorigenesis, organ failure, or mortality (Waters and Fostel, 2004). Whilst most metabolomics studies have been conducted in controlled laboratory experiments (Belivermiş et al., 2020; Gómez-Canela et al., 2018), applications of environmental metabolomics are proving equally useful for evaluating the physiological responses of biota to stressors in the field (Griffiths et al., 2020; Melvin et al., 2018).

In urban areas where mangroves are restored, elevated nutrients from wastewater effluent can promote prolific macrophyte growth, and the subsequent increased food availability may enhance faunal recruitment (Delibes et al., 2001). The concurrent elevation in contaminants means recruiting fauna may be exposed to harmful pollutants. There is thus a risk that restoring mangroves in contaminated sites could be creating ecological traps, whereby animals mistakenly prefer occupying habitats that reduce their fitness relative to other available habitats (i.e., where there is a decoupling of habitat quality and habitat attractiveness; Hale and Swearer, 2016; Robertson and Hutto, 2006). This is in contrast to the creation of a sink, where faunal populations spill over into non-preferred habitats that confer lower fitness outcomes (Kristan and William, 2003). The risk of creating ecological traps or habitat sinks poses a challenge for the restoration of coastal ecosystems in urban settings, and remains a key knowledge gap in marine restoration science (Swearer et al., 2021).

Here, we investigate restoration outcomes by evaluating indicators of animal health. Sediment characteristics, uptake of contaminants by benthic shore crabs, and the resulting biological impact via metabolome analysis were analysed across three sites: an urban restored site, an urban natural site, and a natural reference site. Habitat selection experiments were done to evaluate the capacity of shore crabs to detect and avoid environments with higher contaminant levels. We hypothesised that contaminant concentrations would be greater in the sediment at urban sites than at a reference site despite restoration efforts, and that this would be reflected in crab tissue concentrations. Differences in the metabolome of crabs were also expected to reflect decreased health in the contaminated environments. Additionally, the substrate sourced from these sites was anticipated to influence crab habitat selection under experimental conditions, where preference for contaminated sediments might indicate the potential for an ecological trap. Through the assessment of contaminant sequestration, uptake, and effect, we aim to characterise the risks associated with mangrove restoration in urban environments to help inform restoration planning and assessments of restoration success.

2. Methods

2.1. Experimental design

We investigated the effects of contaminants in restored mangrove environments at three sites in southeast Queensland, Australia; an urban restored habitat (Bulimba Creek; −27.4486, 153.1155), an urban natural habitat (Port of Brisbane; −27.4022, 153.1683) and an undeveloped natural habitat with few contaminants (Currumbin Creek; −28.1334, 153.4769; reference site; Fig. S1). Site selection was based on known contaminant inputs (or lack thereof) and existing studies in adjacent waterways. Several studies in the Brisbane River - the suppling waterway to both urban sites - show that local sediments historically contained elevated concentrations of trace elements (Duodu et al., 2016, 2017; Mackey and Hodgkinson, 1995). The urban restored habitat is the result of extensive excavation, waste removal, and revegetation which has been ongoing since 2001. All sites are in relatively close proximity, so that ecological variability due to latitudinal gradients was avoided. At all sites, *Avicennia marina* was the dominant mangrove species, and the most prevalent shore crab species were *Pseudohelice subquadrata* and *Helograpsus haswellianus*, both in the Verunidae family.

2.2. Sample collection and preparation

2.2.1. Sediment

From each site, surficial sediment samples (0–10 cm) were collected with a hand-operated corer (7 cm diameter) from ten randomly selected locations within the habitat zone occupied by Verunidae crabs. Each sample was placed in a low-density polyethelyne (LDPE) bag for trace element analysis and the determination of physicochemical characteristics. All QA/QC protocols were adhered to, including having different personnel for different core uses, and preventing accidental contamination of samples by avoiding waterproof clothing, insect repellent, and cosmetic lotions. Samples were transported on ice prior to storage in

darkness at −20 °C (DES, 2019). Samples used for trace element analysis and physicochemical parameter investigation were homogenised wet in a nitrogen-filled glove-bag and relevant quantities were subsampled into LDPE sample containers.

2.2.2. Fauna

Crab specimens (*Helograpsus haswellianus* and *Pseudohelice subquadrate)* were captured using baited pitfall traps (Smith et al., 1991) 10 cm deep and 15 cm in diameter, buried flush with the sediment at randomly selected locations within mangrove habitat, in August 2022. These two species have similar morphology and highly variable colouration, therefore it was not feasible to differentiate them in the field at the time of sampling. Efficiency (to preserve the metabolic state of the specimens) was prioritised over species identification, therefore crabs from both species were sampled at random. Both species are detritivores and bioturbators, with similar diets and burrowing behaviour. The first 15 crabs captured from each site were used in habitat selection experiments and were transported to the lab on a slab of local sediment and site water. Crabs collected for trace element analysis ($n = 17$ per site) were stored in snaplock bags, placed immediately into an ice slurry for euthanasia and then stored at −20 °C. Crabs collected for metabolomics analysis (n = 17 per site) were flash-frozen in liquid nitrogen in LDPE sample containers and then stored at −80 °C. Within each analytical group, crab size (carapace width) and sex distribution were standardised (Table S1). To avoid contamination of tissue analysis with ingested sediments, crabs used in trace element analysis were thawed and dissected to remove the digestive organs and contents. Remaining tissues were then weighed and stored at −80 °C prior to processing.

2.3. Analyses

2.3.1. Sediment characteristics

Physicochemical parameters assessed included total organic carbon (TOC), particle size, acid volatile sulphide (AVS), and reactive iron speciation. Total organic carbon analysis followed the procedures outlined by Tran and colleagues Tran et al. (2020). In brief, sediment samples were dried at 105 °C, homogenised, and weighed. Sulphurous acid (1 mol L^{-1}) was added to the subsample in a dropwise manner to liberate inorganic carbon, redried using the above procedure, and then analysed for total organic carbon using a TRUMAC CNS analyser (LECO, USA). For particle size analysis (0.01 mm – 3 mm), wet sediment samples were sieved at 2 mm to remove large detritus prior to laser diffraction spectroscopy using a Malvern Mastersizer 3000 (Malvern Panalytical, United Kingdom). Each sample was measured at least 5 times, and all relative standard deviation (RSD) values were below 5 % (Table S2). Acid volatile sulphides were extracted via cold diffusion and quantified via iodometric titration. Cold diffusion method followed Brouwer & Murphy Brouwer and Murphy (1994), where sediment samples were acidified with 1 mL of ascorbic acid (1 mol L^{-1}) and 9 mL of HCl (1 mol L^{-1}), and liberated AVS were collected in alkaline Zn solution. The extract was mixed with 50 mL deionised water, 10 mL HCl (6 mol L^{-1}), 1 mL of starch solution and 2.0 mL of iodine solution in a 50 mL conical flask, then titrated with a standardised thiosulphate solution. Acid volatile sulphides were quantified following the methods described by Burton and colleagues Burton et al. (2008).

Reactive iron speciation was determined in accordance with Kostka & Luther Kostka and Luther III (1994). Sediment samples (0.1–0.5 g) were extracted with 10 mL HCl (0.5 mol L^{-1}; under anerobic conditions), vortexed, placed on the shaker (200 rpm for 1 h) and centrifuged (3000 x*g* for 5 min). For iron (II) analysis, 0.1 mL of the supernatant (extract) was added to 5 mL of iron (II) buffer (50 mM HEPES adjusted to pH 7.0 with NaOH) and 0.1 mL of Ferrozine reagent, while for total iron analysis, 5 mL of total iron buffer (50 mM HEPES, 144 mM $[NH_3OH]^+Cl^-$, adjusted to pH 7.0 with NaOH) was added to an additional 0.1 mL of sample extract in a separate centrifuge tube. After 20 min, 0.1 mL of Ferrozine reagent was added to the second sample and mixed by inversion, and both tubes were incubated in darkness for 30 min prior to measurement via UV–Vis spectrophotometry at 562 nm. Iron(II) standards (25, 50, and 100 μL; 1 mmol L^{-1}) were prepared and measured alongside each tube to develop a calibration curve. Across all sediment physicochemical analyses, a sample duplicate was analysed for every 10 field samples.

2.3.2. Trace element analysis

Sediment samples were prepared for trace element analysis via weak acid extraction (Simpson and Batley, 2016). This involved adding ~1 g of wet sediment and 1 mol L^{-1} HCl (at a 1:50 sediment to acid ratio) to a 50 mL centrifuge tube for extraction. The samples were placed on shaker for one hour, centrifuged at 3000 x*g* for 5 min, and the extract was aliquoted and diluted 20-fold for ICP-MS analysis. The inclusion of mercury (Hg) analysis necessitated the addition of gold chloride to each sample at 2 mg L^{-1} to maintain Hg in ionic form and prevent loss to the container walls and atmosphere (Feldman, 1974). There are no commercially available sediment reference materials certified for the weak acid extraction of trace elements. However, we subjected the PACS-3 marine sediment reference material to the weak acid extraction protocol to demonstrate the difference in extractability of various metals and to provide some reference data to future studies that utilise the weak acid extraction protocol (Table S3a). Replicate ($n = 3$) extractions of PACS-3 CRM samples were within 1.5–14.5 % agreement. Several studies have found a weak acid digestion to sufficiently predict benthic faunal bioaccumulation from sediments, which is meaningful for the objectives of this study which aim to evaluate differences in contaminant risks among urban mangrove ecosystems, rather than compare absolute contaminant values in sediments (ANZG, 2018; Luoma and Bryan, 1981; Snape et al., 2004; Tessier and Campbell, 1987).

Crab tissue samples were prepared for trace element analysis via microwave-assisted acid digestion (USEPA, 1996). Wet crab tissues were weighed prior to lyophilisation in a CHRIST alpha 2–4 LSCplus freeze dryer and homogenised via mortar and pestle. A subsample of 0.25 mg was weighed into 55 mL perfluoro alkoxy alkane (PFA) digestion vessels (CEM Corporation). Samples were digested with 4.5 mL of distilled concentrated nitric acid, 1.0 mL of concentrated hydrochloric acid (Instrument Quality; Seastar Chemicals), and 0.5 mL of concentrated hydrogen peroxide (Baseline; Seastar Chemicals; USEPA, 1996). The extracts were diluted 50-fold in deionised water for trace element analysis via ICP-MS. As no Hg was detected in the sediment samples, crab tissues were not analysed for Hg. For every microwave digestion (containing 35 sample aliquots), two sample duplicates, two certified reference material (CRM) samples, and four procedural blanks were analysed alongside samples (Willie et al., 2013; Willie et al., 2012; Yang et al., 2014). The majority of DORM-4 and DOLT-5 recoveries were between 75 % and 125 %, except for Pb for DORM-4 and Al, Cr, and Ni for DOLT-5 (Table S3b). The ICP-MS was operated in helium collision mode to remove polyatomic interferences via kinetic energy discrimination. Initial mercury (Hg) and selenium (Se) analysis of sediment samples required a separate run in standard (No Gas) and high energy helium (HEHe) modes, respectively, to achieve maximum sensitivity for these elements. The instrument was externally calibrated with a multi-element calibration solution, ranging from 0.1 to 100 μg L^{-1}, prepared in 2 % (*v*/v) HNO_3 (High Purity Standards). For Hg and Se analysis, external calibration consisted of a mixed Hg and Se solution, ranging from 0.1 to 100 μg L^{-1}, prepared in 2 % (v/v) HNO_3 with 2 mg L^{-1} $AuCl_3$ (High Purity Standards).

2.3.3. Metabolomics

Sample preparation and analysis for metabolite profiling of animal tissues followed the methods of Melvin and colleagues (Melvin et al., 2017). In brief, polar metabolites were extracted via methanol/chloroform extraction (Bligh and Dyer, 1959) prior to analysis using ^1H nuclear magnetic resonance (NMR) spectroscopy. Whole crab specimens were lyophilised in a CHRIST alpha 2–4 LSCplus freeze dryer and

homogenised via mortar and pestle. A 100 μg subsample was placed into a 2 mL centrifuge tube and the exact weight was recorded. A double extraction was performed to ensure maximum yield. Each extraction maintained a 4:8:3 ratio of solvents (MeOH:$CHCl_3$:H2O). The initial extraction involved adding 400 μL of methanol to each sample. The mixture was vortexed and left to incubate for 2 h at −20 °C. 800 μL of chloroform was then added and the sample was briefly vortexed followed by the addition of 300 μL distilled water. The mixture was vortexed thoroughly and centrifuged at 20,627 x*g* for 15 min at 4 °C. The polar metabolite supernatant was aliquoted into an amber vial, and the extraction process was repeated on the remnant cellular debris with some slight modifications (solvent volumes adjusted to 200 μL methanol, 400 μL chloroform and 150 μL of distilled water; incubation period increased to 18 h at −20 °C).

The metabolite fractions were lyophilised and resuspended in 10 mmol L^{-1} phosphate buffer made with deuterium oxide and containing trimethylsilylpropanesulfonate (TSP) as an internal standard. After vortexing and centrifugation, the supernatants were transferred into 3 mm NMR tubes for analysis. An 800 MHz Bruker® Avance III HDX spectrometer (Bruker Pty Ltd., Victoria, Australia) was used to acquire the ^1H spectra of metabolite fractions, following the methods outlined by Melvin and colleaguesMelvin et al. (2018). Post-processing of the NMR spectra was completed using MestReNova (version 14.2.3, 2022), which involved manual phase correction, baseline adjustment (ablative algorithm) and TSP normalisation (^1H δ 0.00). The spectra were stacked, then important features (corresponding to metabolites) were integrated and imported into Microsoft Excel (Version 2303, 2023). This data was normalised to sample weight and analysed using MetaboAnalyst (Xia and Wishart, 2010) and R Studio. A ^1H—^{13}C Heteronuclear Single Quantum Coherence (HSQC) spectrum was acquired for an individual representative sample to contribute to metabolite identification. The HSQC spectra was acquired with 128 scans, 0.8 s relaxation delay, 8.20 μs pulse width and spectral widths of 12.8 kHz (^1H δ −3.23–12.79) and 33.1 kHz (^{13}C δ −9.40–155.2). The Human Metabolome Database (HMDB; Wishart et al., 2022) and Chenomx NMR Suite v9 (ChenomxInc., Edmonton, Canada) were used for metabolite identification and aided interpretation of the results.

2.4. Choice experiments

To investigate habitat selection preferences, thirty patches of surficial sediment (depth 10 cm, area 28 × 20 cm) were collected from each site, with care taken to retain vertical sediment structure. Fifteen crabs were also collected from each site, as described above. The experimental set up included 45 arenas (28 × 40 cm) containing two abutting patches of sediment from different sites, into which a crab was introduced. This allowed for 15 replicates of each choice combination, where 5 crabs from each site were exposed to each combination of sites at random (Fig. S2). Once placed into the container, the burrowing behaviour of the crabs was monitored after a 24-h period. The presence of a burrow allocated a score of 1 to the sediment from that site.

2.5. Data analysis

Statistical analyses were completed using R Studio (R Core Team, 2022). All measurement values within each variable group were assessed for normality and homogeneity using Shapiro-Wilk and Levene's tests. To evaluate differences in sediment characteristics and contaminant concentrations among the three sites, we used non-parametric Kruskal Wallace tests, followed by Wilcoxon signed rank tests, since data did not meet parametric assumptions of normality and homoscedasticity, even after transformation. Principal components analyses were used to assess multivariate differences among locations in trace element concentrations, in sediment and crab tissue, followed by PERMANOVA and pairwise PERMANOVA to evaluate the sources of variation. Differences in the metabolome among locations were analysed both as a multivariate dataset (using a PCA) and for individual metabolites of interest (using a two-way analysis of variance; ANOVA, with a False Discovery Rate correction of 0.01), using the MetaboAnalyst package (Pang et al., 2021) and R Studio. A Chi-squared test of independence was used to investigate the influence of sediment on crab burrowing activity during the choice experiment, including habitat of origin as a factor.

3. Results and discussion

3.1. Sediment characterisation and contaminant load

Sediment characteristics at the three sites aligned with our hypotheses based on the known behaviour of trace elements under different environmental conditions (Horowitz, 1985). Locations with finer sediments generally had higher organic matter, and more reactive iron oxides and acid volatile sulphides, and this corresponded with increased contaminant concentrations (Fig. 1). This is due to the greater surface area of fine sediments, and the binding affinity of acid volatile sulphides, reactive iron, and organic macromolecules, resulting in increased retention of metal ions (Miranda et al., 2021).

The reference site had coarser, sandy sediment, with particles <63 μm accounting for only 18.5 % (± 2.6 % SD, Kruskal Wallace *p*-value <0.001) of the sample composition. Sediment from this site also contained the least organic matter (0.7 % ± 0.1 %, $p < 0.001$) and reactive iron (III) oxides (1.13 μmol/g DM ± 1.39 μmol/g, $p = 0.01$) and the highest AVS:SEM ratio (37.31; see Table S4 for all pairwise results). This corresponded with significantly lower concentrations of most trace elements (except As) in reference site sediments when compared to at least one urban site (Table 1).

In contrast, sediments from the urban restored and urban natural sites contained more fine sediment (45.5 % - 73.3 %) and similar amounts of organic matter, but varied in AVS and Fe(III) concentrations (Fig. 1). The restored site had significantly higher concentrations of iron oxides (29.4 mmol/kg; $p = 0.042$) and the lowest AVS:SEM ratio (8.7, compared to 18.5 in the urban natural site, and 37.7 in the natural reference site), indicating a potentially greater risk of trace element bioavailability when compared to both other sites, however this ratio is still above the threshold where there is an expected threat of trace element toxicity. While AVS and Fe(III) form stable associations with trace elements and prevent interaction with biological systems, there is potential for bound elements to be liberated upon changes in environmental parameters (i.e. acidification and oxidation; Eggleton and Thomas, 2004; Pelfrêne et al., 2011). Such changes can occur due to burrowing bioturbators, or in the stomachs of benthic invertebrates during digestion of detritus, and can contribute to bioaccumulation regardless of the presence of AVS or Fe(III) (De Jonge et al., 2009; Mayer et al., 1996).

Within the sediment, the concentration of most trace elements followed a similar trend (Table 1). Of the sixteen detected trace elements, ten elements exhibited a significant stepwise increase in concentration among the three sites, increasing from Currumbin Creek (reference site) to Port of Brisbane (urban natural) to Bulimba Creek (urban restored; Fig. S3). While the distinction between the reference site and urban sites was expected, the differentiation between the restored and natural urban sites was not. Despite having similar inputs, sediment from the restored site contained far greater trace element concentrations than the natural urban site. This could be a result of the varied tidal influence between the sites, with the Port of Brisbane urban natural mangrove site situated at the mouth of the Brisbane River experiencing greater tidal energy compared to the low energy environment of the restored mangrove area in Bulimba Creek. Furthermore, the additive effects of AVS and iron oxide concentrations at the restored urban site would allow for greater adsorption of trace elements compared to the urban natural site, which had comparably low Fe(III) and AVS. These differences in the sediment characteristics not only influence the retention of

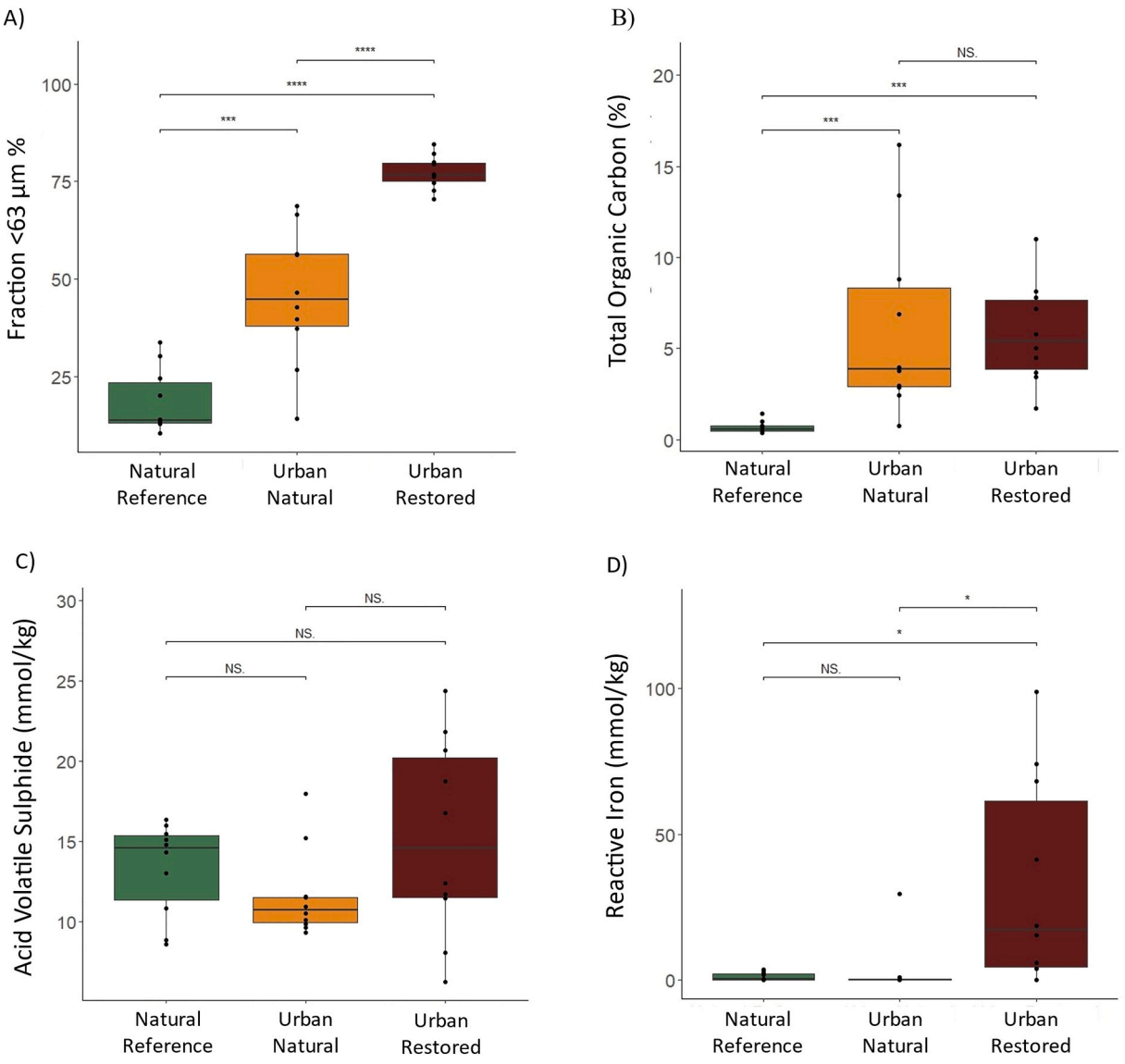

Fig. 1. Boxplots displaying the distribution of data measured for a) Clay and silt (<63 µm) fraction (%); b) total organic carbon content (%); c) AVS concentration (mmol/kg); d) Reactive iron content (mmol/kg). Significant differences between sites were determined via a paired Wilcoxon test ($\alpha = 0.05$) and are represented by stars; * difference is significant at the 0.01 level (two-tailed, $p < 0.05$); ** difference is significant at the 0.001 level (two-tailed, $p < 0.001$); *** difference is significant at the 0.0001 level (two-tailed, $p < 0.0001$).

trace elements, but the potential bioavailability of contaminants and habitat preferences among shore crabs.

Referencing the ANZG guidelines, all elements at all sites, except for one measurement of Pb at the urban restored site (86.5 mg/kg DM), were below the default guideline values (ANZG, 2018). These values are based upon the 10th percentile distribution value of data collected from a range of field and laboratory toxicity assessments (MacDonald et al., 2000; Simpson and Batley, 2016). Despite concentrations below these guidelines, our contaminated sites may still exhibit toxicity depending on the physiological targets of the trace elements and the magnitude of exposure experienced by wildlife (which is specific to their metabolism, habitat, and dietary preferences). Further, these guideline concentrations are based on single-contaminant studies, but synergistic interactions between multiple contaminants may confer impacts at concentrations below default guidelines values (Bao et al., 2008; Castelhano Gebara et al., 2021; Cedergreen, 2014; Sinclair et al., 2019).

3.2. Bioaccumulation of trace elements in crabs

There were varying patterns of trace element bioaccumulation in crabs that differed to what was expected based on the sediment contaminant profiles of each site, with the contaminant profiles of both urban populations most similar compared to the reference site ($p = 0.004$; Fig. 2). Crabs from the urban restored site contained the highest concentrations of elements such as Cd, Co, Cr, Mn and Ni (Table 1). While crabs from the natural reference site contained the highest concentrations of Mo, Se, Sb and U, the concentrations of these were relatively low in crabs from all three populations (Mo and Se <1 mg/kg DM, Sb <0.01 mg/kg DM, U < 0.5 mg/kg DM). The tissue concentrations of Cr and Cd in crabs from the reference site and the urban natural site were not significantly different, but were significantly lower than those from the urban restored site. Furthermore, the tissue concentrations of Al, Co, Mn and Ni were significantly greater in crab tissues from the urban restored site than both of the natural sites, yet for these trace elements accumulation in crabs from the urban and reference natural sites also differed significantly (Table 1, Fig. S4). This may indicate greater

Table 1
Trace element concentrations in sediment and crab tissues sampled from each site. Values in bold indicate those above ANZG default value guidelines for sediment quality (ANZG, 2018). All concentrations are reported as mg/kg of dry mass (DM).

Trace Elements		Sediment (n = 10 per site)						Crab tissue (n = 17 per site)					
		Reference		Urban natural		Urban restored		Reference		Urban natural		Urban restored	
		Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Al	mg/kg	228	119	1430	585	2320	389	404	62.8	324	172	486	136
As		4.17	1.95	1.85	0.775	4.67	2.27	5.89	3.11	8.26	4.43	2.54	0.867
Cd		0.00798	0.0165	0.125	0.0906	0.0454	0.0218	0.0205	0.0125	0.0303	0.0213	0.0837	0.0337
Co		0.760	0.389	1.82	0.725	5.27	3.65	0.830	0.354	0.500	0.280	1.98	0.720
Cr		1.84	0.619	3.03	1.07	8.05	5.65	0.507	0.130	0.656	0.409	1.02	0.293
Cu		2.41	1.49	3.01	2.03	23.8	13.7	57.8	10.7	56.1	18.7	62.3	28.3
Fe		1940	886	2320	1020	10,300	4850	663	197	836	533	883	270
Mn		10.1	5.97	35.0	13.0	307	246	21.8	6.65	72.0	39.8	138	75.9
Mo		0.789	0.498	0.870	0.515	0.550	0.264	0.416	0.105	0.189	0.048	0.217	0.0440
Ni		0.276	1.06	2.46	0.913	5.07	2.78	1.48	0.694	0.705	0.289	3.63	1.60
Pb		8.26	5.75	7.33	2.39	**28.0**	**22.0**	0.671	0.301	0.616	0.230	1.02	0.778
Sr		23.9	5.91	27.6	4.10	49.1	22.1	–	–	–	–	–	–
Ti		37.7	13.7	108	29.4	84.1	28.1	–	–	–	–	–	–
U		1.95	1.69	1.56	0.713	1.83	1.04	0.138	0.0483	0.0663	0.0230	0.101	0.0319
V		15.6	7.07	26.0	5.56	30.6	13.2	1.81	0.748	1.54	0.795	1.81	0.574
Zn		18.0	6.44	32.0	9.49	75.2	29.2	84.4	10.6	66.6	10.4	78.1	16.4

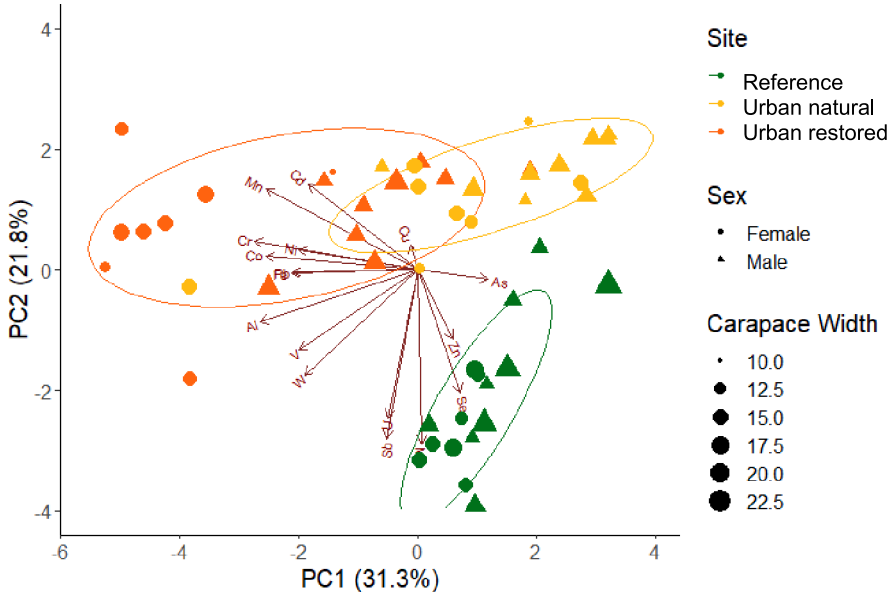

Fig. 2. Biplot of the principal components analysis for trace elements in crab tissues (n = 17 per site), grouped by site and plotted against qualitative measures (size, sex). Vectors show the explanatory variables, with vector length indicating eigen values. Pairwise PERMANOVA analysis revealed the difference between sites was significant between Reference and Urban restored (p adj = 0.003).

bioaccumulation of these trace elements in crabs at the urban restored habitat compared to both the urban and reference natural habitats.

The shore crabs sampled in this study are deposit feeders, and are likely exposed to trace elements bound to the sediment through ingestion and burrowing (Hare et al., 1989). Shore crab particle ingestion has been shown to negatively correlate with particle size, thus in environments where the sediment is finer (and adsorbs more trace elements), the potential for crabs to ingest particles is greater which could increase their exposure to sediment-bound contaminants (Taghon, 1982). Bioaccumulation of trace elements has been observed previously in crabs and other benthic invertebrates (De Cock et al., 2023; Reed et al., 2010; Shaiek et al., 2018), with impacts including changes to behaviour, moulting cycles, fecundity, and the histology of vital organs (Olsén, 2010; Penha-Lopes et al., 2009; Saaristo et al., 2018). As an abundant prey item, shore crabs are an effective model organism to explore the contaminant risk to the wider food web (Carvalho Neta et al., 2019; Saher and Siddiqui, 2019).

The trends of bioaccumulation in shore crab tissues from the urban restored mangroves varied compared to similar studies of estuarine crustaceans from around the world depending on the trace element. Typically, Cu, Zn, As, and Ni accumulated in higher concentrations, while Cd and Pb were lower, in the crabs collected from the urban restored site compared to small crustaceans from polluted mangrove habitats in Southern Australia (Maher, 1986), Niger Delta, Nigeria (Gbaruko and Friday, 2007), and Korea (Na and Park, 2012). Fiddler crabs (*Austruca sindensis*) sampled from Hawks Bay, Pakistan – which receives metropolitan and industrial wastes and is considered highly polluted – contained similar levels of trace element bioaccumulation to shore crabs collected in this study (Siddiqui and Saher, 2015). In an experimental laboratory setting, *Minuca rapax* exposed to varying concentrations of settleable atmospheric metallic particulates accumulated greater concentrations of Cr, Cu and As in their tissues (under the highest exposure scenario; 1 g/L), but less Al, Fe and Zn than shore crabs collected from both urban sites of our study (Maraschi et al., 2024).

M. rapax exhibited sub-lethal physiological changes at these concentrations, and at lower levels of bioaccumulation, including decreased metabolic rate with consequences to natural behaviour (Capparelli et al., 2024; Maraschi et al., 2024). Large predatory crabs collected from the Guayas estuary in Ecuador had 3–36 times lower concentrations of As, Cr, Cu and Ni in their tissues compared to the shore crabs sampled from the restored urban site, despite being a longer lived species in a higher trophic position (De Cock et al., 2021). The consequences of these levels of bioaccumulation, and rates or modes of depuration (such as moulting; Bergey and Weis, 2007), is not yet described for *P. subquadrata* or *H. haswellianus,* thus interpreting the relevance of these concentrations is speculative. These trends in trace element accumulation in crab tissues may indicate that the effects of urban contaminants can be more severe in restored habitats than natural habitats due to a lack of established ecosystem services and ecological resilience, despite the compensative physicochemical properties of the sediment at the restored site (Sinclair et al., 2018; Su et al., 2021). This highlights the need to consider biological metrics when evaluating toxicity risks, rather than relying on absolute sediment concentrations (De Jonge et al., 2009; Peijnenburg et al., 1997; Remaili et al., 2018).

The observed relationship between sediment contaminant concentrations and those in the crab tissue can partly be explained by the methods selected for analysis. A weak acid digestion extracts labile elements from the sediment, which is representative of the potentially bioavailable fraction (Luoma and Bryan, 1981; Simpson and Batley, 2016). Extracting only the labile portion of trace elements presents a more realistic value for the amount of contaminants available to interact with biological systems through respiration or digestion, and is useful for comparing sites with varying physicochemical properties (Miranda et al., 2021; Snape et al., 2004; Tessier and Campbell, 1987). While many elements were significantly higher in the sediment of both urban sites compared to the reference site, the increased grain size and AVS:SEM ratio at the urban natural site may be responsible for the lower bioaccumulation of some trace elements in crabs from this population, when compared to those from the urban restored site (De Jonge et al., 2010; Zhang et al., 2014).

3.3. Physiological stress in crabs

A total of 44 unique polar metabolites was identified in crab tissues, consisting of amino acids (50 %), carbohydrates (sugars and carboxylic acids, 20.5 %), nucleosides (11.4 %) and other molecules (vitamins, small enzymes, and structural components, 18.2 %). Of these metabolites, 25 differed significantly in abundances among sites (Fig. S5, Table S6). The trends observed for these metabolites aligned with contaminant data for crabs and sediments, with the abundance of 34 % ($n = 15$) of the detected metabolites significantly higher in crabs from the urban restored mangroves (Fig. S5). Crabs sampled from this site had markedly greater abundances of most metabolites except trigonelline, homarine, formic acid, and beta-alanine. Homarine and beta-alanine are both non-essential amino acids that exhibited an inverse relationship with contaminant concentrations, with the lowest abundances in crabs from the restored site compared to the natural and reference populations. Formic acid and trigonelline are dietary metabolites that each exhibited a different pattern of abundance across the three populations (Fig. S5). The increase in metabolite abundance may be linked with an increased burden of some key trace elements (including Cd, Co, Mn, and Ni) that were found in higher concentrations in the urban restored population. However, we acknowledge the variation in bioaccumulation trends across the trace elements detected in crab tissues. While our results depict a clear metabolome shift for Verunidae crabs collected from sites with higher sediment contaminant concentrations and levels of bioaccumulation, this may be influenced by other factors specific to food availability, sediment characteristics (granulometry and organic matter), and stressors at this site. Direct causal links cannot be determined without targeted experimental assays, however, other studies have used metabolites as bioindicators of environmental stressors (Schock et al., 2010), and key patterns in our field data lend evidence to existing theories.

Typical biomarkers of trace element detoxification (metallothionine, methionine, glutamine, and glutathione) were either not detected, or were similar in crab populations across sites. However, amino acids that work in cooperation to reduce metal stress (precursors of glutathione synthesis [glutamate], and proline) were significantly higher in crabs from the contaminated populations (Fig. 3). The abundance of proline and glutamate help to offset cellular stress, maintain osmoregulation, and control redox regulation in the presence of increased trace element concentrations and reactive oxygen species (Liang et al., 2013; Shen et al., 2023; Wang et al., 2012).

There was also a prominent shift in the proportions of other amino acids, carboxylic acids, and nucleic acids that have substantial roles in processes such as fatty acid synthesis, osmotic regulation, and immune function (Fig. 3). Branched chain amino acids (BCAAs) such as valine, leucine, and isoleucine perform roles in immune function and studies show that the relative abundance of these compounds can be affected in crabs from polluted field sites (Gago-Tinoco et al., 2014; Lu et al., 2020; Yu et al., 2020). In this study, all BCAAs were more abundant in crabs from locations with higher trace element concentrations. This may indicate an increased immunological response in crabs from the contaminated sites, or perhaps heightened adaptation and resilience mechanisms for exposed crab populations. Free amino acids such as lysine, alanine, tryptophan and phenylalanine were also more abundant. This response could indicate changes in osmoregulation and is often observed when both osmotic stress and trace element concentrations are high (Capparelli et al., 2024; Shen et al., 2023; Viant et al., 2003). Further, homarine (an amino acid specific to marine invertebrates) is typically mobilised in tissues under osmotic stress, evidenced here as a depletion of homarine in crabs from the urban restored site (Wu et al., 2017). These results could point towards osmotic stress and altered immune function in crabs from the contaminated sites, with those from the restored mangroves being the most affected. However, we acknowledge the varied physiological roles and biochemical origins of these compounds, and that fluctuations in amino acid abundance may also be influenced by diet, developmental state, and moulting stage, despite attempts made to standardise this during sample selection (Table S6) (Li et al., 2021).

The increased abundance of carboxylic acids (malonic acid and dimethylmalonic acid) could indicate decreased fatty acid synthesis and a shift in energy metabolism for crabs from the contaminated sites compared to those from the reference site. Trace elements and organic contaminants can both interfere with fatty acid synthesis, by either binding to carboxylic acids and preventing their function (resulting in increased fatty acid synthesis) or by competitively inhibiting enzymes such as succinate dehydrogenase and Acetyl-CoA synthetase (resulting in fatty acid synthesis inhibition and excess carboxylic acid accumulation; Filimonova et al., 2016). This may indicate decreased energy reserves for crabs from contaminated sites (Maraschi et al., 2024). These links to biochemical pathways remain subject to speculation, as the unique physicochemical and environmental parameters of each ecosystem sampled could contribute to the metabolome shift observed, highlighting the benefits of complementing field observations with lab-based ecotoxicology testing. However, such clear differences in the metabolome of crabs from the three sites, along with differences in trace element bioaccumulation, indicate that there may be an effect of contaminants on the physiology of crabs at the urban restored site.

3.4. Habitat selection: Ecological trap or sink?

In the habitat selection experiments, there was a significant effect of arena scenario on burrow selection ($c^2 = 25.24$; $p < 0.001$), with crabs preferentially selecting sediment from the reference site when burrowing (or the urban natural site when between urban natural and urban

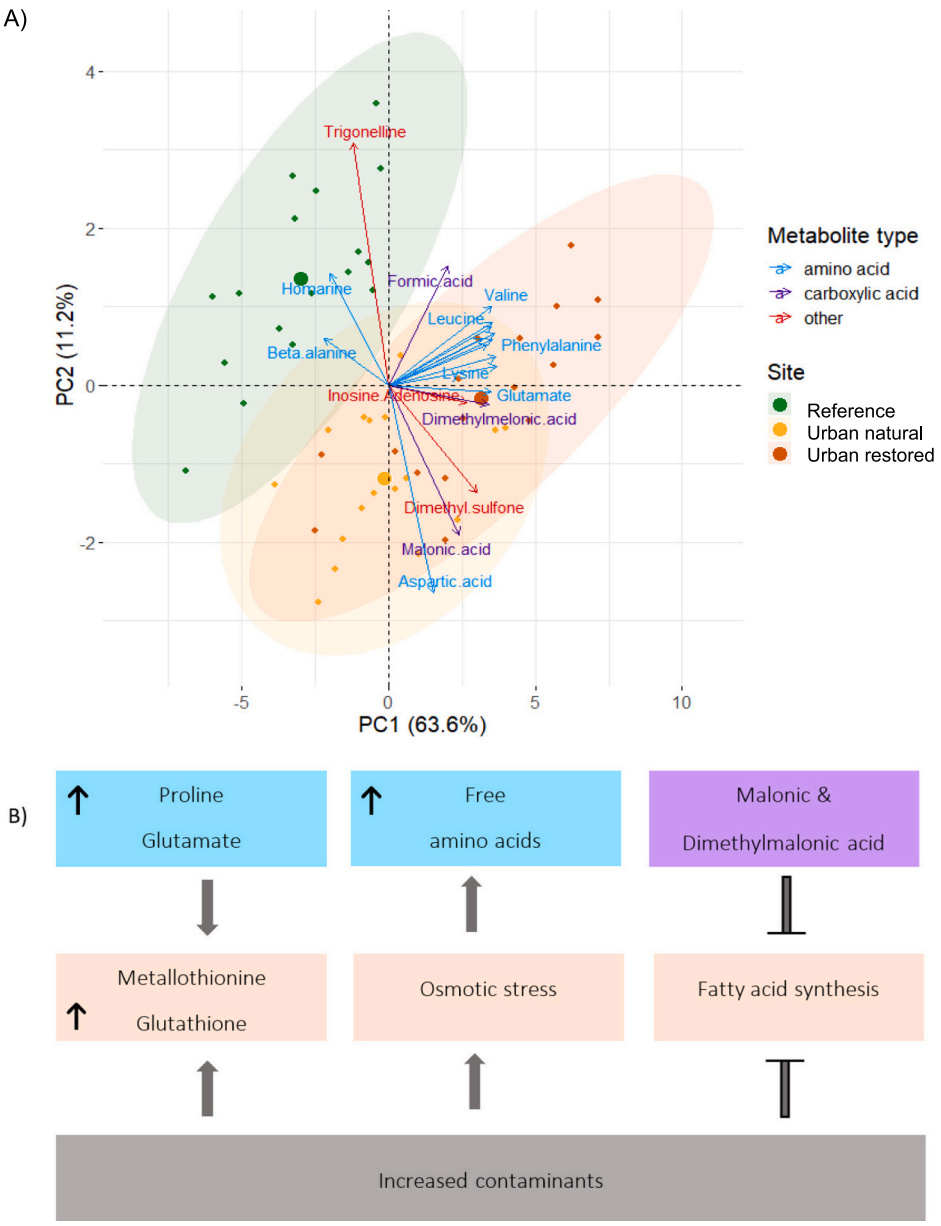

Fig. 3. A) Top panel: biplot of multivariate analysis (PCA) of metabolites in crabs from the three locations, with the most important explanatory variables shown with length representing eigenvalues (only the strongest relationships shown). The largest points for each site represent median. B) Bottom panel: a conceptual schematic of the biochemical pathways potentially altered by contaminant stress, linking our metabolomics data with the observed contaminant concentrations in crab tissue. Arrows show direction of effect, and blind ends show direct inhibition.

restored; Fig. 4). Since the order of burrowing preference was directly inverse to that of contaminant concentrations for most trace elements, crabs may be able to use chemical cues in the sediment when selecting suitable habitats. Alternatively, the burrow selection may be influenced by other sediment parameters, such as granularity and the presence of organic matter (Leoville et al., 2021). Thus, these results do not support the existence of an ecological trap. However, recruitment of fauna to the restored site may evidence a sink, where animals 'spill-over' from other habitats, and reside in poor quality habitats due to a lack of available alternatives. This is probable in the highly urbanised setting of Brisbane, Australia, where large losses of natural mangroves have occurred (Lovelock et al., 2019). If animals are avoiding urban restored sites, or experiencing reduced health when living in contaminated restored mangroves, then these restoration projects may be unsuccessful in compensating for the functionality lost in degraded ecosystems (Hale and Swearer, 2017).

Further research is required to substantiate the links between specific contaminants and a metabolome shift in crabs, thereby underpinning the biochemical pathways directly affected by contaminant exposure. Tissue-specific metabolomics would further aid in refining these results, for example, by identifying compensative energy shifts. While such sensitive metrics are useful in understanding the mechanisms of contaminant impact, to increase the ecological relevance of findings efforts should be made to monitor fitness metrics (e.g., survival), population demographics, and community dynamics. Repeated sampling at multiple trophic levels and expansion of the study to other areas would be important future steps to achieve this goal. Finally, different taxa have different capacities to detect and avoid contaminated sites that reduce their fitness (Hale et al., 2018; Sievers et al., 2018). Therefore, habitat selection experimentation could be conducted for different species to more completely assess the risk of creating ecological traps when restoring mangroves in urban environments.

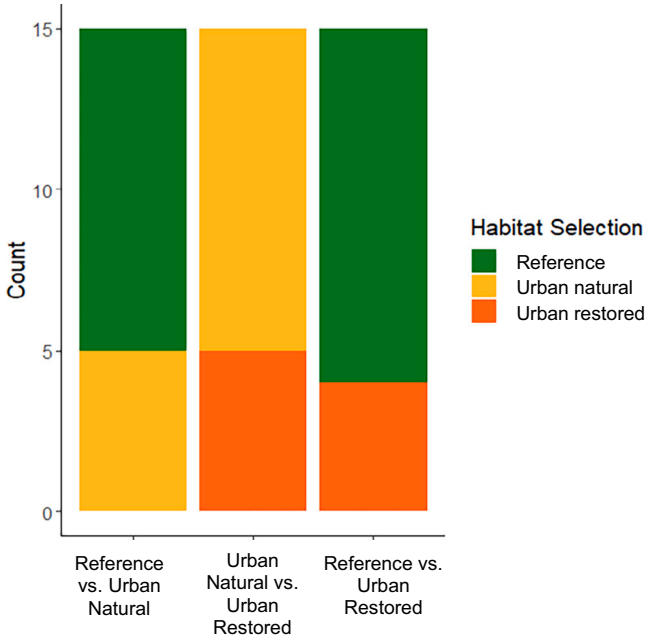

Fig. 4. Burrow counts recorded from the choice experiments, with preference for sediment from the reference site, followed by the sediment from the urban natural and restored sites, respectively. $n = 5$ crabs from each sample were exposed to each arena scenario, total of $n = 15$ crabs per scenario, for overall total of $n = 45$ crabs.

Our results highlight the need to address knowledge gaps in ecotoxicology assessment during restoration planning, particularly in the context of increasing competition for space along coastlines. In addition to the metrics evaluated in this study, quantifying a wider suite of contaminants and environmental parameters, as well as incorporating multi-omic analysis, would provide a comprehensive framework for restoration practitioners to adopt in post-restoration monitoring (Beale et al., 2022). 'Omics-based ecosurveillance' is a multi-disciplinary approach to ecosystem monitoring and, if applied to restored ecosystems, could elucidate more definitive links between faunal health and restoration outcomes. Increased effort to remediate contaminated sites and manage pollutant sources is warranted to complement active revegetation efforts. Implementing these shifts in restoration assessment and approaches is expected to help to increase the ecological and economic benefit of restoration.

This study addressed multiple layers of the complex processes involved in contaminant cycling in an estuarine ecosystem, transcending most contaminant studies that focus only on one aspect. We found that, despite the compensative characteristics of contaminated sediments, biota are still susceptible to bioaccumulation and the associated biological impacts from contaminant exposure. Furthermore, the restored site exhibited significantly higher contaminant burdens and consequential effects to the metabolome of shore crabs when compared to natural mangroves in both urbanised and non-urbanised environments. While the restored habitat was not preferred in an experimental setting, these findings still highlight the importance of considering toxicological risks to wildlife associated with restoring urban environments. Future restoration projects should therefore consider the threats of contaminants when establishing new habitat and invest resources into monitoring the impacts on colonising fauna over time to avoid creating ecological sinks. Including metrics related to animal health in the assessment of restoration outcomes will prioritise ecosystem functionality and increase overall restoration success.

Animal ethics statement

Use and number of animals in this study complies with Griffith University's animal ethics guidelines and the NHMRC 'Australian code for the care and use of animals for scientific purposes'. This includes the consideration of the '3R's'; replacement, refinement, and reduction of animal use. The ARRIVE guidelines have been addressed throughout this manuscript. Animal use for this project was approved by the Griffith University Animal Ethics committee (ENV/08/22/AEC, 2022).

CRediT authorship contribution statement

Jasmine A. Rasmussen: Writing – review & editing, Writing – original draft, Visualization, Software, Resources, Project administration, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **William W. Bennett:** Writing – review & editing, Supervision, Methodology, Conceptualization. **Steve D. Melvin:** Writing – review & editing, Investigation, Data curation. **Michael Sievers:** Writing – review & editing, Methodology, Funding acquisition, Conceptualization. **Charlotte A. McAneney:** Writing – review & editing, Investigation. **Ainsley Leaning:** Writing – review & editing, Investigation. **Rod M. Connolly:** Writing – review & editing, Supervision, Methodology, Funding acquisition, Conceptualization.

Declaration of competing interest

The 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. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2024.177064.

Data availability

Data will be made available on request.

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