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Distinct population-wide differences in contaminants and blood parameters in foraging green sea turtles

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ABSTRACT

The rising diversity and concentration of contaminants have surpassed ecological thresholds, threatening marine ecosystems. The effects of pollutants on marine animals, particularly sea turtles, are receiving increased attention due to their role as indicators of human impacts. This study examined the health implications of contaminant exposure in three green turtle (*Chelonia mydas*) foraging sites in the southern Great Barrier Reef, Australia. Assessments were performed on 45 immature turtles from offshore (Heron, Lady Elliot Island) and inshore (Hervey Bay) foraging sites, hypothesising greater anthropogenic exposure inshore. A cytotoxicity assay tested blood toxicity, while trace element concentrations were compared with baseline reference intervals. Interestingly, this analysis revealed elevated cobalt and manganese levels in Hervey Bay turtles, and offshore turtles showed higher cytotoxicity despite appearing healthier, contrasting with low cytotoxicity and low body condition in Hervey Bay. These findings highlight the complexities of ecotoxicology and the need for comprehensive data on contaminant impacts.

1. Introduction

Exploring relationships between environmental contaminants and associated health impacts on the marine environment is vital for conservation and management efforts (López-Berenguer et al., 2020). Contaminants in the environment have exceeded what is considered to be "safe" for humans and animals alike (Persson et al., 2022). It is therefore important that the impacts of contaminants on key species are assessed. The use of charismatic species as environmental proxies for anthropogenic impacts has increased in popularity across a variety of ecosystems, due to their ability to bioaccumulate contaminants and increased community engagement (Fariñas-Franco et al., 2013; González et al., 2014; Rodriguez-Perez et al., 2019). For example, sea turtles have been used as environmental indicators of the impacts of climate change, fisheries bycatch, urbanisation and pollutants (Labrada-Martagón et al., 2011; Villa et al., 2019; Barraza et al., 2021). Sea turtles are regularly used as bioindicators of ecosystem health, particularly relating to pollution, as they bioaccumulate chemical contaminants, are long-lived, and have strong foraging site fidelity, and large body size (Arienzo, 2023).

Sea turtles foraging in coastal areas are exposed to a diversity of chemicals due to runoff from nearby terrestrial catchments (Gallen et al., 2019). The intensity and type of chemical risk differs greatly over spatial scales and are dependent on the type of terrestrial land use (e.g. urban, agricultural, industrial, natural) adjacent to the coastal area (Dogruer et al., 2018). Therefore, the risk of sea turtles accumulating contaminants will usually be dependent on their foraging location. Several studies have explored the accumulation of organic and inorganic contaminants and related health effects in sea turtles foraging adjacent to industrial and agricultural areas, particularly in Queensland, Australia (Villa et al., 2017, 2019; Flint et al., 2019; Gaus et al., 2019; Leusch et al., 2020). However, the effects of these contaminants on individual turtles and their surrounding environments remain unknown in many settings. As such, environmental pollutants have been identified as a major, but hitherto poorly understood, threat to sea turtle populations globally (Fuentes et al., 2023).

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Understanding the health implications of contaminant exposure in sea turtles poses a considerable challenge due to their susceptibility to multiple simultaneous stressors, making it difficult to disentangle root causes of poor population health (Fuentes et al., 2023; Smith et al., 2023). To navigate this complexity, researchers can quantify physiological, biochemical, and haematological parameters in concert. The array of potential analyses available for a comprehensive understanding of animal health is extensive, with the choice contingent upon the specific research questions (Barraza et al., 2021; Zhang et al., 2021). In addition, some researchers have expressed concerns about relying solely on visual health assessments and biochemical parameters to gauge contaminant impact in sea turtles as variation in these parameters can be caused by numerous variables, advocating instead for a focus on cellular function and physiological responses following chemical exposure (Roman et al., 2021).

The first step in understanding impacts of chemical pollution on animal health is often to measure contaminant accumulation. Both targeted and non-targeted analyses can identify organic contaminants in biological samples (Barraza et al., 2021). A non-targeted approach to chemical analysis can identify the presence of a wide range of compounds within the environmental or biological sample, facilitating efficient management strategies in containing or improving contaminant exposure (Gaus et al., 2019). However, this method is limited by its costliness and inability to accurately quantify individual contaminant concentrations. Conversely, targeted chemical analysis can accurately measure the presence and concentration of specific compounds (Sapozhnikova and Lehotay, 2013). However, this method is limited by the number of chemicals that can be measured, potentially overlooking contaminants that have deleterious effects on species and ecosystem health. In addition to chemical analyses, species-specific cell-based bioassays offer a complementary perspective by investigating the cellular impacts of contaminant exposure on individual species (Johnson et al., 2022). Unlike targeted and non-targeted chemical analyses, bioassays do not capture compound identification, instead providing insights into direct species-level impacts of individual chemicals of interest, and combined effects of chemical mixtures present in the environment (Dogruer et al., 2018; Jin et al., 2015). Exposing species-specific cell cultures to extracted contaminant mixtures enables the quantitative assessment of diverse parameters such as cytotoxicity, genotoxicity, and oxidative stress (Finlayson et al., 2016; Johnson et al., 2022).

The coastal waters of Queensland, Australia supports two genetically distinct populations of green sea turtles, *Chelonia mydas*, that have been well studied and have health, toxicity and trace element reference intervals reported (Flint et al., 2010, 2019; Villa et al., 2017). Flood events in southern Queensland, Australia during the La Niña period from 2019 to 2023 inundated coastal areas, had significant and widespread impact on foraging green sea turtle habitats (Healthy Land and Water, 2022). These events brought a substantial volume of freshwater and land-based sediment to coastal environments, leading to a notable rise in marine wildlife strandings and mortality events, particularly sea turtles in the Hervey Bay region (*pers. comms.* Turtles in Trouble Rescue). This is similar to historical flooding events in this region, which caused an increase in turbidity and nutrients, as well as decreased salinity, leading to a 40 % reduction in coral cover on nearshore reefs (Butler et al., 2013). While the reasons underlying recent increases in strandings and mortality is under ongoing investigation by local authorities, it is likely that increased organic and trace element contaminants in coastal waters could be a significant but unquantified contributor across the region (Cebula and Ciba, 2005; Foulds et al., 2014).

Using species-specific cell-based bioassays in combination with chemical analyses improves the depth of our understanding regarding the physiological consequences of contaminant exposure, increasing the knowledge base required for future research directions and pollution management strategies. There have been various studies evaluating contaminant concentrations in green turtles along the Queensland coast, which highlight the increase in trace elements and organic contaminant exposure inshore foraging sites (Villa et al., 2019; Finlayson et al., 2021). Reference intervals for trace elements have been established for comparative analysis on a spatial and temporal scale (Villa et al., 2017). In addition, a species-specific cytotoxicity bioassay has recently been effective at determining temporal and spatial variations in organic contaminant loads between foraging aggregations of green turtles throughout Queensland (Finlayson et al., 2020, 2022). Combining these cell-based assays with trace element analyses has been validated as an accurate method of quantifying both organic and inorganic contaminants and allows researchers to more effectively disentangle the effects of chemical accumulation (Barraza et al., 2023).

Immature green turtles are often used as representatives for their foraging grounds as they have not yet entered the migratory portion of their lifecycle and therefore are indicative of contaminants that are present in these coastal ecosystems (Arthur et al., 2008). This study aimed to examine contaminant levels, and associated blood parameters in immature green sea turtles from chemically distinct foraging locations. It was hypothesised that individuals from the inshore foraging site would have increased exposure to contaminants and therefore have subsequent variations in blood parameters in comparison to offshore foraging sites. This was achieved by collecting blood samples from three foraging locations in the southern Great Barrier Reef green sea turtle genetic stock, and analysing 45 individuals for trace elements, cytotoxicity, and various blood parameters to elucidate spatial variation in chemical profiles and subsequent health implications.

2. Material and methods

2.1. Study sites

Blood parameters and contaminant exposure in green turtles was quantified from three foraging locations of the southern Great Barrier Reef genetic stock of green sea turtles. Heron Island, Lady Elliot Island, and Hervey Bay, along the eastern Australia coast, represent chemically distinct areas for the scientific investigation of contaminants and their implications on green sea turtle health (Fig. 1). Heron Island and Lady Elliot Island are two offshore coral cays in the Capricorn bunker group of the Great Barrier Reef that host considerable nesting and foraging aggregations of multiple turtle species (Staines et al., 2022). Despite some similarities between these two islands, such as the presence of resorts, Heron Island has the addition of a research station and is in the direct path of the Fitzroy River plume, ~80 km from the township of Gladstone (Devlin et al., 2001). Lady Elliot Island is within 100 km of the Mary River and was once decimated by guano mining and the introduction of goats (Great Barrier Reef Marine Park Authority, 2020). These offshore islands offer useful comparison to inshore foraging sites as they are not exposed to the same level of anthropogenic impacts as inshore foraging aggregations. Hervey Bay is an inshore shallow, soft bottom foraging location, that is considered one of the major foraging locations of the southern Great Barrier Reef genetic stock, prompting an examination of contaminant dynamics in this critical foraging environment (Finlayson et al., 2021).

2.2. Animal ethics and permits

Data collection at Heron Island and Lady Elliot Island was performed under a Scientific Purposes Permit (SPP18-001035), Marine Parks Permit (MPP18-001041-2) and Great Barrier Reef Marine Park Permit (G18/40908.1) issued by the Queensland Department of Environment and Science (DES) and the Great Barrier Reef Marine Park Authority (GBRMPA). Animal ethics was approved by the University of the Sunshine Coast Animal Ethics Committee, approval number ANS2076. Samples collected in Hervey Bay were taken under the Queensland Turtle Program, Department of Environment and Science permits.

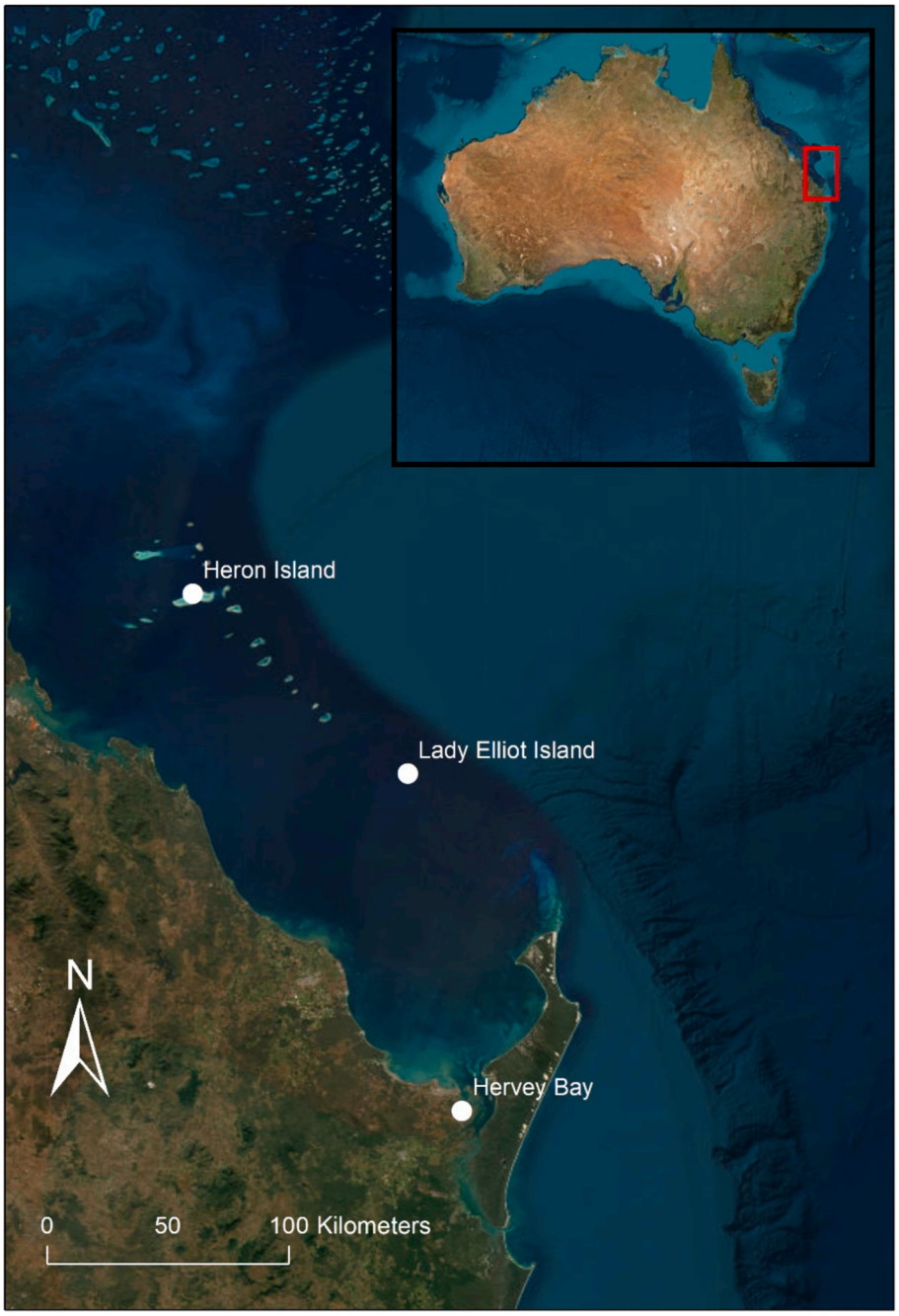

Fig. 1. The localities of the three foraging study sites of Heron Island, Lady Elliot Island and Hervey Bay in Queensland, Australia.

2.3. Sample collection

Fifteen immature (45–85 cm curved carapace length (CCL)) green turtles were collected from each of Lady Elliot Island, Heron Island and Hervey Bay foraging grounds (for a total of 45 individuals), during February, April, and September 2022, respectively. This sample size has been demonstrated to provide sufficient statistical power in similar toxicological and biomarker studies involving other marine species (Gagnon and Hodson, 2012). Moreover, the high cost and significant time required to collect and analyse toxicological samples further justify the practicality of this approach. This size class of green turtle is typically more representative of chemical pollution in the surrounding environment as they have not yet entered the migratory portion of their lifecycle and have been residents of the area for several years. There is evidence of variation in contaminant exposure between immature recruits that have recently returned from their post-hatchling pelagic stage, otherwise known as 'new recruits', and individuals that have been in the foraging ground for an extended period of time (Perkins et al., 2022). Therefore, 'new recruit' individuals characterised by a CCL <50 cm, as well as a white plastron with the presence of ventral ridges not worn down by inshore foraging were not selected for sampling (Arthur et al., 2008). Turtle collection was conducted by hand capture at both low and high tide. Unique titanium tags, supplied by the Department of Environment and Science, Queensland Government, were applied on the trailing edge on the left and right front flippers of each turtle, and registered in the Queensland Turtle Tagging Database. Weight (kg) and CCL (cm) were measured to the nearest g and mm, respectively, and epibiota including barnacle number (>1 cm diameter) and algal growth (% cover) recorded. An external examination assessed the general health condition of each turtle, noting any tissue, bone or carapace loss or

injury, any signs of disease, and/or dehydration. Plastron concavity or malnourishment severity was also noted by observing any sinking between the plastron ridges.

2.4. Blood collection

Blood was collected via the external jugular vein located on the neck of the turtle, using either a 21- or 23- gauge 1 ¼ inch Terumo™ needle (dependent on the individual's size) and a 20 mL Terumo™ syringe. Total blood collection amount was a maximum of 20 mL and did not exceed 1 mL/kg of each turtle. Blood was aliquoted for various analyses, detailed below.

2.5. Trace element extractions

Analysis of trace elements in whole blood samples was conducted through inductively coupled plasma mass spectrometry (ICP-MS). Blood samples were stored in 10 mL BD™ lithium heparinised vacutainers in a −20 °C freezer until analysis. Once thawed to room temperature, 1 mL of whole blood was acid digested with 4 mL nitric acid, 1 mL hydrochloric acid, and 1 mL hydrogen peroxide in pressurised Teflon vessels, using microwave irradiation (CEM Mars 6; model 910905) following EPA method 3052 (United States Environmental Protection Agency, 1995). Each batch of samples included three duplicates (random samples analysed twice), laboratory blanks (MilliQ water) and the standard reference material, Seronorm™ whole blood. Digested samples were diluted with MilliQ water (1:50) and analysed using ICP-MS (Agilent Technologies; 7900). Data was processed using Agilent Chemstation software (ICP-MS MassHunter version 4.3), and all concentrations were blank corrected and reported as μg/L of whole blood. The limit of detection (LOD) was calculated for each element as three times the standard deviation of the blanks, and elements that did not pass the quality control and assurance (80–120 % of the certified reference concentrations, and <20 % relative standard deviation of the duplicates) were excluded from further analyses.

2.6. QuEChERS extraction method

For the extraction of organic contaminants from whole blood samples, the QuEChERS (quick, easy, cheap, effective, rugged, and safe) methodology was employed (Dogruer et al., 2018; Perestrelo et al., 2019; Finlayson et al., 2019, 2022). Briefly, whole blood samples stored in BD™ lithium heparin vacutainers were thawed, and 5 mL were transferred into 50 mL falcon tubes. Three solvent washed ceramic homogenizers were added to the falcon tubes and vortexed for 1 min, followed by 15 mL of acetonitrile and vortexed for 1 min. Five grams of magnesium sulfate ($MgSO_4$) and 1 g of sodium chloride (NaCl) were added into the falcon tube and hand shaken for 2 min. Mixtures were then centrifuged at 1900*g* for 8 min at 10 °C, and the supernatant was aliquoted into glass test tubes and evaporated under a gentle stream of nitrogen gas. Dried extracts were reconstituted in 1 mL methanol and transferred into glass amber vials with Teflon lids. This resulted in a relative enrichment factor (REF), the amount that the blood was concentrated in the extract, of 5. Extracts were stored at −20 °C until use in the cytotoxicity bioassay.

The extracts obtained were used in in vitro cell-based bioassays to comprehensively assess the effects of organic contaminants in these blood samples (Finlayson et al., 2021). This extraction method, coupled with cytotoxicity bioassays, has been applied to evaluate organic contaminants in foraging green sea turtles along the eastern Australian coast (Finlayson et al., 2022).

2.7. Species-specific cytotoxicity bioassay

Organic contaminants in the blood extracts were measured using a cytotoxicity resazurin assay on green turtle primary skin fibroblasts. Briefly, green sea turtle primary skin fibroblasts were grown in RPMI-1640 medium with 10 % foetal bovine serum (FBS) and incubated at 30 °C with 5 % CO_2. An aliquot of methanol extracts were evaporated using nitrogen gas and reconstituted in RPMI-1640 medium with 5 % FBS, resulting in a REF of 2, corresponding to twice the contaminant concentration in blood samples (Finlayson et al., 2020).

A black/clear bottom 96-well microtiter plate was seeded at 3×10^4 cells per well and incubated for 24 h at 30 °C with 5 % CO_2. After 24 h, a separate plate was used to prepare extracts in a serial dilution (2-fold, 4 point) along with: Triton X-100 (0.1 %) as a positive control, chromium as a reference compound (7-point 2-fold dilution standard curve), and a negative control of RPMI medium with 5 % FBS. All controls and extracts were tested in duplicate. Medium from the test plate was replaced with medium containing samples and controls for a 24-hr exposure. After 24 h, 20 μL of 0.15 mg/mL resazurin in phosphate buffered saline and 80 μL of RPMI medium with 5 % FBS was added to each well for a further 24-hr incubation. Following resazurin incubation, each well's fluorescence was measured at $\lambda_{ex} = 544$ nm and $\lambda_{em} = 590$ nm using a Spark plate reader (Tecan, serial number: 1912004413). Each plate had 10 extracts, including quality assurance and quality controls, and extracts were tested in at least three independent trials. The percent mortality of cells for each sample/reference dilution within an assay was calculated using the following equation, as described by Finlayson et al. (2020):

$$\text{Mortality } (\%) = \frac{F - NC}{PC - NC} * 100$$

where F signifies the mean fluorescence of sample/reference duplicates, NC signifies mean fluorescence of the negative control duplicates, and PC signifies the mean fluorescence of the positive control duplicates.

The percent mortality of cells was calculated in GraphPad Prism 5 (GraphPad Software Inc.). Each sample/reference dilution was plotted against the log concentration, and the Hill Slope equation was employed to determine the concentration required to produce 20 % cell mortality (EC_{20}), expressed as a REF. The Toxicity Unit (TU), the reciprocal of the EC_{20} value, was then calculated to indicate the relative toxicity of each sample, with higher TU values signifying greater toxicity. For instance, a TU of 1 corresponds to a 20 % effect of the turtle blood on cell death; values below 1 indicate a lower risk, whereas values above 1 suggest an increased risk of cytotoxicity.

2.8. Health analysis

Immediately post-collection, approximately 0.1 mL of whole blood was added to a CG8+ iSTAT™ cartridge, which were stored between +2 °C and + 8 °C, until within 2 h of use, then kept at room temperature until the time of use. An iSTAT Portable Clinical Analyser (Heska Corporation, Fort Collins, Colorado, USA) was used to obtain in-field results. To avoid overheating while in the field, cartridges and the analyser unit were stored in an insulated box with ice packs to maintain temperature below 25 °C. The CG8+ cartridge provided values for the following parameters: sodium (Na; mmol/L), potassium (K; mmol/L), ionised calcium (iCa; mmol/L), and glucose (Glu; mmol/L) iSTAT values were used.

Following the iSTAT analysis, the remaining blood was transferred into 10 mL lithium heparin BD™ vacutainers and stored in a cooler until the blood was centrifuged or frozen for storage. Hematocrit % or packed cell volume (PCV) was measured using heparinised whole blood, which was transferred by capillary action into microhematocrit tubes, and centrifuged at 12,000 rpm for 3 mins (JorVet ZipCombo). The percentage of red blood cells was calculated using a microhematocrit reader. Heparinised blood (2 mL) was also transferred into Eppendorf™ tubes and centrifuged for 3 mins at 12,000 rpm. The plasma was then pipetted into a separate Eppendorf™ tube, and, along with the remaining red blood cells, was frozen at −20 °C and archived.

An IDEXX catalyst One Laboratory Analyser™ was used to establish

ranges of blood biochemistry of the plasma samples. Analysis was carried out at SeaLife Aquarium Mooloolaba, a facility that regularly rehabilitates sick and injured sea turtles. *IDEXX Chem 17*™ clips were used to calculate values of biochemical parameters including; creatinine, calcium, alanine aminotransferase, albumin, globulin, blood urea nitrogen, phosphorus, calcium, urea, cholesterol, amylase, and lipase. Samples where haemolysis occurred were excluded from blood parameter analysis.

Scaled mass index (SMI) of each turtle was calculated following Bell et al. (2019):

$$\text{Scaled mass index (SMI)} = W_i \left[\frac{L_O}{L_i}\right]^{b_{SMA}}$$

Using a log transformed standardised major axis regression (SMA) of weight (kg) versus curved carapace length (cm), the slope of the linear regression provides the allometric scaling exponent (b_{SMA}). L_i and W_i are the curved carapace length (CCL; cm) and weight (kg) for each individual, respectively, and L_O is the CCL to which the index is standardised. This study used the overall curved carapace length mean of both sites (55.5 cm) as L_O, similarly to Bell et al. (2019). This method was used to standardise the body condition for the purpose of population comparison.

The sub-adult biochemical values from Hamann et al. (2006), the Gulf of Carpentaria, and Bolten and Bjorndal (1992), the Bahamas, were used as reference standards to compare against the sub-adult individuals sampled in this study. These two studies represent very different populations in terms of habitat use, diet and genetic variation.

2.9. Statistical analyses

Statistical analyses were performed with an α of 0.05 using R (v. 4.2.0), in Rstudio (v. 2022.06.0), and GraphPad Prism 5 (GraphPad Software Inc.). Samples where elements were below the limit of detection were adjusted by replacing the value with three times the standard deviation of laboratory blanks (Barraza et al., 2023). Trace element concentrations were compared to reference intervals established from a remote clinically healthy population of turtles (Villa et al., 2017). Contaminant and blood parameter data were assessed for normal distribution using the Shapiro-Wilk test. The majority of contaminant and health variables were not normally distributed therefore the differences between sites were determined using a Kruskal-Wallis test and a post hoc Dunn test, means are reported ± standard error. The relationships between contaminant concentrations, blood parameters and body condition were assessed using generalised additive models (GAMs) in the *mgcv* package (Wood, 2011) of R. Variables were tested for collinearity using Spearman correlation. Pairs of variables with a correlation >0.6 or less than −0.6 were excluded from the analysis. Among the correlated variables, one was retained based on the strength of the underlying hypothesis. Two streams of analyses occurred, the first explored the variation in blood parameters in relation to body condition, and the second contaminant influence on body condition. Model overfitting was minimised by limiting GAM relationships to three knots or fewer (i.e. k = 3) and by calculating GAMs with all possible combinations of four variables or fewer using the *MuMIn* package (Barton, 2023). The best fit models were the resulting models with the lowest Akaike's Information Criterion (AIC) value.

3. Results

3.1. Trace elements

The mean concentration of trace elements found in the whole blood of green turtles collected from the foraging individuals of Hervey Bay, Heron Island and Lady Elliot Island showed considerable variation between sites, and many were above the reference intervals (Figs. 2 and 3; SM1). All trace elements were significantly different at one or more sites. Cobalt (Fig. 2A), manganese (Fig. 2B), copper (Fig. 2D), iron (Fig. 2F), zinc (Fig. 2G), lead (Fig. 3B), and uranium (Fig. 3G) were all significantly higher in the Hervey Bay aggregation in comparison to Heron and Lady Elliot Island. Trace element concentrations in the two offshore island sites were very similar apart from copper (Fig. 2D), vanadium (Fig. 3D), and nickel (Fig. 3F), with Heron Island having a higher copper and nickel concentration and a lower vanadium concentration. Cobalt and manganese concentrations were approximately 57 and 4.3 times higher, respectively, in Hervey Bay than Heron and Lady Elliot Island (SM1). The mean concentration for cobalt, manganese and molybdenum were 6.4, 1.9, 1.06 times higher, respectively, than the maximum reference interval in the Hervey Bay aggregation. Heron and Lady Elliot Islands had mean concentrations higher than the maximum reference interval for selenium, arsenic, and cadmium (SM1). Lady Elliot Island exceeded the maximum reference interval for vanadium, and all sites were above the reference range for antimony.

3.2. Organic contaminants

The mean TU for Hervey Bay, Heron Island and Lady Elliot Island were statistically significant at a site level ($\chi^2 = 27.6$, $p < 0.001$). The mean TU recorded for the Hervey Bay aggregation (0.35 ± 0.05) was significantly lower than that of Heron (4.33 ± 0.83) and Lady Elliot Islands (3.89 ± 0.57; Fig. 4). Hervey Bay was significantly different from both Heron and Lady Elliot Island aggregations ($z = 4.63$, $p < 0.001$; $z = 4.47$, $p < 0.001$, respectively), however the Heron and Lady Elliot aggregations did not show a difference between TU ($z = -0.167$, $p = 0.86$). The Hervey Bay individuals produced consistently low TU (< 1 TU), whereas the Heron and Lady Elliot populations have four high outliers (>6 TU) that may be increasing the mean toxicity at these sites (Fig. 4). When these outliers are removed the mean toxicity reduces to 3.24 ± 0.45 (Heron Island) and 3.30 ± 0.45 (Lady Elliot Island).

3.3. Body condition variation

The scaled mass index of green sea turtles was significantly different between sites (Fig. 5; $\chi^2 = 34.3$, $p = 3.46 \times 10^{-8}$). According to the post hoc Dunn test, all three sites were statistically significant from each other, with Heron Island having a higher scaled mass index (22.0 ± 2.42), followed by Lady Elliot Island (18.1 ± 1.79) and Hervey Bay (15.4 ± 1.09). Epibiota was not recorded in either offshore location, and epibiota was only recorded in the Hervey Bay individuals.

Blood parameters that are indicative of health impacts were extracted from the dataset and analysed. Several of the blood parameters have a significant variation between foraging locations and published ranges (Table 1). Generalised additive modelling showed that the combination of four blood parameters were significant in influencing scaled mass index (Fig. 6; AIC = 183.6, $R^2 = 0.47$). These were calcium ($F = 3.2$, $p = 0.08$), albumin ($F = 2.44$, $p = 0.12$), hematocrit/packed cell volume ($F = 2.58$, $p = 0.11$), and urea ($F = 3.4$, $p = 0.05$).

Generalised additive modelling observed significant relationships between trace element concentrations and scaled mass index (Fig. 7; AIC = 214.1, $R^2 = 0.53$). Cobalt ($F = 22.43$, $p < 0.001$) and molybdenum ($F = 4.117$, $p = 0.03$) were highlighted as the two contaminant variables that contributed the most to variation in scaled mass index.

4. Discussion

The complex relationship between ecotoxicology and sub-lethal health implications on marine life is difficult to disentangle due to the diverse environmental and ecological contributors to physiological variation. Therefore, understanding the impacts of pollution on ecosystems to optimising their condition and ecological resilience. This study aimed to quantify contaminant concentrations in three foraging locations of green sea turtles in the southern Great Barrier Reef and

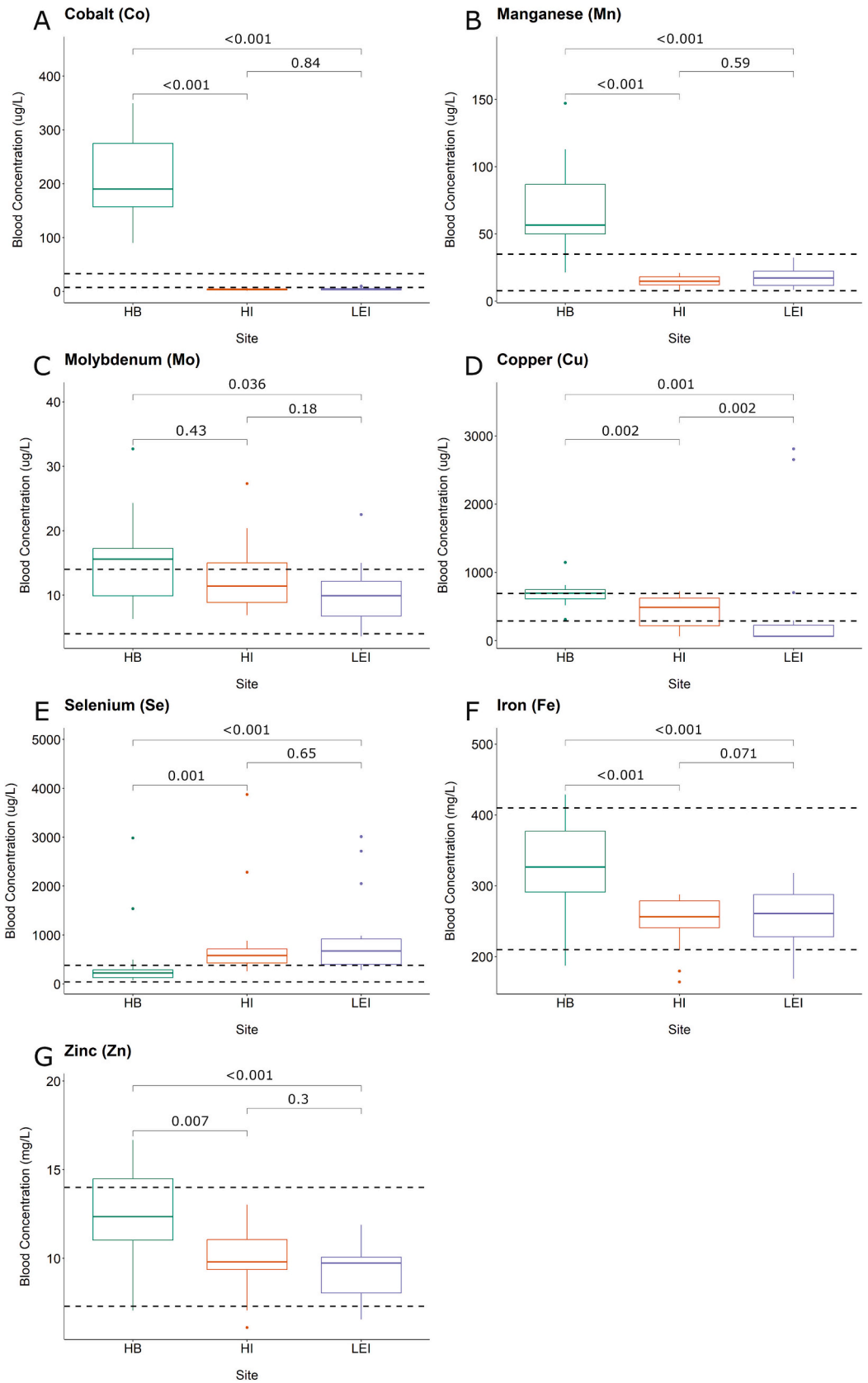

Fig. 2. Concentrations of essential trace elements in the whole blood of green turtles from Hervey Bay (HB), Heron Island (HI) and Lady Elliot Island (LEI). Significance is denoted by annotations. Reference intervals, as reported by Villa et al. (2017), are depicted by the black dotted horizontal lines. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

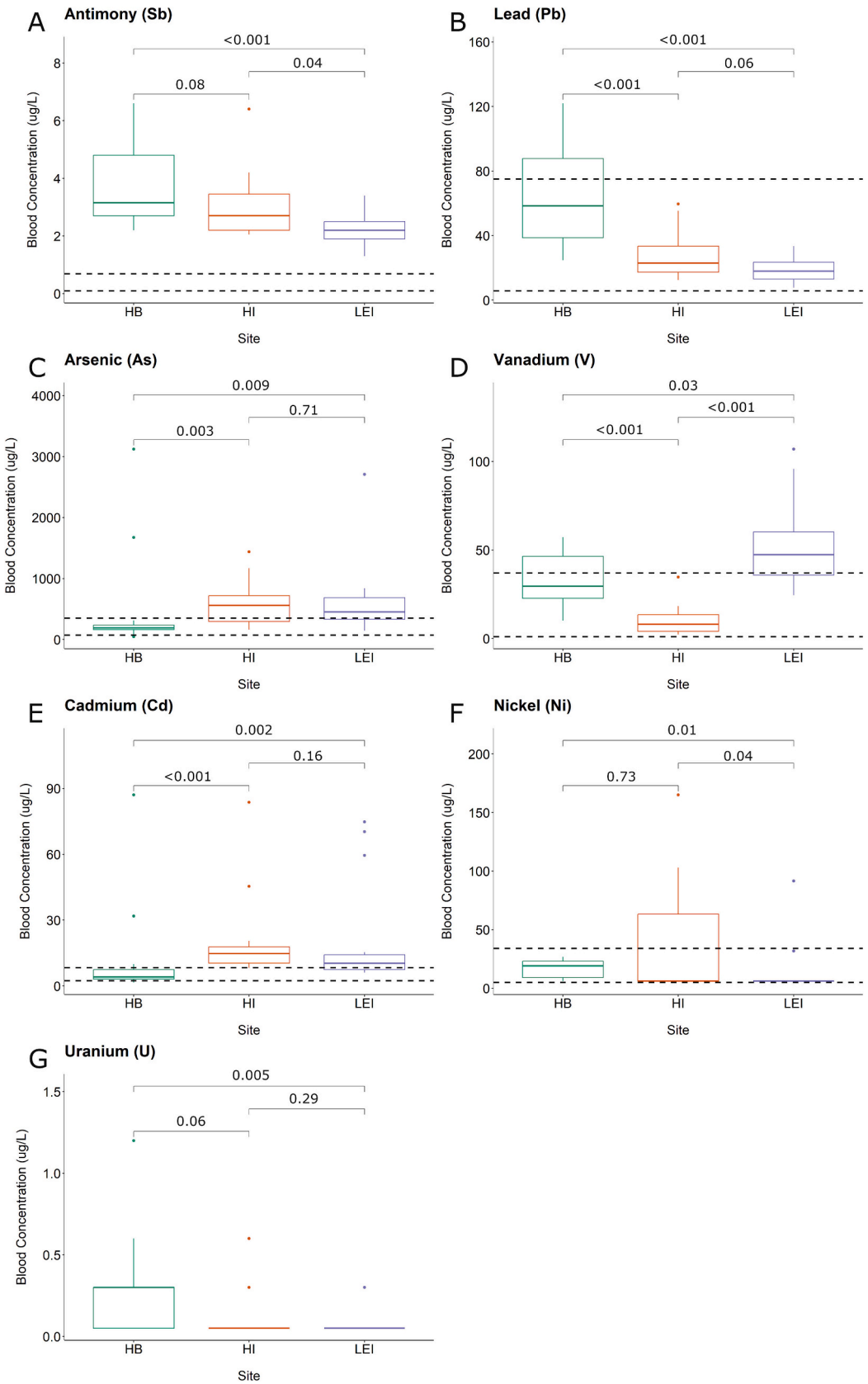

Fig. 3. Concentrations of non-essential trace elements in the whole blood of green turtles from Hervey Bay (HB), Heron Island (HI) and Lady Elliot Island (LEI). Significance is denoted by annotations. Reference intervals, as reported by Villa et al. (2017), are depicted by the black dotted horizontal lines. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

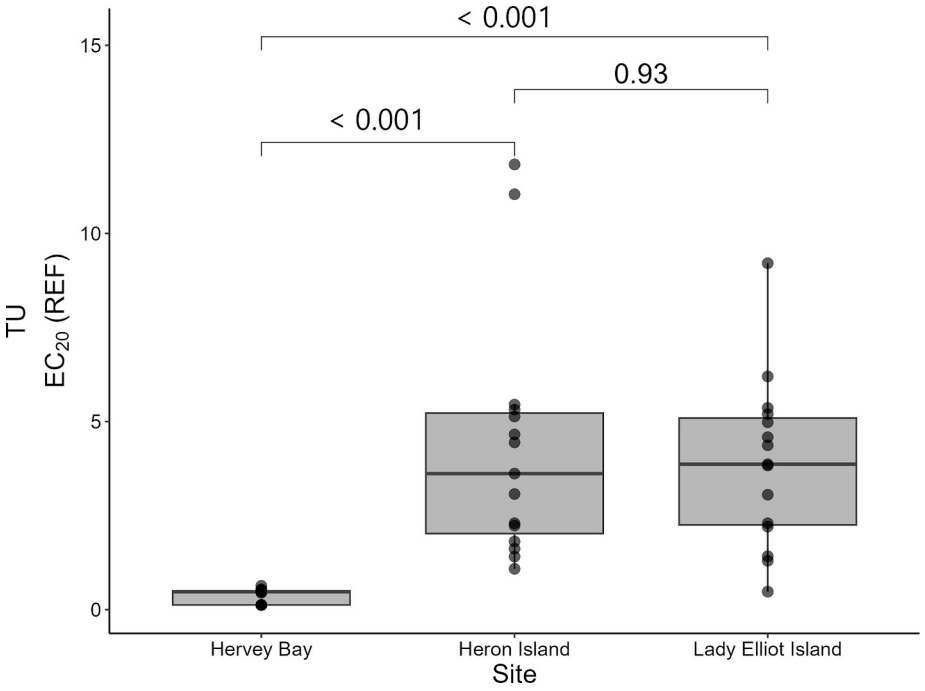

Fig. 4. Toxicity units (TU) denoting the reciprocal of the effective concentration for 20 % cell death (EC_{20}) with the relative enrichment factor (REF) for the Hervey Bay, Heron Island and Lady Elliot Island immature green turtle foraging populations (n = 15 per site). Significance is denoted by annotations. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

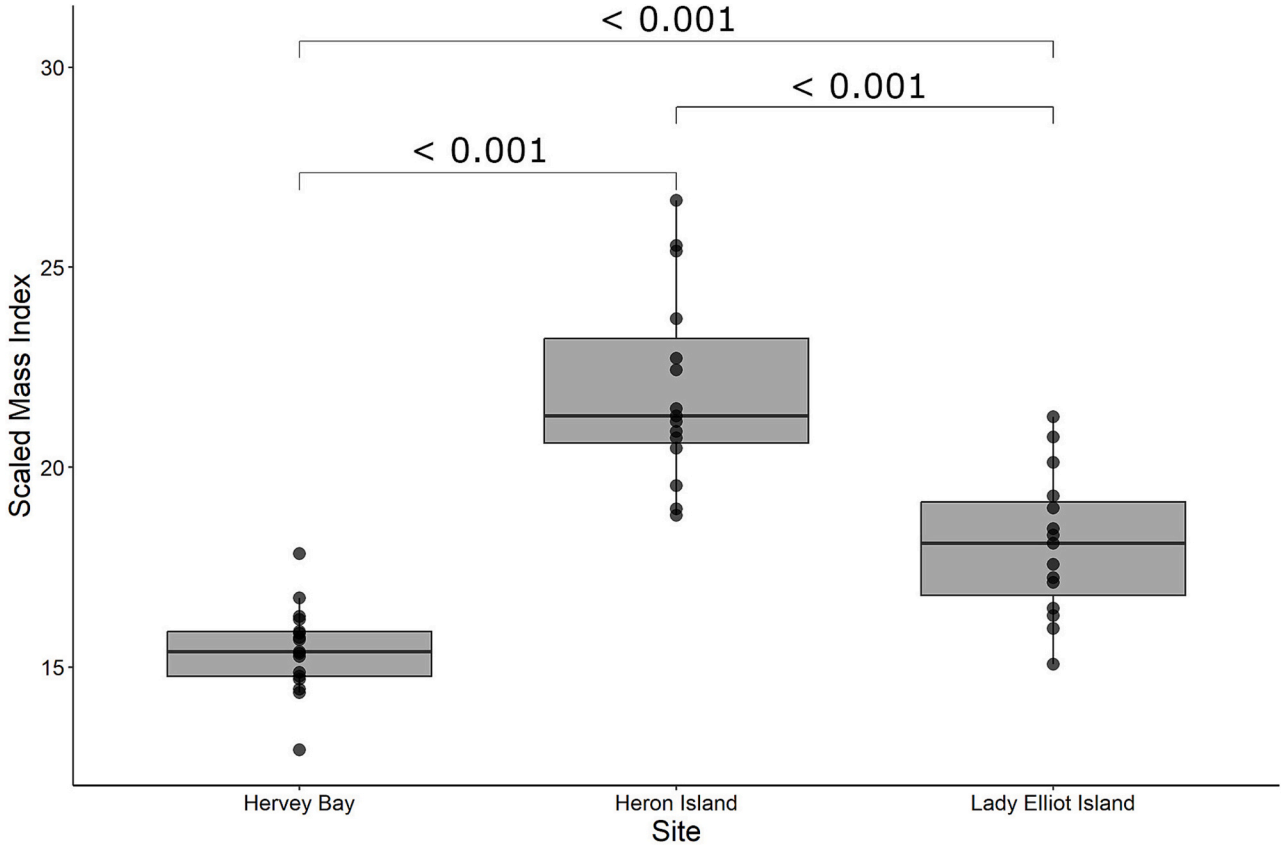

Fig. 5. Scaled mass index of immature green sea turtles at three distinct foraging grounds in the southern Great Barrier Reef genetic stock. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 1
Mean ± standard deviation (minimum and maximum values) of biochemical parameters in the plasma of immature green turtles from the Gulf of Carpentaria (Hamann et al., 2006) and Bahamas (Bolten and Bjorndal, 1992) and the southern Great Barrier Reef genetic stock (this study). Some values were transformed from μg/L to mmol/L for comparison. Plasma samples that were haemolysed were excluded from the table below. Only values that overlapped between these three studies were used for analysis, '*' denotes significance between sites in this study.

Parameter	Smith et al., this study			Hamann et al., 2006	Bolten and Bjorndal, 1992
Foraging location	Hervey Bay ($n = 15$)	Heron Island ($n = 11$)	Lady Elliot Island ($n = 10$)	Gulf of Carpentaria	Bahamas
Creatinine (μmol/L) *	11.14 ± 8.1 (9–39)	18.81 ± 12.4 (9–39)	9.9 ± 1.9 (9–14)	22.03 ± 3.66 (15.8–27.8)	44.2 ± 8.84 (26.52–79.56)
Urea (mmol/L) *	12.9 ± 15.05 (0.9–48.4)	1.37 ± 0.5 (0.9–2.6)	3.59 ± 6.48 (0.9–21.8)	0.94 ± 0.75 (0.2–2.3)	NA
Glucose (mmol/L) *	3.22 ± 0.89 (1.45–5.23)	3.34 ± 0.71 (2.28–4.37)	3.78 ± 0.37 (3.16–4.51)	1.94 ± 0.79 (0.5–3.0)	6.3 ± 0.8 (4.8–9.3)
Alanine aminotransferase (U/L) *	10.28 ± 0.72 (10−12)	17.09 ± 4.96 (10−30)	19.3 ± 6.83 (10–26)	9.3 ± 8.98 (0.0–32.0)	6.0 ± 3.0 (1.0–17.0)
Total protein (g/L) *	34.8 ± 8.38 (20–53)	40.4 ± 4.45 (26–49)	41.2 ± 7.36 (30–46)	39.94 ± 14.93 (5.4–66.7)	51.0 ± 8.0 (26–69)
Albumin (g/L) *	9.64 ± 2.3 (6–14)	12.36 ± 1.56 (9–16)	13.2 ± 2.2 (9–15)	18.31 ± 7.11 (3.2–29.1)	15 ± 2 (6–21)
Globulin (g/L)	25.3 ± 6.23 (13–39)	28.09 ± 3.78 (17–35)	28.1 ± 5.34 (20−33)	18.4 ± 2.82 (13.7–21.4)	36 ± 7 (19–52)
Albumin/globulin ratio*	0.38 ± 0.05 (0.3–0.5)	0.44 ± 0.06 (0.4–0.5)	0.47 ± 0.04 (0.3–0.5)	1.04 ± 0.36 (0.5–1.6)	0.4 ± 0.1 (0.3–0.7)
Calcium (mmol/L) *	1.48 ± 0.45 (0.74–2.2)	2.11 ± 0.59 (0.79–2.2)	1.54 ± 0.41 (1.41–3.69)	2.12 ± 0.39 (1.43–2.72)	0.5 ± 0.1 (0.1–0.7)
Phosphorus (mmol/L) *	1.54 ± 0.39 (0.91–2.31)	2.05 ± 0.42 (1.2–2.43)	1.93 ± 0.35 (1.41–2.53)	1.51 ± 0.4 (0.58–2.02)	0.4 ± 0.1 (0.2–0.6)

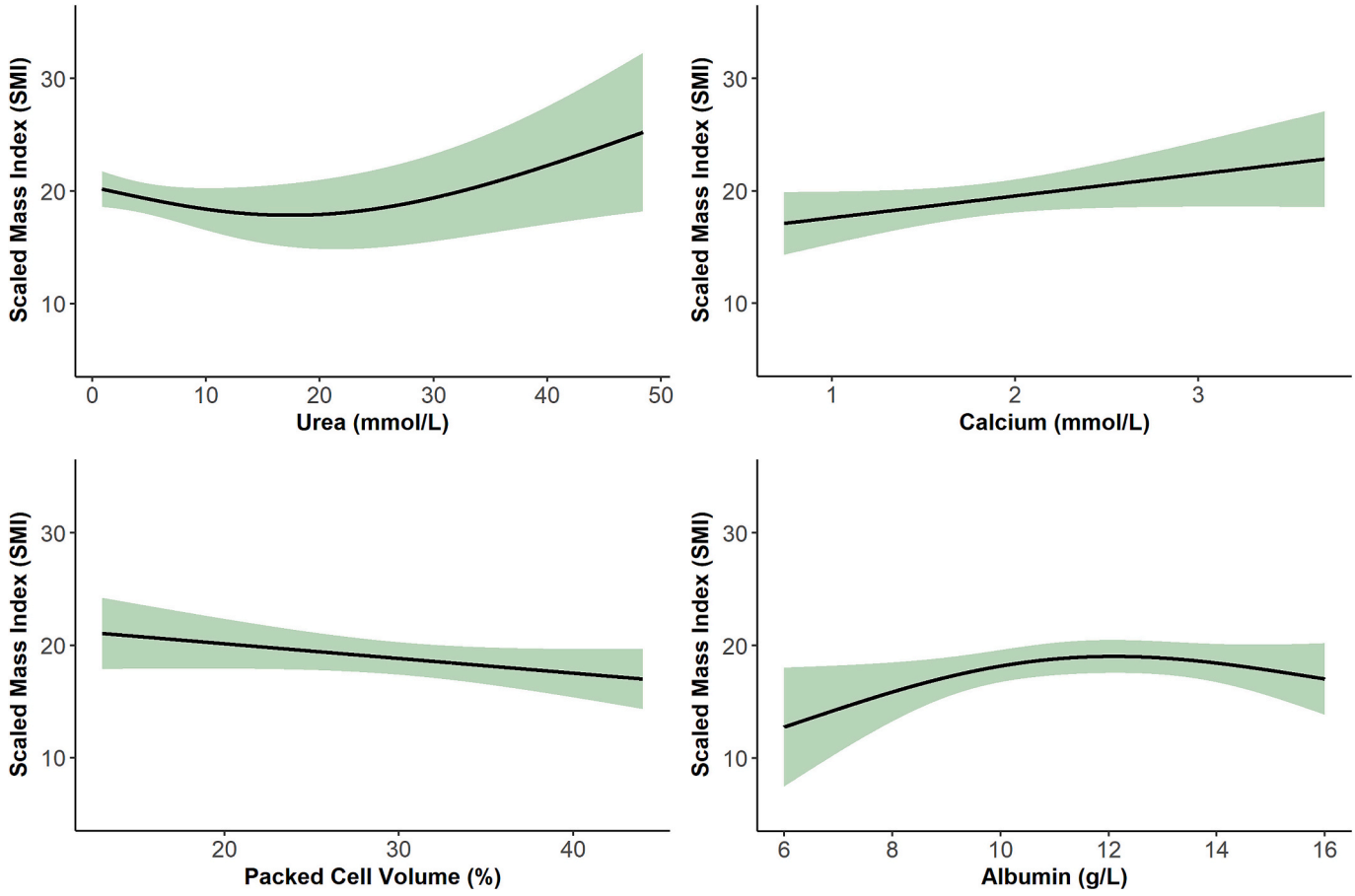

Fig. 6. GAM explaining the relationships between body condition (SMI) and the concentration of urea (mmol/L), calcium (mmol/L), albumin (g/L) and packed cell volume (%) in the blood of immature foraging green sea turtles. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

elucidate health parameter variations due to contaminant exposure. It was hypothesised that the inshore foraging site, Hervey Bay, would have higher contaminant exposure and poorer health in comparison to the two offshore foraging sites, Heron and Lady Elliot Island. This study uncovered various relationships between contaminant concentration, blood parameters and body condition, with distinct differences observed between inshore and offshore sites.

Body condition was linked to variations in urea (mmol/L), albumin (g/L) levels, packed cell volume (%), and calcium (mmol/L) highlighting the potential susceptibility to renal disease, dehydration, catabolism, and chronic debilitation due to contaminant exposure (Johnson III and Watson, 2020; Wilkinson and Divers, 2020). This highlights the importance of continuing this research to further understand the drivers of poor environmental health and employ effective management strategies to mitigate anthropogenic impacts. Data collected throughout this project identifies statistical relationships between pollutants and blood parameters, providing insights that can guide stakeholders in mitigating contamination sources and supporting the resilience of marine ecosystems.

This study observed trace element concentrations above the

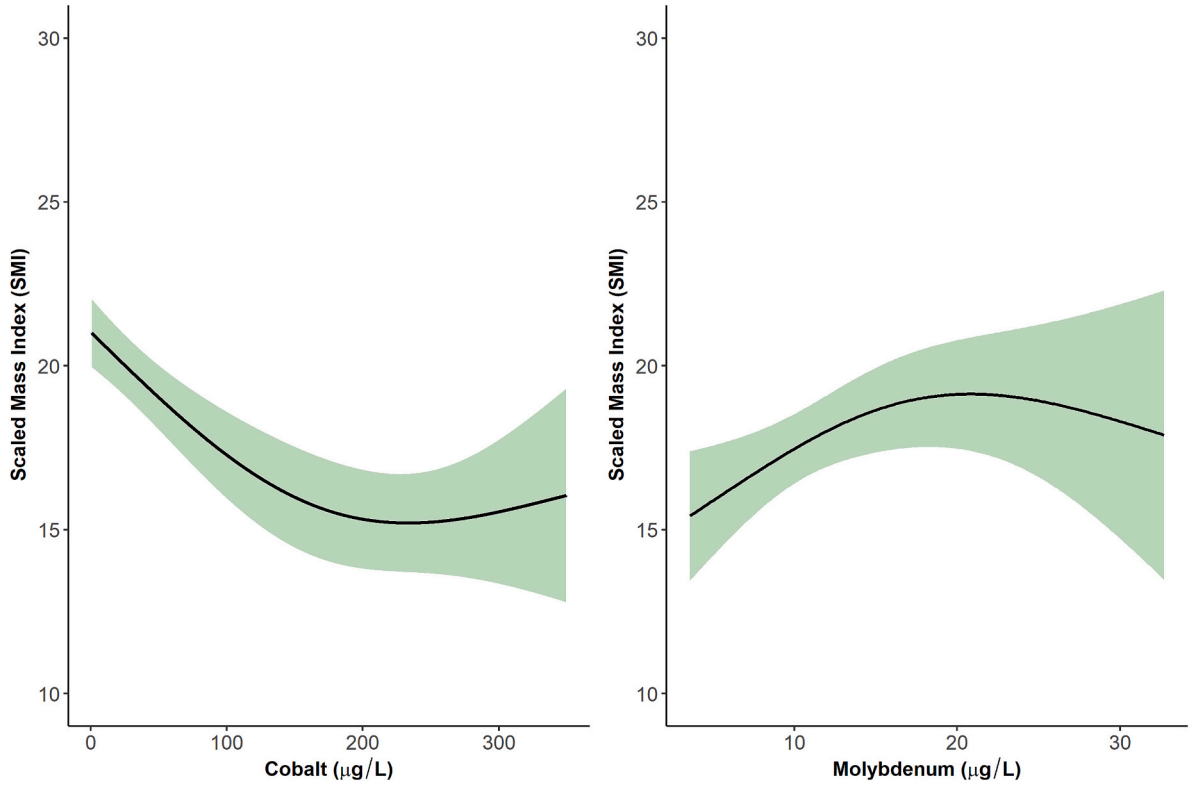

Fig. 7. GAM explaining the relationships between body condition (SMI) and the concentration of cobalt (μg/L) and molybdenum (μg/L) in the blood of immature foraging green sea turtles. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

reference intervals reported by Villa et al. (2017) within offshore foraging sites. Elements that were above the reference intervals were selenium, vanadium, cadmium, and antimony. High mean trace element concentrations, outside the standard reference intervals, were found to be common within the inshore foraging site, Hervey Bay. Noticeably the elements that were outside of these "normal" ranges for green sea turtles were cobalt, manganese, molybdenum, and antimony. Uranium was also higher in Hervey Bay individuals compared to the offshore sites, although no standard reference interval exists for this element. While reference intervals for trace element concentrations in juvenile green turtles are currently unavailable, the established intervals (Villa et al., 2017) for larger size classes of green turtles provide a useful comparison. Although differences in trace element concentrations across size classes are noted, these patterns are not always consistent; for instance, cadmium levels have been reported to be higher in juveniles (Dogruer et al., 2021).

Barraza et al. (2023) found that contaminants in Heron Island green sea turtle hatchling livers had significant effects on sex ratio deviation, finding that an increase in lead, antimony, and cadmium were correlated to increased female hatchling production. Heron Island hatchlings were also found to have cadmium concentrations outside of the reference intervals, consistent with the findings in this study, suggesting that cadmium may be present in high quantities in these reef systems (Barraza et al., 2023). Few studies have explored the impacts of cadmium exposure on reptile species, however Canzanella et al. (2021) highlighted that cadmium could have renal implications as cadmium is transferred from the liver to the kidneys and is typically bioaccumulated. Concentrations of trace elements and organic contaminant loads have been positively correlated to age and size of multiple sea turtle species (Faust et al., 2014), however, there were no relationships between the CCL and contaminant concentrations observed in this study. Antimony was found to be significantly higher than the reference interval in all three foraging sites. Lithium heparinised vacutainers are an industry standard when ensuring that whole blood samples taken in the field do not clot. However, antimony contamination has been a concern in the BD™ vacutainers and have been found to have false elevated antimony concentrations and therefore, we cannot be certain that the antimony values reported here are accurate (Yang et al., 2023). Yang et al. (2023) states that BD™ vacutainers are not suitable for assessing antimony concentrations and suggests the use of glass vacutainers. In future, the authors suggest taking a blank of MilliQ in the vacutainer while taking samples in the field if glass vacutainers are not efficient, so that the baseline antimony contamination can be removed.

High cobalt and manganese concentrations, with means up to four times the maximum reference interval, were found for the Hervey Bay aggregation. Higher cytotoxicity in the two offshore foraging sites of Heron and Lady Elliot Island (4.33 ± 0.83 and 3.89 ± 0.57 TU respectively) despite turtles showing no visible signs of poor health at these locations. Finlayson et al. (2021) reported trace element concentrations and organic toxicity from whole blood samples collected in Hervey Bay in 2016. From these data there was an observed decrease from 2016 to 2022 for cobalt concentrations (from 310.0 ± 120.0 μg/L to 212.28 ± 21.25 μg/L), and an increase in manganese concentrations (58.0 ± 33.0 μg/L to 68.12 ± 9.02 μg/L). However, the large variation between individual values, denoted by the standard error, shows a statistically insignificant difference between the two means.

Port Curtis, Queensland is a similar inshore foraging site, that undergoes frequent sampling, is located within a mining port and is approximately 80 km from Heron Island. Port Curtis has been observed to have high cobalt concentrations, with a mean of 420.0 ± 130.0 μg/L (Finlayson et al., 2021). This could potentially indicate that an increased level of cobalt above the established reference interval is common for these inshore foraging aggregations due to the common abundance in these coastal systems (Munro-Smith, 2006). The relative concentration distribution for cobalt in green turtles reported by Faust et al. (2014) suggests that the majority of cobalt is stored in the liver and kidney tissues. The strong relationship observed between increased cobalt concentration and biochemical indicators for reduced liver function in

this study could be a result of prolonged exposure to these high concentrations of trace elements in Hervey Bay, as high concentrations of cobalt have been found to be cytotoxic to green sea turtle skin fibroblasts (Finlayson et al., 2020). The Hervey Bay population may have tolerated prolonged exposure to this level of cobalt concentration, due to increased baselines of trace elements in coastal areas. However, when coupled with decreased seagrass availability (Bryant et al., 2024), this sustained high concentration overwhelms the liver's processing capacity without an adequate food supply. Cobalt deposits in the Hervey Bay region were discovered in the 1870's, including a significant deposit called Mt. Cobalt (Munro-Smith, 2006). The increase in sediment ouow as a result of recent flooding events is consistent with an increase in trace element concentration as a result of the release of historical trace elements trapped in sediment (Shaheen et al., 2014). According to the healthy land and sea report card, the flooding in 2020–2022 resulted in extremely high levels of sediment in several catchments in southeast Queensland (Healthy Land and Water, 2022). High cobalt concentrations should be prioritised for water quality monitoring in inshore areas to detect variation that may be harmful for marine animals.

The varying dietary items present and consumed at each of the foraging locations could explain the variation in trace elements in offshore foraging sites. Increased trace elements have been found elevated in offshore foraging locations due to their preference for algal species known for high selenium (Fuentes et al., 2006; Komoroske et al., 2012). Turtles at the two offshore foraging sites typically rely on algal species and ascidians, whereas the Hervey Bay population has historically relied on seagrass beds, and occasionally ascidians and mangrove leaves. Vanadium was significantly higher in the Lady Elliot Island population and exceeded the maximum reference interval. Despite this, there was no observed relationship between vanadium and body condition or blood parameters. Vanadium levels in these offshore populations could be a natural occurrence and exist outside of the baseline reference intervals.

Surprisingly, our findings indicated higher cytotoxicity in the two offshore foraging sites of Heron and Lady Elliot Island in contrast to Hervey Bay, where turtles showed lower cytotoxicity. The cytotoxicity of green turtle blood observed in Hervey Bay has decreased between 2016 and 2022, from 1.25 ± 1.37 TU (Finlayson et al., 2021) to 0.35 ± 0.05 TU. The prolonged La Niña period prevalent during the early 2020s, coupled with climate change-induced storm intensification and frequency, increased water flow from adjacent catchments, potentially reducing overall organic contaminant loads via dilution.

A potential source of organic contaminants for the Heron and Lady Elliot Island aggregations are the resorts present on the islands. Both islands have wastewater treatment plants, a high influx of tourists. In addition to this, Lady Elliot Island was decimated by guano mining and the introduction of goats leading to a total loss of vegetation, leading to a higher loss of ground water and increased island runoff. Heron and Lady Elliot Islands are somewhat removed from direct anthropogenic impacts, such as agricultural, industrial, and urban contaminants as they are >80 km away from the mainland. However, these offshore islands may be exposed to organic pollutants via shipping traffic, recreational boating, discharge of wastewater (from onsite resorts) and flood plumes from nearby river systems. A 1991 flooding event of the Fitzroy River found green tree frogs and freshwater turtles observed floating on clumps of vegetation around marker buoys surrounding Heron Reef, with some of the freshwater turtles coming ashore (Limpus et al., 1999, 2011). It is hypothesised that if these animals can travel ~80 km to Heron Island via flood plumes, then the potential for land-based contaminants to reach this site is highly likely. Further investigations into the transport and pathways of organic contaminants because of flooding in these catchments are integral in our understanding of impacts of contaminants on the marine environment.

Sea turtles are also susceptible to biological toxins such as diarrhetic shellfish toxins, toxic species of dinoflagellates and benthic and planktonic microalgae (Moreira-González et al., 2023). The cytotoxicity bioassay cannot distinguish between synthetic and naturally occurring cytotoxic compounds such as those found in harmful algal blooms and other biological toxins. Therefore, if there was a harmful algal bloom prior to sampling in the offshore sites this could potentially influence the level of organic contaminants in the blood leading to an increase in cytotoxicity. Sea turtles mostly exist at the surface water/air boundary, leading them to be exposed to these impacts via ingestion when feeding or breathing. *Gracilaria* spp. are a group of red algae species that is high in selenium, and have cytotoxic properties (Nabil-Adam et al., 2020). Immature Queensland green sea turtle populations have shown a preference for *Gracilaria* algae species (Fuentes et al., 2006). A potential scenario is that a main dietary source on Heron and Lady Elliot Islands are *Gracilaria* potentially explaining why increased selenium concentration and cytotoxicity values were observed at these sites. The dietary preferences of green sea turtles on Heron and Lady Elliot Islands have not been published in any peer-reviewed journals. During the blooming stage of toxic algae, there is a considerable amount that is dissolved into the surrounding seawater, meaning that animals that ingest this can succumb to subsequent health impacts. Toxic marine microalgae species occur globally, there is increasing concern that the amount of harmful algal blooms may be increasing due to human based impacts such as eutrophication and climate change. Saxitoxins are readily available in the Pacific Ocean, and multiple sea turtle strandings and mortality have been linked to blooms of *Pyrodinium bahamense*, a species of saxitoxin algae (Barrientos et al., 2019). Saxitoxins have been found to bind to sodium and potassium channels and therefore can influence sodium and potassium levels in the blood of organisms that have ingested the algae (Llewellyn, 2006). While there were no algal blooms reported during the sampling periods, this could be a potential hypothesis that may have influenced the bioassay results. In future, sediment and water sampling should be employed during turtle sampling events so that this could be investigated.

The Heron and Lady Elliot Island sites showed no negative health signs visually or biochemically, which is interesting considering their high TU comparative to the Hervey Bay population. Meanwhile, the Hervey Bay population had low toxicity, was emaciated, and had variable trace element concentrations that were outside of published reference intervals, suggesting that this population was not clinically healthy. As stated previously perhaps the high cytotoxicity in the offshore sites is attributed to biological toxins that are regular occurrences in these locations (Kretzschmar et al., 2019), or the cytotoxic contaminants causing the effect haven't been present in the body long enough to have any realised implications on biochemical parameters and visual health. In addition, we hypothesise that wild caught turtles in chemically distinct foraging locations could have a different tolerance to certain contaminants than the cell lines used in the bioassay.

The Hervey Bay aggregation had higher variation outside of other published ranges in blood parameters than at Heron and Lady Elliot Island, and could be considered in “poor” condition, in particular relating to scaled mass index, urea, albumin, packed cell volume, and calcium. The variation in blood parameters could be due to a myriad of factors, such as the decrease in seagrass availability, leading to starvation and susceptibility to other anthropogenic impacts and diseases. The loss of seagrass has led to a shift in diet in the Hervey Bay population, and the turtles have had to move to an ascidian and mangrove-based diet to supplement their feeding (*pers. comms.* Turtles in Trouble Turtle Rescue). The observed visible poor health and blood parameter variation cannot be attributed to one cause and is likely to be a culmination of synergistic pressures, suggesting that clinical health is not an accurate measure of sublethal impacts of contaminant exposure (Roman et al., 2021).

It is probable that the temporal variation in sampling the three foraging sites may have accounted for variation in contaminant exposure as the two offshore populations were sampled shortly after major flooding events, whereas the inshore site as sampled several months after major flooding. In future, sampling the different foraging sites

should be carried out as simultaneously as possible. In this case, sampling at these sites was dictated by funding availability and government department annual sampling. Future research directions for ecotoxicological studies should prioritise annual sampling at various sites to unveil temporal variations crucial for understanding contaminant impacts and subsequent health effects. In addition, sampling after impactful events, such as flooding, should be included to monitor populations response. If funding permits, adopting a non-targeted approach to chemical analysis in addition to species-specific bioassays could provide a more comprehensive understanding of the toxicity observed on Heron and Lady Elliot Islands, distinguishing between anthropogenic contaminants and biological toxins. Additionally, including other species-specific bioassays that quantify other cellular processes, such as endocrine disruption, genotoxicity and aryl hydrocarbon receptor activation, would be useful in disentangling contaminant impacts. Expanding these study sites to explore spatial patterns and habitat characteristics driving cytotoxicity levels can further enhance our understanding of environmental impacts on marine organisms.

5. Conclusion

In conclusion, the complex relationship between sea turtles, contaminants and sub-lethal health implications is difficult to disentangle. By elucidating the relationship between contaminants and green turtle health, this investigation provides crucial insights that extend beyond the immediate findings, contributing to the larger field of ecotoxicology and promoting informed conservation practices. The increased organic toxicity observed for Lady Elliot and Heron Island green sea turtles could be potentially driven by biological toxins present in the surface waters or forage items of these reef systems, as there were no major variations to blood parameters or visual signs of decreased health such as poor body condition or epibionts. The long exposure to human contaminants from the resorts on the two offshore islands could also be causing an increase in organic contaminants in the surrounding water. The decreased organic contaminant load observed in the Hervey Bay population is likely due to the increased water flow from the Mary River, diluting the pollutants from the surrounding terrestrial environments and pushing this out to sea.

The Hervey Bay population seems to be impacted by the concentration of several trace elements including cobalt, manganese, uranium, with prolonged exposure to these levels could be contributing to the variation in urea (mmol/L), albumin (g/L) levels, packed cell volume (%), and calcium (mmol/L) highlighting the potential susceptibility to renal disease, dehydration, catabolism, and chronic debilitation due to contaminant exposure. In contrast, the offshore foraging sites were observed to have concentrations of selenium, arsenic, cadmium, and vanadium at levels higher than the maximum reference interval. As a result, more in depth and frequent investigations into the health and toxicology of both inshore and offshore populations is required. These analyses contribute to our understanding of the consequences of contaminant accumulation in the marine environment. Insights gathered from these datasets contribute significantly to shaping effective management strategies and informing legislative changes aimed at mitigating and combatting the deleterious effects of pollutants.

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CRediT authorship contribution statement

Caitlin E. Smith: Writing – review & editing, Writing – original draft, Visualization, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Kimberly Finlayson:** Writing – review & editing, Methodology, Formal analysis. **Arthur Barraza:** Writing – review & editing, Methodology, Formal analysis. **Erina J. Young:** Writing – review & editing, Formal analysis. **Ben L. Gilby:** Writing – review & editing, Supervision, Conceptualization. **Jason P. van de Merwe:** Writing – review & editing, Supervision, Methodology. **Kathy A. Townsend:** Writing – review & editing, Supervision, Conceptualization.

Declaration of Generative AI and AI-assisted technologies in the writing process

During the preparation of this work the author(s) used Chat GPT 4.0 to improve readability and overall writing flow. After using this tool/service, the author(s) reviewed and edited the content as needed and take(s) full responsibility for the content of the publication.

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Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Caitlin E. Smith reports financial support was provided by Holsworth Wildlife Research Endowment. If there are other authors, they 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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Data availability

Data will be made available on request.

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