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Green Turtle (*Chelonia mydas*) Blood and Scute Trace Element Concentrations in the Northern Great Barrier Reef

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Abstract: Marine turtles face numerous anthropogenic threats, including that of chemical contaminant exposure. The ecotoxicological impact of toxic metals is a global issue facing *Chelonia mydas* in coastal sites. Local investigation of *C. mydas* short-term blood metal profiles is an emerging field, while little research has been conducted on scute metal loads as potential indicators of long-term exposure. The aim of the present study was to investigate and describe *C. mydas* blood and scute metal profiles in coastal and offshore populations of the Great Barrier Reef. This was achieved by analyzing blood and scute material sampled from local *C. mydas* populations in five field sites, for a suite of ecologically relevant metals. By applying principal component analysis and comparing coastal sample data with those of reference intervals derived from the control site, insight was gleaned on local metal profiles of each population. Blood metal concentrations in turtles from coastal sites were typically elevated when compared with levels recorded in the offshore control population (Howick Island Group). Scute metal profiles were similar in Cockle Bay, Upstart Bay, and Edgecumbe Bay, all of which were distinct from that of Toolakea. Some elements were reported at similar concentrations in blood and scutes, but most were higher in scute samples, indicative of temporal accumulation. Coastal *C. mydas* populations may be at risk of toxic effects from metals such as Co, which was consistently found to be at concentrations magnitudes above region-specific reference intervals. *Environ Toxicol Chem* 2023;00:1–14. © 2023 The Authors. *Environmental Toxicology and Chemistry* published by Wiley Periodicals LLC on behalf of SETAC.

Keywords: Ecotoxicology; Environmental toxicology; Metal accumulation; Tropical ecotoxicology; Water quality

INTRODUCTION

Marine turtles are reptiles that are distributed globally throughout tropical and subtropical regions (Bowen et al., 1992), with six of the seven extant species inhabiting the Great Barrier Reef. The global status of marine turtles, determined by the International Union for Conservation of Nature Red List, varies between vulnerable (such as loggerhead, *Caretta caretta*; Casale & Tucker, 2017) and critically endangered (including hawksbill, *Eretmochelys imbricata*; Mortimer & Donnelly, 2008). Such variation in status is likely influenced by diverse threats (often localized) and complex life histories demonstrated by these species. All marine turtle species, excluding the flatback turtle (*Natator depressus*), are migratory species, traveling hundreds of kilometers yearly to breed as adults (Aguirre & Lutz, 2004) and inhabiting a diverse connection of habitats and marine environments as they transition through life stages from posthatchling to adult green turtles (*Chelonia mydas*); they are long-lived, slow-growing macrograzers that demonstrate strict foraging site fidelity as juveniles and adults (Hazel et al., 2013). Strict fidelity only occurs on recruitment to coastal foraging grounds, from the pelagic zone, following the posthatchling phase (Hamann et al., 2002), as juveniles, where they will remain until sexually mature (Colman et al., 2015; Velez-Zuazo et al., 2014). Life-history strategies, such as long life and narrow home ranges, increase the susceptibility of *C. mydas* to stressors, including exposure to and bioaccumulation of ecotoxic contaminants (da Silva et al., 2016; Faust et al., 2014; Gaus et al., 2012). These factors likely contribute to the globally endangered status of *C. mydas* (Seminoff, 2004). Anthropogenic contamination is considered a priority risk to marine turtle health and is a factor in the global decline of *C. mydas* populations (Hamann et al., 2010). Because

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of the proximity of nearshore environments to anthropogenic land activities (agricultural, industrial, and urban practices), foraging turtles are regularly exposed to harmful contaminants, including xenobiotic organic chemicals such as pesticides, herbicides, and toxic trace metals (see Kroon et al., 2015; Lewis & Devereux, 2009), positioning coastal-dwelling marine turtles as good sentinel species to help monitor long-term local environmental conditions (Aguirre & Lutz, 2004; Aguirre & Tabor, 2004; Tabor & Aguirre, 2004).

Acute and long-term exposure to toxic levels of some metals incurs detrimental health impacts in *C. mydas* (Camacho et al., 2013; da Silva et al., 2016; Faust et al., 2014; Flint et al., 2015). Such impacts include endocrine disruption (Iavicoli et al., 2009), enzyme inhibition (da Silva et al., 2016; Ercal et al., 2001), and oxidative stress and immunosuppression, which likely increase susceptibility to infections and diseases (da Silva et al., 2016). Elevated metal concentrations are transported to the coastal zone and enter the marine environment via several pathways and sources, including use in industrial and agricultural land practices and from several processes associated with urban settlement, such as crop production, fuel burning, and metal processing and transport (Pacyna & Pacyna, 2001; Pacyna et al., 1995; Strzelec et al., 2020; Taylor, 2015). Naturally geoavailable metals also influence the metal profile of a local area. These elements can often be essential, as micronutrients, whereas others are nonessential and likely toxic at very low concentrations (Aggett et al., 2015; da Silva et al., 2016; de Souza Machado et al., 2016).

It is important to monitor metal concentrations not only in *C. mydas* populations but also in the wider environment, to efficiently detect when contaminant levels may be elevated and potentially toxic. In the absence of ecotoxicologically defined endpoints or thresholds for the protection of *C. mydas* populations, one way to detect elevated levels of metal contamination is to compare data from target sites against predefined natural baseline reference ranges, obtained from a remote offshore region that is minimally impacted by anthropogenic pollution (i.e., Howick Island Group in the present study; Flint et al., 2019; Villa et al., 2017).

Blood collection is a nonlethal and minimally invasive method of measuring metal concentrations in sea turtles and is representative of recent exposure (2–3 weeks), dependent on metal elimination rates (Villa et al., 2017). Although blood sampling and analysis for metals are informative in indicating whether a foraging population of turtles is exposed to elevated metals, they are somewhat limited when investigating long-term metal concentrations. Internal tissues, such as the liver and kidney, are known to be long-term stores of metals and have previously been opportunistically sampled from stranded or moribund turtles (Gaus et al., 2012; Grillitsch & Schiesari, 2010). This approach is limited because it relies on sporadic access to deceased animals and is not likely representative of average metal concentrations in free-ranging populations. A better approach, using scute, has been recently implemented and validated (Villa et al., 2019). Like blood collection, *C. mydas* scute sampling is minimally invasive and, as such, is potentially a suitable alternative to sampling internal organs as an indication of past metal exposure (1.4–2.8 years prior to capture; Vander Zanden et al., 2013; Villa et al., 2019). Scutes are made of keratin (as is the case of human hair and nails) and can be sampled with little risk of harm to the animal (Day et al., 2005; Villa et al., 2019). Each scute layer provides data on metal concentrations at the time of formation, though no known method is available to pinpoint the timing of exposure events. Calculating the metal concentrations in scute samples is still informative because it indicates whether levels have been elevated at a given study site in recent history. Few studies have measured element concentrations in paired blood and scute samples (Day et al., 2005; Villa et al., 2019), and thus reference values are not currently available for scute metal concentrations in green turtles (Villa et al., 2019). Further work, therefore, is necessary to understand long-term exposure of green turtles to elevated trace metals.

The aim of the present study was to measure metal concentrations in blood and scute samples of *C. mydas* from five foraging grounds within the Great Barrier Reef, to assess whether exposure to a suite of ecologically relevant metals exceeded locally defined reference intervals (RI) for blood concentrations and whether the scute samples represented a more time-integrated indication of past metal exposure. A broader aim of the present study was to demonstrate the value in the validation of alternative sample collection methods for the analysis of long-term chemical contamination storage in reptiles and other animals, where conventional sampling is often invasive, lethal, or opportunistic in nature (which is often the case when relying on internal organ sampling). The scute sampling methods demonstrated in the present study were noninvasive and can be used to collect valuable samples from animals of high conservation status, without detriment. It was hypothesized that turtles sampled from coastal sites would be influenced by land-sourced anthropogenic contamination, and therefore have greater blood metal concentrations than turtles sampled from the reference study site. In addition, it was hypothesized that metal concentrations would be higher in scute samples than in blood and that mean concentrations would vary between coastal sites, dependent on local land uses and exposure to anthropogenic contamination.

METHODS

All sampling was performed in accordance with the James Cook University Animal Ethics Committee (A2396), the Department of Environment and Science (WISP18586417, WISP1859817), and the Great Barrier Reef Marine Parks authority (G17/39429.1).

Study locations

Five geographically distinct green turtle foraging areas were sampled along the east coast of Queensland, Australia, adjacent to the Great Barrier Reef catchment area (Figure 1). First, Howick Island Group is a midshelf group of remote reefs found in the northern region of the Great Barrier Reef (14°30′11″S, 144°58′26″E). Located over 130 km from the nearest human

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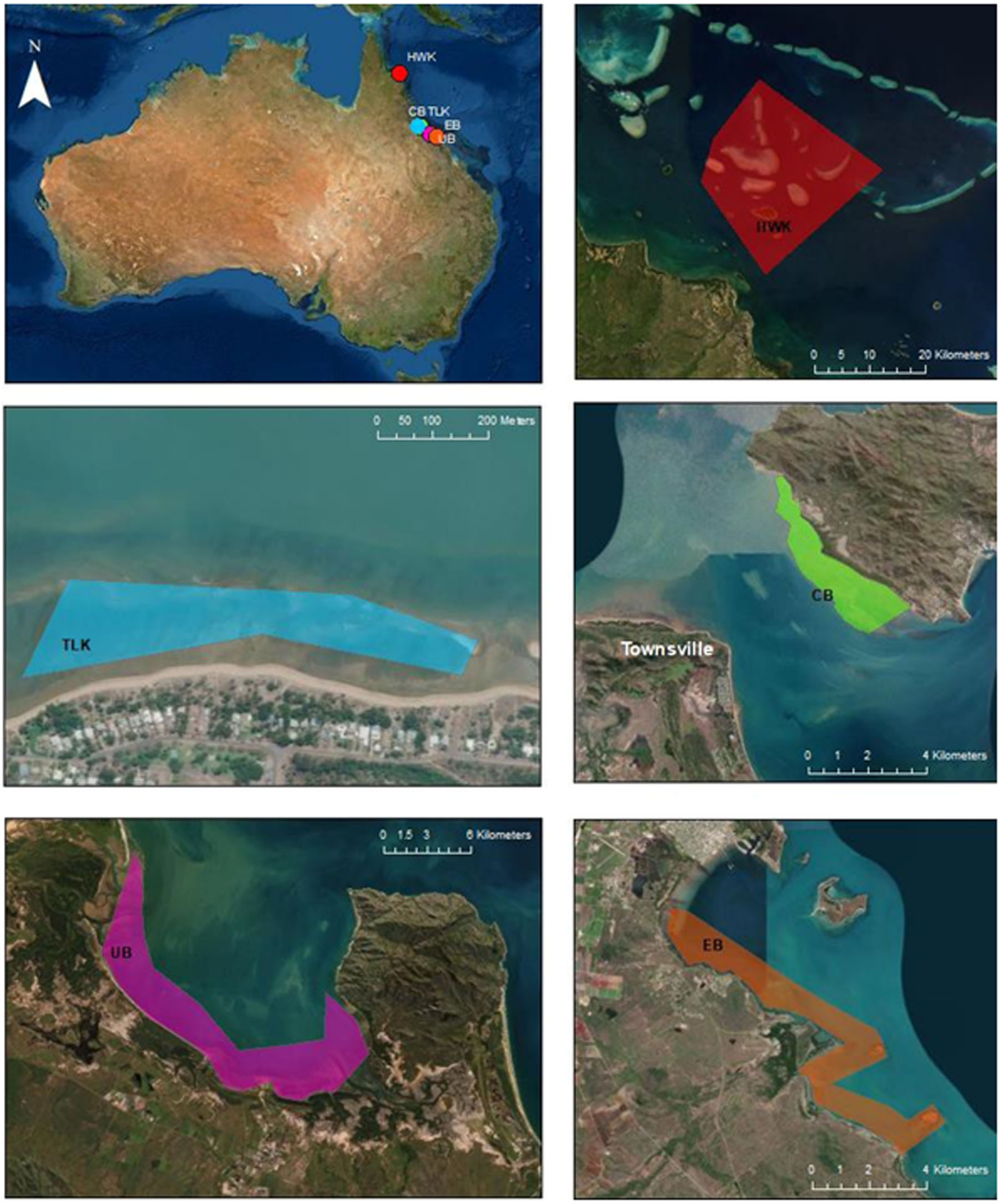

FIGURE 1: Overview of all study sites sampled. Map of five foraging areas where *Chelonia mydas* were captured and sampled. Each site is color-coordinated between the national overview and localized maps. Toolakea Beach and Cockle Bay appear overlapped in the national overview but are geographically distinct on the local scale. HWK = Howick Island Group; CB = Cockle Bay; UB = Upstart Bay; EB = Edgecumbe Bay; TLK = Toolakea Beach.

populace (Cooktown) and at least 15 km from the nearest coastal catchment, Howick Island Group was considered relatively removed from land-sourced anthropogenic chemical loads (Bell et al., 2018; Senior et al., 2015; Villa et al., 2017). Second, Toolakea (19°08′36″S, 146°34′56″E) is located 30 km north of Townsville and is predominantly inhabited by juvenile *C. mydas*, recently recruited from the pelagic zone. Third, Cockle Bay (19°10′26.7″S, 146°49′32.1″E) is a westerly facing bay of Magnetic Island, 13 km off the coast of Townsville, and forms a part of Cleveland Bay. Industrial practices such as metal processing (including Zn, Cu, Ni, and Co), urban runoff from the city of Townsville (population ~200 000), and major sea port practices (including regular channel dredging) take place within the region (Esslemont, 2000) and potentially influence both Cockle Bay and Toolakea. Fourth, Upstart Bay (19°44′44.4″S, 147°36′03.8″E) is a north-facing bay located 150 km south of Townsville and receives

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river discharges from a major catchment, the Burdekin, dominated by agricultural and grazing practices and with a prominent mining background. The Burdekin catchment is one of the two largest catchments in the Great Barrier Reef catchment area (the other being the Fitzroy) with an area of 140 000 km^2. Finally, Edgecumbe Bay (20°6′49″S, 148°23′25″E) is located just south of Bowen, which is approximately 200 km south of Townsville. Within the catchment draining into Edgecumbe Bay, there are a number of sources of potential contaminants, including a secondary wastewater-treatment plant (for the town of Bowen, which has a population of ~10 000), cokeworks and sugarcane farms (mostly on the catchment of the Gregory River in the south of the bay), and seasonal (during the wet season) discharge plumes from the Burdekin River (Clark et al., 2016). The genetic composition of each distinct population is prodominantly comprised of the same three genetic stocks: northern Great Barrier Reef (nGBR), southern Great Barrier Reef (sGBR), and Coral Sea stocks. The proportions, which account for the total population, shift from nGBR dominant stock in Howick Island Group to sGBR dominance from north to south (Dethmers et al., 2006; Jensen et al., 2016; Jones et al., 2018).

C. mydas capture and morphometric sampling

During numerous trips between 2017 and 2019, a total of 122 green turtles were captured across the five study sites (Table 1). All turtles were captured using the turtle rodeo technique described by Limpus & Read (1985). Individually numbered titanium flipper tags (Department of Environmental Sciences, Queensland government) were applied as described by Eckert et al. (1999). Curved carapace length (CCL) was measured for all turtles captured, using a plastic tape measure (in centimeters) calibrated against metal calipers. Measurements were taken along the midline ridge of the carapace, from the notch of the supracaudal scutes to the posterior edge of the carapace where it joins the skin of the neck, to an accuracy of ±0.1 cm. Large barnacles were removed if their location obstructed accurate CCL measurements. Animal weight was measured using digital scales (in kilograms) and a custom harness that allowed for balanced weight distribution. The CCL and weight ranges of turtles sampled at each site are detailed in Table 1, with most animals captured classed as juveniles (<65 cm; Table 1). Juvenile green turtles dominated the population composition of captured individuals at each coastal site sampled. This size class is one where turtles do not migrate and, instead, demonstrate strict site fidelity (Hazel et al., 2013); thus, migration was not considered a significant influencer on metal loads.

Blood sample collection

Nonlethal blood samples were collected from the jugular artery located in the dorsal cervical sinus of *C. mydas*. Up to 10 mL of blood was collected per turtle (<1% of the turtle's body weight). Whole blood was sampled using a 10-mL syringe fitted with 21- to 20-gauge needles, as described by Owens & Ruiz, (1980). Whole blood was transferred directly to sodium heparin–coated evacuated vacutainer tubes (BD Vacutainer; Becton Dickinson). All blood samples were stored under chilled conditions (4–10 °C) until return to the laboratory, where they were refrigerated at 4 °C until analysis.

Scute sample collection

Scute samples (~0.5–1.0 g each) were collected from the radial edge of the posterior marginal scutes of 64 *C. mydas*. Scute samples were not collected from turtles captured at Howick Island Group (sample collection from Howick Island Group was conducted by researchers other than the authors of the present study, and permits did not allow for scute collection), but samples were obtained from each of the coastal sites (see Table 1). The sampling area was first prepared by cleaning with 70% isopropanol swabs to remove any epibionts and other fouling. All scute samples were collected using a sterile diamond-tipped hollow drill bit (12 mm diameter). The surrounding area of carapace was again swabbed with isopropanol, and pressure was applied in the rare instances where minimal bleeding occurred. Samples were placed in sterile 1.5-mL microfuge tubes and stored on ice until return from the field. Once at the laboratory, keratin samples were soaked in 70% isopropanol for 5 min to remove any remaining epibionts. To minimize environmental contamination of each sample, the outermost scute layer (visible as carapace) was removed prior to storage and was not included in analysis. All keratin samples were then stored at –20 °C until analysis.

Trace metal analysis

A fully quantitative, multielement approach was applied to analyze the concentrations of a suite of essential (Co, Cu, Fe,

TABLE 1: Morphometric data of all turtles sampled

Study location	Blood	Scute	CCL range (cm)	Juvenile:subadult:adult	Weight range (kg)
Howick	*n* = 30	–	42.5–72.0	27:3:0	7.8–45.0
Toolakea Beach	*n* = 9	*n* = 9	36.8–49.2	9:0:0	6.3–10.8
Cockle Bay	*n* = 35	*n* = 17	40.1–61.8	35:0:0	7.2–32.0
Upstart Bay	*n* = 24	*n* = 24	42.0–111.5	20:2:2	8.2–29.0
Edgecumbe Bay	*n* = 24	*n* = 14	43.2–108.5	20:2:2	7.2–>100

Sample sizes (*n*) for both blood and scute analysis, and curved carapace length (CCL) and weight of all *Chelonia mydas* included in the present study. A weight of >100 kg was assigned when the turtle was heavier than the maximum weight capacity of the scale used (100 kg). Size class composition of turtles sampled at each site is broken down into juvenile (CCL < 65 cm), subadult (CCL 66–90 cm), and adult (CCL > 90 cm), per Chaloupka and Limpus (2001).

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Mg, Mn, Zn) and nonessential (Al, Cd, Ni, and Pb) elements (Table 2). Whole-blood samples were digested using a microwave oven (Burghof Speedwave). Briefly, all samples (~0.5 g) were accurately weighed into a microwave digestion vessel, and 4 mL of double-distilled HNO_3 and 0.5 mL of analytical reagent (AR)–grade H_2O_2 were added. Samples were then heated to 180 °C for 10 min in the microwave oven until fully digested. Once cooled, the digested samples were quantitatively transferred into 25-mL volumetric flasks and diluted to the mark using Milli-Q water. No further dilution was carried out before inductively coupled plasma–mass spectrometric (ICP-MS) analysis. Dry and clean scute material was digested similarly to the blood by adding 4 mL of double-distilled HNO_3 and 0.5 mL of AR-grade H_2O_2. Once the reaction was complete, samples were heated to 185 °C for 10 min in the microwave oven. Once cooled, the digested samples were quantitatively transferred into 25-mL volumetric flasks and diluted to the mark using Milli-Q water. No further dilution was carried out before ICP-MS analysis.

Trace element analysis was carried out by a Varian 820-MS ICP-MS. Indium acted as the internal standard to correct for the matrix effects and instrument drift, and for quantification, a series of multielement standard solutions were used to calibrate the instrument. A series of multielement standard solutions containing all elements of interest were included to ensure that external calibration was met. Nitric acid and H_2O_2 (acids used in sample digestion, minus the sample) were used as procedure blanks, and three times the standard deviation of the blanks was used as the limit of detection (LOD), for all elements. Randomly selected samples (three in total) were duplicated to check for consistency in metal concentrations throughout the analysis. To ensure instrument calibration, numerous independent standards (1 ppm) were tested, with reported recoveries ranging from 83% (Co) to 120% (Al). Two certified reference materials (GBW07605 tea leaves and NIST 1566 oyster tissues) were analyzed to validate the analytical method, and recoveries ranged from 92% (Cu) to 118% (Cd).

Data analysis

Blood and scute metal concentrations were reported as milligrams per kilogram, and all concentrations that were <LOD were assigned a value half of the LOD (Villa et al., 2019; Wendelberger & Campbell, 1994). Blood concentrations of Cd, Ni, and Pb were included in the calculation of site-specific mean concentrations for all sites except Toolakea (all concentrations were reported as <LOD) but not included in multivariate analysis because >40% of samples were <LOD. Blood metal concentrations (for all metals except Al) at five sites were compared with the blood metal RIs reported in Villa et al. (2017). Mean metal concentrations were compared between blood samples collected in 2014 (Villa et al., 2017) and 2017–2019 (present study) for the three sites included in both studies (Howick Island Group, Cockle Bay, and Upstart Bay). Edgecombe Bay and Toolakea were not included in this analysis because investigation was not conducted within those sites during the reference study. Reference intervals were not available for Al, and therefore, no comparison was made between studies.

To reduce the complexity of potential associations between variables (metal elements) and to best analyze whether similarities exist between metals in either blood or scute samples, multivariate analysis was conducted. Dimension reduction of all data was achieved by applying a multivariate principal component analysis (PCA). By applying PCA, spatial and temporal variations between study locations and sampling events were investigated. To determine the most important dimensions in the data, two dimension-reduction protocols, scree plots and quality of representation measurements (cos2), were used. Because no scutes were collected from Howick Island Group, this site is not represented in the scute PCAs. Statistical analysis and plotting of PCA were conducted in the R statistical program (R Core Team, 2019), using the R packages "Tidyverse" (data exploration), "Factoshiny" (multivariate analysis and plotting; Husson et al., 2017), and "FactoMineR" (factor analysis; Husson et al., 2013).

RESULTS

C. mydas blood metal concentrations

When comparing coastal *C. mydas* blood metal concentrations to the established RIs from the control site it was found that concentrations were similar between Howick Island Group and coastal sites for most elements analyzed (Table 2). This was

TABLE 2: Table of mean *Chelonia mydas* blood metal concentrations

Element	HWK	TLK	CB	UB	EB
Al	0.7 ± 0.5	0.2 ± 0.1	0.5 ± 0.6	0.6 ± 0.5	1.2 ± 2.4
Cd	0.01 ± 0.01	–	0.02 ± 0.02	0.03 ± 0.04	0.01 ± 0.01
Co	0.02 ± 0.02	0.1 ± 0.2	0.1 ± 0.2	0.4 ± 0.3	0.3 ± 0.2
Cu	0.6 ± 0.1	0.5 ± 0.1	0.7 ± 0.5	0.6 ± 0.2	0.6 ± 0.2
Fe	267 ± 55	193 ± 88	199 ± 104	267 ± 100	303 ± 204
Mg	73 ± 8.5	94 ± 9.9	84 ± 11	88 ± 14	111 ± 57
Mn	0.1 ± 0.1	0.03 ± 0.02	0.1 ± 0.1	0.1 ± 0.04	0.1 ± 0.2
Ni	0.002 ± 0.01	–	0.01 ± 0.01	0.02 ± 0.04	0.02 ± 0.02
Pb	0.04 ± 0.02	–	0.08 ± 0.06	0.03 ± 0.02	0.04 ± 0.03
Zn	8.8 ± 2.2	6.5 ± 2.6	7.8 ± 4.1	8.8 ± 2.9	11 ± 6.7

Average blood concentration (milligrams per kilogram) and standard deviation for each metal. All data are arranged by site, from north to south.
CB =Cockle Bay; EB = Edgecumbe Bay; HWK = Howick Island Group; TLK = Toolakea Beach; UB = Upstart Bay.

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particularly true for the essential elements Cu, Fe, and Zn, where concentrations were comparative between data from Howick Island Group and the majority, if not all, of coastal site concentrations. Aluminum and Mg were detected at greater concentrations at Edgecumbe Bay when compared with the Howick Island Group, but at the other sites they were at similar concentrations to Howick Island Group. Concentrations of Co were observed to be greatest in Upstart Bay ($0.4 \pm 0.3\,mg\,kg^{-1}$) and decreased the farther north the sites were located. A higher mean Co concentration (compared with Howick Island Group, Toolakea, and Cockle Bay) was also detected in samples from Edgecumbe Bay ($0.3 \pm 0.2\,mg\,kg^{-1}$), although not quite as high as in Upstart Bay.

Temporal changes in blood metal concentrations

The mean blood metal concentrations reported for Howick Island Group, Cockle Bay, and Upstart Bay (collected 2017–2019) were compared with those analyzed in Villa et al. (2017), collected in 2014 (Table 3). For Howick Island Group, all elements, except Mn, were within standard deviation ranges between studies. In Howick Island Group blood samples Mn was detected at a mean concentration five times greater in the present study ($0.1 \pm 0.05\,mg\,kg^{-1}$) when compared against the reference study ($0.02 \pm 0.01\,mg\,kg^{-1}$). For Cockle Bay (Cleveland Bay [CLV] in reference study), all elements except Co were within standard deviation range between studies, with Co detected at a higher mean concentration in 2014 ($0.2 \pm 0.1\,mg\,kg^{-1}$), double the concentration compared to the present study ($0.1 \pm 0.2\,mg\,kg^{-1}$). For Upstart Bay, all nine elements were similar and within standard deviation range between studies. For each of the compared sites, most metals included were within or below RI values derived from Howick Island Group blood metal data in Villa et al. (2017). Concentrations of Mn were greater than the RI at Howick Island Group and exceeded concentrations in blood collected from Cockle Bay, Upstart Bay, and Edgecumbe Bay. Similarly, Cd mean concentrations exceeded RIs at all sites, but standard deviation ranges were wide enough to encompass the RI in each instance. Concentrations of Mg exceeded the RI at Upstart Bay, Edgecumbe Bay, and Toolakea but was within the RI at Cockle Bay. Only at Cockle Bay did Cu exceed the RI. Concentrations of Co at Cockle Bay, Upstart Bay, Edgecumbe Bay, and Toolakea exceeded the RI, by up to 14-fold. Concentrations of Fe, Ni, Pb, and Zn were within RIs at all sites.

C. mydas scute metal concentrations

Like metal concentrations analyzed in blood samples (Table 2), most elements in scute samples were detected at comparable concentrations between coastal study sites, particularly when considering the large standard deviations of most mean metal concentrations (Table 4). Despite the large variation in metal concentrations within sites, some patterns in metal concentrations can be observed between sites. For instance, Co was similar in Cockle Bay and Toolakea, which were both lower than Upstart Bay and Edgecumbe Bay. This finding differs from that for Co measured in blood, where concentrations were higher in Upstart Bay than at all sites. Mean scute Cd, Mg, and Zn concentrations were higher in Toolakea compared to all other sites. Mean concentrations of Fe and Ni were two to three times higher in Cockle Bay compared to all other sites.

When comparing blood metal concentrations to scute metal concentrations at each site, scute element concentrations were often greater than in blood samples. This was particularly the case for Al, Mg, Mn, Ni, and Zn, where scute concentrations were greater by several orders of magnitude at all coastal sites sampled (Table 5).

Multivariate dimension reduction of blood metal data

Principal component analysis was conducted to investigate differences in total blood metal profiles between locations and to assess which metals most influenced differences between sites (Supporting Information, S1). The scree plot (Supporting Information) indicated that the first two data dimensions adequately represented most of the variation in the data. Therefore, the suite of seven metals (variables or dimensions)

TABLE 3: Table of mean *Chelonia mydas* blood metal concentrations and published reference intervals

	HWK		CB		UB		
Element	2017–2019	2014	2017–2019	2014	2017–2019	2014	Reference intervals
Cd	0.01 ± 0.01	0.005 ± 0.002	0.02 ± 0.02	0.007 ± 0.006	0.03 ± 0.04	0.004 ± 0.002	0.003–0.008
Co	0.02 ± 0.02	0.02 ± 0.001	0.1 ± 0.2	0.2 ± 0.1	0.4 ± 0.3	0.5 ± 0.2	0.007–0.03
Cu	0.6 ± 0.1	0.5 ± 0.1	0.7 ± 0.5	0.7 ± 0.2	0.6 ± 0.2	0.6 ± 0.2	0.3–0.7
Fe	267 ± 55	290 ± 49	200 ± 104	260 ± 67	267 ± 100	300 ± 85	210–410
Mg	73 ± 9	69 ± 7	84 ± 11	91 ± 11	88 ± 14	99 ±12	55–85
Mn	0.1 ± 0.05	0.02 ± 0.01	0.1 ± 0.1	0.05 ± 0.02	0.09 ± 0.04	0.07 ± 0.04	0.008–0.04
Ni	0.002 ± 0.01	0.01 ± 0.007	0.01 ± 0.01	0.04 ± 0.04	0.02 ± 0.04	0.01 ± 0.08	0.005–0.03
Pb	0.04 ± 0.02	0.03 ± 0.02	0.08 ± 0.06	0.02 ± 0.01	0.03 ± 0.02	0.02 ± 0.01	0.006–0.08
Zn	8.8 ± 2.2	10 ± 1.7	7.7 ± 4.1	9.6 ± 2.7	8.9 ± 2.9	11 ± 2.5	7.3–14

Mean metal concentrations (milligrams per kilogram) and standard deviation for *Chelonia mydas* blood collected from Howick Island Group, Cockle Bay, and Upstart Bay in 2017–2019 (present study) and 2014 (Villa et al., 2017), including established *Chelonia mydas* blood reference intervals calculated by Villa et al. (2017). Data for Al were not included because no published reference intervals are available for this element currently.
HWK = Howick Island Group; CB = Cockle Bay; UB = Upstart Bay.

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TABLE 4: Table of mean *Chelonia mydas* scute metal concentrations

Element	TLK	CB	UB	EB
Al	48 ± 36	262 ± 230	138 ± 140	60 ± 92
Cd	0.1 ± 0.03	0.07 ± 0.03	0.05 ± 0.04	0.07 ± 0.03
Co	0.1 ± 0.02	0.1 ± 0.1	0.2 ± 0.1	0.2 ± 0.1
Cu	0.1 ± 0.03	0.3 ± 0.4	0.3 ± 0.6	0.3 ± 0.3
Fe	48 ± 30	171 ± 148	82 ± 94	52 ± 65
Mg	7400 ± 700	5500 ± 1490	5810 ± 1260	5400 ± 1500
Mn	6 ± 2	8 ± 4	13 ± 9	10 ± 6
Ni	1.1 ± 1.0	3.3 ± 7.3	1.3 ± 1.6	0.9 ± 1.0
Pb	0.3 ± 0.1	0.3 ± 0.1	0.2 ± 0.1	0.2 ± 0.07
Zn	302 ± 104	181 ± 58	165 ± 67	148 ± 80

Mean scute element concentrations (milligrams per kilogram) and standard deviation for each coastal site. All data are arranged by site, from north to south. Scutes were not collected from the Howick Island Group; thus, it is not presented in Table 4.
CB = Cockle Bay; EB = Edgecumbe Bay; HWK = Howick Island Group; TLK = Toolakea Beach; UB, Upstart Bay.

was reduced to two principal components (Dim 1 and 2), which together represented 71.86% of the total variation of the data. Data were represented by two distinct clusters of metals (variables), Fe–Zn–Co–Cu (*Fe cluster*) and Al–Mg–Mn (*Mg cluster*), indicative of associations between elements, with loadings near one another (Supporting Information, S1). Squared cosine (Cos^2) indicated the importance of a metal element to a particular principal component (or the quality of representation) and supported the reduction to two principal components. A $Cos^2 > 0.60$ was true for all metals in the analysis suite, excluding Co and Cu, which were represented by the first four dimensions, indicating that most of the data variation was indeed accounted for by the first two dimensions. The Fe cluster (top right) aligned closely to Dim 2, whereas the Mg cluster (bottom right) aligned closer to Dim 1. Thus, each cluster influences the respective components, and therefore the entire data set, when analyzing the results in a reduced space.

When looking at individual sample PCA data per study location (Figure 2), it was apparent that variation within each site (confidence ellipses) was closely associated between all sites, with ellipses overlapping and individual points converging in the center of the plot, indicative of similar blood metal profiles between sites. Variation is comparatively low in all sites other than Edgecumbe Bay, with a wider spread in the data and a larger elliptical area. These findings support the data in

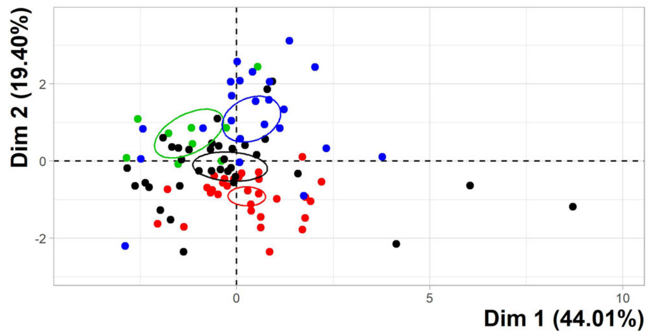

FIGURE 2: Principal component analysis individual plot depicting *Chelonia mydas* blood metal profiles (excluding Edgecumbe Bay data), reduced to two principal dimensions. Points indicate individual turtles, which are categorized by study site and represented as different colors: Howick Island Group = red, Toolakea Beach = green, Cockle Bay = black, and Upstart Bay = blue. Data presented in Figure 2 are the same as in Figure 3, excluding data from Edgecumbe Bay. Confidence ellipses are color-coordinated with the individual sample points. Dim = dimension.

Table 2, whereby metal concentrations were generally similar between sites and Edgecumbe Bay metal concentrations noticeably more variable than at other sites.

In Figure 3, Edgecumbe Bay data dominate the plot, with the widest spread of individual points, causing a loss in resolution regarding the distribution of data from each of the other included sites. To remove this impact, the PCA was reanalyzed with Edgecumbe Bay data excluded (Figure 2; Supporting Information, S2). The Cos^2 of the first two principal components (Dim 1 and 2) was satisfied (>0.6) for all elements except Co and Cu, which implied that the reduction of data to two dimensions was justified. However, the scree plot for this data set indicated that the first four dimensions were required for representation of the majority of variation present. As seen in Supporting Information, S1, the variables (metals) influenced the data in two distinct clusters (when reducing the data to two dimensions), though differing between analyses (Mg–Co and Al–Cu–Fe–Mn–Zn). Strong associations between metals in each cluster are suggested by the proximity of each loading, and influence was equally shared between all variables (length of the loadings).

Figure 2 represents the same data as Supporting Information, S2, and thus the position of loadings in Supporting

TABLE 5: Table of coastal *Chelonia mydas* mean blood and scute metal concentrations and blood:scute ratios

	Al		Cd		Co		Cu		Fe		Mg		Mn		Ni		Pb		Zi	
Site	B	S	B	S	B	S	B	S	B	S	B	S	B	S	B	S	B	S	B	S
TLK	0.2	48	–	0.1	0.1	0.1	0.5	0.1	193	48	94	7400	0.03	6	–	1.1	–	0.3	6.5	302
B:S	**240**		–		**0.0**		**0.2**		**4.0**		**78.7**		**200**		–		–		**46.5**	
CB	0.5	262	0.02	0.07	0.1	0.1	0.7	0.3	199	171	84	5500	0.1	8	0.01	3.3	0.08	0.3	7.8	181
B:S	**524**		**3.5**		**0.0**		**0.4**		**0.9**		**65.5**		**80**		**330**		**3.8**		**23.2**	
UB	0.6	138	0.03	0.05	0.4	0.2	0.6	0.3	267	82	88	5810	0.1	13	0.02	1.3	0.03	0.2	8.8	165
B:S	**230**		**1.7**		**0.5**		**0.5**		**3.3**		**66**		**130**		**65**		**6.7**		**18.8**	
EB	1.2	60	0.01	0.07	0.3	0.2	0.6	0.3	303	52	111	5400	0.1	10	0.02	0.9	0.04	0.2	11	148
B:S	**50**		**0.7**		**0.67**		**0.5**		**0.2**		**48.6**		**100**		**45**		**5**		**13.5**	

Mean metal concentrations (milligrams per kilogram) in *Chelonia mydas* blood and scute samples and blood:scute ratio calculated for the suite of metals analyzed at each study site. Where blood concentrations for certain metals were not detected, ratios were not calculated (–).
B = blood; S, scute; TLK = Toolakea Beach; CB = Cockle Bay; UB = Upstart Bay; EB = Edgecumbe Bay.

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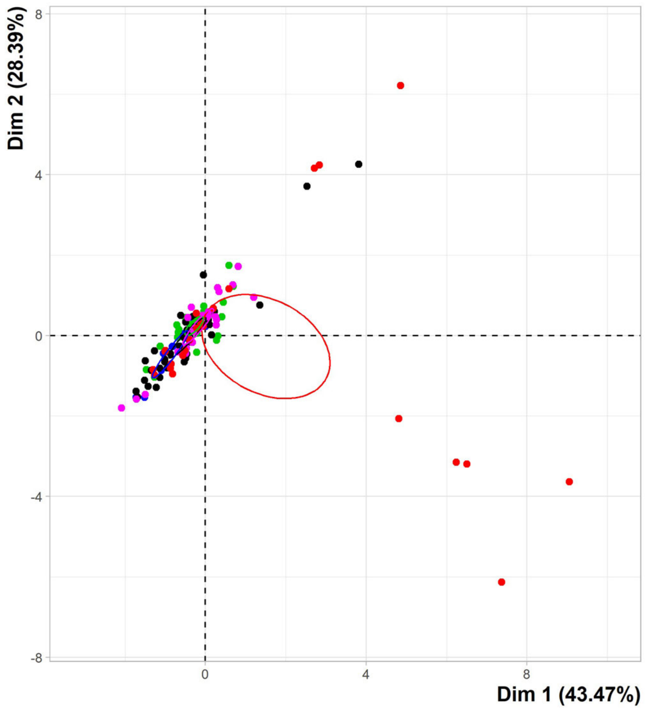

FIGURE 3: Principal component analysis individual plot depicting *Chelonia mydas* blood metal profiles, reduced to two principal dimensions. Points indicate individual turtles, which are categorized by study site and represented as different colors: Howick Island Group = green, Toolakea Beach = blue, Cockle Bay = black, Upstart Bay = pink, and Edgecumbe Bay = red. Confidence ellipses are color-coordinated with the individual sample points. Dim = dimension.

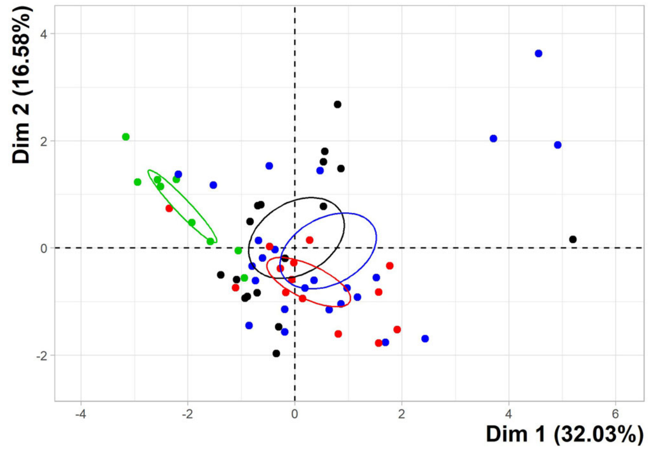

FIGURE 4: Principal component analysis individual plot depicting *Chelonia mydas* scute metal profiles, reduced to two principal dimensions. Points indicate individual turtles, which are categorized by study site and represented as different colors: Toolakea Beach = green, Cockle Bay = black, Upstart Bay = blue, and Edgecumbe Bay = red. Confidence ellipses are color-coordinated with the individual sample points. Dim = dimension.

Information, S2, corresponds to the position of individual points in Figure 2. Combined, these figures indicate that the metal profile of blood samples collected in Upstart Bay were most strongly influenced by elements Co and Mg, whereas Cockle Bay and Howick Island Group were primarily influenced by the Al cluster. Overlap of confidence ellipses (and thus close associations in metal profiles) was observed between Toolakea and Upstart Bay only, and all other coastal sites were distinct from one another, although some overlap between individual sample profiles occurred between sites.

Multivariate dimension reduction of scute metal data

Principal component analysis was also conducted to investigate differences in total metal profile in *C. mydas* scute samples between locations and to assess which metals most influenced differences between sites (Figure 4; Supporting Information, S3). The scree plot indicated that the first two data dimensions adequately represented most of the variation in the data. Therefore, the suite of metals (variables) was reduced to two principal components (Dim 1 and 2), which together represented 48.61% of the total variation of the data. Unlike the blood metal data set, for scute metal data the Cos^2 of the first two principal components (Dim 1 and 2) was not satisfied (>0.6) for most elements (only for Co, Mg, and Zn), though the scree plot showed that most of the variation was represented by the first two dimensions. As previously observed in blood metal PCA findings, variables (metals) influenced the scute metal data in two distinct clusters, Cd–Mg–Zn and Al–Co–Cu–Mn, with Ni and Fe negatively correlated to one another and influencing the data to a lesser degree (smaller loadings). Strong associations between metals in each cluster were suggested by the proximity of each loading, and influence was equally shared between all variables (length of the loadings).

When looking at individual scute sample PCA data per study location (Figure 4), it was apparent that variation within each site (confidence ellipses) was closely associated between Cockle Bay, Upstart Bay, and Edgecumbe Bay, with ellipses overlapping and individual points converging in the center of the plot, indicative of similar scute metal profiles between sites. The Toolakea metal profile was distinct from all other sites, as indicated by isolation of the confidence ellipse and less mixing of individual points. Variation was similar in all sites, though lower in Toolakea data. These findings support the data in Table 4, whereby Toolakea scute metal concentrations tended to be different when compared to the other sites, with element concentrations being higher or lower dependent on site. When looking at both Figure 4 and Supporting Information, S3, together, it was apparent that the Toolakea profile was most influenced by Cd, Mg, and Zn concentrations (all of which were highest at Toolakea; Table 4), whereas the loadings of the Al cluster of elements (also Ni and Fe less so) were most representative of the metal profiles of Cockle Bay, Upstart Bay, and Edgecumbe Bay.

DISCUSSION

Spatial variation in C. mydas metal concentrations

It was predicted that *C. mydas* blood metal concentrations would be lower at the control site (Howick Island Group)

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compared with each coastal site studied. Previous research in this region showed that metal concentrations were consistently higher in coastal *C. mydas* blood (Villa et al., 2017) and forage material (seagrass; Thomas et al., 2020; Wilkinson et al., 2022) compared with the natural baseline metal levels in an area minimally influenced by land-based contaminants and industrial activity, Howick Island Group (Villa et al., 2017). In the present study, several elements (Al, Co, Mg, and Mn) were occasionally higher in blood collected from one or more coastal sites compared with the Howick Island Group, though most elements were detected at similar concentrations between sites. This was true particularly for essential elements such as Cu, Fe, and Mn, though variability between blood samples was lower at the offshore site, as predicted. Essential elements tend to be maintained within optimal ranges through homeostatic processes (Aggett et al., 2015; Bury et al., 2003), and therefore variability would be expected to be lower in a population consistently exposed to minimal concentrations, as expected at a remote site, with little influence from anthropogenic nonessential element inputs, such as Howick Island Group (Flint et al., 2019; Villa et al., 2017). Like blood concentrations, most scute concentrations were similar between coastal sites, particularly between Cockle Bay, Upstart Bay, and Edgecumbe Bay (Table 4 and Figure 4; Supporting Information, S3); Toolakea was slightly different, with several essential elements (Al, Fe, and Mn) being lower there than at the other sites. However, this finding was comparative to the means from the blood analyses, where it was suggested that these elements were likely within natural baselines.

When comparing blood and scute metal mean concentrations, Cd, Cu, Fe, and Co concentrations were similar between sampling type, inferring that these elements have remained at steady-state concentrations within the region in recent years (Villa et al., 2019). In contrast, elements such as Al, Mg, Ni, Pb, and Zn were detected at concentrations greater in scutes than blood (by several orders of magnitude; Table 5), which may be indicative of past exposure to elevated elements (up to 2.8 years prior to sampling; Vander Zanden et al., 2013; Villa et al., 2019) or bioaccumulation over time (Grillitsch & Schiesari, 2010). Further toxicokinetic investigation is encouraged to better understand the affinity of some metals to different storage tissues in marine turtles, including absorption to keratinous scute tissue.

No explanation as to historical contamination events or sources that may have influenced this trend are offered in the present study. Unlike blood sampling, which is an established and widely applied method of metal monitoring in turtles, scute metal analysis is still a novel approach not readily adopted as an alternative long-term metal store. Because of this, no published RIs are currently available for scute metal concentrations (Villa et al., 2019); thus, no indication as to whether current coastal concentrations are within expected ranges can be stated. Unlike Villa et al. (2019), the scope of the present study did not allow for scute sample collection from a control site such as Howick Island Group. Therefore, RIs could not be calculated, and steady-state relationships between blood and scute concentrations in control animals (Howick Island Group) could not be defined. If such information was available, deviations from the steady state in the coastal sites would have been possible and would have further elucidated whether elevation in scute metals not seen in blood was indeed due to past exposure to elevated concentrations (Villa et al., 2019).

Chemical contaminants are known to interact in complex mixtures, which could have synergistic, antagonistic, or additive influences on one another (Gatidou et al., 2015; Wilkinson et al., 2015); thus, a univariate analysis is weak when variable mixture interactions are undefined. To account for limitations encountered when comparing single metal elements, multivariate analysis allows for the analysis of metals as covariables within the overall metal profile, rather than distinct stressors that act in isolation of any others. The multivariate PCA implemented in the present study (following removal of Edgecumbe Bay data, which skewed initial analyses) illustrated a distinction between metal profiles at each coastal site. Individual sample profiles of blood collected from Upstart Bay were particularly distinct from the other sites (lack of data point mixing) and influenced primarily by Co, supported by the elevated mean Co concentrations detected at Upstart Bay when compared to the other sites (Table 2). Similarly, the metal profile of Howick Island Group was distinct from all coastal sites and was most influenced by concentrations of Al, which was the only element to be found at greater blood concentrations at Howick Island Group compared to all coastal sites during univariate analysis. The multivariate outcome further supports the univariate data in this instance because the lower variation often (but not always) observed in data reported in the Howick Island Group samples was depicted with a narrower confidence ellipse in the PCA in comparison with all other sites.

Individual scute sample data and overall confidence ellipses for most coastal populations overlapped consistently except for Toolakea, which was distinct from all other sites. The multivariate analysis suggested that the scute metal profile of Toolakea was likely influenced by concentrations of Cd, Mg, and Zn, all of which, in the univariate analysis, were detected at the greatest concentrations in scute samples from Toolakea. Conversely, Cockle Bay, Upstart Bay, and Edgecumbe Bay profiles were more strongly influenced by Al, Cu, Mn, and Co, which were consistently detected at concentrations somewhat higher than the same elements at Toolakea, hence the distinction in multivariate analysis of scute data between Toolakea and all other coast sites sampled.

Coastal zone ecosystems and offshore regions vary in a range of geochemical characteristics, which can influence the bioavailability and geoavailability of certain elements at a particular locality (Namieśnik & Rabajczyk, 2010). The proximity to river discharge catchments, sediment type, and grain size are fundamental characteristics that play a role in a site's metal profile. Freshwater runoff of contaminated sediments and water from land-based practices into creeks and river systems are major transport pathways of elevated land-based, nonessential and potentially harmful metals (Pacyna & Pacyna, 2001; Pacyna et al., 1995). Further confounding the risk, fine clay–based sediment types, which are dominant in estuarine and intertidal zones

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(including Cockle Bay and Upstart Bay), often carry higher trace metal concentrations than larger and coarser bicarbonate sediments found offshore (Weber et al., 2006). As such, making a direct comparison between sites is unreliable, and therefore further emphasis was not placed on comparisons of metal concentrations between coastal sites. The finding that trace metal concentrations were similar between coastal populations and the offshore control population is again indicative of those particular essential elements at the coastal sites likely being within their respective natural and optimal homeostatic ranges (at the time of sampling; Aggett et al., 2015; Bury et al., 2003), rather than at elevated levels, as predicted (Villa et al., 2017), where nonessential elements are not metabolically maintained within a concentration range, being potentially toxic at low concentrations (Aggett et al., 2015; da Silva et al., 2016; de Souza Machado et al., 2016).

Concentrations of Mg and Zn were significantly greater in *C. mydas* scutes from Toolakea than any other coastal site, including Cockle Bay (the study area closest to the Zn refinery), though Zn is also naturally geoavailable in certain substrates (Scott et al., 2013). Like Zn, Mg is an essential element and is associated with carbonate-based sediments found in bays not dominated by river discharge, such as Toolakea and Edgecumbe Bay. Variation in sediment composition between sites is known to play a role in local natural metal geoavailability (Thomas et al., 2020) but likely does not lend an explanation for significant fluctuation in metal loads over time.

As previously discussed, numerous site-specific confounding factors play a role in the bioavailability and abundance of metal elements, making robust comparisons between regions and data obtained previously very difficult. Reliable comparisons may be further hindered by the lack of universal sampling and analytical methods across studies, though some limited insight may be gleaned as to ecologically relevant metal profiles of other *C. mydas* foraging habitats elsewhere and how local environments along the Great Barrier Reef compare. Concentrations of Co in the blood of *C. mydas* from populations in Brazil (Prioste et al., 2015), Australia (Gaus et al., 2012), and California (Barraza et al., 2019) were lower than at all coastal sites in the present study (Supporting Information, Table S6). Cobalt was not detected at all in other populations, such as those studied in Thailand (Chomchat et al., 2023) and Hawaii (Shaw et al., 2021). In contrast, Ni was detected at greater levels in the same populations in Thailand (Chomchat et al., 2023) and California (Barraza et al., 2019) but within standard deviations of all sites sampled in Hawaii (Shaw et al., 2021) and Gladstone, Australia (Gaus et al., 2012). Likewise, Pb was also detected at higher concentrations within populations in the Thailand (Chomchat et al., 2023) and Californian (Barraza et al., 2019; Supporting Information, Table S6).

Limited research has been conducted on scute metal concentrations of *C. mydas*, and thus comparison with other geographically distinct populations was difficult. Cobalt and Pb were detected at greater concentrations in *C. mydas* scute samples from one of the two California populations studied (Barraza et al., 2019) and one Hawaii population (Shaw et al., 2023) than all local populations in the present study and were not detected at any load in two of three Hawaiian (Shaw et al., 2021) or Chinese (Ng et al., 2018) populations (Supporting Information, Table S6).

Temporal variation in C. mydas metal concentration

Blood samples are considered a short-term store for metal concentrations and are representative of recent exposure on or close to (2–3 weeks) the point of capture (Villa et al., 2017). Because no turtles were recaptured during the present study, temporal differences within sites could not be accurately made. To address this limitation, the study design ensured that similar methodologies to those conducted by Villa et al. (2017) were applied for the collection, storage, and analysis of blood and scute data. Such a universal study design allowed for direct comparison between data collected at different times but from the same locations. The study areas that were measured in both sites and compared were Howick Island Group, Cockle Bay, and Upstart Bay. Blood metal concentrations detected in *C. mydas* from Howick Island Group were similar between studies, with most mean concentrations being within range of one another. Similarly, when comparing mean concentrations at both Cockle Bay and Upstart Bay, most elements were similar or within standard deviation range between studies. The association between metal profiles of Cockle Bay and Upstart Bay was similar to that reported in Villa et al. (2017), which is further indicative of the absence of any significant contamination event in recent years (since 2014) at any sites in the present study (or that all sites may have been impacted by contamination in similar ways throughout the study region).

Cobalt was the sole element to be detected at higher concentrations in blood between studies, with concentrations up to double that of current levels in 2014 in Cockle Bay samples. This finding suggests that concentrations are currently lower than previously reported and that recent exposure to Co at Cockle Bay is reduced in the local *C. mydas* population. The scope of the present study did not allow for inference to be made as to why this may be the case.

Elevated Co concentrations

Cobalt was consistently higher not only in coastal *C. mydas* populations but particularly in Upstart Bay turtles. Such findings are true not only for the blood and scute analyses conducted in the present study but also in previous analyses of *C. mydas* blood (Villa et al., 2017), scutes (Villa et al., 2019), and seagrass species within the same study region (Thomas et al., 2020; Wilkinson et al., 2022). Concentrations of Co reported in Upstart Bay are purportedly the highest reported in blood collected from marine turtles or other vertebrates from anywhere else in the world (Villa et al., 2017). The consistently elevated Co profile observed in Upstart Bay is not deemed to be the result of direct anthropogenic land activity or industrial practices but is due to natural deposits of Ni and Co that saw the establishment of industrial practices such as intensive mining

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and refining industries to process the available ore. Furthermore, the Burdekin River (which outlets into Upstart Bay) and adjacent catchment area has seen significant increases in erosion rate since the mid-1800s, associated with agricultural land practices such as beef grazing and the cultivation of rangeland for cattle (Lewis et al., 2007; Wilkinson et al., 2013), likely leading to the distribution and transport of enriched sediment into waterways. As previously discussed, estuarine and river-borne sediment tend to be fine in grain size, and metals have a high affinity for such surfaces. Therefore, it is feasible that elevated Co and Ni concentrations have likely been consistently transported over time to the coastal environment in this region (Kroon et al., 2012).

Elevated Co concentrations in the present study are of concern because Co is considered a threat and may be linked to several negative health impacts in exposed marine species, such as *C. mydas* (Gaus et al., 2012; Villa et al., 2017, 2019). The biochemical implications of elevated Co concentrations are poorly understood, though recent research has found that Co may induce liver dysfunction (Villa et al., 2017), inflammatory responses (Gaus et al., 2019; Villa et al., 2017), and oxidative stress (Finlayson, Leusch, & van de Merwe, 2019a, 2019b; Permenter et al., 2014; Pulido & Parrish, 2003). Such risks are of particular concern because the blood concentrations reported at Upstart Bay in the present study exceeded the upper limit of the reported RI (0.007–0.03 mg kg^{-1}) by nearly 13-fold. Metal-induced oxidative stress via the production of toxic reactive oxygen species is of particular interest in the investigation of fibropapillomatosis etiology. Chronic damage caused by oxidative stress has the potential to induce immunosuppression (da Silva et al., 2016; Gaus et al., 2019), which is believed to increase susceptibility to further infection (such as ChHV5; Gaus et al., 2019), possibly associated with the expression of fibropapillomatosis (da Silva et al., 2016; Jones et al., 2016; Page-Karjian, 2019). Unfortunately, it was not possible to determine whether Co, or any other metals in the present study, were associated with fibropapillomatosis because most elements were at concentrations that presented little toxicological risk to local *C. mydas* populations. Furthermore, no turtles captured in the present study presented with visible fibropapillomatosis tumors, and thus comparisons could not be made between clinically healthy and diseased individuals to investigate differences in metal loads. While causation could not be drawn between fibropapillomatosis and toxic metal exposure, it is highly recommended that Co should be considered a priority element in future investigation. Further study should be conducted into the ecotoxicological implications of elevated exposure to toxic metal loads on *C. mydas* and to research how metal-induced immunosuppression may play a role in the development of fibropapillomatosis clinical signs and other toxicity-induced health complications in susceptible *C. mydas* populations. Although in vivo investigation of toxic metals in marine turtles is not possible for obvious ethical concerns, an in vitro cytotoxic assay has been recently validated to measure the toxic effects of metal concentrations on *C. mydas* primary skin fibroblasts (Finlayson, Leusch, & van de Merwe, 2019a; Finlayson, Leusch, Limpus, & van de Merwe, 2019). It is strongly suggested that future effort should be placed on applying this and other such sensitive ecotoxicological assays to investigate the potential toxic effects of environmentally relevant metals that may be of most concern.

CONCLUSION

A suite of ecologically relevant metal elements was measured in blood and scute samples from resident *C. mydas* at four coastal sites and compared with a reference site within the nGBR. Metal profiles were similar between coastal study sites, all of which differed from the control population. Blood metal concentrations at all sites (coastal and control) were generally within published RIs (except Co), suggesting that metal loads at the time of sampling were not at concentrations deemed a major concern. In contrast, scute concentrations of some elements were greater than those in blood within the same sites, which may be indicative of previous exposure of local *C. mydas* to certain elements at elevated levels (Mg, Mn, and Zn). Furthermore, some blood metal concentrations differed temporally when compared with previous research. Particularly, concentrations of Co were found to be magnitudes greater than baseline values and may be of concern as a possible etiological agent of disease expression and other health conditions in *C. mydas*. Further study investigating overall risk and susceptibility of *C. mydas* to elevated metal elements, such as Co, is required to better manage and protect populations inhabiting at-risk coastal areas. As more sensitive methods are validated and implemented in marine turtle ecotoxicology, future study design should strive to promote comparison between multiple study findings where possible. Such an approach will greatly improve baseline data and thus the sensitivity of the analysis. Further effort should be applied to develop the use of scute material as a long-term indication of metal exposure in individuals. Development of region-specific natural baseline RIs is an essential step that would aid comparison between regions and allow the development of a holistic view of areas of particular concern.

Supporting Information—The Supporting Information is available on the Wiley Online Library at https://doi.org/10.1002/etc.5718.

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field and sampling efforts throughout the course of this research.

Conflict of Interest—The authors declare no conflict of interest.

Author Contributions Statement—**Adam Wilkinson:** Conceptualization; Formal analysis; Funding acquisition; Investigation; Methodology; Project administration; Writing—original draft; Writing—review & editing. **Ellen Ariel:** Conceptualization; Funding acquisition; Investigation; Supervision; Writing—review & editing. **Jason van de Merwe:** Methodology; Software; Supervision; Visualization; Writing—review & editing. **Jon Brodie:** Funding acquisition; Methodology; Supervision; Writing—review & editing.

Data Availability Statement—Blood and scute metal concentration raw data are available as Supporting Information.

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