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Heavy metal concentrations suggest pollution risk varies between sea turtle species in the northwest Atlantic Ocean

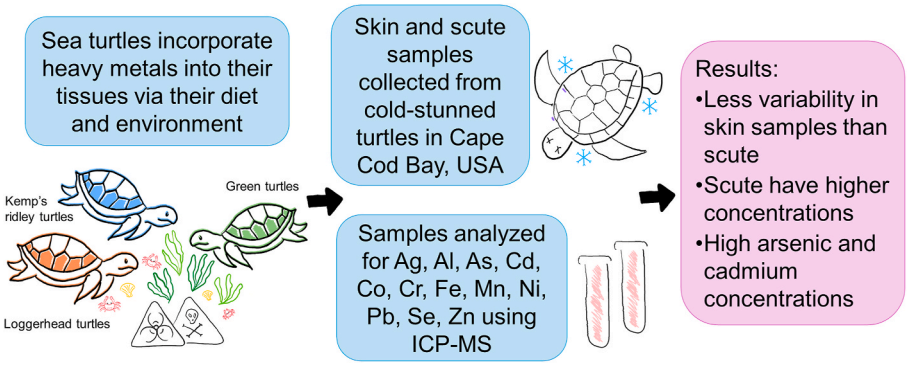
YiWynn Chan [a], Nathan J. Robinson [b,c,*], Karen Dourdeville [d], Heather L. Haas [e], James Nielsen [d], Frank V. Paladino [a,f], Robert Prescott [d], Samir H. Patel [g]

[a] Department of Biological Sciences, Purdue University Fort Wayne, 2101 E Coliseum Blvd, Fort Wayne, Indiana, 46805, USA
[b] Institut de Ciències del Mar, Spanish National Research Council - Consejo Superior de Investigaciones Científicas, Passeig Marítim de la Barceloneta, 37-49., 08003, Barcelona, Spain
[c] Fundación Oceanogràfic de la Comunitat Valenciana, Ciudad de las Artes y las Ciencias, C/ d'Eduardo Primo Yúfera, 1B, Quatre Carreres, 46013, València, Spain
[d] Mass Audubon Wellfleet Bay Wildlife Sanctuary, 291 US-6, South Wellfleet, Massachusetts, 02663, USA
[e] NOAA Fisheries Northeast Fisheries Science Center, 166 Water Street, Woods Hole, MA, 02543, USA
[f] The Leatherback Trust, Edificio Los Yoses, Oficina No. 4, Avenida Central, Calle 35, San José, Costa Rica
[g] Coonamessett Farm Foundation, Inc.233 Hatchville Rd, East Falmouth, MA, 02536, USA

HIGHLIGHTS

- First study of heavy metal concentrations in green and loggerhead turtles in northwest Atlantic.
- Metal concentrations in scute are more variable than in skin.
- Loggerheads had less variability in metal concentrations relative to green and Kemp's ridleys.
- As and Cd pose potential health risk to sea turtles in the northwest Atlantic.

GRAPHICAL ABSTRACT



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ABSTRACT

Heavy metal pollution poses an increasing threat to marine life globally. Due to bioaccumulation, the risks of heavy metal pollution are particularly acute for large species at high trophic levels although this will vary based on a species' diet and foraging location. Here, we assessed exposure risk to heavy metal pollution in three sea turtle species: the green (*Chelonia mydas*), Kemp's ridley (Lepidochelys kempii), and loggerhead (*Caretta caretta*) turtles. Specifically, we collected skin and scute samples from deceased turtles found after cold-stunning in Cape Cod Bay, Massachusetts, USA (green: n = 8, Kemp's ridley: n = 30, loggerhead: n = 17). Using ICP-MS, we analyzed samples for aluminum, arsenic, cadmium, cobalt, chromium, iron, manganese, nickel, lead, selenium, silver, and zinc concentrations. Across all species, heavy metal concentrations were predominantly higher and

* Corresponding author. Institut de Ciències del Mar - Consejo Superior De Investigaciones Científicas, Passeig Marítim de la Barceloneta, 37-49, 08003, Barcelona, Spain.
E-mail addresses: chany02@pfw.edu (Y. Chan), nathan@icm.csic.es (N.J. Robinson), kdourdeville@massaudubon.org (K. Dourdeville), heather.haas@noaa.gov (H.L. Haas), jnielsen@massaudubon.org (J. Nielsen), paladino@pfw.edu (F.V. Paladino), rprescott@massaudubon.org (R. Prescott), spatel@cfarm.org (S.H. Patel).

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Loggerhead turtles
Kemp's ridley turtles

more variable in scute than skin. When comparing species, PCA analysis revealed loggerhead turtles had the least variability in metal heavy concentrations, potentially driven by a generalist foraging strategy, relative to green and Kemp's ridley turtles. Nevertheless, all three species had concentrations of As and Cd near values considered toxic in vertebrates with loggerhead turtles having the highest concentrations. These findings underscore the importance of considering inter-specific differences when assessing the risks of heavy metal exposure in sea turtles and highlight As and Cd as key pollutants of concern in the northwest Atlantic.

1. Introduction

The accumulation of pollution in our oceans and atmosphere is one of the nine planetary boundaries that currently exceeds the safe operating space for humanity (Richardson et al., 2023). Key pollutants that are of growing concern, especially in marine habitats, are heavy metals. When concentrations of these heavy metals exceed certain thresholds, they can have detrimental effects on the health and fitness of marine wildlife (Catania et al., 2020; Sun et al., 2020). Furthermore, many heavy metals (i.e. Hg, Pb, and Zn) bio-magnify along trophic pathways, meaning that their concentrations are elevated in organisms at higher trophic levels, such as sea turtles (Bjorndal, 1985; Lambiase et al., 2021).

Sea turtles are a long-lived taxon that often conduct long-distance migrations (Bjorndal, 1985). As such, their tissues may incorporate environmental pollutants that they have been exposed to over their wide-geographic ranges (Barraza et al., 2019; Canzanella et al., 2021). However, each sea turtle species exhibits unique foraging preferences (Bjorndal, 1985), and this may alter the susceptibility of each species to pollutant exposure. For example, juvenile green turtles are typically herbivorous (Esteban et al., 2020) while juvenile loggerhead and Kemp's ridley turtles are predominantly carnivorous (Seney and Musick, 2007; Standora et al., 1994). Thus, by feeding at higher trophic levels, loggerhead and Kemp's ridley turtles exhibit higher concentrations of heavy metals than green turtles (Escobedo Mondragón et al., 2023). This could be especially true for heavy metals that are associated with a carnivorous diet, such as As and Cd (Bustamante et al., 1998; Storelli and Marcotrigiano, 2003).

As heavy metal pollution varies geographically, one way to assess the overall risk posed to each species is to sample species in the same environment, such as in the northwest Atlantic. Juvenile green, Kemp's ridley, and loggerhead turtles typically migrate to the coastal waters along the east coast of USA after completing their oceanic development stage in the north Atlantic gyre (Bolten, 2003). After recruiting to neritic waters, these different-species-turtles largely occupy similar habitats (Robinson et al., 2020), with turtles foraging in the Northwest Atlantic Ocean during the summer and fall when the surface water is warm (Morreale et al., 1992) and migrating southward to the Southwest Atlantic during winter (Musick et al., 1994). This migratory cycle may expose them to pollutants from a large portion of the northwestern Atlantic continental shelf. Interestingly, there is also the current and ongoing construction of offshore wind turbines, which are known to be a source of heavy metals including Al, Zn, In, Cd, Pb and Cu ("Offshore Wind | Mass.gov," n.d., Federal Maritime et al., 2022).

While the number of studies quantifying heavy metals in sea turtles have grown in recent decades (Robinson et al., 2023), the only studies in the northwest Atlantic focused on leatherback (Perrault, 2012; Perrault, 2014; Perrault et al., 2019) and Kemp's ridley turtles (Innis et al., 2008). Specifically, Innis et al. (2008) analyzed Hg in scute, blood, and liver, and Cu, Zn, and Se in plasma. As this study analyzed tissues that are difficult to obtain (e.g. liver, blood), we proposed investigating tissues that can collected with minimum harm from live animals, such as skin and scute (St. Andrews et al., 2021). In addition, different tissues might incorporate heavy metal over different timescales. Using stable isotope turnover rates as a proxy for heavy metal bioaccumulation and depuration, sea turtles skin have a higher turnover rate, reflecting exposure over approximately 1 year (Seminoff et al., 2006), while scutes, which have a slower turnover rate, offer insights into dietary and environmental information from the past 1.4–2.8 years (Vander Zanden et al., 2013). These tissues, therefore, offer complementary information on both recent and past environmental exposure.

Here, we measured the concentrations of seven essential heavy metals (Cr, Co, Fe, Mn, Ni, Se, and Zn) and five non-essential heavy metals (As, Al, Cd, Pb, and Ag) in green, Kemp's ridley, and loggerhead sea turtles that were sampled after being cold stunned in the waters of Cape Cod Bay, Massachusetts, USA. Furthermore, little is known about the concentrations of Al and Se in sea turtle scute samples, and no studies have analyzed Ag and Al in sea turtle skin samples (Barraza et al., 2019; Komoroske et al., 2011). Like other heavy metals, Ag, Al, and Se have also been shown to cause physiological and reproductive impacts in laboratory animals (ATSDR, 1999; ATSDR, 2003; ATSDR, 2008). With these data, we aimed to: (1) assess variation in skin and scute tissues between individuals to determine the different heavy metals they accumulate at different stages of their lives, (2) determine how exposure to heavy metal pollution varies between three sea turtle species occupying similar habitats, and (3) assess whether heavy metal concentrations for each species are approaching toxic levels. We predict that scute samples would have overall higher heavy metal concentrations than skin samples due to lower turnover rates (Seminoff et al., 2006; Vander Zanden et al., 2013). We also predict that loggerhead and Kemp's ridley turtles, as higher trophic level species, will have higher heavy metal concentrations than green turtles (Vander Zanden et al., 2010). However, as individual loggerhead turtles can exhibit specific prey preferences (Vander Zanden et al., 2010), we predict that the sampled loggerhead turtle population will have the highest variation of heavy metal concentrations in their tissue samples.

2. Methods

2.1. Field sample collection

This study took place in Cape Cod Bay, Massachusetts, USA - a 1100 km^2 semi-enclosed bay in the southern Gulf of Maine (Fig. 1). As the local water temperatures decline below ~10 °C in mid-autumn, turtles that do not migrate away begin cold stunning (Still et al., 2005). Mass Audubon Wellfleet Bay Wildlife Sanctuary (WBWS) annually rescues and collects these cold-stunned sea turtles from the beaches of Cape Cod Bay. The cause of death in these turtles is assumed to be exclusively due to cold-stunning, with no other contributing pathological factors (Innis et al., 2009). Turtles that are deceased on collection or cannot be rehabilitated are frozen and retained for necropsies within 2–4 months. From 2019 to 2021, skin and scute samples were collected during necropsies of green (n = 8), Kemp's ridley (n = 30), and loggerhead (n = 17) turtles. Skin samples (~0.5 g) were collected using a 6 mm biopsy punch on the right shoulder by sterilizing the area between the neck and right flipper. Scute samples (~0.5 g) were collected using separate 6 mm biopsy punches along the posterior end of the first lateral scute. All work was conducted under United States Endangered Species Act Permits #60415D, 23639, and 22218 issued to Mass Audubon Wellfleet Bay Wildlife Sanctuary, Coonamessett Farm Foundation, and Northeast Fisheries Science Center respectively.

2.2. Heavy metal analysis

We analyzed skin and scute samples without separating tissue layers.

Samples were analyzed for Ag, Al, As, Cd, Co, Cr, Fe, Mn, Ni, Pb, Se and Zn at [Said lab has been removed for double blind purposes], following standard protocols (N. Gou, personal communication), using an Inductively Coupled Plasma Mass Spectrometry (ICP-MS) (Thermo Scientific Element 2) equipped with a Teledyne Cetac Aridus II nebulizer and Thermon Element software. The ICP-MS parameters were set as follows: Radio frequency set as 1114 W; cool gas as argon at 16 L/min, sample gas at 1.1 L/min. The Aridus II nebulizer had spray chamber set at 110 °C, desolvation temperature set at 160 °C, sweep gas as argon at 2.88 L/min, and nitrogen gas at 3 m L/min. In brief, we weighed 0.2–0.5 g of each sample before adding 2 mL of ultra-high purity nitric acid and 0.5 mL of ultrapure water into borosilicate digestion vessels (Anton Paar 179436). Samples were then digested along with method blanks in a microwave digestor (Anton Paar 7000 Microwave Digestion System) using the preconfigured 'Organic' program. Next, samples and blanks were diluted using ultrapure water to a final volume of 50 mL and we added 125 μL of 0.005 ppm indium as an internal standard. Each sample and blank solution was analyzed 10 times, and the mean results are reported as μg per g wet weight of tissue.

2.3. Quality assurance and control

We used reagents and solvents of analytical grade to reduce the chances of impurities. Ultrapure water was obtained through using Barnstead MicroPure water purification system (Thermo Scientific). We used Ultra-high purity nitric acid (Aristar Ultra, VWR) for sample digestion and rinsing apparatus. After each use, we added ~5 mL of 2% nitric acid to all borosilicate digestion vessels (Anton Paar 179436) and cleaned them using the microwave digester, before rinsing them with ultrapure water.

In each batch of samples, a process blank was digested and analyzed to check for contamination or background interference. 125 μL of 0.005 ppm indium was added to each sample and blank as an internal standard. Elemental Standard solutions (0.00001–10 ppm) was prepared by diluting a 10 ppm elemental stock solution (Inorganic Venture) containing all 12 heavy metals. These standard solutions were used to plot standard calibration curves with correlation coefficients (R) that were greater than 0.99 for all heavy metals.

The limits of detection (LOD) of each heavy metal were calculated as three times the standard deviation of the 10-blank measurement, divided by the slope of the calibration curve. As we analyzed 110 samples for each of the 12 heavy metals, we recalibrated the ICP-MS between runs based off the indium internal standard readings. The range of LODs (ppm) of each heavy metal were: Ag: 0.00001–0.00007, Al: 0.00012–0.00246, As: 0.00001–0.00007, Cd: 0.00002–0.00008, Co: 0.00001–0.00011, Cr: 0.00001–0.00006, Fe: 0.00894–0.01387, Mn: 0.00003–0.00014, Ni: 0.00003–0.00029, Pb: 0.00001–0.00004, Se: 0.00032–0.00153, Zn: 0.00006–0.00051.

2.4. Statistical analysis

Statistical analyses were conducted using R 4.3.0 (R Core Team, 2021) and considering statistical significance when $p < 0.05$. To compare heavy metal concentrations between species (green, Kemp's ridley, and loggerhead turtles) and sample types (skin, scute), we used two-way ANOVA with post-hoc Tukey HSD test and PCA analysis.

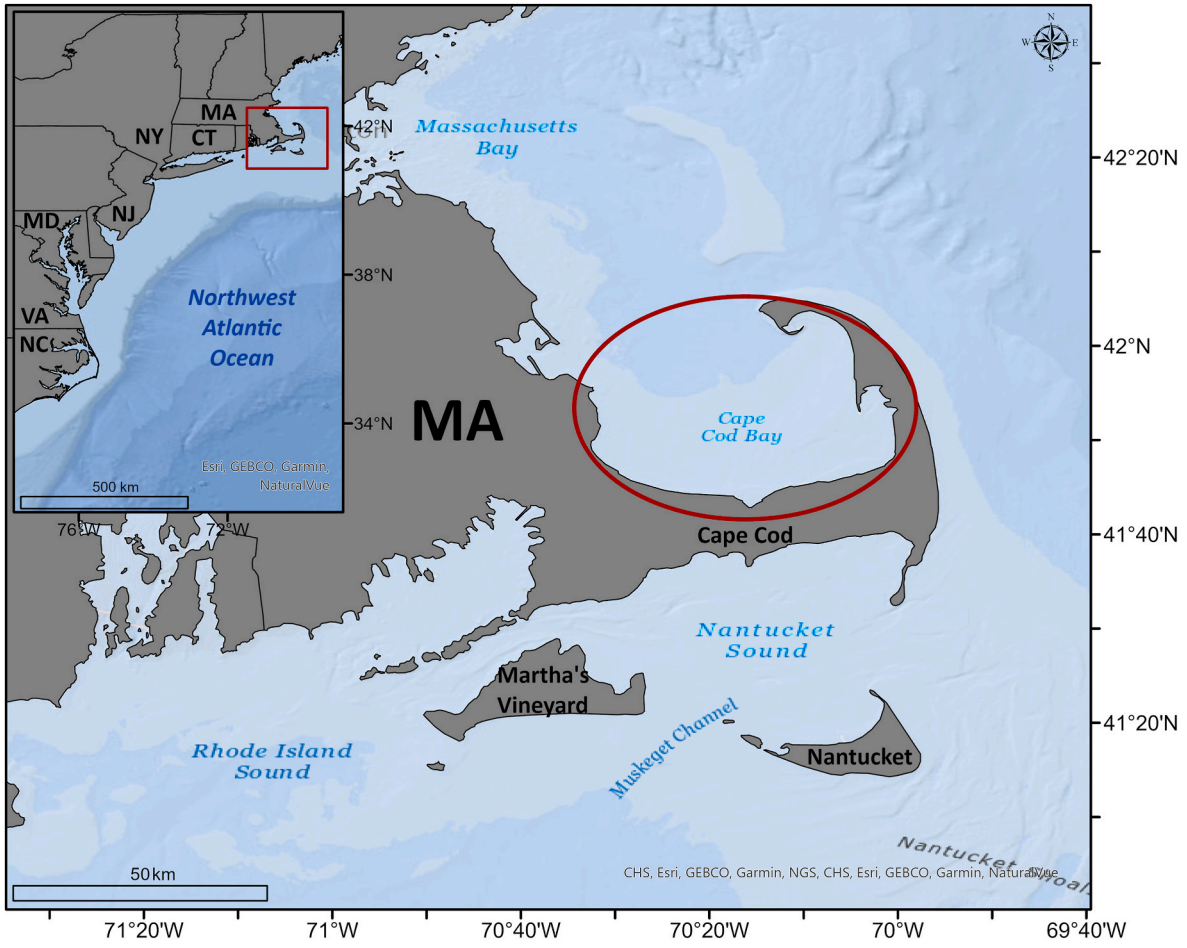

Fig. 1. Map of study area in the USA. The red circle is Cape Cod Bay, highlighting the hook-shaped bay which results in the entrapment of numerous turtles as they migrate south every winter. Map made using ESRI ArcMap 10.8.2.

Two-way ANOVA was employed for objective (1), to analyze the variation between sample types and species for each heavy metal individually, whereas principal component analysis (PCA) was used for objective (2), to provide a comprehensive assessment by considering all heavy metals simultaneously. By combining both methods, we were able to determine statistically significant differences as well as multivariate relationships of the data. When the assumptions of normality was not met, we log transformed the non-parametric heavy metal concentrations to normalize the data. The only heavy metals that did not need to be normalized was As. If heavy metal concentrations were below the LOD, we substituted these non-readings with the lowest value from associated range of LODs (i.e. Ag's < LOD would be substituted with 0.0001) when calculating mean values and conducting statistical analyses.

3. Results

We collected skin and scute samples from 8 green, 30 Kemp's ridley, and 17 loggerhead turtles. Mean SCL for green turtles was 28.7 cm (2.33SD) (26.5 cm–33.9 cm), for Kemp's ridleys was 25.8 cm (2.84SD) (18.6 cm–32.8 cm), and loggerhead turtles was 51.7 cm (9.7SD) (28.5 cm–69.2 cm).

3.1. Heavy metal concentrations between skin and scute samples for each species

Across all species, scute samples had higher concentrations than skin samples for seven out of twelve heavy metals (Al, Cd, Cr, Fe, Mn, Pb, and Zn). Kemp's ridley turtles scute samples had two elements that were significantly higher than skin samples (Co ($p < 0.01$), and Zn ($p < 0.01$)); loggerhead turtles had one element (Zn ($p < 0.01$)); green turtles had two elements (Al ($p < 0.01$), and Zn ($p < 0.01$)) (see Table 1). In contrast, Kemp's ridley turtles skin samples had one element that was significantly higher than scute samples (Ni ($p < 0.01$), and loggerhead turtles had four (Ag ($p = 0.02$), (As ($p < 0.01$), and (Co ($p < 0.01$)) (see Table 1).

PCA analysis showed that heavy metal concentrations in scute samples had greater variability than in skin samples, illustrated by the larger ellipses (Fig. 2). The ellipses for skin samples were much smaller, with most data points concentrated in Dim 2. PCA analysis revealed two reduced dimensions that accounted for at least 48% of the variance in PCA between skin and scute samples of each species (Fig. 2). For loggerhead turtles, the first two dimensions explained 57% of the variance (Fig. 2), with Zn (0.83) and Cd (0.82) having the strongest loading factors for Dim 1, and Co (0.70) and Al (0.61) for Dim 2. For Kemp's ridley turtles, the first two reduced dimensions accounted for 48% of the variance (Fig. 2), with Fe (0.90) and Mn (0.87) having the strongest loading factors for Dim 1, and Zn (−0.73) for Dim 2. For green turtles, the first two reduced dimensions accounted for 65% of the variance in

Table 1
Heavy metal concentrations in skin and scute samples of green ($n = 8$), Kemp's ridley ($n = 17$) and loggerhead ($n = 30$) turtles from Cape Cod Bay, Massachusetts, USA. Skin and scute heavy metal concentration values are reported in μg g^{-1} wet weight; *n = total number of samples analyzed; N** = number of samples that had a measurable detection for respective heavy metals; [a] indicates significant differences between different tissues of the same species; [b/c] indicates significant differences between tissues of different species.

Elements	Species	*n*	Skin		Scute	
			*N**	mean ± SD (range)	*N**	mean ± SD (range)
Non-essential elements						
Ag	Green	*8*	*6*	0.005 ± 0.006 (<LOD-0.017)	*1*	0.004 ± 0.012 (<LOD-0.034)
	Kemp's ridley	*30*	*11*	0.003 ± 0.008 (<LOD-0.036)	*4*	0.007 ± 0.017 (<LOD-0.066)
	Loggerhead	*17*	*11*	0.006 ± 0.006 [a] (<LOD-0.020)	*2*	0.003 ± 0.010 [a] (<LOD-0.036)
Al	Green	*8*	*8*	22.911 ± 33.710 [a] (1.002–101.809)	*7*	122.739 ± 213.918 [a] (<LOD-635.246)
	Kemp's ridley	*30*	*30*	25.013 ± 38.247 (1.547–137.335)	*28*	63.076 ± 76.818 [a] (<LOD-387.228)
	Loggerhead	*17*	*17*	19.015 ± 14.860 (1.801–62.315)	*17*	43.696 ± 35.238 (2.561–111.387)
As	Green	*8*	*8*	3.614 ± 2.569 (1.286–9.261)	*7*	1.935 ± 1.680 [b] (<LOD-5.205)
	Kemp's ridley	*30*	*30*	4.580 ± 1.753 (2.125–8.290)	*30*	4.678 ± 2.536 [b, c] (1.405–14.288)
	Loggerhead	*17*	*17*	5.069 ± 2.259 [a] (1.448–10.033)	*17*	1.792 ± 0.849 [a, c] (0.952–4.550)
Cd	Green	*8*	*8*	0.075 ± 0.052 (0.235-0.187)	*5*	0.090 ± 0.082 [b, c] (<LOD-0.193)
	Kemp's ridley	*30*	*29*	0.056 ± 0.034 (<LOD-0.184)	*26*	0.279 ± 0.199 [b] (<LOD-0.844)
	Loggerhead	*17*	*17*	0.092 ± 0.025 (0.060–0.162)	*17*	0.256 ± 0.150 [c] (0.077–0.593)
Pb	Green	*8*	*8*	0.050 ± 0.044 (0.011–0.134)	*5*	0.205 ± 0.389 (<LOD-1.146)
	Kemp's ridley	*30*	*28*	0.057 ± 0.076 (<LOD-0.352)	*19*	0.251 ± 0.317 [b] (<LOD-1.201)
	Loggerhead	*17*	*17*	0.077 ± 0.083 (0.011–0.347)	*12*	0.139 ± 0.237 [b] (<LOD-0.972)
Essential elements						
Co	Green	*8*	*8*	0.051 ± 0.021 (0.022–0.077)	*4*	0.058 ± 0.096 (<LOD-0.273)
	Kemp's ridley	*30*	*27*	0.024 ± 0.028 [a] (<LOD-0.145)	*8*	0.064 ± 0.215 [a] (<LOD-1.171)
	Loggerhead	*17*	*17*	0.016 ± 0.008 [a] (0.006–0.034)	*3*	0.006 ± 0.013 [a] (<LOD-0.044)
Cr	Green	*8*	*8*	0.291 ± 0.345 (0.017–1.044)	*6*	0.519 ± 0.802 (<LOD-2.399)
	Kemp's ridley	*30*	*30*	0.262 ± 0.350 (0.025–1.727)	*26*	0.936 ± 1.370 (<LOD-5.384)
	Loggerhead	*17*	*17*	0.153 ± 0.088 [a] (0.052–0.309)	*16*	0.804 ± 0.960 [a] (<LOD-3.550)
Fe	Green	*8*	*8*	46.239 ± 46.222 (3.583–132.315)	*5*	219.849 ± 423.011 (<LOD-1243.723)
	Kemp's ridley	*30*	*30*	44.331 ± 60.660 (3.000–279.505)	*26*	131.562 ± 120.327 (<LOD-499.501)
	Loggerhead	*17*	*17*	28.849 ± 52.200 (2.024–108.634)	*17*	73.884 ± 52.190 (13.048–178.620)
Mn	Green	*8*	*8*	0.529 ± 0.655 (0.085–2.054)	*7*	4.558 ± 8.650 (<LOD-25.625)
	Kemp's ridley	*30*	*30*	0.635 ± 0.931 (0.043–3.330)	*28*	2.878 ± 3.069 (<LOD-11.479)
	Loggerhead	*17*	*17*	0.510 ± 0.410 (0.048–1.362)	*17*	1.302 ± 1.480 (0.070–5.827)
Ni	Green	*8*	*8*	3.743 ± 6.497 (0.114–18.185)	*8*	2.497 ± 1.799 (0.242–5.316)
	Kemp's ridley	*30*	*30*	3.330 ± 12.226 [a] (0.051–67.355)	*30*	2.290 ± 1.441 [a] (0.271–6.023)
	Loggerhead	*17*	*17*	0.589 ± 0.363 (0.193–1.528)	*17*	1.528 ± 1.690 (0.368–7.380)
Se	Green	*8*	*8*	1.364 ± 2.497 (0.0003–7.295)	*2*	0.058 ± 0.108 (<LOD-0.242)
	Kemp's ridley	*30*	*4*	0.094 ± 0.281 [b] (<LOD-1.342)	*2*	0.134 ± 0.558 (<LOD-2.876)
	Loggerhead	*17*	*8*	0.443 ± 0.764 [b] (<LOD-2.762)	*2*	0.030 ± 0.086 (<LOD-0.290)
Zn	Green	*8*	*8*	21.561 ± 9.226 [a, b] (7.542–38.734)	*8*	108.095 ± 33.384 [a, b] (74.959–182.283)
	Kemp's ridley	*30*	*30*	19.980 ± 6.822 [a, c] (10.947–40.235)	*30*	166.972 ± 72.265 [a] (42.363–358.102)
	Loggerhead	*17*	*17*	11.271 ± 4.850 [a, b, c] (5.975–24.409)	*17*	201.786 ± 50.971 [a, b] (129.379–283.557)

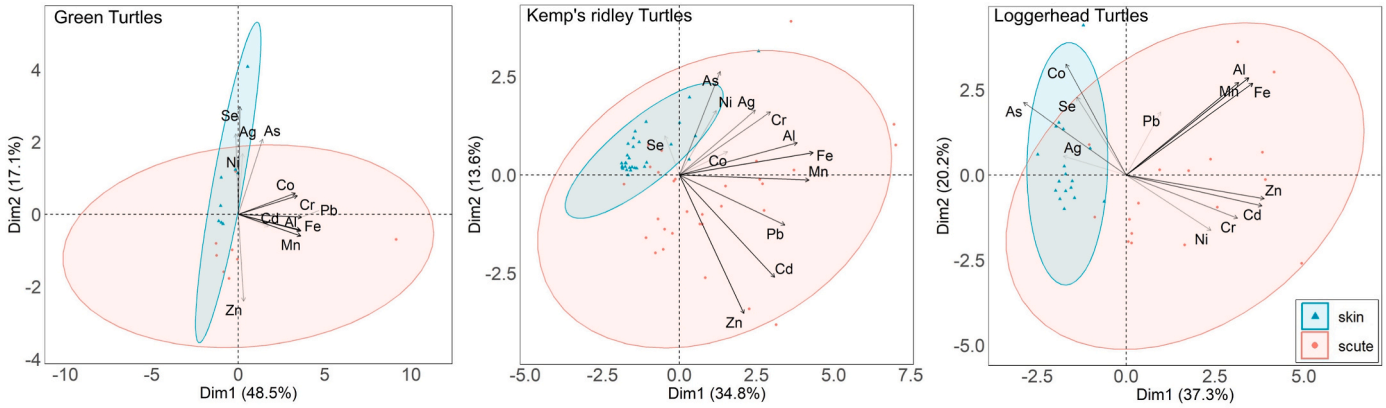

Fig. 2. Principal component analysis of heavy metals detected in scute and skin samples of green turtles (n = 8), Kemp's ridley turtles (n = 30), and loggerhead turtles (n = 17). Colored ellipses indicate 95% confidence ellipses. Heavy metal elements are depicted in scientific abbreviations.

the PCA analysis (Fig. 2), with Pb (0.99), Fe (0.98), Al (0.97), and Mn (0.97) having the strongest loading factors for Dim 1, and Se (0.80) for Dim 2.

3.2. Interspecific patterns of heavy metal concentrations

For skin samples, there were no statistical differences between species in heavy metal concentrations with the exception of Se and Zn. Specifically, loggerhead turtles had higher Se (0.443 ± 0.764 μg g^{-1}) than Kemp's ridley turtles (0.094 ± 0.281 μg g^{-1}, $p = 0.04$), although there was no difference between loggerhead and green turtles (1.363 ± 2.497 μg g^{-1}, $p > 0.05$). On the other hand, loggerhead turtles had significantly lower Zn concentrations (11.271 ± 4.850 μg g^{-1}) than green turtles (21.561 ± 9.226 μg g^{-1}, $p < 0.01$) and Kemp's ridley turtles (19.980 ± 6.822 μg g^{-1}, $p < 0.01$).

For scute samples, there were no statistical differences between species in heavy metal concentrations with the exception of As, Cd, Pb, and Zn concentrations. Specifically, Kemp's ridley turtles had higher concentrations of As (4.678 ± 2.536 μg g^{-1}) than both green (1.935 ± 1.680 μg g^{-1}, $p = 0.01$) and loggerhead (1.792 ± 0.849 μg g^{-1}, $p < 0.01$) turtles, and higher Pb (0.251 ± 0.317 μg g^{-1}) than loggerhead (0.139 ± 0.237 μg g^{-1}, $p < 0.01$) turtles. Furthermore, Kemp's ridley (0.279 ± 0.199 μg g^{-1}, $p = 0.04$) and loggerhead turtles (0.256 ± 0.150 μg g^{-1}, $p < 0.01$) both had significantly higher concentrations of Cd than green turtles (0.090 ± 0.082 μg g^{-1}). Loggerhead turtles (201.786 ± 50.971 μg g^{-1}, $p < 0.01$) also had higher Zn concentrations than green turtles (108.095 ± 33.384 μg g^{-1}).

PCA analyses revealed, via the area of the bounding ellipses, that loggerhead turtle samples exhibited less variability than those of Kemp's ridley and green turtles in both skin and scute (Fig. 3), and loggerhead turtle ellipse was also fully encompassed by Kemp's ridley turtles' ellipses. It should be noted that the size of the green ellipses, that were much bigger than Kemp's ridley ellipses, was extended by a single outlier. With this removed, the ellipse was similar size to Kemp's ridley turtle ellipse for skin and smaller for scute (Supplementary Fig. S1).

For skin samples, PCA detected two reduced dimensions that accounted for 48% of the variance (Fig. 3), with Fe (0.89) and Mn (0.89) having the strongest loading factors for Dim 1, and Ni (0.66) and Zn (−0.63) for Dim 2. For scutes, PCA exhibited two reduced dimensions that accounted for 57% of the variance (Fig. 3), with Fe (0.96), Al (0.91), and Cr (0.90) having the strongest loading factors for Dim 1, and Ag (0.75) for Dim 2.

4. Discussion

This study is the first to investigate heavy metal concentrations in green and loggerhead turtles in the northwest Atlantic. Additionally, it expands on Innis et al.'s (2008) study that only analyzed Hg in scute, blood, and liver, and Cu, Zn, and Se in plasma, in Kemp's ridley turtles from the region. In the following discussion, we explore the variation in

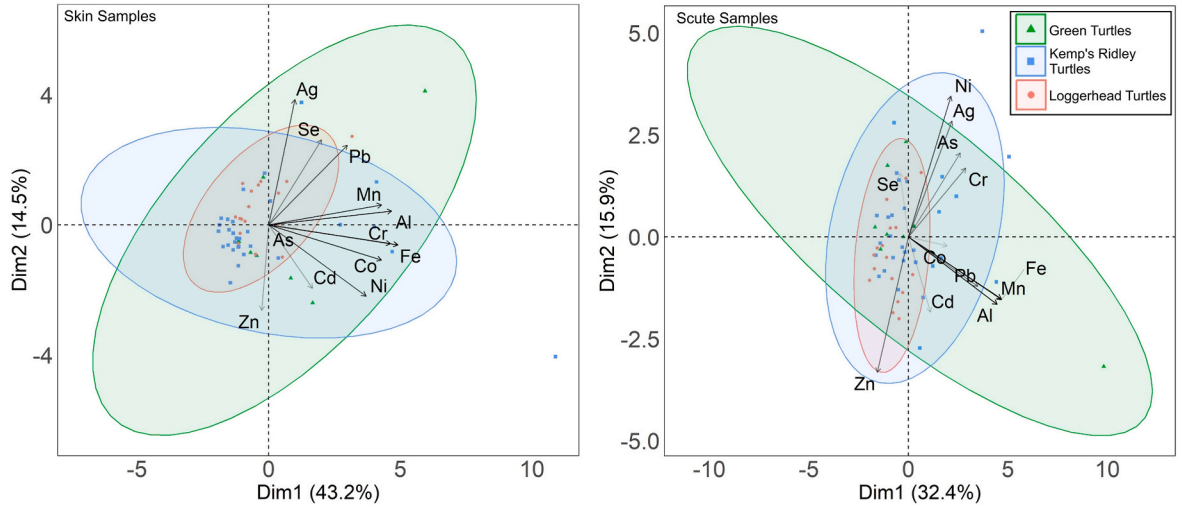

Fig. 3. Principal component analysis of heavy metals detected in skin and scute samples of green turtles (n = 8), Kemp's ridley turtles (n = 30), and loggerhead turtles (n = 17). Colored ellipses indicate 95% confidence ellipses. Heavy metal elements are depicted using scientific abbreviation.

heavy metal concentrations between skin and scute tissues as indicators of exposure in sea turtles. We then provide an interspecies comparison before concluding with an evaluation of the heavy metals that may pose particular concern for sea turtles in the region.

4.1. Skin vs. scute

The stable isotope turnover rates of skin samples are generally shorter than those of scute samples (Seminoff et al., 2006; Vander Zanden et al., 2013), which led to the prediction that skin samples would exhibit lower heavy metal concentrations. This study supports that prediction, finding higher concentrations of heavy metals in scute samples in 7 of 12 heavy metals tested. Additionally, other studies have shown that heavy metals may accumulate in the feathers of birds and skins of amphibians and non-turtle reptiles (Escobedo Mondragón et al., 2023; Martín et al., 2022). It therefore is possible that turtles use their scutes as an inert "deposit" to remove pollutants from more sensitive tissues.

In the PCA analysis, skin samples were more clustered suggesting less variability in heavy metal concentrations compared to scutes. As these turtles have been found to migrate over across the entire east coast of the USA and also into offshore waters (Robinson et al., 2020), this finding supports the assumption that skin has a higher turnover rate are probably reflecting heavy metals incorporated from a more localized area (Seminoff et al., 2006), while scute samples with lower turnover rates would reflect heavy metals incorporated from a wider geographic range (Vander Zanden et al., 2013). However, it is important to note we assumed turnover rates for heavy metals in skin and scute samples to be similar to those inferred in stable isotope studies (Seminoff et al., 2006; Vander Zanden et al., 2013), which may not perfectly align with actual heavy metal turnover dynamics. Furthermore, the collected scute samples may have included additional soft tissue depending on the depth of collection that could also contribute to the observed variability, resulting in both older and some newer tissue; however, the distribution of heavy metals in loggerheads is typically uniform across the central portion of the carapace (vertebral scutes and adjacent portions of the costal scutes) (Mattei et al., 2015).

4.2. Inter-specific variation between sea turtle species

PCA analysis revealed that loggerhead turtles exhibited less variability in heavy metal concentrations in both skin and scute tissues compared to green and Kemp's ridley turtles, contradicting our prediction. As they have been previously identified as specialists in a population of generalist foragers (Vander Zanden et al., 2010), we predicted that their different individual prey preferences would result in high variability in the PCA analysis. Our PCA results' small cluster suggest that this group of loggerheads may not be specialists but are all individual generalists instead. It is also possible that the lack of variation in the PCA analysis might be due to the loggerhead turtles feeding in more geographically focused areas, or simply the concentration of heavy metals in tissue does not follow the same pathways as stable isotopes.

Applying the same concept, we postulate that the green and Kemp's ridley turtles in this study might be individual specialists as they exhibited high variability. Green turtles displayed the widest ellipse in the PCA. This finding leads us to postulate that these green turtles may still be exhibiting omnivorous diet typical of juvenile green turtles, which is consistent with their SCL (28.7 ± 2.33 cm) (Bjorndal, 1985). It is also possible that these green turtles are foraging over a wider geographic area and are therefore incorporating varying heavy metal concentrations through their diet and environment. However, it is important to note that there was a single outlier among the green turtles. This outlier could be attributed to this individual turtle preferring a specific seagrass species, originating from a different green turtle cohort, or potential sampling and laboratory error. Even after removing the outlier, the ellipse remained much larger than loggerhead turtles', comparable to that of Kemp's ridley turtles. It is also important to highlight that it is difficult to draw conclusions from the green turtles' data due to the small sample size ($n = 8$).

We predicted that loggerhead and Kemp's ridley turtles, by feeding at higher trophic levels than green turtles, would have the highest heavy metal concentrations due to biomagnificiation. Loggerhead turtles were found to have higher Se concentration than Kemp's ridley turtle skin samples. As for scute samples, Kemp's ridley turtles and loggerhead turtles were found to have two heavy metals (As, Cd and Cd, Zn respectively) that were significantly higher compared green turtles. We postulate that biomagnification is probably only occurring in these certain elements in these species. This observation aligns with the findings of Bean and Logan (2019), who suggested that Kemp's ridley turtles in this region may feed at similar trophic levels to loggerhead turtles. Furthermore, Servis et al. (2015) noted that Kemp's ridley turtles eat fish and horseshoe crabs, which could have contributed to the biomagnification of heavy metals in Kemp's ridley turtles. It is likely that both Kemp's ridley and loggerhead turtles may be storing excess heavy metals in this inert tissue, likely as a form of detoxification, as described in Martín et al. (2022).

4.3. Heavy metals of concern

Worryingly, loggerhead turtles had higher concentrations of As (not significant) and Cd (significant for green turtles) than both green and Kemp's ridley turtles, both non-essential and particularly toxic metals. Furthermore, loggerhead turtle skin samples had significantly higher As concentrations than their scute samples. This could be attributed to loggerhead turtles' diet, which primarily consists of crustaceans, such as cephalopods and mollusks — organisms known to accumulate high levels of As and Cd through biomagnification (Bustamante et al., 1998; Storelli and Marcotrigiano, 2003). Furthermore, the elevated As and Cd concentrations in loggerhead turtle skin samples may also reflect their recent foraging in benthic habitats along the northeast coast of USA. This region has a history of industrial pollution, particularly from activities such as smelting and insufficient sewage treatment, leading to contamination of benthic ecosystems (Bothner et al., 1998; Eckel et al., 2001). Nevertheless, the loggerhead turtles' postulated generalist diet may act as a mitigating factor, potentially reducing the risk of over-accumulation of As and Cd from a single dietary source.

As concentrations of both skin and scute tissues of all three turtle species in this study were higher than that of normal levels (<1 ppm, synonymous with $\mu g\ g^{-1}$) in human keratin (Choucair and Ajax, 1988; Franzblau and Lilis, 1989) and safe concentrations for Lanzhou catfish (1.288 ppm) (Lian and Wu, 2017). Finlayson et al. (2020) also found As to be cytotoxic to green turtle skin cells. Nevertheless, the As concentrations in the skin of green and loggerhead turtles in this study were lower than that of turtles in Laguna Madre, USA (Faust et al., 2014) and Murcia, Spain (Jerez et al., 2010) (Supplementary Table S2). However, the scute samples from loggerhead turtles in this study had higher As concentrations than those from Brazil, where turtles were exposed to mining tailings (Miguel et al., 2022). These regional differences highlight the variability in environmental exposure and underscore the importance of ongoing monitoring.

Cd is associated with respiratory damage, cancer, liver disease, and neurological impairment (ATSDR, 2012). Studies have found Cd to cause toxicity in vertebrates when Cd concentrations are above 2 ppm (Eisler, 1985). However, 10% of humans with occupational exposure to Cd have been found to have signs of tubular damage when their blood concentrations were as low as 0.005 6 ppm (ATSDR, 2008). As these loggerhead turtles are chronically exposed to Cd and have skin tissue with 0.092 ± 0.025 ppm Cd concentration, we postulate that these loggerhead turtles could potentially be at risk for Cd toxicity due to chronic exposure. Furthermore, Cd concentrations in skin samples from loggerhead and green turtles in this study were higher than those reported in Murcia, Spain, and Texas, USA, respectively (Supplementary

Table S2) . As Cd has been shown to accumulate in human and sea turtle liver and kidneys (Esposito et al., 2020), we suggest that future studies should analyze Cd concentrations in liver and kidney samples of these cold-stunned turtles.

While these regional differences highlight the potential influence of environmental factors on heavy metal exposure in sea turtles, it is important to note that differences in sample processing (e.g., drying of skin samples) may have contributed to geographic variations in metal concentrations. To standardize the comparison, we converted the heavy metal concentrations of loggerhead scutes of other studies from μg g^{-1} dry weight to μg g^{-1} wet weight, using the value of 29.1% moisture content (Rodriguez et al., 2022). However, to the best of our knowledge, there are no known moisture values for sea turtle skin samples that could help us standardize heavy metal concentrations reported in dry weight.

Se was detected in all skin samples from all the green turtles, but only in half of the skin samples from Kemp's ridley and loggerhead turtles. Se was found in only 25%, 6.7% and 11.8% of scute samples from green, Kemp's ridley, and loggerhead turtles respectively. This is critical as Se is essential in maintaining cellular redox balance and keratinocyte function in the epidermis (Sengupta et al., 2010; Thiry et al., 2013). The lower Se concentrations in scute samples could suggest that Se has been used to regulate Hg, preventing its deposition in the scute. Although Hg concentrations were not measured in this study, Innis et al. (2008) observed that Kemp's ridley turtles with low blood Hg concentrations had higher Hg concentrations in their keratinized tissues.

We found statistically significant differences in Ag and Al concentrations between skin and scute tissues of green and loggerhead turtles respectively. However, there were no other significant differences in Ag or Al concentrations between the different species and tissue types. The concentrations of Ag and Al in these turtles were lower than known safe concentrations for other species, such as ilish fish fingerlings (*Tenualosa ilisha*) (1.450 ppm) for Ag (Sadat Sadeghi and Peery, 2018) and Atlantic salmon (*Salmo salar*) (92.051 ppm) for Al (GEI Consultants, Inc. 2011), indicating that these heavy metals may not be a health concern in these turtles.

5. Conclusions

The loggerhead turtle population in this study appear to be the most vulnerable species based on their high arsenic (As) and cadmium (Cd) concentrations in their skin samples. Since skin samples reflect local exposure, along the northeast US coast in this case, ongoing monitoring is crucial, especially with the recent development of offshore wind farms known to release metals. Although Kemp's ridley turtles also showed elevated levels of heavy metals, their population may not be as vulnerable as they appear to forage on various prey and seem to deposit high concentrations of excess metals in their inert keratin tissue. These findings underscore the importance of considering inter-specific differences when assessing the risks of heavy metal exposure in sea turtles and highlight As and Cd as key pollutants of concern in the northwest Atlantic.

CRediT authorship contribution statement

YiWynn Chan: Writing – original draft, Visualization, Methodology, Investigation, Formal analysis, Data curation. **Nathan J. Robinson:** Writing – review & editing, Supervision, Methodology, Conceptualization. **Karen Dourdeville:** Writing – review & editing, Resources. **Heather L. Haas:** Writing – review & editing, Resources. **James Nielsen:** Resources. **Frank V. Paladino:** Supervision, Resources. **Robert Prescott:** Resources. **Samir H. Patel:** Writing – review & editing, Supervision, Resources, Project administration, Conceptualization.

Declaration of competing interest

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

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.chemosphere.2025.144190.

Data availability

Data will be provided via email request to the corresponding author.

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