

1 **TITLE: Heavy metal concentrations suggest pollution risk varies between sea turtle**
2 **species in the northwest Atlantic Ocean.**

3

4 **ABSTRACT**

5 Heavy metal pollution poses an increasing threat to marine life globally. Due to
6 bioaccumulation, the risks of heavy metal pollution are particularly acute for large species at
7 high trophic levels although this will vary based on a species' diet and foraging location. Here,
8 we assessed exposure risk to heavy metal pollution in three sea turtle species: the green
9 (*Chelonia mydas*), Kemp's ridley (*Lepidochelys kempii*), and loggerhead (*Caretta caretta*)
10 turtles. Specifically, we collected skin and scute samples from deceased turtles found after cold-
11 stunning in Cape Cod Bay, Massachusetts, USA (green: n=8, Kemp's ridley: n=30, loggerhead:
12 n=17). Using ICP-MS, we analyzed samples for aluminum, arsenic, cadmium, cobalt, chromium,
13 iron, manganese, nickel, lead, selenium, silver, and zinc concentrations. Across all species,
14 heavy metal concentrations were predominantly higher and more variable in scute than skin.
15 When comparing species, PCA analysis revealed loggerhead turtles had the least variability in
16 metal heavy concentrations, potentially driven by a generalist foraging strategy, relative to green
17 and Kemp's ridley turtles. Nevertheless, all three species had concentrations of As and Cd near
18 values considered toxic in vertebrates with loggerhead turtles having the highest concentrations.
19 These findings underscore the importance of considering inter-specific differences when
20 assessing the risks of heavy metal exposure in sea turtles and highlight As and Cd as key
21 pollutants of concern in the northwest Atlantic.

22

23 **Keywords:** Trace elements, pollution, marine turtles, green turtles, loggerhead turtles, Kemp's
24 ridley turtles

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27 **1. INTRODUCTION**

28 The accumulation of pollution in our oceans and atmosphere is one of the nine planetary
29 boundaries that currently exceeds the safe operating space for humanity ([Richardson et al.](#)
30 [2023](#)). Key pollutants that are of growing concern, especially in marine habitats, are heavy
31 metals. When concentrations of these heavy metals exceed certain thresholds, they can have
32 detrimental effects on the health and fitness of marine wildlife ([Catania et al. 2020](#), [Sun et al.](#)
33 [2020](#)). Furthermore, many heavy metals (i.e. Hg, Pb, and Zn) bio-magnify along trophic
34 pathways, meaning that their concentrations are elevated in organisms at higher trophic levels,
35 such as sea turtles ([Bjorndal 1985](#), [Lambiase et al. 2021](#)).

36 Sea turtles are a long-lived taxon that often conduct long-distance migrations ([Bjorndal](#)
37 [1985](#)). As such, their tissues may incorporate environmental pollutants that they have been
38 exposed to over their wide-geographic ranges ([Baraza et al. 2019](#), [Canzanella et al. 2021](#)).
39 However, each sea turtle species exhibits unique foraging preferences ([Bjorndal 1985](#)), and this
40 may alter the susceptibility of each species to pollutant exposure. For example, juvenile green
41 turtles are typically herbivorous ([Esteban et al. 2020](#)) while juvenile loggerhead and Kemp's
42 ridley turtles are predominantly carnivorous ([Seney and Musick 2007](#), [Standora et al. 1994](#)).
43 Thus, by feeding at higher trophic levels, loggerhead and Kemp's ridley turtles exhibit higher
44 concentrations of heavy metals than green turtles ([Escobedo Mondragón et al. 2023](#)). This could
45 be especially true for heavy metals that are associated with a carnivorous diet, such as As and
46 Cd ([Bustamente et al. 1998](#), [Storelli and Marcotrigiano 2003](#)).

47 As heavy metal pollution varies geographically, one way to assess the overall risk posed
48 to each species is to sample species in the same environment, such as in the northwest
49 Atlantic. Juvenile green, Kemp's ridley, and loggerhead turtles typically migrate to the coastal
50 waters along the east coast of USA after completing their oceanic development stage in the
51 north Atlantic gyre ([Bolten 2003](#)). After recruiting to neritic waters, these different-species-turtles

52 largely occupy similar habitats ([Robinson et al. 2020](#)), with turtles foraging in the Northwest
53 Atlantic Ocean during the summer and fall when the surface water is warm ([Morreale et al.](#)
54 [1992](#)) and migrating southward to the Southwest Atlantic during winter ([Musick et al. 1994](#)). This
55 migratory cycle may expose them to pollutants from a large portion of the northwestern Atlantic
56 continental shelf. Interestingly, there is also the current and ongoing construction of offshore
57 wind turbines, which are known to be a source of heavy metals including Al, Zn, In, Cd, Pb and
58 Cu (["Offshore Wind | Mass.gov," n.d.](#), [Federal Maritime and Hydrographic Agency and](#)
59 [Helmholtz-Zentrum Hereon, 2022](#)).

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

73 Here, we measured the concentrations of seven essential heavy metals (Cr, Co, Fe, Mn,
74 Ni, Se, and Zn) and five non-essential heavy metals (As, Al, Cd, Pb, and Ag) in green, Kemp's
75 ridley, and loggerhead sea turtles that were sampled after being cold stunned in the waters of
76 Cape Cod Bay, Massachusetts, USA. Furthermore, little is known about the concentrations of Al
77 and Se in sea turtle scute samples, and no studies have analyzed Ag and Al in sea turtle skin

78 samples ([Barraza et al. 2019](#), [Komoroske et al. 2011](#)). Like other heavy metals, Ag, Al, and Se
79 have also been shown to cause physiological and reproductive impacts in laboratory animals
80 ([ATSDR 1999](#), [ATSDR 2003](#), [ATSDR 2008](#)). With these data, we aimed to: (1) assess variation
81 in skin and scute tissues between individuals to determine the different heavy metals they
82 accumulate at different stages of their lives, (2) determine how exposure to heavy metal
83 pollution varies between three sea turtle species occupying similar habitats, and (3) assess
84 whether heavy metal concentrations for each species are approaching toxic levels. We predict
85 that scute samples would have overall higher heavy metal concentrations than skin samples
86 due to lower turnover rates ([Seminoff et al. 2006](#), [Vander Zanden et al. 2013](#)). We also predict
87 that loggerhead and Kemp's ridley turtles, as higher trophic level species, will have higher heavy
88 metal concentrations than green turtles ([Vander Zanden et al. 2010](#)). However, as individual
89 loggerhead turtles can exhibit specific prey preferences ([Vander Zanden et al. 2010](#)), we predict
90 that the sampled loggerhead turtle population will have the highest variation of heavy metal
91 concentrations in their tissue samples.

92

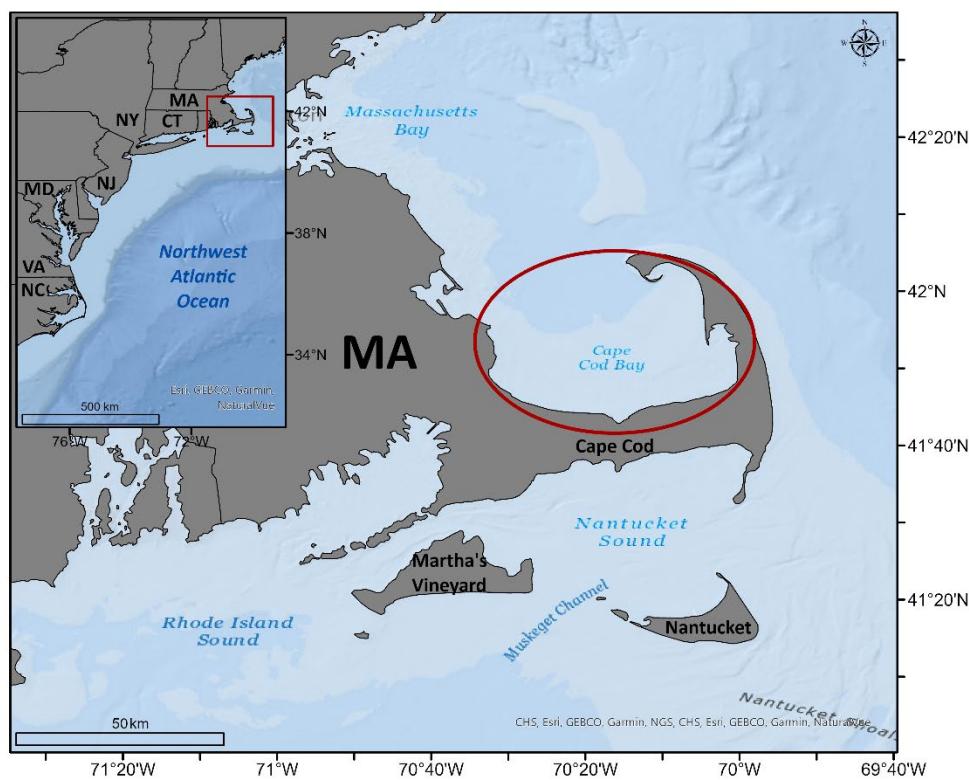
93 **2. METHODS**

94 *2.1 Field Sample Collection*

95 This study took place in Cape Cod Bay, Massachusetts, USA - a 1100 km² semi-
96 enclosed bay in the southern Gulf of Maine (**Figure 1**). As the local water temperatures decline
97 below ~10 °C in mid-autumn, turtles that do not migrate away begin cold stunning ([Still et al.](#)
98 [2005](#)). Mass Audubon Wellfleet Bay Wildlife Sanctuary (WBWS) annually rescues and collects
99 these cold-stunned sea turtles from the beaches of Cape Cod Bay. The cause of death in these
100 turtles is assumed to be exclusively due to cold-stunning, with no other contributing pathological
101 factors ([Innis et al. 2009](#)). Turtles that are deceased on collection or cannot be rehabilitated are
102 frozen and retained for necropsies within 2 – 4 months. From 2019 to 2021, skin and scute
103 samples were collected during necropsies of green (n=8), Kemp's ridley (n=30), and loggerhead

104 (n=17) turtles. Skin samples (~0.5 g) were collected using a 6mm biopsy punch on the right
105 shoulder by sterilizing the area between the neck and right flipper. Scute samples (~0.5 g) were
106 collected using separate 6mm biopsy punches along the posterior end of the first lateral scute.
107 All work was conducted under United States Endangered Species Act Permits #60415D, 23639,
108 and 22218 issued to Mass Audubon Wellfleet Bay Wildlife Sanctuary, Coonamessett Farm
109 Foundation, and Northeast Fisheries Science Center respectively.

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

117

118 *2.2 Heavy Metal Analysis*

119 We analyzed skin and scute samples without separating tissue layers. Samples were
120 analyzed for Ag, Al, As, Cd, Co, Cr, Fe, Mn, Ni, Pb, Se and Zn at [Said lab has been removed
121 for double blind purposes], following standard protocols (N. Gou, personal communication),
122 using an Inductively Coupled Plasma Mass Spectrometry (ICP-MS) (Thermo Scientific Element
123 2) equipped with a Teledyne Cetac Aridus II nebulizer and Thermon Element software. The ICP-
124 MS parameters were set as follows: Radio frequency set as 1114 W; cool gas as argon at 16
125 L/min, sample gas at 1.1 L/min. The Aridus II nebulizer had spray chamber set at 110 °C,
126 desolvation temperature set at 160 °C, sweep gas as argon at 2.88 L/min, and nitrogen gas at
127 3m L/min. In brief, we weighed 0.2-0.5 g of each sample before adding 2 mL of ultra-high purity
128 nitric acid and 0.5 mL of ultrapure water into borosilicate digestion vessels (Anton Paar 179436).
129 Samples were then digested along with method blanks in a microwave digestor (Anton Paar
130 7000 Microwave Digestion System) using the preconfigured 'Organic' program. Next, samples
131 and blanks were diluted using ultrapure water to a final volume of 50 mL and we added 125 µL
132 of 0.005 ppm indium as an internal standard. Each sample and blank solution was analyzed 10
133 times, and the mean results are reported as µg per g wet weight of tissue.

134

135 *2.3 Quality assurance and control*

136 We used reagents and solvents of analytical grade to reduce the chances of impurities.
137 Ultrapure water was obtained through using Barnstead MicroPure water purification system
138 (Thermo Scientific). We used Ultra-high purity nitric acid (Aristar Ultra, VWR) for sample

139 digestion and rinsing apparatus. After each use, we added ~5 mL of 2% nitric acid to all
140 borosilicate digestion vessels (Anton Paar 179436) and cleaned them using the microwave
141 digester, before rinsing them with ultrapure water.

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

148 The limits of detection (LOD) of each heavy metal were calculated as three times the
149 standard deviation of the 10-blank measurement, divided by the slope of the calibration curve.

150 As we analyzed 110 samples for each of the 12 heavy metals, we recalibrated the ICP-MS
151 between runs based off the indium internal standard readings. The range of LODs (ppm) of
152 each heavy metal were: Ag: 0.00001-0.00007, Al: 0.00012-0.00246, As: 0.00001-0.00007, Cd:
153 0.00002-0.00008, Co: 0.00001-0.00011, Cr: 0.00001-0.00006, Fe: 0.00894-0.01387, Mn:
154 0.00003-0.00014, Ni: 0.00003-0.00029, Pb: 0.00001-0.00004, Se: 0.00032-0.00153, Zn:
155 0.00006-0.00051.

156

157 *2.4 Statistical analysis*

158 Statistical analyses were conducted using R 4.3.0 ([R Core Team 2021](#)) and considering
159 statistical significance when $p < 0.05$. To compare heavy metal concentrations between species
160 (green, Kemp's ridley, and loggerhead turtles) and sample types (skin, scute), we used two-way
161 ANOVA with post-hoc Tukey HSD test and PCA analysis. Two-way ANOVA was employed for
162 objective (1), to analyze the variation between sample types and species for each heavy metal
163 individually, whereas principal component analysis (PCA) was used for objective (2), to provide
164 a comprehensive assessment by considering all heavy metals simultaneously. By combining

165 both methods, we were able to determine statistically significant differences as well as
166 multivariate relationships of the data. When the assumptions of normality was not met, we log
167 transformed the non-parametric heavy metal concentrations to normalize the data. The only
168 heavy metals that did not need to be normalized was As. If heavy metal concentrations were
169 below the LOD, we substituted these non-readings with the lowest value from associated range
170 of LODs (i.e. Ag's <LOD would be substituted with 0.0001) when calculating mean values and
171 conducting statistical analyses.

172

173 **3. RESULTS**

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

178

179 *3.1 Heavy metal concentrations between skin and scute samples for each species*

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

187

188 **Table 1.** Heavy metal concentrations in skin and scute samples of green ($n=8$), Kemp's ridley
189 ($n=17$) and loggerhead ($n=30$) turtles from Cape Cod Bay, Massachusetts, USA. Skin and scute

190 heavy metal concentration values are reported in $\mu\text{g g}^{-1}$ wet weight; n = *total number of samples*
 191 *analyzed*; N^* = number of samples that had a measurable detection for respective heavy
 192 metals; ^a indicates significant differences between different tissues of the same species; ^{b/c}
 193 indicates significant differences between tissues of different species.

Elements	Species	<i>n</i>	Skin		Scute	
			N^*	mean \pm SD (range)	N^*	mean \pm SD (range)
<i>Non-essential elements</i>						
Ag	Green	8	6	0.005 \pm 0.006 (<LOD-0.017)	1	0.004 \pm 0.012 (<LOD-0.034)
	Kemp's ridley	30	11	0.003 \pm 0.008 (<LOD-0.036)	4	0.007 \pm 0.017 (<LOD-0.066)
	Loggerhead	17	11	0.006 \pm 0.006 ^a (<LOD-0.020)	2	0.003 \pm 0.010 ^a (<LOD-0.036)
Al	Green	8	8	22.911 \pm 33.710 ^a (1.002-101.809)	7	122.739 \pm 213.918 ^a (<LOD-635.246)
	Kemp's ridley	30	30	25.013 \pm 38.247 (1.547-137.335)	28	63.076 \pm 76.818 ^a (<LOD-387.228)
	Loggerhead	17	17	19.015 \pm 14.860 (1.801-62.315)	17	43.696 \pm 35.238 (2.561-111.387)
As	Green	8	8	3.614 \pm 2.569 (1.286-9.261)	7	1.935 \pm 1.680 ^b (<LOD-5.205)
	Kemp's ridley	30	30	4.580 \pm 1.753 (2.125-8.290)	30	4.678 \pm 2.536 ^{b, c} (1.405-14.288)
	Loggerhead	17	17	5.069 \pm 2.259 ^a	17	1.792 \pm 0.849 ^{a, c}

				(1.448-10.033)		(0.952-4.550)
Cd	Green	8	8	0.075 ± 0.052	5	0.090 ± 0.082 ^{b, c}
				(0.235-0.187)		(<LOD-0.193)
	Kemp's ridley	30	29	0.056 ± 0.034	26	0.279 ± 0.199 ^b
Pb	Loggerhead	17	17	0.092 ± 0.025	17	0.256 ± 0.150 ^c
				(0.060-0.162)		(0.077-0.593)
	Kemp's ridley	30	28	0.057 ± 0.076	19	0.251 ± 0.317 ^b
Pb	Loggerhead	17	17	0.077 ± 0.083	12	0.139 ± 0.237 ^b
				(0.011-0.347)		(<LOD-0.972)

Essential elements

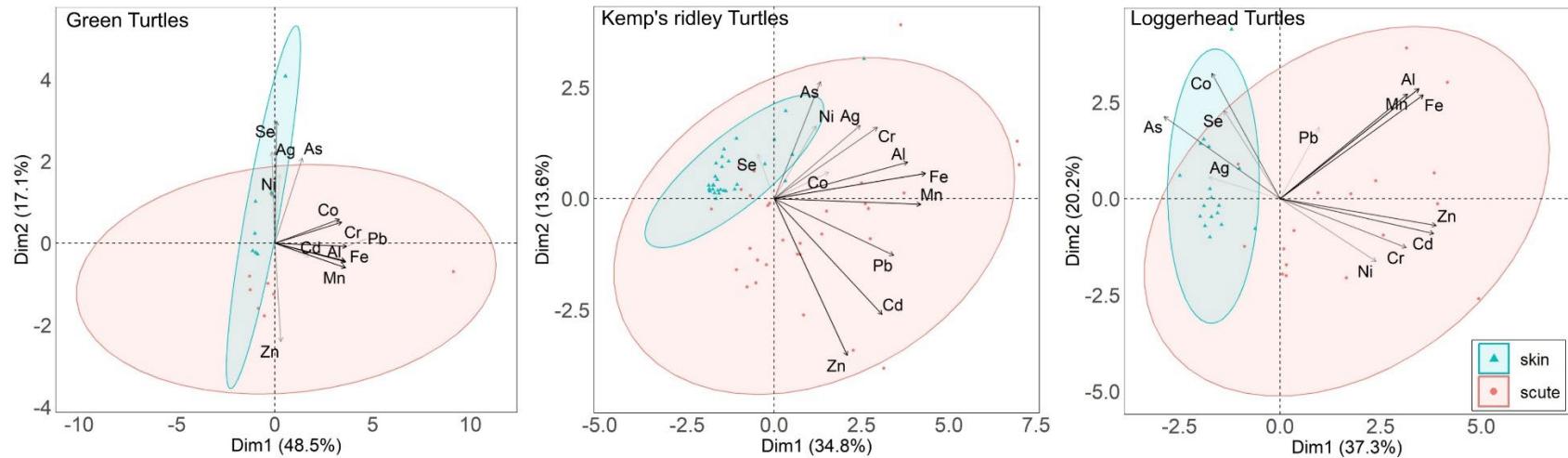
Co	Green	8	8	0.051 ± 0.021	4	0.058 ± 0.096
				(0.022-0.077)		(<LOD-0.273)
	Kemp's ridley	30	27	0.024 ± 0.028 ^a	8	0.064 ± 0.215 ^a
Cr	Loggerhead	17	17	0.016 ± 0.008 ^a	3	0.006 ± 0.013 ^a
				(0.006-0.034)		(<LOD-0.044)
	Kemp's ridley	30	30	0.291 ± 0.345	6	0.519 ± 0.802
Cr	Loggerhead	17	17	(0.017-1.044)	26	(<LOD-2.399)
				0.262 ± 0.350		0.936 ± 1.370
				(0.025-1.727)		(<LOD-5.384)
Cr	Green	8	8	0.153 ± 0.088 ^a	16	0.804 ± 0.960 ^a

				(0.052-0.309)		(<LOD-3.550)
Fe	Green	8	8	46.239 ± 46.222	5	219.849 ± 423.011
				(3.583-132.315)		(<LOD-1243.723)
	Kemp's ridley	30	30	44.331 ± 60.660	26	131.562 ± 120.327
Mn	Loggerhead	17	17	28.849 ± 52.200	17	73.884 ± 52.190
				(2.024-108.634)		(13.048-178.620)
	Kemp's ridley	30	30	0.635 ± 0.931	28	2.878 ± 3.069
Ni	Loggerhead	17	17	0.510 ± 0.410	17	1.302 ± 1.480
				(0.048-1.362)		(0.070-5.827)
	Green	8	8	3.743 ± 6.497	8	2.497 ± 1.799
Se	Kemp's ridley	30	30	3.330 ± 12.226 ^a	30	2.290 ± 1.441 ^a
				(0.051-67.355)		(0.271-6.023)
	Loggerhead	17	17	0.589 ± 0.363	17	1.528 ± 1.690
	Green	8	8	1.364 ± 2.497	2	0.058 ± 0.108
				(0.0003-7.295)		(<LOD-0.242)
	Kemp's ridley	30	4	0.094 ± 0.281 ^b	2	0.134 ± 0.558
	Loggerhead	17	8	0.443 ± 0.764 ^b	2	0.030 ± 0.086
				(<LOD-2.762)		(<LOD-0.290)

Zn	Green	8	8	$21.561 \pm 9.226^{\text{a, b}}$ (7.542-38.734)	8	$108.095 \pm 33.384^{\text{a, b}}$ (74.959-182.283)
	Kemp's ridley	30	30	$19.980 \pm 6.822^{\text{a, c}}$ (10.947-40.235)	30	$166.972 \pm 72.265^{\text{a}}$ (42.363-358.102)
	Loggerhead	17	17	$11.271 \pm 4.850^{\text{a, b, c}}$ (5.975-24.409)	17	$201.786 \pm 50.971^{\text{a, b}}$ (129.379-283.557)

194

195 PCA analysis showed that heavy metal concentrations in scute samples had greater
 196 variability than in skin samples, illustrated by the larger ellipses (**Figure 2**). The ellipses for skin
 197 samples were much smaller, with most data points concentrated in Dim 2. PCA analysis
 198 revealed two reduced dimensions that accounted for at least 48% of the variance in PCA
 199 between skin and scute samples of each species (**Figure 2**). For loggerhead turtles, the first two
 200 dimensions explained 57% of the variance (**Figure 2**), with Zn (0.83) and Cd (0.82) having the
 201 strongest loading factors for Dim 1, and Co (0.70) and Al (0.61) for Dim 2. For Kemp's ridley
 202 turtles, the first two reduced dimensions accounted for 48% of the variance (**Figure 2**), with Fe
 203 (0.90) and Mn (0.87) having the strongest loading factors for Dim 1, and Zn (-0.73) for Dim 2.
 204 For green turtles, the first two reduced dimensions accounted for 65% of the variance in the
 205 PCA analysis (**Figure 2**), with Pb (0.99), Fe (0.98), Al (0.97), and Mn (0.97) having the strongest
 206 loading factors for Dim 1, and Se (0.80) for Dim 2.



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

212 3.2 *Interspecific patterns of heavy metal concentrations*

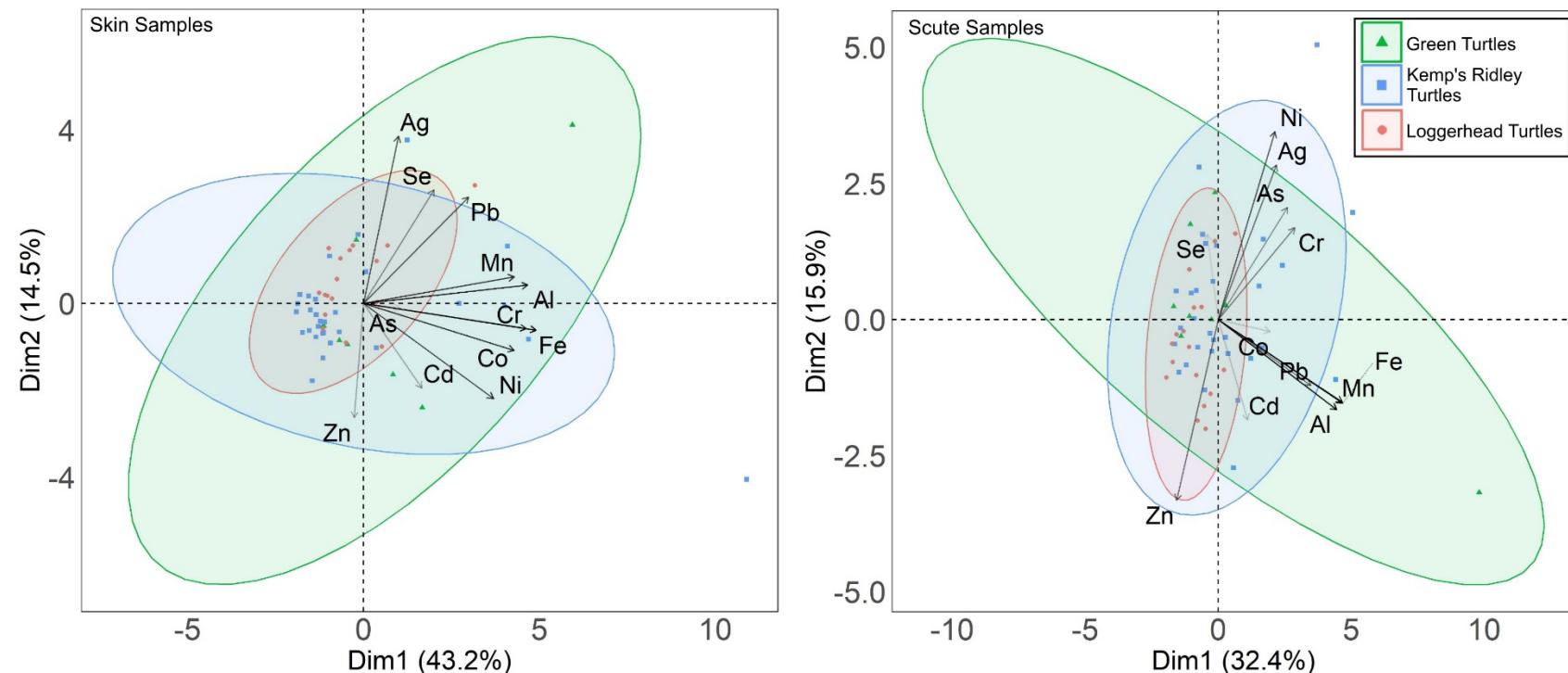
213 For skin samples, there were no statistical differences between species in heavy metal
214 concentrations with the exception of Se and Zn. Specifically, loggerhead turtles had higher Se
215 ($0.443 \pm 0.764 \mu\text{g g}^{-1}$) than Kemp's ridley turtles ($0.094 \pm 0.281 \mu\text{g g}^{-1}$, $p=0.04$), although there was
216 no difference between loggerhead and green turtles ($1.363 \pm 2.497 \mu\text{g g}^{-1}$, $p>0.05$). On the other
217 hand, loggerhead turtles had significantly lower Zn concentrations ($11.271 \pm 4.850 \mu\text{g g}^{-1}$) than
218 green turtles ($21.561 \pm 9.226 \mu\text{g g}^{-1}$, $p<0.01$) and Kemp's ridley turtles ($19.980 \pm 6.822 \mu\text{g g}^{-1}$,
219 $p<0.01$).

220 For scute samples, there were no statistical differences between species in heavy metal
221 concentrations with the exception of As, Cd, Pb, and Zn concentrations. Specifically, Kemp's
222 ridley turtles had higher concentrations of As ($4.678 \pm 2.536 \mu\text{g g}^{-1}$) than both green (1.935 ± 1.680
223 $\mu\text{g g}^{-1}$, $p=0.01$) and loggerhead ($1.792 \pm 0.849 \mu\text{g g}^{-1}$, $p<0.01$) turtles, and higher Pb
224 ($0.251 \pm 0.317 \mu\text{g g}^{-1}$) than loggerhead ($0.139 \pm 0.237 \mu\text{g g}^{-1}$, $p<0.01$) turtles. Furthermore, Kemp's
225 ridley ($0.279 \pm 0.199 \mu\text{g g}^{-1}$, $p=0.04$) and loggerhead turtles ($0.256 \pm 0.150 \mu\text{g g}^{-1}$, $p<0.01$) both
226 had significantly higher concentrations of Cd than green turtles ($0.090 \pm 0.082 \mu\text{g g}^{-1}$).
227 Loggerhead turtles ($201.786 \pm 50.971 \mu\text{g g}^{-1}$, $p<0.01$) also had higher Zn concentrations than
228 green turtles ($108.095 \pm 33.384 \mu\text{g g}^{-1}$).

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

236 For skin samples, PCA detected two reduced dimensions that accounted for 48% of the
237 variance (**Figure 3**), with Fe (0.89) and Mn (0.89) having the strongest loading factors for Dim 1,

238 and Ni (0.66) and Zn (-0.63) for Dim 2. For scutes, PCA exhibited two reduced dimensions that
239 accounted for 57% of the variance (**Figure 3**), with Fe (0.96), Al (0.91), and Cr (0.90) having the
240 strongest loading factors for Dim 1, and Ag (0.75) for Dim 2.



241

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

245

246

247 **4. DISCUSSION**

248 This study is the first to investigate heavy metal concentrations in green and loggerhead
249 turtles in the northwest Atlantic. Additionally, it expands on [Innis et al.](#)'s (2008) study that only
250 analyzed Hg in scute, blood, and liver, and Cu, Zn, and Se in plasma, in Kemp's ridley turtles
251 from the region. In the following discussion, we explore the variation in heavy metal
252 concentrations between skin and scute tissues as indicators of exposure in sea turtles. We then
253 provide an interspecies comparison before concluding with an evaluation of the heavy metals
254 that may pose particular concern for sea turtles in the region.

255

256 *4.1 Skin vs. scute*

257 The stable isotope turnover rates of skin samples are generally shorter than those of
258 scute samples ([Seminoff et al. 2006](#), [Vander Zanden et al. 2013](#)), which led to the prediction
259 that skin samples would exhibit lower heavy metal concentrations. This study supports that
260 prediction, finding higher concentrations of heavy metals in scute samples in 7 of 12 heavy
261 metals tested. Additionally, other studies have shown that heavy metals may accumulate in the
262 feathers of birds and skins of amphibians and non-turtle reptiles ([Escobedo Mondragón et al.](#)
263 [2023](#), [Martín et al. 2022](#)). It therefore is possible that turtles use their scutes as an inert
264 "deposit" to remove pollutants from more sensitive tissues.

265 In the PCA analysis, skin samples were more clustered suggesting less variability in
266 heavy metal concentrations compared to scutes. As these turtles have been found to migrate
267 over a fairly wide region ([Robinson et al. 2020](#)), this finding supports the assumption that skin
268 has a higher turnover rate are probably reflecting heavy metals incorporated from a more
269 localized area ([Seminoff et al. 2006](#)), while scute samples with lower turnover rates would
270 reflect heavy metals incorporated from a wider geographic range ([Vander Zanden et al. 2013](#)).
271 However, it is important to note we assumed turnover rates for heavy metals in skin and scute

272 samples to be similar to those inferred in stable isotope studies ([Seminoff et al. 2006](#), [Vander](#)
273 [Zanden et al. 2013](#)), which may not perfectly align with actual heavy metal turnover dynamics.
274 Furthermore, the collected scute samples may have included additional soft tissue depending
275 on the depth of collection that could also contribute to the observed variability, resulting in both
276 older and some newer tissue; however, the distribution of heavy metals in loggerheads is
277 typically uniform across the central portion of the carapace (vertebral scutes and adjacent
278 portions of the costal scutes) ([Mattei et al. 2015](#)).

279

280 *4.2 Inter-specific variation between sea turtle species*

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

290 Applying the same concept, we postulate that the green and Kemp's ridley turtles in this
291 study might be individual specialists as they exhibited high variability. Green turtles displayed
292 the widest ellipse in the PCA. This finding leads us to postulate that these green turtles may still
293 be exhibiting omnivorous diet typical of juvenile green turtles, which is consistent with their SCL
294 (28.7 ± 2.33 cm) ([Bjorndal 1985](#)). It is also possible that these green turtles are foraging over a
295 wider geographic area and are therefore incorporating varying heavy metal concentrations
296 through their diet and environment. However, it is important to note that there was a single

297 outlier among the green turtles. This outlier could be attributed to this individual turtle preferring
298 a specific seagrass species, originating from a different green turtle cohort, or potential sampling
299 and laboratory error. Even after removing the outlier, the ellipse remained much larger than
300 loggerhead turtles', comparable to that of Kemp's ridley turtles. It is also important to highlight
301 that it is difficult to draw conclusions from the green turtles' data due to the small sample size
302 ($n=8$).

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

316

317 *4.3 Heavy metals of concern*

318 Worryingly, loggerhead turtles had higher concentrations of As (not significant) and Cd
319 (significant for green turtles) than both green and Kemp's ridley turtles, both non-essential and
320 particularly toxic metals. Furthermore, loggerhead turtle skin samples had significantly higher As
321 concentrations than their scute samples. This could be attributed to loggerhead turtles' diet,

322 which primarily consists of crustaceans, such as cephalopods and mollusks — organisms
323 known to accumulate high levels of As and Cd through biomagnification ([Bustamente et al.](#)
324 [1998](#), [Storelli and Marcotrigiano 2003](#)). Furthermore, the elevated As and Cd concentrations in
325 loggerhead turtle skin samples may also reflect their recent foraging in benthic habitats along
326 the northeast coast of USA. This region has a history of industrial pollution, particularly from
327 activities such as smelting and insufficient sewage treatment, leading to contamination of
328 benthic ecosystems ([Bothner et al. 1998](#), [Eckel et al. 2001](#)). Nevertheless, the loggerhead
329 turtles' postulated generalist diet may act as a mitigating factor, potentially reducing the risk of
330 overaccumulation of As and Cd from a single dietary source.

331 As concentrations of both skin and scute tissues of all three turtle species in this study
332 were higher than that of normal levels (<1 ppm, synonymous with $\mu\text{g g}^{-1}$) in human keratin
333 ([Choucair and Ajax 1988](#), [Franzblau and Lilis 1989](#)) and safe concentrations for Lanzhou catfish
334 (1.288 ppm) ([Lian and Wu 2017](#)). [Finlayson et al. \(2020\)](#) also found As to be cytotoxic to green
335 turtle skin cells. Nevertheless, the As concentrations in the skin of green and loggerhead turtles
336 in this study were lower than that of turtles in Laguna Madre, USA ([Faust et al. 2014](#)) and
337 Murcia, Spain ([Jerez et al. 2010](#)) (**Supplementary Table S2**). However, the scute samples from
338 loggerhead turtles in this study had higher As concentrations than those from Brazil, where
339 turtles were exposed to mining tailings ([Miguel et al. 2022](#)). These regional differences highlight
340 the variability in environmental exposure and underscore the importance of ongoing monitoring.

341 Cd is associated with respiratory damage, cancer, liver disease, and neurological
342 impairment ([ATSDR 2012](#)). Studies have found Cd to cause toxicity in vertebrates when Cd
343 concentrations are above 2 ppm ([Eisler 1985](#)). However, 10% of humans with occupational
344 exposure to Cd have been found to have signs of tubular damage when their blood
345 concentrations were as low as 0.005 6ppm ([ATSDR 2008](#)). As these loggerhead turtles are
346 chronically exposed to Cd and have skin tissue with 0.092 ± 0.025 ppm Cd concentration, we
347 postulate that these loggerhead turtles could potentially be at risk for Cd toxicity due to chronic

348 exposure. Furthermore, Cd concentrations in skin samples from loggerhead and green turtles in
349 this study were higher than those reported in Murcia, Spain, and Texas, USA, respectively
350 (**Supplementary Table S2**). As Cd has been shown to accumulate in human and sea turtle
351 liver and kidneys ([Esposito et al. 2020](#)), we suggest that future studies should analyze Cd
352 concentrations in liver and kidney samples of these cold-stunned turtles.

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

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

370 We found statistically significant differences in Ag and Al concentrations between skin
371 and scute tissues of green and loggerhead turtles respectively. However, there were no other
372 significant differences in Ag or Al concentrations between the different species and tissue types.
373 The concentrations of Ag and Al in these turtles were lower than known safe concentrations for

374 other species, such as ilish fish fingerlings (*Tenualosa ilisha*) (1.450 ppm) for Ag ([Sadat](#)
375 [Sadeghi and Peery 2018](#)) and Atlantic salmon (*Salmo salar*) (92.051 ppm) for Al ([GEI](#)
376 [Consultants, Inc. 2011](#)), indicating that these heavy metals may not be a health concern in
377 these turtles.

378

379 **5. Conclusions**

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

390

391 **Data availability**

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

393

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