HEAVY METAL CONCENTRATIONS IN SEA TURTLES AND THEIR PREY IN THE NORTHWEST ATLANTIC

by

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Mum, Dad

Thank you for having Lyn, Meng, and Zhen first

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LIST OF ABBREVIATIONS

Abbreviation	Definition
Ag	Silver
Al	Aluminum
As	Arsenic
Cd	Cadmium
Co	Cobalt
Cr	Chromium
Fe	Iron
MA	Cape Cod Bay, Massachusetts
MAB	Mid-Atlantic Bight
Mn	Manganese
NC	North Carolina
Ni	Nickel
Pb	Lead
Se	Selenium
Zn	Zinc

ABSTRACT

The Northwest Atlantic Ocean, which surrounds the US eastern coastline, is an area rich in marine life. The US eastern coastline is also highly urbanized, resulting in a lot of pollutants (like heavy metals) entering the marine environment. This is of concern for long-lived marine species like sea turtles. Since sea turtles are long-lived and highly migratory, their tissues can often incorporate these pollutants through environmental and dietary exposure. I collected tissue samples from 5 different sea turtle populations in the Northwest Atlantic and analyzed them for concentrations of silver (Ag), aluminum (Al), arsenic (As), cadmium (Cd), cobalt (Co), chromium (Cr), iron (Fe), manganese (Mn), nickel (Ni), lead (Pb), selenium (Se) and zinc (Zn) using an Inductively Coupled Plasma Mass Spectrometry (ICP-MS). The first chapter looks at skin (reflects exposure ~1 year ago) and scute (reflects exposure from 4-6 years ago) samples collected during necropsies of juvenile green (*Chelonia mydas*) (n=8), Kemp's ridley (*Lepidochelys kempii*) (n=30) and loggerhead (*Caretta caretta*) (n=17) turtles that were found cold-stunned in Cape Cod Bay, Massachusetts. In scute samples, the heavy metal with the highest concentration for green turtles was iron, zinc for loggerhead turtles, and arsenic for Kemp's ridley turtles. In skin samples, the heavy metal with the highest concentration for green turtles was iron, arsenic for loggerhead turtles, and aluminum for Kemp's ridley turtles. Overall, I found scute samples to have higher heavy metal concentrations than skin samples. The second chapter looks at scute samples collected from loggerhead turtles of different life stages. These samples were collected during necropsies of cold-stunned loggerhead turtles from Cape Cod Bay, Massachusetts (CCB; n=17), as well as from live loggerhead turtles in the Mid-Atlantic Bight (MAB; n=37) and off the coast of North Carolina (NC; n=9). We also collected commonly known loggerhead turtle prey items including whelk (*Buccinum undatum*) (n=12), Atlantic scallop (*Placopecten magellanicus)* (n=10) and Jonah crab (*Cancer borealis*) (n=5) from the Mid-Atlantic Bight region to study the occurrence of biomagnification through trophic pathways. NC loggerhead turtles had higher heavy metal concentrations than other locations except for cadmium and zinc, where CCB loggerhead turtles were higher. I found that all heavy metals except silver, cadmium, and lead appear to be biomagnified (TTF>1) in loggerhead turtles. These two chapters provided baseline information on heavy metal concentrations in sea turtles in east coast US.

1. GENERAL INTRODUCTION

1.1 Introduction

The Northwest Atlantic Ocean, off the east coast of USA, is a coastal zone rich in resources. Among the valuable fisheries species are the Atlantic Sea Scallop and American lobster fishery (National Marine and Fisheries Service 2017, Seidov et al. 2022). The NW Atlantic coast is also an important recruitment area for juvenile Atlantic green turtles (*Chelonia mydas*), Kemp's ridley turtles (*Lepidochelys kempii*), and loggerhead turtles (*Caretta caretta*) after their oceanic development stage. Other adult turtles like loggerhead and Kemp's ridley turtles also forage in the warmer months of summer and fall (Morreale et al. 1992) and migrate towards the Southwest Atlantic Ocean in the colder winter months (Musick et al. 1994).

Apart from being important natural habitats for flora and fauna, the US east coast is also home to numerous development projects and technological advancements. This has resulted in a lot of runoff pollution from agriculture, farmland, industrialization, and roads to enter the NW Atlantic Ocean (NRC 2000, Howarth et al. 2002, Valiela and Bowen 2002). Some of the known pollutants are plastic, rare earth elements, and heavy metals (Herbst and Klein 1995, da Silva et al. 2014).

Heavy metals are naturally occurring inorganic elements. They can be found at low concentrations in rocks, water, and soil in non-polluted ecosystems. However, anthropogenic activities have led to an increase in concentration of these elements in the environment. For example, smelting activities release cadmium and arsenic into terrestrial, aquatic, and marine environments (ATSDR 2003, ATSDR 2004, ATSDR 2007). While they are harmless and can even be beneficial to organisms at low concentrations, they become toxic at high concentrations (ATSDR 2004, 2012). The release of heavy metals into marine environments is especially true in the NW Atlantic as this area undergoes a lot of development both on land and in the coastal area.

As the NW Atlantic Ocean is an area where environmental pollution and ecological habitats intersect, it is important to be able to measure pollution levels in the area as well as its organisms. Previous studies have found that the long-lived and highly migratory nature of sea turtles make them possible biomarkers for marine pollution (Bjorndal 1985, Omedes et al. 2024). This is because they incorporate elements from their diet and environment into their body tissues

(Seminoff et al. 2006, Vander Zanden et al. 2013, Barraza et al. 2019, Franzellitti et al. 2004). For example, skin samples have a quicker formation rate, which is likely to reflect heavy metal exposure of sea turtles within approximately 1 year (Seminoff et al. 2006). On the other hand, scute samples (from the carapace) have a longer turnover rate and have been found to reflect heavy metal exposure from 4-6 years ago (Vander Zanden et al. 2013).

We analyzed heavy metal concentrations in a few different populations of sea turtles found in the NW Atlantic Ocean. In the second chapter of my thesis, I analyzed cold-stunned juvenile green, loggerhead, and Kemp's ridley turtles that were encountered in Cape Cod Bay, Massachusetts. These are turtles that did not migrate southward early enough after foraging in the north over summer and were cold stunned (Henwood and Ogren 1987, Keinath 1993, Still et al. 2005). These turtles were juveniles and were probably just transitioning their diet, with loggerhead and Kemp's ridley turtles transitioning to a predominantly carnivorous diet (Nelson 1988, Reyes-López et al. 2021) and green turtles transitioning to a predominantly herbivorous diet (Bjorndal 1985), We collected skin and scute samples to investigate if the different tissues reflect heavy metals from different diet types.

The third chapter of my thesis focuses on loggerhead turtle scutes sampled from different sites within the NW Atlantic Ocean. These turtles were also of different life stages — Cape Cod Bay turtles were the smallest juveniles, North Carolina turtles were late-stage juveniles, and Mid-Atlantic Bight turtles were considered as sub-adults. I also collected loggerhead turtle prey to study the occurrence of biomagnification through their trophic pathways. These preys are whelk (*Buccinum undatum*), Atlantic scallop (*Placopecten magellanicus*) and Jonah crab (*Cancer borealis*). Biomagnification was calculated as a ratio of an element in the tissue compared to the element in the prey item (DeForest et al. 2007). Biomagnification is observed when the ration, which is denoted as Trophic Transfer Factor (TTF), is greater than 1 (Matthews and Fisher 2008). As loggerhead turtles are considered as high-level predators, they are susceptible to the effects of biomagnification of heavy metals.

To date, no other studies have conducted an extensive study on heavy metal concentrations in sea turtles from the NW Atlantic Ocean. I measured seven essential heavy metals (chromium, cobalt, iron, manganese, nickel, selenium, and zinc) to see if the different sea turtle populations were obtaining similar concentrations. I also measured five non-essential heavy metals (arsenic,

aluminum, cadmium, lead, and silver) to better understand the state of our turtles' health and possible physiological implications of the heavy metals on our turtles.

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2. HEAVY METAL CONCENTRATIONS IN SKIN AND SCUTE OF COLD-STUNNED GREEN, KEMP'S RIDLEY AND LOGGERHEAD SEA TURTLES IN CAPE COD BAY, MASSACHUSETTS

2.1 Abstract

Heavy metal pollution is a growing threat to marine life worldwide. As sea turtles are a long-lived and migratory species, their tissues often incorporate these pollutants over vast ocean habitats. In turn, this means that sea turtles can function as broad-scale indicators of heavy metal pollution. To determine heavy metal concentrations in the tissues of sea turtles in the Northwest Atlantic, we collected skin and scute samples during necropsies of green (*Chelonia mydas*) (n=8), Kemp's ridley (*Lepidochelys kempii*) (n=30) and loggerhead (*Caretta caretta*) (n=17) that were found cold-stunned in Cape Cod Bay, Massachusetts. These sea turtle species have different diets, with their skin reflecting heavy metal exposure within approximately 1 year and their scute reflecting exposure from 4-6 years ago. We analyzed the concentrations of silver (Ag), aluminum (Al), arsenic (As), cadmium (Cd), cobalt (Co), chromium (Cr), iron (Fe), manganese (Mn), nickel (Ni), lead (Pb), selenium (Se) and zinc (Zn) using an Inductively Coupled Plasma Mass Spectrometry (ICP-MS). We found different turtle species to have different heavy metals that were of the highest concentrations. In scute samples, the heavy metal with the highest concentration for green turtles was iron (mean ± SD wet weight; 351.8 ± 505.1 μg g^{-1}), zinc (202.8 ± 51.0 μg g^{-1}) for loggerhead turtles, and arsenic (4.68 ± 2.54 μg g^{-1}) for Kemp's ridley turtles. In skin samples, the heavy metal with the highest concentration for green turtles was iron (46.2 ± 46.2 μg g^{-1}), arsenic (5.07 ± 2.26 μg g^{-1}) for loggerhead turtles, and aluminum (25.0 ± 38.2 μg g^{-1}) for Kemp's ridley turtles. Across all species and heavy metals, scute samples had higher heavy metal concentrations compared to skin samples. This is likely due to the accumulation of unwanted heavy metals in the keratinized tissues and their longer turnover rate. Arsenic, cadmium, and cobalt concentrations found in tissues of these stranded turtles are above normal levels found in most other living organisms, including humans.

2.2 Introduction

Modern day anthropogenic activities have resulted in an increase in pollutants in the environment (Borrelle et al. 2020). While much media attention often focuses on plastic pollution, other pollutants such as heavy metals that are typically invisible to the human eye are also of increasing concern (Herbst and Klein 1995, da Silva et al. 2014). These heavy metals are often naturally occurring in non-polluted ecosystems and the organisms that inhabit them; however, as their concentrations rise, they can become toxic to wildlife (ATSDR 2004, 2006, 2012).

Heavy metals can be found at all trophic levels. At the base of the food chain, plants are exposed to heavy metals from uptake of the environment. This leads to some plants, like seaweed, having high heavy metal concentrations in their tissues (Zhou et al. 2008). Despite organisms at the base of the food chain being exposed to heavy metals, different heavy metals biomagnify through trophic levels differently. Some heavy metals (i.e. mercury, lead, and zinc) increase in concentration along trophic pathways, some (i.e. arsenic and nickel) are not passed on through trophic levels, and some (i.e. cadmium, chromium, and copper) remain constant (Sun et al. 2020). With regards to heavy metal that biomagnify, organisms that occupy higher trophic levels are likely to have higher heavy metal concentrations (Jakimska et al. 2011). Thus, the biomagnification of certain heavy metals often poses a threat to high-level predators, like sea turtles. It is therefore important to identify species that can be used as indicators to assess heavy metal concentrations within the marine environment.

Heavy metals can be divided into essential elements (i.e. chromium, iron, selenium, and zinc) that play key roles in physiological and biochemical pathways and non-essential elements (i.e. cadmium and lead) that are not commonly useful to most organisms (Brown and Depledge 1985, Nordberg et al. 2007). Most heavy metals like cobalt, arsenic, and selenium are naturally found in low concentrations. Some of these natural sources include rocks, soil, water, and air (ATSDR 2003, ATSDR 2004, ATSDR 2007). However, many of these elements are entering the environment as by-products of anthropogenic activities. For example, smelting facilities and coal-fired powerplants release cobalt (ATSDR 2004) and pesticides, farm animal feed, and electrical conductors release arsenic (ATSDR 2007). Even heavy metals like cadmium which are not as commonly found make its way into the environment through activities such as electroplating and smelting (ATSDR 2008). As a result, many of these elements dissolve and are deposited into the environmental soil, sediment and water columns (ATSDR 2007).

Sea turtles are long-lived, migratory species (Bjorndal 1985). As they migrate over wide geographic areas, the elements that they are exposed to may be incorporated into their bodily tissues (Seminoff et al. 2006, Vander Zanden et al. 2013, Barraza et al. 2019, Franzellitti et al. 2004). Therefore, sea turtles can serve as possible biomarkers for pollution in the marine ecosystem (Bjorndal 1985, Omedes Martínez et al. 2024). In the northwest Atlantic coast, juvenile Atlantic green turtles (*Chelonia mydas*), Kemp's ridley turtles (*Lepidochelys kempii*), and loggerhead turtles (*Caretta caretta*) recruit to the Atlantic coast after their oceanic development stage. These juvenile turtles forage in the Northwest Atlantic Ocean during the summer and fall when the surface water is warm (Morreale et al. 1992), then, as the water temperature drops, they migrate southward to the Southwest Atlantic Ocean for the winter (Musick et al. 1994). However, if sea turtles do not migrate southward early enough, they are susceptible to cold-stunning (Henwood and Ogren 1987, Keinath 1993, Still et al. 2005). Within a migratory cycle, these turtles may therefore be exposed to pollution from most of the continental shelf of the northwestern Atlantic Ocean.

The recruitment of these turtles to the Atlantic shelf is usually accompanied by a transition in diet. This ontogenetic shift occurs at >20 cm straight carapace length in green and Kemp's ridley turtles, and >25cm in loggerhead turtles. While all three species are omnivorous in their oceanic habitats, when recruiting to coastal habitats, green turtles transition to a predominantly herbivorous diet (Bjorndal 1985), while loggerhead and Kemp's ridley turtles transition to a predominantly carnivorous diet (Nelson 1988, Reyes-López et al. 2021). As the turtles transition their diet, the elements which they were exposed to at different life stages are reflected in different bodily tissues. It has been demonstrated that skin samples have a quicker formation rate, reflecting exposure within approximately 1 year (Seminoff et al. 2006), whereas scute samples reflect exposure from 4-6 years ago (Vander Zanden et al. 2013). Therefore, skin samples are likely to reflect more recent (and possibly local to the NW Atlantic) heavy metal exposure of sea turtles to their environment and diet, whereas scute samples are likely to reflect diet and environmental exposure from their oceanic phase.

Numerous studies have analyzed heavy metal concentrations in sea turtle tissues, and primarily in the organs of dead sea turtles (Sakai et al. 2000, van de Merwe et al. 2010). We propose that skin and scute samples are suitable indicators of turtle diet and habitat at a given period relative to the rate of tissue formation. To date, within the NW Atlantic, there has only been

one heavy metal study conducted on cold-stunned sea turtles in Cape Cod Bay and no other studies in the Mid-Atlantic Bight region. While Innis et al. (2008) investigated heavy metal concentrations in Kemp's ridley sea turtles from the region, no other studies have analyzed heavy metal concentrations in green and loggerhead turtles even though they occupy very similar habitats (Robinson et al. 2020).

The primary goal of our study was to analyze the heavy metal concentrations in the skin and scute samples of green, loggerhead, and Kemp's ridley turtles that cold-stunned in Cape Cod Bay, Massachusetts. We measured seven essential heavy metals (chromium, cobalt, iron, manganese, nickel, selenium, and zinc) to see if the different sea turtle species were obtaining similar concentrations. We measured five non-essential heavy metals (arsenic, aluminum, cadmium, lead, and silver) to better understand the state of our turtles' health and possible physiological implications of the heavy metals on our turtles. Apart from selecting most heavy metals to compare NW Atlantic values to other parts of the world (Faust et al. 2014, Barraza et al. 2019, Jerez et al. 2010, Sakai et al. 2000), few studies have measured aluminum and selenium and sea turtle scute samples (Komoroske et al. 2011, Rossi et al. 2015, Mondragón et al. 2023, Barraza et al. 2019) and no other known studies have analyzed silver and aluminum and sea turtle skin samples.

The objectives of this study were 1) to investigate the difference in heavy metal concentration between the different sea turtle species and 2) to investigate the changes in exposure of sea turtles to heavy metals in recent years through comparative tissue analysis. 3) to compare values obtained from our studies to studies conducted in other parts of the world. We predict that green turtle skin would exhibit higher zinc and cobalt concentrations as these are heavy metals associated with a more herbivorous diet (Smith and Carson 1981). Conversely, we predict that loggerhead and Kemp's ridley turtles' skin would exhibit higher arsenic and cadmium concentrations which are found in cephalopods (Bustamante et al. 1998, Storelli and Marcotrigiano 2003). We also predict that scute samples would have overall higher heavy metal concentrations as keratinized tissue are often used to deposit unwanted inorganic elements (Mondragón et al. 2023) and that heavy metals linger in keratinized tissue longer than skin tissue (Seminoff et al. 2006 and Vander Zanden et al. 2013). We also predict that sea turtles from NW Atlantic are likely to have higher heavy metal concentrations when compared to studies conducted in more pristine environments.

2.3 Methods

2.3.1 Field Sample Collection

This study took place in Cape Cod Bay, Massachusetts, USA, a 1100 km^2 semi-enclosed bay in the south of the Gulf of Maine (Figure 2-1). During the necropsies organized by Massachusetts Audubon Wellfleet Bay Wildlife Sanctuary (WBWS) after the winters of 2019 and 2021, researchers collected skin and scute samples from cold-stunned, recently deceased green (n=8), loggerhead (n=17), and Kemp's ridley (n=30) sea turtles. We collected skin samples from the right shoulder after sterilizing the area between the neck and flipper by twisting a 6mm biopsy punch about 2mm deep to collect 0.5g of tissue (Eckert et al. 1999). We collected scute samples (~0.5g) by scraping the biopsy punch along the rear of the first lateral scute of each turtle (Day et al. 2005).

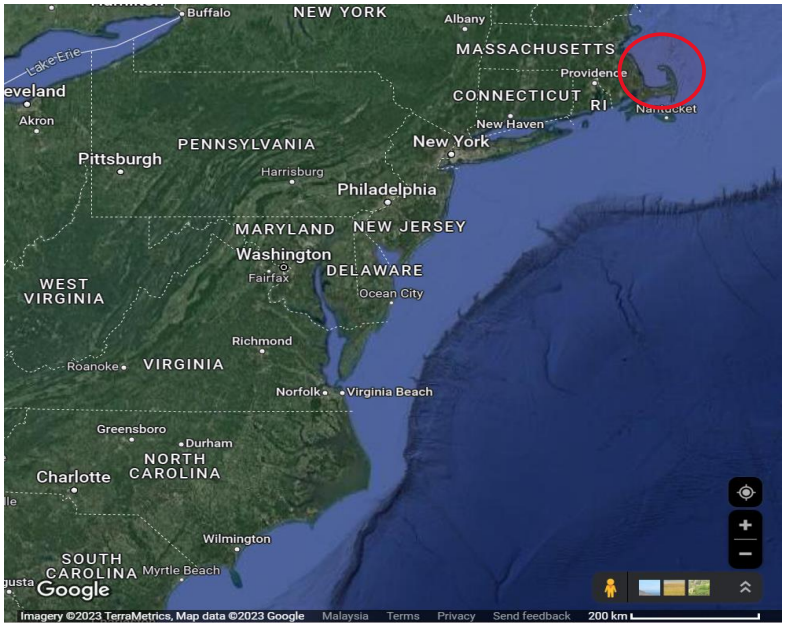

Figure 2-1 Map of study area in the USA. The red circle is Cape Cod Bay, highlighting the hook-shaped bay which results in the entrapment of numerous turtles as they migrate south every winter.

2.3.2 Heavy Metal Analysis

We analyzed both skin and scute samples for silver (Ag), aluminum (Al), arsenic (As), cadmium (Cd), cobalt (Co), chromium (Cr), iron (Fe), manganese (Mn), nickel (Ni), lead (Pb),

selenium (Se) and zinc (Zn) at Purdue University West Lafayette. Heavy metal concentrations were determined using an Inductively Coupled Plasma Mass Spectrometry (ICP-MS) (Thermo Scientific Element 2) equipped with a Teledyne Cetac Aridus II nebulizer following their standard protocols (N. Gou, personal communication, September 15, 2022). We weighed out 0.2-0.5g of each sample and added 2mL of ultra-high purity nitric acid and 0.5mL of ultrapure water into borosilicate digestion vessels (Anton Paar 179436). We digested these samples along with method blanks in a microwave digestor (Anton Paar 7000 Microwave Digestion System) using the preconfigured 'Organic' program. After digestion, we diluted the samples and blanks to a final volume of 50mL using ultrapure water and added 125 µL of 5 ppb indium as an internal standard. We prepared standard solutions ranging from 0.01-1000ppb for all heavy metals from 10 ppm standard solutions purchased from Inorganic Ventures. The limits of detection (LOD) of each heavy metal were calculated as three times the standard deviation of the ten independent measurements of the blank, divided by the slope of the calibration curve. As we were analyzing 110 samples for 12 heavy metals each, we recalibrated the ICP-MS between runs, resulting in a range of LODs. The range of LODs (µg mL^{-1}) of each heavy metal are as follows: 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. We considered heavy metal concentrations below the LOD as undetectable (i.e. 0). We report heavy metal concentrations in µg g^{-1} wet weight of the tissue samples.

2.3.3 Statistical Analysis

We conducted statistical analyses using R (R Core Team, 2020). We executed a two-way ANOVA with post-hoc Tukey HSD tests to compare the concentrations of each heavy metal between species (green, loggerhead, Kemp's ridley) and sample types (skin, scute). When the assumption of normality was not met for ANOVA and post-hoc Tukey HSD, we log transformed the values of non-parametric heavy metal concentrations to normalize the data. For running correlations, we used Pearson's correlation to investigate the significance between heavy metals in skin and scute samples for parametric datasets, and Spearman's correlation for non-parametric datasets. We used Pearson's and Spearman's correlation to assess the relationships between heavy metal concentrations and turtle body size. When needed, 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.

2.4 Results

We sampled 55 juvenile to subadult sea turtles for skin and scute tissues (n=8 green; n=30 Kemp's ridley; n=17 loggerhead; Table 2-1).

Table 2-1 Range, mean and standard deviation of straight carapace length (SCL) of the three different sea turtle species collected during cold-stunned events from Cape Cod Bay, Massachusetts, USA.

Species	*n*	SCL (cm)	
		Range	Mean ± SD
Green *Chelonia mydas*	8	26.5-33.9	28.7 ± 2.33cm
Kemp's ridley *Lepidochelys kempii*	30	18.6-32.8	25.8 ± 2.84cm
Loggerhead *Caretta caretta*	17	28.5-69.2	51.7 ± 9.7cm

2.4.1 Heavy metal concentrations between skin and scute samples within each species

For all species, eight out of twelve heavy metals were found in higher concentrations in scute samples compared to skin samples (silver, aluminum, cadmium, cobalt, chromium, iron, manganese, and zinc). On the other hand, arsenic and selenium concentrations in green turtles and loggerhead turtles as well as nickel concentrations in green turtles and Kemp's ridley turtles were found to be higher in skin samples compared to scute samples. Kemp's ridley turtles had nine elements that were significantly different ($p<0.05$) between its skin and scute samples, loggerhead turtles had four and green turtles had two elements (

Table 2-2). For Kemp's ridley turtles, they were aluminum (skin=25.013±38.247 µg g^{-1}; scute=67.581±77.604 µg g^{-1}, p=0.0004), cadmium (skin=0.058±0.033 µg g^{-1}; scute=0.322±0.178µg g^{-1}, p<0.0001), cobalt (skin=0.027±0.029 µg g^{-1}; scute=0.238±0.379 µg g^{-1}, p<0.0001), chromium (skin=0.262±0.350 µg g^{-1}; scute=1.080±1.419 µg g^{-1}, p<0.0001), iron (skin=44.331±60.660 µg g^{-1}; scute=151.801±116.620 µg g^{-1}, p<0.0001), manganese (skin=0.635±0.931 µg g^{-1}; scute=3.083±3.076 µg g^{-1}, p<0.0001), nickel (skin=3.330±12.226 µg g^{-1}; scute=2.290 ±1.441 µg g^{-1}, p=0.003), lead (skin=0.061±0.077 µg g^{-1}; scute=0.397±0.318 µg g^{-1}, p<0.0001), and zinc (skin=19.980±6.822 µg g^{-1}; scute=166.972±72.265 µg g^{-1}, p<0.0001). For loggerhead turtles, they were arsenic (skin=5.069±2.258 µg g^{-1}; scute=1.792±0.849 µg g^{-1}, p=0.0001), cadmium (skin=0.092±0.025 µg g^{-1}; scute=0.256±0.150 µg g^{-1}, p<0.0001), chromium (skin=0.153±0.088 µg g^{-1}; scute=0.854 ± 0.968 µg g^{-1}, p=0.002) and zinc (skin=11.271±4.850 µg g^{-1}; scute=201.786±50.971 µg g^{-1}, p=0). As for green turtles, they were manganese (skin=0.529±0.655 µg g^{-1}; scute=5.209±9.128µg g^{-1}, p=0.04) and zinc (skin=21.561±9.226 µg g^{-1}; scute=108.095±33.384 µg g^{-1}, p=0).\

Table 2-2 Heavy metal concentrations detected in blood, skin and scute samples of green (*n*=8), Kemp's ridley (*n*=30) and loggerhead (*n*=17) turtles from Cape Cod Bay, Massachusetts, USA.

		Skin		Scute	
Elements	Species	*n*	mean ± SD	*n*	mean ± SD
Non-essential elements					
Silver	Green	*6*	0.006 ± 0.007	*1*	0.034
	Kemp's ridley	*11*	0.009 ± 0.011	*4*	0.049 ± 0.014
	Loggerhead	*11*	0.009 ± 0.006	*2*	0.028 ± 0.010
Aluminum	Green	*8*	22.911 ± 33.710	*7*	140.273±224.762
	Kemp's ridley	*30*	25.013 ± 38.247 [a]	*28*	67.581 ± 77.604 [a]
	Loggerhead	*17*	19.015 ± 14.860	*17*	43.695 ± 35.238
Arsenic	Green	*8*	3.614 ± 2.569	*7*	2.212 ± 1.606
	Kemp's ridley	*30*	4.580 ± 1.753	*30*	4.678 ± 2.536 [b]
	Loggerhead	*17*	5.069 ± 2.258 [a]	*17*	1.792 ± 0.849 [a, b]
Cadmium	Green	*8*	0.075 ± 0.052	*5*	0.144 ± 0.046
	Kemp's ridley	*29*	0.058 ± 0.033 [a, b]	*26*	0.322 ± 0.178 [a]
	Loggerhead	*17*	0.092 ± 0.025 [a, b]	*17*	0.256 ± 0.150 [a]
Lead	Green	*8*	0.050 ± 0.044	*5*	0.328 ± 0.462
	Kemp's ridley	*28*	0.061 ± 0.077 [a]	*19*	0.397 ± 0.318 [a]
	Loggerhead	*17*	0.077 ± 0.083	*12*	0.197 ± 0.264
Essential elements					
Cobalt	Green	*8*	0.051 ± 0.021 [b]	*4*	0.117 ± 0.111
	Kemp's ridley	*27*	0.027 ± 0.029 [a, b]	*8*	0.238 ± 0.379 [a, b]
	Loggerhead	*17*	0.016 ± 0.008 [b]	*3*	0.032 ± 0.012 [b]
Chromium	Green	*8*	0.291 ± 0.345	*6*	0.692 ± 0.869
	Kemp's ridley	*30*	0.262 ± 0.350 [a]	*26*	1.080 ± 1.419 [a]
	Loggerhead	*17*	0.153 ± 0.088 [a]	*16*	0.854 ± 0.968 [a]
Iron	Green	*8*	46.239 ± 46.222	*5*	351.753±505.120
	Kemp's ridley	*30*	44.331 ± 60.660 [a]	*26*	151.801 ± 116.620 [a]
	Loggerhead	*17*	28.849 ± 23.796	*17*	73.884 ± 52.190
Manganese	Green	*8*	0.529 ± 0.655 [a]	*7*	5.209 ± 9.128 [a]
	Kemp's ridley	*30*	0.635 ± 0.931 [a]	*28*	3.083 ± 3.076 [a, b]
	Loggerhead	*17*	0.510 ± 0.410	*17*	1.302 ± 1.480 [b]
Nickel	Green	*8*	3.743 ± 6.497	*8*	2.497 ± 1.799
	Kemp's ridley	*30*	3.330 ± 12.226 [a]	*30*	2.290 ± 1.441 [a]
	Loggerhead	*17*	0.589 ± 0.363	*17*	1.528 ± 1.691
Selenium	Green	*8*	2.181 ± 2.950	*2*	0.233 ± 0.013
	Kemp's ridley	*4*	0.700 ± 0.446	*2*	2.009 ± 1.226
	Loggerhead	*8*	0.940 ± 0.895	*2*	0.257 ± 0.047
Zinc	Green	*8*	21.561 ± 9.226 [a]	*8*	108.095±33.384 [a, b]
	Kemp's ridley	*30*	19.980 ± 6.822 [a]	*30*	166.972 ± 72.265 [a]
	Loggerhead	*17*	11.271 ± 4.850 [a]	*17*	201.786±50.971 [a, b]

NOTE: Skin and scute heavy metal concentration values are µg g^{-1} wet weight
n = number of samples that were detected for respective heavy metals
[a] indicates significant differences between different tissues of the same species
[b] indicates significant differences between tissues of different species

In Kemp's ridley turtles, we observed significant positive correlations between skin and scute samples for aluminum (R=0.38, p=0.04), cadmium (R=0.66, p=0.0004), iron (R=0.46, p=0.02), manganese (R=0.51, p=0.007), nickel (R=0.48, p=0.008) (Figure A-7). In green turtles and loggerhead turtles, we did not observe any significant positive correlations between skin and scute samples (Figure A-8; Figure A-9).

2.4.2 Interspecific patterns of heavy metal concentrations

When comparing interspecific patterns of heavy metal concentrations in skin samples, we observed significantly higher cobalt concentrations in green turtles (0.0.051±0.021 µg g^{-1}) compared to loggerhead turtles (0.016±0.008 µg g^{-1}, p=0.004) and Kemp's ridley turtles (0.027±0.029 µg g^{-1}, p=0.04). We observed significantly higher cadmium concentrations in loggerhead turtles (0.092±0.025 µg g^{-1}) compared to Kemp's ridley turtles (0.058±0.033 µg g^{-1}, p=0.004), but not for green turtles (0.075±0.052 µg g^{-1}, p>0.05).

When comparing interspecific pattens of heavy metal concentrations in scute samples, we observed significantly higher arsenic (p=0.0001), cobalt (p=0.04) and manganese (p=0.05) concentrations in Kemp's ridley turtles (4.68±2.54 µg g^{-1}; 0.238±0.379 µg g^{-1}; 3.08±3.08 µg g^{-1} respectively) compared to loggerhead turtles (1.79±0.85 µg g^{-1}; 0.032±0.012 µg g^{-1}; 1.3±1.5 µg g^{-1} respectively), but not for green turtles (2.21±1.61 µg g^{-1}; 0.117±0.111 µg g^{-1}; 5.21±9.13 µg g^{-1}; p>0.0.5 respectively). We also observed significantly higher zinc in loggerhead turtles (202.8±51.0 µg g^{-1}, p=0.004) than green turtles (108.1±33.4 µg g^{-1}), but not for Kemp's ridley turtles (167.0±72.3 µg g^{-1}, p>0.05).

2.4.3 Correlating skin, scute, and SCL

Linear correlation revealed only silver concentrations in green turtles' skin had a significant positive correlation with increasing carapace size (R=0.81, p=0.05) (Figure A-1). Although there were no significant relationships for all the other heavy metals when running correlations between green turtles' tissues and increasing carapace size, two greens CS.CM.6 (SCL: 28.6cm) and CS.CM.8 (SCL: 28.4cm) were outliers for both skin and scute samples. CS.CM.6 had the highest skin concentrations for aluminum, cobalt, chromium, iron, manganese, and lead (Figure A-1) and was the only green scute sample with silver above the LOD (

Table 2-2). CS.CM.8 on the other hand had the highest skin concentrations for arsenic, cadmium, and zinc (Figure A-1) and the highest scute concentrations for all heavy metals except nickel (Figure A-2). Kemp's ridleys had an overall more clustered heavy metal concentrations in both its skin and scute samples (Figure A-3, Figure A-4), whereas loggerheads heavy metal concentrations were more scattered (Figure A-5, Figure A-6).

2.5 Discussion

The cold-stunned green and Kemp's ridley turtles in our study are of similar sizes and are still considered as juveniles, whereas the loggerhead turtles in our study are slightly bigger, ranging from juveniles to subadults. All our turtles were undergoing or have undergone an ontogenetic shift where they recruit to neritic developmental waters and transition their diets — Kemp's ridley and loggerhead turtles towards a predominantly carnivorous diet (Nelson 1988, Reyes-López et al. 2021), green turtles towards a predominantly herbivorous diet (Bjorndal 1985). Skin samples will reflect more recent exposure (~1 year; Seminoff et al. 2006) whereas scute samples will reflect exposure prior to their ontogenetic shift (4-6 years ago; Vander Zanden et al. 2013). Through the heavy metals we analyzed, we see the shifts in green turtle diet through higher cobalt and zinc concentrations in their skin samples. Furthermore, the changes in arsenic and cadmium concentrations of loggerhead turtles provide insights to elements in which they are exposed to through the environment they forage in. Kemp's ridley turtles' scute samples have 9 (out of 12) heavy metals that are significantly higher than their skin samples highlight the changes in their environmental exposure as well as the possibility of different physiological reaction to unwanted inorganic elements.

2.5.1 Interspecies comparison of heavy metal concentrations – Diet and Environment

Green turtle skin samples had higher cobalt concentration than Kemp's ridley and loggerhead turtles (

Table 2-2). As cobalt is associated with herbivorous diet, this suggests the highly herbivorous diet of green turtles as they approach sexual maturity. In a review by Smith and Carson (1981), they found that cobalt concentrations in algae have a wide range between 0.1ppm to 100ppm, whereas marine shells typically have cobalt concentrations between 0.15ppm to 1.2ppm, far below that of algae. This is supported by the fact that these green turtles' stomachs were found to contain mostly seagrass during necropsies (S. Patel, personal communication, September 19, 2023). Furthermore, the lower cobalt concentrations in Kemp's ridley and loggerhead turtles' skin samples are probable indications of their transition away from algae and seagrass to a more carnivorous diet (Shaver 1991).

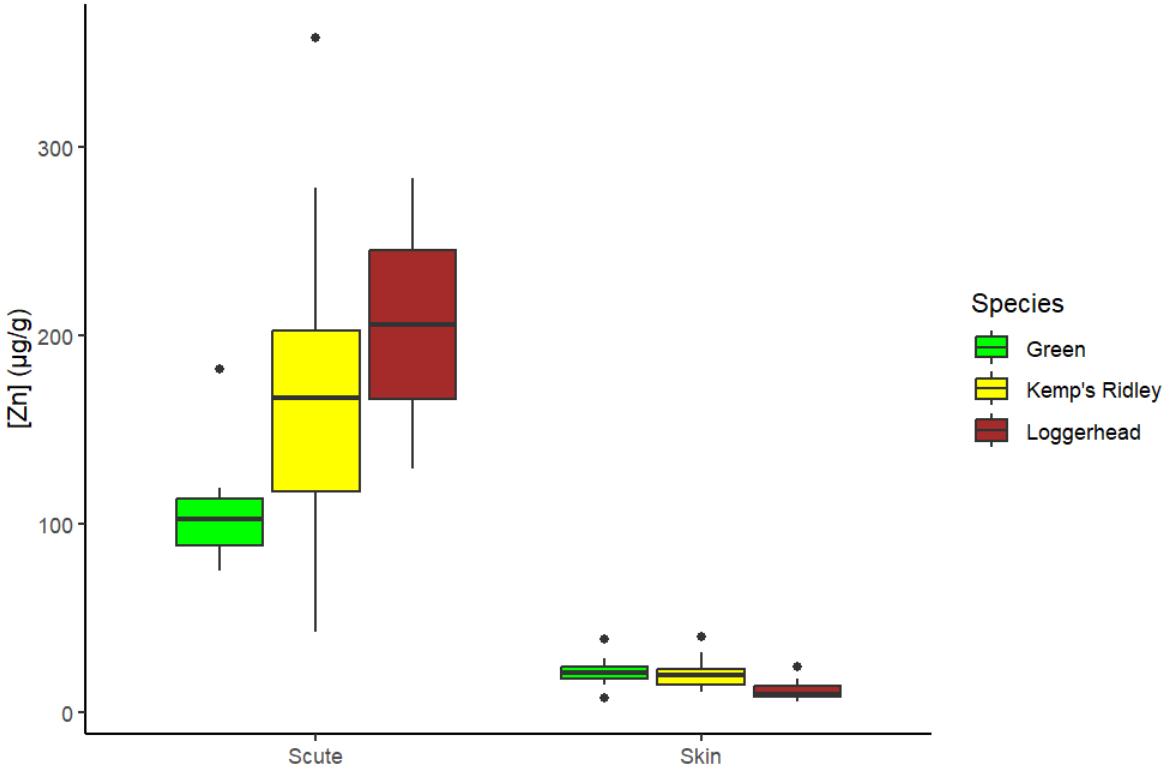

Figure 2-2 Boxplots comparing zinc concentration of scute and skin samples found in greens (n=8), Kemp's ridley (n=30) and loggerhead (n=17) sea turtles in Cape Cod Bay, Massachusetts. Please note the values of the Y axes vary between plots. Boxes are the middle 50% quartiles, lines are median, whiskers are the range of the minimum and maximum values.

Green turtle scute samples had lower zinc concentrations than loggerhead and Kemp's ridley turtle scute samples. However, green turtle skin samples had higher zinc concentrations than

the other two species (Figure 2-2). This inverse pattern likely reflects the dietary transition of green turtles towards herbivory and loggerhead and Kemp's ridley turtles towards carnivory as they reach sexual maturity. Although Mondragón et al. (2023) also found that green turtle scute had lower zinc concentrations compared to other species nesting along the Northeast Coast of Quintana Roo State, Mexico, this finding is interesting as green turtle hatchlings are omnivorous and consume mollusks which have a relatively high zinc concentration (76µg g^{-1} dry weight) compared to the diets of loggerhead turtle hatchlings which consume relatively more algae (zinc: 51.3µg g^{-1} dry weight) (Conti et al. 2007). It is possible that these green sea turtles were consuming more zooplankton in the pelagic waters and could result in lower zinc concentrations in their scute samples. Achary et al. (2020) compiled global zooplankton zinc concentrations that had a wide range of 53-5800.3 µg g^{-1} wet weight. Furthermore, according to Balthis et al. (2009), Cooksey et al. (2010), Balthis et al. (2013), and Cooksey et al. (2014), zinc concentrations in the Gulf of Mexico, South Atlantic Bight, and Mid-Atlantic Bight are similar. As these locations are the likely migratory routes of our sampled turtles, it is unlikely that the varying zinc concentrations in the scute samples of the different species are due to environmental exposure.

Our Kemp's ridley turtles had nine heavy metal concentrations which were significantly higher in the scute samples compared to the skin samples — aluminum, cadmium, cobalt, chromium, iron, manganese, nickel, lead, and zinc. This observed difference could be due to hatchlings being exposed to elevated heavy metals in their early developmental days or due to maternal transfer of heavy metals from nesting Kemp's ridley turtles. Kemp's ridley turtles are the only species which exclusively nest and hatch on the nesting beaches in the Gulf of Mexico. The hatchlings enter the Gulf of Mexico before entering current systems of the Gulf of Mexico or the Northwest Atlantic's Gulf Streams, where they enter their oceanic feeding phase (Reyes-López et al. 2021). The heavy metal levels in the waters of the Gulf of Mexico are generally higher than that of the Atlantic Ocean (Vázquez-Botello et al. Unpublished, Wang et al. 2023). Vázquez-Botello et al. (Unpublished) compiled data from a few studies that revealed the seawater in the Gulf of Mexico had chromium concentrations of 5.2-7.4ppm back in the 1990s. These concentrations are much higher compared to that of the seawater in the Atlantic Ocean where chromium is only found at a concentration of 0.00012ppm (Wang et al. 2023). Furthermore, aluminum and lead levels in northwestern Gulf of Mexico sediments (3.440 ± 1.587% dry weight and 16.622 ± 4.502 µg g^{-1} dry weight respectively) were found to be higher than that of the Mid-

Atlantic Bight (1.374 ± 0.680% dry weight and 9.348 ± 5.858 µg g^{-1} dry weight respectively) (Balthis et al. 2009, Balthis et al. 2013). The elevated heavy metal levels in the Gulf of Mexico are probably a result of the offshore oil and gas activities, which occasionally result in catastrophic events, including the Deep Horizon Oil Spill in 2010. We postulate that the difference in heavy metal concentrations in Kemp's ridley turtles' skin and scute samples indicates that they were exposed to high levels of heavy metals as hatchlings. While this exposure could be a result of them moving through the Gulf of Mexico for a brief period before entering the Northwest Atlantic's current system, it is more likely that their elevated levels of heavy metals came from maternal transfer, where nesting females transfer the excess chemicals in their body to their eggs (Camacho et al. 2017). The higher heavy metal concentrations in Kemp's ridley turtles' scute samples possibly indicate that they deposit unnecessary elements in their scutes as a detoxifying mechanism (Martín et al. 2021).

Kemp's ridley turtles' scute samples had higher arsenic concentrations than loggerhead turtles' scute samples. Maher and Butler (1988) noted that diet is a big contributing factor towards the accumulation of arsenic in marine animals. Apart from crustaceans as a source of arsenic which both loggerheads and Kemp's ridleys have in their diet (Storelli and Marcotrigiano 2003), this higher arsenic concentration in Kemp's ridley turtles' scute is likely due to the algae that the hatchlings consume in their oceanic days. Francesconi and Edmonds (1993) reported that algae can accumulate arsenic up to 50000 times their surrounding seawater. This, coupled with higher arsenic concentrations in the Gulf of Mexico, likely resulted in high exposure of Kemp's ridleys to arsenic as hatchlings. While green and loggerhead turtles do nest in the Gulf of Mexico, it is possible that the cohorts we sampled in the NW Atlantic came from other nesting beaches.

The cadmium and arsenic concentrations in loggerhead turtles' skin samples are likely due to heavy metals found in their diet as well as the environment that they forage in. Loggerhead turtles' skin samples had higher cadmium concentrations when compared to Kemp's ridley turtles. Although not statistically significant, loggerhead turtles' skin samples also had higher arsenic concentrations when compared to the other two species. Loggerhead turtles are likely accumulating arsenic and cadmium from consuming crustaceans such as cephalopods and mollusks (Bustamante et al. 1998, Clark 1992, Storelli and Marcotrigiano 2003). The overall higher arsenic and cadmium concentrations in loggerhead turtles' skin samples are possibly due to loggerhead turtles foraging for benthic organisms in Massachusetts which is a highly polluted area

due to smelting activities (Eckel et al. 2001). Despite the more recent exposure to possibly higher cadmium concentrations as loggerhead turtles forage in Massachusetts benthic environment, loggerhead turtles' skin samples (recent exposure) had lower cadmium concentrations than their scute samples. Since cadmium is not an essential element, the accumulation of cadmium in the scute might be a detoxifying mechanism (Martín et al. 2021).

2.5.2 Comparing heavy metal concentrations to other studies and their implications

Our green turtles' skin samples had higher cobalt and zinc concentrations than loggerhead and Kemp's ridley turtles. While this is likely attributed to a predominantly herbivorous diet (seagrass), our green turtles' skin cobalt concentration (0.051 ± 0.021 µg g^{-1}) was higher than that of other studies (Table 2-3). To the best of our knowledge, Faust et al. (2014) is the only other paper to have studied cobalt concentrations in green turtle skin samples, and all 12 of their turtles had cobalt concentrations below detectable limits. While cobalt is necessary for the development of numerous organisms, excessive exposure to cobalt has been found to cause negative behavioral and physiological effects and could possibly be carcinogenic to humans (ATSDR 2004). Finlayson et al. (2020) also found that high cobalt concentrations are cytotoxic to green turtle skin cells and alter their Glutathione-S-transferase activity, which are enzymes that protect cellular macromolecules. Despite the cobalt concentrations in all species' skin samples being much higher than normal human blood concentrations that range from 0.00005-0.0027 ppm (synonymous with µg g^{-1}) (Catalani et al. 2011), they are much lower than acute toxicity values (LC50) of rainbow trout (1.343-1.704 ppm) (Stubblefield et al. 2020). This indicates that our green turtles may have elevated cobalt levels, but it is probably still far from acute toxicity levels. However, we suggest that future studies should collect blood samples for a more direct comparison to known human blood cobalt concentrations.

While our green and loggerhead turtles had a much lower concentration of arsenic in their skin samples (3.614 ± 2.569 µg g^{-1} and 5.069 ± 2.258 µg g^{-1} respectively) compared to studies conducted in Laguna Madre, USA (Faust et al. 2014) and Murcia, Spain (Jerez et al. 2010) (Table 2-3), our loggerhead turtles' scute samples (1.792 ± 0.849 µg g^{-1}) had higher arsenic concentrations than that of loggerhead turtles (0.96 ± 0.98 µg g^{-1}) sampled in an area affected by mining tailings in Brazil (Miguel et al. 2022). It is worth noting that while Faust et al. (2014) and Jerez et al. (2010) also conducted their studies on juvenile turtles, their skin samples were dried prior to analysis.

From a mathematical perspective, the lack of moisture content makes the skin samples lighter, resulting in the calculation of higher arsenic concentrations. To the best of our knowledge, there are no known moisture values for sea turtle skin samples that could help us standardize these calculations. Furthermore, since arsenic is not an essential element, it is likely that our higher arsenic concentrations compared to that of Miguel et al.'s (2022) study is due to Mid-Atlantic Bight having higher levels of arsenic pollutants, despite Miguel et al.'s (2022) study site being affected by mining tailings. As arsenic is not an essential element, exposure to arsenic could result in negative effects such as digestive irritation, decreased white and red blood cell production, and cancer (ATSDR 2007). Finlayson et al. (2020) found arsenic to be cytotoxic to green turtle skin cells, which might also be the case for loggerhead turtles. Other living organisms, including humans, usually have less than 1.00 ppm of arsenic in their tissue (Eisler 1988, Gomez-Caminero et al. 2001). Furthermore, Lian and Wu (2017) found that safe arsenic concentrations for Lanzhou catfish is 1.288 ppm. As all species' skin and scute samples are above these values, it is probable that they are being exposed to concerning amounts of arsenic throughout their migratory paths and diets as they approach adulthood.

Cadmium concentrations in our turtle skins were higher than that of other studies. Our loggerhead turtles had (0.092 ± 0.025 μg g^{-1}) that is double the concentration (0.04 ± 0.02 μg g^{-1}) of loggerhead turtles' skin sample in Murcia, Spain (Jerez et al. 2010). As mentioned in discussing arsenic concentrations, Jerez et al. (2010) used dried skin samples for their study. This means that their arsenic concentrations would be much lower if they used wet samples. Our green turtles' skin samples also had higher cadmium concentration (0.075 ± 0.052 μg g^{-1}) when comparing to Faust et al. (2014) study in Texas, USA, whose cadmium concentrations were below method detection limits. As Massachusetts is an area with high smelting activities (Eckel et al. 2001), it is likely that these elevated cadmium concentrations are a result of environmental exposure in the area. Cadmium is a non-essential element and has been found to cause respiratory damage, cancer, liver disease, and neural damage in animals (ATSDR 2012). Despite higher cadmium concentrations in our turtles, evidence of contamination is only considered when whole body tissues exceed 2ppm in vertebrates and is only considered life-threatening at 5ppm (Eisler 1985). However, 10% of occupationally exposed humans have shown signs of tubular damage in chronic blood concentrations as low as 0.0056 (ATSDR 2008). This means that our turtles' skin samples are showing higher levels of cadmium concentration compared to occupationally exposed humans,

but lower than that of dangerous levels for vertebrates. As cadmium has been shown to accumulate in human and sea turtle liver and kidneys (Sakai et al. 2000, Storelli et al. 2005), we suggest that future studies should collect liver and kidney samples from the cold-stunned turtles to determine if the turtles have been exposed to excessive cadmium concentrations in the environment.

Our selenium concentrations were comparable to other studies (Faust et al. 2014, Barraza et al. 2019, Jerez et al. 2010). Despite selenium being detected in all our green turtles' skin samples, Kemp's ridley and loggerhead turtles only had less than half of their skin samples which were above detectable levels. Furthermore, selenium concentrations were detected in only 25%, 6.7% and 11.8% of green, Kemp's ridley and loggerhead turtles' scute samples. This is critical as selenium is essential for regulating seleno-aminoacid functions (Thiry et al. 2013). Furthermore, selenium has been found to reduce mercury toxicity impacts in mammals and aquatic biota (Raymond and Ralston 2020). One possible explanation of the lack of selenium in our scute samples is that selenium has been used in mercury regulation mechanisms, and therefore was not deposited in the scute. However, we did not conduct analysis on mercury concentrations.

2.5.3 Correlating Skin, Scute and SCL

We found higher concentrations of heavy metals in scute samples than skin samples across all species. This is likely due to both the accumulation of unwanted inorganic elements in the keratinized tissues (Mondragón et al. 2023) as well as keratinized tissue having lower turnover rates compared to skin tissue (Seminoff et al. 2006 and Vander Zanden et al. 2013). Since keratinized tissue (scute) have lower turnover rates and reflect exposure of 4-6 years (Vander Zanden et al. 2013) and skin tissue only reflect exposure of ~1 year (Seminoff et al. 2006), scute samples tend to accumulate a greater concentration of heavy metals when compared to skin samples. Furthermore, Martín et al. 2021 described such detoxifying mechanisms in feathers of birds and skins of amphibians and reptiles. Despite cobalt, chromium, manganese, nickel, selenium, zinc, silver, arsenic, cadmium, and lead having found to bioaccumulate in organisms (ATSDR 1990, ATSDR 2005, ATSDR 2007, ATSDR 2023, Fatima et al. 2014, Lemly and Smith 1987), we however only found silver to bioaccumulate in green sea turtle skin (Figure A-4). This opposes the findings of Komoroske et al. (2011) where aluminum, manganese, copper, and lead correlated moderately and positively with increasing CCL while mercury had a strong negative correlation.

We did not observe any correlation between the size of the sea turtles with increasing heavy metal concentrations (Figure A-1, Figure A-2, Figure A-3). Larger turtles did not appear to have higher heavy metal concentrations in their skin and scute samples. Despite the significant differences of chromium, manganese, and zinc concentrations between skin and scute samples, we did not find any significant differences between the skin samples of different species. As these are essential elements, this finding indicates that our sea turtles are obtaining these elements from their environment.

We did not find any correlations between skin and scute samples for any of the heavy metals analyzed (Figure A-7, Figure A-8, Figure A-9). Other studies have found correlations between different sea turtle tissues for certain heavy metals. For example, van de Merwe (2010) found strong correlations in cobalt concentrations between blood and liver, kidney, and muscle tissue of green turtles and Bezerra et al. (2013) found correlations in mercury concentrations between muscles and scute samples of green turtles. These correlations indicate that blood and scute samples are useful non-invasive methods for predicting cobalt and mercury concentrations in the green turtles. The fact that we did not find any correlation between skin and scute samples suggests that scute samples (which are relatively less invasive) cannot be used as a replacement for skin biopsies in juvenile green, loggerhead, and Kemp's ridley turtles.

The two green turtle outliers (CS.CM.6 and CS.CM.8) which had higher concentrations of heavy metals in their skin or scute samples are indicative of these two turtles originating from different cohorts compared to the other cold-stunned green turtles (Figure A-4, Figure A-7). The higher levels of heavy metal concentrations tested in these two green turtles (CS.CM.6 skin - Al, Co, Cr, Fe, Mn, Pb; CS.CM.8 skin – As, Cd, Zn; CS.CM.8 scute – Al, As, Cd, Co, Cr, Fe, Mn, Pb) are likely due to these two turtles originating from different nesting populations. On the other hand, the clustered data points of heavy metal concentrations in Kemp's ridley turtle's skin (Figure A-5) and scute samples (Figure A-8) are indicative that most of these turtles have similar diets and have foraged and migrated through similar environments. This contrasts the spread-out data points of heavy metal concentrations in loggerhead turtles' skin (Figure A-6) and scute (Figure A-9) samples, which might be a result of loggerheads having high fidelity to their individual preferences of prey, habitat, and geographical location (Vander Zanden et al. 2010).

Table 2-3 Heavy metal concentrations of blood, skin and scute samples in green, Kemp's ridley and loggerhead sea turtles found in the present study and other studies.

Species	Elements	Skin		Scute		References
		n	mean ± SD	*n*	mean ± SD	
Green	Silver	*6*	0.006 ± 0.007	*1*	0.034	Present Study
				8	0.06 ± 0.02	Barraza et al. (2019)
	Aluminum	*8*	22.9 ± 33.7	*7*	140.3 ± 224.8	Present Study
				20	209.99 ± 40.18	Barraza et al. (2019)
	Arsenic	*8*	3.61 ± 2.57	*7*	2.21 ± 1.61	Present Study
		12	17.6 ± 1.5 [1] *d.w.*	*20*	0.58 ± 0.07 [2]	[1]Faust et al. (2014) [2]Barraza et al. (2019)
	Cadmium	*8*	0.075 ± 0.052	*5*	0.144 ± 0.046	Present Study
		0	<LOD [1]	*20*	0.3 ± 0.06 [2]	[1]Faust et al. (2014) [2]Barraza et al. (2019)
	Lead	*8*	0.050 ± 0.044	*5*	0.328 ± 0.462	Present Study
		0	<LOQ [1]	*20*	3.10 ± 0.96 [2]	[1]Faust et al. (2014) [2]Barraza et al. (2019)
	Cobalt	*8*	0.051 ± 0.021	*4*	0.117 ± 0.111	Present Study
		0	<LOQ [1]	*20*	0.4 ± 0.09 [2]	[1]Faust et al. (2014) [2]Barraza et al. (2019)

Table 2-3 continued

Chromium	*8*	0.29 ± 0.35	*6*	0.69 ± 0.87	Present Study
	12	46.9 ± 4.7 [1] *d.w.*	*20*	0.61 ± 0.1 [2]	[1]Faust et al. (2014) [2]Barraza et al. (2019)
Iron	*8*	46.2 ± 46.2	*5*	351.8 ± 505.1	Present Study
			20	340.53 ± 56.92	Barraza et al. (2019)
Manganese	*8*	0.53 ± 0.66	*7*	5.21 ± 9.1	Present Study
	12	1.15 ± 0.23 [1] *d.w.*	*20*	15.81 ± 4.3	[1]Faust et al. (2014) [2]Barraza et al. (2019)
Nickel	*8*	3.74 ± 6.50	*8*	2.50 ± 1.80	Present Study
	0	<LOD [1]	*20*	2.37 ± 0.44 [2]	[1]Faust et al. (2014) [2]Barraza et al. (2019)
Selenium	*8*	2.18 ± 2.95	*2*	0.23 ± 0.01	Present Study
	12	2.01 ± 0.18 [1] *d.w.*	*20*	0.48 ± 0.06 [2]	[1]Faust et al. (2014) [2]Barraza et al. (2019)
Zinc	*8*	21.6 ± 9.2	*8*	108.1 ± 33.4	Present Study
	12	43.8 ± 4.6 [1] *d.w.*	*20*	225.39 ± 19.15 [2]	[1]Faust et al. (2014) [2]Barraza et al. (2019)

Table 2-3 continued

Loggerhead	Arsenic	*17*	5.07 ± 2.26	*17*	1.79 ± 0.85	Present Study
		2	52.13 ± 6.1 *d.w.*			Jerez et al. (2010)
	Cadmium	*17*	0.092 ± 0.025	*17*	0.256 ± 0.15	Present Study
		2	0.04 ± 0.02 [1] *d.w.*	*6*	0.129 ± 0.034 [2]	[1]Jerez et al. (2010) [2]Sakai et al. (2000)
	Lead	*17*	0.077 ± 0.083	*12*	0.197 ± 0.264	Present Study
		2	0.02 ± 0.03 [1] *d.w.*	*6*	2.42 ± 0.52 [2]	[1]Jerez et al. (2010) [2]Sakai et al. (2000)
	Iron	*17*	28.8 ± 23.8	*17*	73.9 ± 52.2	Present Study
				6	26.2 ± 19.1	Sakai et al. (2000)
	Manganese	*17*	0.51 ± 0.41	*17*	1.3 ± 1.	Present Study
				6	7.01 ± 4.49	Sakai et al. (2000)
	Nickel	*17*	0.59 ± 0.36	*17*	1.53 ± 1.69	Present Study
				6	0.0094 ± 0.022	Sakai et al. (2000)
	Selenium	*8*	0.94 ± 0.90	*2*	0.26 ± 0.05	Present Study
		2	2.25 ± 0.93 *d.w.*			Jerez et al. (2010)
	Zinc	*17*	11.3 ± 4.9	*17*	202.8 ± 51.0	Present Study
		2	13.9 ± 8.6 [1] *d.w.*	*6*	198 ± 37.2	[1]Jerez et al. (2010) [2] Sakai et al. (2000)

Table 2-3 continued

Kemp's ridley	Silver	*11*	0.009 ± 0.011	*4*	0.049 ± 0.014	Present Study
				61	0.102 ± 0.113*	Wang (2005)
	Cadmium	*29*	0.058 ± 0.033	*26*	0.322 ± 0.178	Present Study
				61	0.092 ± 0.087*	Wang (2005)
	Lead	*28*	0.061 ± 0.077	*19*	0.397 ± 0.318	Present Study
				61	0.865 ± 1.219*	Wang (2005)
	Chromium	*30*	0.26 ± 0.3	*26*	1.08 ± 1.42	Present Study
				61	0.23 ± 0.25*	Wang (2005)
	Zinc	*30*	20.0 ± 6.8	*30*	167.0 ± 72.3	Present Study
				61	233.3 ± 208.5*	Wang (2005)

NOTE: Skin and scute heavy metal concentration values are µg g^{-1} wet weight, unless stated otherwise.
** were converted from dry weight to wet weight using the value of 29.1% moisture content as seen in Rodriguez et al. 2022*
d.w. = dry weight; <LOQ = below limit of quantification; <LOD = below method detection limit

2.6 Conclusion

As indicated by the number of heavy metal concentrations that are significantly different between different sea turtle species and their tissue types, it is important to assess this population for essential and non-essential heavy metals that they are exposed to as they transition into adulthood. As there is potential to use skin and scute samples to indicate physiological impacts of elevated heavy metal concentrations on sea turtles, it would be beneficial to also collect organ samples from these cold-stunned turtles to determine if there is a correlation between heavy metal loads that internal organs deal with before they are deposited into skin and scute tissue.

The ability to use skin and scute samples as bioindicators of sea turtle health is of great importance considering the environmental stressors that they are exposed to throughout their lives. Climate change is a pressing issue that is of concern for the turtles in the NW Atlantic. According to thermal models by Patel et al. (2021), thermal windows in the NW Atlantic are expected to increase. This will likely increase the period in which loggerheads (and potentially other turtles) would spend foraging further up north, where we know they are potentially being overexposed to arsenic, cadmium, and cobalt concentrations. If it is true that turtles are accumulating higher heavy metal levels in their scute samples as a way of disposing of unwanted inorganic materials, perhaps this projected prolonged foraging period in the Mid-Atlantic Bight is not of that great a concern. However, this assumption is based off studies on amphibian and avian species (Martín et al. 2021) and it is very likely that the elevated heavy metals are impacting physiological functions of sea turtles in the region negatively.

Determining baseline heavy metal concentrations in Mid-Atlantic Bight turtles allows us to better understand their exposure to pollutants via diet and environment throughout the past 6 years of their lives. Such information is useful in monitoring the health status of the turtles which could be useful information for implementing sustainable development efforts. It is however important to note that different species may metabolize different heavy metals differently (Swarthout et al. 2010) and that different tissues accumulate different heavy metals differently too (Camacho et al. 2017). Therefore, to be able to confidently use sea turtle tissue as indicators of the environment, future analysis would have to compare same tissues from turtles of the same species, similar size and from the same site to our baseline heavy metal concentrations.

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3. COMPARING HEAVY METAL CONCENTRATIONS OF LOGGERHEAD TURTLES AND THEIR PREY ALONG THE US EAST COAST

3.1 Abstract

The eastern coastline of the USA is highly urbanized, which has contributed to a significant anthropogenic output of pollutants (such as heavy metals) entering the environment and washing out to the ocean. This is of particular concern for long-lived marine species like sea turtles. Sea turtles, therefore, make useful indicator species because they incorporate environmental and dietary heavy metals as they migrate through marine habitats. Scute samples (from the carapace) can be collected in a relatively non-invasive manner and can reflect the environment and diet of sea turtles within 4-6 years of their life. To better understand trophic accumulation of heavy metals in loggerhead sea turtles (*Caretta caretta*), we collected scute samples during necropsies of cold-stunned loggerhead turtles from Cape Cod Bay, Massachusetts (CCB; n=17), as well as from live loggerhead turtles in the Mid-Atlantic Bight (MAB; n=37) and off the coast of North Carolina (NC; n=9). The three loggerhead turtle groups are of different life stages and exposure duration, with CCB having the smallest loggerhead turtles and MAB having the largest loggerhead turtles. Therefore, the heavy metal concentrations in their scutes act as indicators of what these sea turtles were exposed to in the environments they experienced and their diet at different stages of their lives. We also collected several commonly known prey items of loggerhead turtles including whelk (*Buccinum undatum*) (n=12), Atlantic scallop (*Placopecten magellanicus)* (n=10) and Jonah crab (*Cancer borealis*) (n=5) from the Mid-Atlantic Bight region. The concentrations of silver (Ag), aluminum (Al), arsenic (As), cadmium (Cd), cobalt (Co), chromium (Cr), iron (Fe), manganese (Mn), nickel (Ni), lead (Pb), selenium (Se) and zinc (Zn) were analyzed using an Inductively Coupled Plasma Mass Spectrometry (ICP-MS). NC loggerhead turtles had higher heavy metal concentrations than other locations except for cadmium (mean ± SD μg g^{-1} wet weight; CCB 0.256 ± 0.150; NC=0.103 ± 0.042; MAB=0.095 ± 0.040) and zinc (CCB=201.79 ± 0.50.97; NC=184.66 ± 70.85; MAB=172.92 ± 52.16), where CCB loggerhead turtles were higher. As NC and CCB loggerhead turtles' scute samples are probably still reflecting heavy metal concentrations from their juvenile omnivorous diets, the higher NC heavy metal concentrations are likely indicative of the heavy metals bioaccumulating in the larger NC turtles. On the other hand, NC turtles having

higher heavy metal concentrations than MAB turtles indicates that MAB turtles' scute samples are probably reflecting heavy metal concentrations from their carnivorous adult loggerhead diet. We found that all heavy metals except silver, cadmium, and lead appear to be biomagnified (TTF>1) in loggerhead turtles. This study provided baseline information on heavy metal concentrations in loggerhead scute samples and their prey in east coast US.

3.2 Introduction

Environmental pollution has been an issue of growing concern in recent years. Advancements in technology, especially in the realms of industrialization, agriculture, and medicine, have resulted in a lot of pollutants entering the marine ecosystem. Heavy metals are one of the concerning groups of pollutants that enter the environment. Most heavy metals like arsenic, selenium, and cobalt are found naturally in rocks, water, and soil at low concentrations (ATSDR 2003, ATSDR 2004, ATSDR 2007). However, these elements are also entering the environment due to anthropogenic activities such as smelting facilities, agriculture, and electroplating (ATSDR 2003, ATSDR 2004, ATSDR 2007). At high concentrations, these elements can become toxic to flora and fauna (ATSDR 2004, ATSDR 2012).

Caretta caretta (loggerhead) turtle is a widely distributed sea turtle species. While it is found abundantly in the Mediterranean Sea, it can also be found in other basins including the Atlantic, Indian, and Pacific Ocean (Caurant et al. 1999, Day et al. 2005, Mingozzi et al. 2007, Nagelkerken et al. 2003). As loggerhead turtles are long-lived and highly migratory, it is likely that their tissue accumulates heavy metals that they are exposed to through diet and the environment (Bjorndal 1985, Vander Zanden et al. 2013, Barraza et al. 2019). While heavy metals can be found at all trophic levels, some elements are biomagnified through trophic levels differently. For example, mercury, lead, and zinc are known to increase along trophic pathways whereas arsenic and nickel do not get passed on (Sun et al. 2020). Since loggerheads are generalist predators and occupy a higher trophic level, they are more susceptible to the biomagnification of heavy metals (Jakimska et al. 2011). The trophic transfer factor (TTF) is used as an indicator of biomagnification in an organism's diet and is the ratio of the specific elemental concentration in an organism's tissue compared to the concentration of the food items (DeForest et al. 2007). Thus, a TTF value >1 suggests biomagnification is occurring (Matthews and Fisher 2008).

The northwest Atlantic Ocean, off the east coast of the USA, is a popular foraging and migratory route for loggerhead turtles (Patel et al. 2016, TEWG 2009, Winton et al. 2018). In summer and early fall, loggerhead turtles are found in regions spanning from Cape Hatteras, North Carolina to Long Island, New York. In late fall to spring, they inhabit more southerly waters along the coast of Florida (TEWG 2009). Loggerhead turtles in this region have been found to feed on gelatinous prey (e.g., Lion's mane jellies, comb jellies and salps) in the pelagic waters, and on benthic crustaceans (e.g., rock crabs and Atlantic Sea scallops) in the coastal shelves (Smolowitz et al. 2015). As we know the diets of loggerhead turtles in this area, they are good target species for understanding the occurrence of heavy metal biomagnification through their diets.

Several studies have assessed heavy metal concentrations in the tissues of loggerhead turtles (Sakai et al. 2000, van de Merwe et al. 2010), including those using scute samples (Casini et al. 2018, Miguel et al. 2022, Mondragón et al. 2023); however, no studies have analyzed heavy metal concentrations in loggerhead turtles from the NW Atlantic. Scute samples have become an increasingly popular tool to study trace metals in sea turtles as it is a relatively easy and non-invasive process. (Seminoff et al. 2006). Furthermore, scute samples have slower isotopic turnover rates that reflect the environment and diet of loggerhead turtles for their last 4-6 years (vander Zanden et al. 2013).

The main goal of our study was to determine the differential exposure to heavy metals as reflected in scute samples and prey items of loggerhead turtles in the NW Atlantic Ocean. We collected scute samples of loggerhead turtles from 3 different locations (Cape Cod Bay Massachusetts, Mid-Atlantic Bight, North Carolina) within the northwestern Atlantic Ocean, as well as loggerhead prey — whelk (*Buccinum undatum*), Atlantic scallop (*Placopecten magellanicus*) and Jonah crab (*Cancer borealis*). To investigate if our loggerhead turtles were accumulating similar concentrations of essential heavy metals, we measured chromium, cobalt, iron, manganese, nickel, selenium, and zinc. To obtain an overview of our loggerhead turtles' health and possible physiological implications of non-essential heavy metals, we measured the concentrations of arsenic, aluminum, cadmium, lead, and silver.

The objectives of this study were 1) to investigate the difference in heavy metal concentrations between loggerhead turtles sampled from different locations and life stages within the NW Atlantic 2) to investigate if heavy metals are biomagnified through trophic pathways of loggerhead sea turtles. 3) to compare heavy metal concentrations in our loggerhead turtles with

studies conducted in other regions of the world. We predict that the bigger sized turtles from Mid-Atlantic Bight would have higher concentrations of arsenic and cadmium which are found in cephalopods associated with adult diets (Bustamante et al. 1998, Storelli and Marcotrigiano 2003). We also predict that lead and zinc would have a TTF>1 as these are heavy metals associated with biomagnification (Sun et al. 2020). Lastly, we predict that our loggerhead turtles in the NW Atlantic would have higher heavy metal concentrations when compared to other studies conducted in more pristine environments.

3.3 Methods

3.3.1 Field Sample Collection

This study took place in three areas off the east coast of the United States of America Cape Cod Bay in Massachusetts (CCB), Mid-Atlantic Bight (MAB) and the coast of North Carolina (NC) (Figure 3-1). CCB is a 1100 km^2 semi-enclosed bay where cold-stunned sea turtles wash up in the winter. During the necropsies organized by Massachusetts Audubon Wellfleet Bay Wildlife Sanctuary (WBWS) after the winters of 2019 and 2021, researchers collected scute samples from cold-stunned, recently deceased loggerhead sea turtles (n=17). MAB is a coastal shelf located 40-100km off the shores of New Jersey through Virginia, USA (latitudinal range = 37.0° to 40.0°; longitudinal range = -75.5° to -73.0°) and NC is on the coastal shelf less than 10km off the shores of North Carolina (latitudinal range = 35.10° to 35.14°; longitudinal range = -75.71° to -75.66°). For MAB and NC sites, researchers from the Coonamessett Farm Foundation collected samples from live loggerheads (n=37; n=9 respectively) (under ESA permit 23639). See Patel et al. 2018 for details on loggerhead captures at-sea. These scute samples (~0.5g) were collected by scraping the biopsy punch along the rear of the first lateral scute of each turtle (Day et al. 2005). Scute samples from live turtles were collected only if the turtles exhibited no external injuries (Barraza et al. 2019, Bean and Logan 2019, Day et al. 2005).

Commercial scallop fishermen in the area where *C. caretta* are known to forage (Patel et al. 2016) provided samples of several prey taxa for loggerhead turtles, including scallops, whelks, and crabs – from the Mid-Atlantic Bight region. To prepare the prey samples for heavy metal analysis, we separated the scallop and whelk meat from the shell, and the operculum of the whelk was also processed separately. All samples were weighed and placed in the oven at 60°C until

constant weight was obtained. Upon samples being completely dried, we crushed the samples using a mortar and pestle until the samples were in powder form.

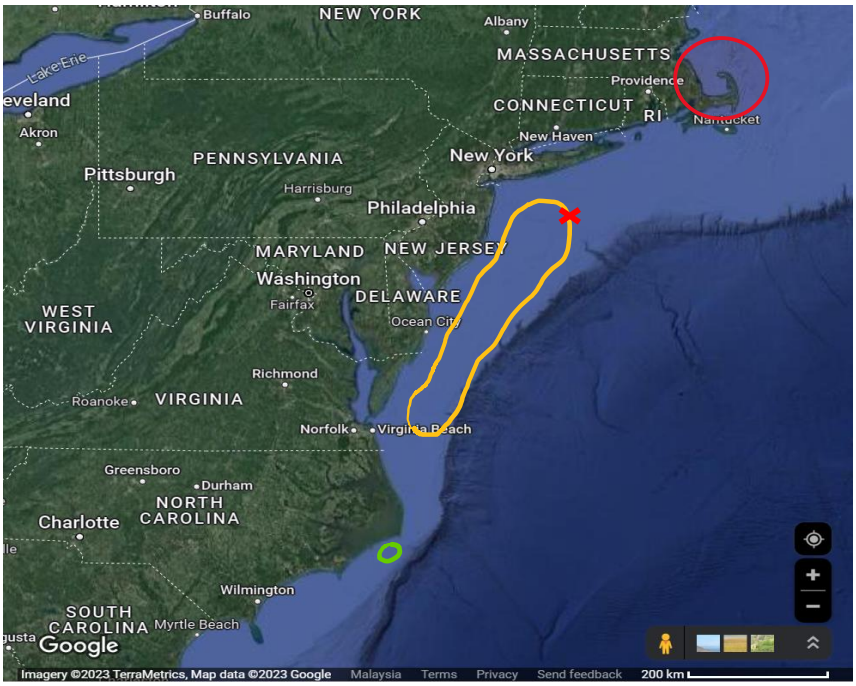

Figure 3-1 Map of study area in the USA. The red circle is Cape Cod Bay, highlighting the hook-shaped bay which results in the entrapment of numerous turtles as they migrate south every winter. The orange shape is the mid-Atlantic bight region, 40-100km from the shore, and the green circle is the coastal area off the shores of North Carolina, less than 10km from shore. The red cross is the commercial scallop fishing site where our prey samples were collected from.

3.3.2 Heavy Metal Analysis

We analyzed both skin and scute samples for silver (Ag), aluminum (Al), arsenic (As), cadmium (Cd), cobalt (Co), chromium (Cr), iron (Fe), manganese (Mn), nickel (Ni), lead (Pb), selenium (Se) and zinc (Zn) at Purdue University West Lafayette. Heavy metal concentrations were determined using an Inductively Coupled Plasma Mass Spectrometry (ICP-MS) (Thermo Scientific Element 2) equipped with a Teledyne Cetac Aridus II nebulizer following their standard protocols (N. Gou, personal communication, September 15, 2022). We weighed out 0.2-0.5g of each sample and added 2mL of ultra-high purity nitric acid and 0.5mL of ultrapure water into borosilicate digestion vessels (Anton Paar 179436). We digested these samples along with method blanks in a microwave digestor (Anton Paar 7000 Microwave Digestion System) using the preconfigured ‘Organic’ program. After digestion, we diluted the samples and blanks to a final

volume of 50mL using ultrapure water and added 125 µL of 5 ppb indium as an internal standard. We prepared standard solutions ranging from 0.01-1000ppb for all heavy metals from 10 ppm standard solutions purchased from Inorganic Ventures. Due to the different sample preparation methods, the heavy metal concentrations of scute samples were expressed in µg g^{-1} wet weight, whereas the prey samples were expressed in µg g^{-1} dry weight. The limits of detection (LOD) of each heavy metal were calculated as three times the standard deviation of the ten independent measurements of the blank, divided by the slope of the calibration curve. As we were analyzing 90 samples for 12 heavy metals each, we recalibrated the ICP-MS between runs, resulting in a range of LODs. The range of LODs (µg mL^{-1}) of each heavy metal are as follows: Ag: 0.000007-0.00027, Al: 0.00012-0.03014, As: 0.000007-0.0001, Cd: 0.000008-0.00009, Co: 0.00001-0.00011, Cr: 0.000008-0.00056, Fe: 0.00168-0.0168, Mn: 0.00003-0.00063, Ni: 0.00003-0.00153, Pb: 0.00001-0.00008, Se: 0.00016-0.00153, Zn: 0.00006-0.00072. We considered heavy metal concentrations below the LOD as undetectable (i.e. 0). We report heavy metal concentrations in µg g^{-1} wet weight of the tissue samples.

3.3.3 Statistical Analysis

We conducted statistical analyses using R (R Core Team, 2020). We executed a one-way ANOVA to compare the concentrations of each heavy metal between loggerhead turtles from different locations (CCB, MAB, NC). When the assumption of normality was not met for ANOVA, we log transformed the values of non-parametric heavy metal concentrations to normalize the data. For running correlations, we used Pearson's correlation to investigate the significance between heavy metals in skin and scute samples for parametric datasets, and Spearman's correlation for non-parametric datasets. We used Pearson's and Spearman's correlation to assess the relationships between heavy metal concentrations and turtle body size. When needed, 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).

For the prey samples, we ran two subsets of crushed prey samples per prey. When the difference between heavy metal concentrations of both subsets were greater than 1 magnitude, we assumed that the crushed samples were not well mixed and ran a third subset for analysis. We also converted the heavy metal concentrations of loggerhead scutes from µg g^{-1} wet weight to µg g^{-1}

dry weight, using the value of 29.1% moisture content (Rodriguez et al. 2022). We calculated the TTF as a ratio of the median concentration of each heavy metal in the loggerhead scute per location to the median concentration of each prey sample (DeForest et al. 2007).

3.4 Results

We sampled 63 loggerhead sea turtles for scute tissue (n=17 MA; n=9 NC; n=37 MAB). TEWG (2009) classifies loggerhead life stages based on their straight carapace length (SCL) — Stage II juveniles range between 41-82cm SCL, Stage III juveniles range between 63-100cm SCL, and adults are greater than 82cm SCL. Due to the overlap in the groupings, our loggerhead turtles from CCB and NC are classified as Stage II or Stage III juveniles, and MAB loggerhead turtles are classified as Stage III juvenile or adults (Table 3-1). It is unlikely that the CCB and NC turtles have reached sexual maturity while some of the MAB turtles have possibly reached sexual maturity. Heavy metal concentrations of the scute samples are reported in

Table 3-2.

Table 3-1 Range, mean and standard deviation of straight carapace length (SCL) of the loggerheads collected during cold-stunned events from Cape Cod Bay in Massachusetts, Mid-Atlantic Bight and North Carolina coast in the USA.

Location	*n*	SCL (cm)	
		Range	Mean ± SD
Cape Cod Bay (CCB)	17	28.5-69.2	51.7 ± 9.7cm
North Carolina Coast (NC)	9	53.2-82	68.7 ± 10.3cm
Mid-Atlantic Bight (MAB)	37	64.6-95.6	82.9 ± 8.4cm

Table 3-2 Heavy metal concentrations detected in scute samples of loggerheads from Cape Cod Bay, Massachusetts (*n*=17), North Carolina (*n*=9) and Mid-Atlantic Bight (*n*=37).

	Cape Cod, MA		North Carolina		Mid-Atlantic Bight	
Elements	*n*	mean ± SD	*n*	mean ± SD	*n*	mean ± SD
Essential elements						
Silver	*2*	0.028 ± 0.010	*0*	<LOD	*3*	0.030 ± 0.015
Aluminum*	*17*	43.70 ± 35.24	*9*	246.67 ± 229.79	*37*	68.21 ± 68.79
Arsenic*	*17*	1.79 ± 0.85	*9*	2.78 ± 1.52	*32*	0.83 ± 0.56
Cadmium*	*17*	0.256 ± 0.150	*4*	0.103 ± 0.042	*20*	0.095 ± 0.040
Lead*	*12*	0.197 ± 0.264	*7*	0.509 ± 0.350	*16*	0.125 ± 0.100
Non-essential elements						
Cobalt	*3*	0.032 ± 0.012	*8*	0.124 ± 0.130	*10*	0.095 ± 0.059
Chromium*	*16*	0.854 ± 0.968	*7*	4.187 ± 2.517	*35*	3.189 ± 3.977
Iron*	*17*	73.89 ± 52.19	*9*	425.17 ± 361.99	*37*	245.65 ± 271.21
Manganese*	*17*	1.302 ± 1.480	*9*	5.392 ± 4.589	*37*	2.962 ± 3.038
Nickel	*17*	1.528 ± 1.691	*1*	3.258	*37*	1.097 ± 0.511
Selenium*	*2*	0.257 ± 0.047	*1*	63.357	*6*	11.383 ± 11.066
Zinc	*17*	201.79 ± 50.97	*9*	184.66 ± 70.85	*37*	172.92 ± 52.16

NOTE: Scute heavy metal concentration values are reported both in µg g^{-1} wet weight.
**indicates significant differences between different locations*
<LOD = below method detection limit

3.4.1 Heavy metal concentrations of scute samples from loggerhead turtles in different locations

We found scute samples collected from turtles in different locations to differ statistically in aluminum (p<0.0001), arsenic (p<0.0001), cadmium (p<0.0001), chromium (p=0.002), iron (p<0.0001), manganese (p<0.0001), lead (p=0.008) and selenium (p=0.004). Across the different locations, we found iron (CCB=73.88±52.19 µg g^{-1}; NC=425.17±361.99 µg g^{-1}; MAB=245.65±271.21 µg g^{-1}) and selenium (CCB=0.257±0.047 µg g^{-1}; NC=63.357 (n=1) µg g^{-1}; MAB=11.383±11.066 µg g^{-1}) to vary significantly. We also found aluminum to be much higher in NC samples (CCB=43.70±35.24 µg g^{-1}; NC=246.67±229.80 µg g^{-1}; MAB=68.21±68.79 µg g^{-1}) and chromium to be much lower in CCB samples (CCB=0.854±0.968 µg g^{-1}; NC=4.187±2.517 µg g^{-1}; MAB=3.189±3.977 µg g^{-1}).

We did not observe any strong correlations between heavy metals and increasing carapace size (Figure B-1). However, there were a few heavy metals that did have significant relationships but weak correlations when heavy metal concentrations and increasing carapace size were compared. Arsenic (R=-0.5, p<0.0001) and cadmium (R=-0.55, p<0.0001) decreased with increasing carapace size, whereas chromium (R=0.37, p=0.0058), iron (R=0.34, p=0.0079) and manganese (R=0.27, p=0.0037) increased with increasing carapace size (Figure B-1). We found that zinc concentrations across the three loggerhead locations were not consistent, and the data was varied over a wide range (87.42-304.69 µg g^{-1}).

It is of interest to note that one turtle from North Carolina, NC.CC.2 (SCL: 60.0cm) was an outlier for numerous heavy metals across all sites. When we compared this turtle to other NC turtles, we found it to have the highest concentrations for aluminum, cobalt, iron, manganese, and selenium. When compared to turtles from all sites, we found it to have the highest concentrations for aluminum (CCB=43.70±35.24 µg g^{-1}; NC=246.67±229.79 µg g^{-1}; MAB=68.21±68.79 µg g^{-1}) and selenium (CCB=0.257±0.047 µg g^{-1}; NC=63.357 µg g^{-1}; MAB=11.383±11.066 µg g^{-1}), and second highest concentrations for manganese (CCB=1.302±1.480 µg g^{-1}; NC=5.392±4.589 µg g^{-1}; MAB=2.962±3.038 µg g^{-1}), iron (CCB=73.89±52.19 µg g^{-1}; NC=425.17±361.99 µg g^{-1}; MAB=245.65±271.21 µg g^{-1}), and cobalt (CCB=0.032±0.012 µg g^{-1}; NC=0.124±0.130 µg g^{-1}; MAB=0.095±0.059 µg g^{-1}).

3.4.2 Heavy metal concentrations in loggerhead turtles and their prey

We analyzed a total of 27 prey samples for heavy metals (n=5 crab; n=10 scallop; n=13 whelk) (Table 3-3). However, one of the whelk samples was empty and so it's flesh or operculum could not sampled, only the shell (Table 3-4).

Table 3-3 Range, mean and standard deviation of prey dry weight collected off the coast of New Jersey by commercial scallop fishermen.

Prey	*n*	Weight (g)	
		Range	Mean ± SD
Crab *Cancer borealis*	5	53.0-158.7	90.8 ± 40.4cm
Scallop *Placopecten magellanicus*	10	61.2-131.7	91.5 ± 23.2cm
Whelk *Buccinum undatum*	12	16.8-35.5	23.9 ± 45.36cm

Table 3-4 Heavy metal concentrations (μg g^{-1} dry weight) detected in whole crabs (n=5), scallop flesh (n=10) and shell (n=10), whelk flesh (n=12), shell (n=13) and operculum (n=12) collected from the coast of New Jersey, USA.

	Crab		Scallop				Whelk					
			Flesh		Shell		Flesh		Shell		Operculum	
Elements	*n*	mean ± SD	*n*	mean ± SD	*n*	mean ± SD	*n*	mean ± SD	*n*	mean ± SD	*n*	mean ± SD
Non-essential elements												
Silver	*5*	0.842 ± 0.37	*5*	0.286 ± 0.097	*2*	0.358 ± 0.255	*12*	2.922 ± 1.471	*4*	0.447 ± 0.200	*1*	0.395
Aluminum	*5*	592.14 ± 401.49	*10*	1607.503 ± 661.01	*10*	87.47 ± 43.99	*12*	76.59 ± 44.81	*13*	169.76 ± 119.98	*12*	795.10 ± 489.42
Arsenic	*5*	18.31 ± 13.19	*10*	6.22 ± 0.68	*10*	1.68 ± 0.68	*12*	31.86 ± 12.82	*13*	1.63 ± 1.28	*12*	4.66 ± 3.90
Cadmium	*5*	4.69 ± 4.86	*10*	29.47 ± 6.16	*10*	0.30 ± 0.20	*12*	22.00 ± 15.64	*13*	1.10 ± 1.84	*11*	2.39 ± 2.88
Lead	*5*	1.59 ± 0.87	*10*	2.196 ± 1.004	*10*	0.252 ± 0.175	*12*	0.182 ± 0.047	*13*	0.332 ± 0.280	*12*	1.95 ± 1.45
Essential elements												
Cobalt	*5*	0.446 ± 0.269	*10*	0.781 ± 0.223	*4*	0.301 ± 0.379	*12*	0.133 ± 0.051	*8*	0.145 ± 0.217	*11*	0.44 ± 0.24
Chromium	*5*	1.77 ± 1.34	*10*	3.17 ± 1.42	*1*	1.33	*0*	<LOD	*5*	0.58 ± 0.39	*4*	2.97 ± 1.35
Iron	*5*	1242.07 ± 1047.27	*10*	2336.57 ± 964.94	*10*	105.71 ± 53.86	*12*	113.33 ± 31.52	*13*	174.13 ± 163.13	*12*	968.12 ± 756.34
Manganese	*5*	246.70 ± 131.17	*10*	51.70 ± 19.86	*10*	20.34 ± 7.06	*12*	6.71 ± 1.94	*13*	23.19 ± 14.26	*12*	153.92 ± 12.72
Nickel	*4*	0.956 ± 0.401	*6*	1.587 ± 0.380	*0*	<LOD	*0*	<LOD	*0*	<LOD	*3*	3.073 ± 0.872
Selenium	*5*	8.80 ± 5.64	*10*	8.61 ± 8.99	*10*	19.37 ± 4.81	*10*	1.54 ± 0.65	*13*	10.42 ± 9.20	*12*	11.79 ± 5.91
Zinc	*5*	27.64 ± 22.66	*10*	43.86 ± 7.48	*3*	2.36 ± 1.87	*12*	178.38 ± 68.89	*7*	4.22 ± 4.82	*12*	38.19 ± 38.22

NOTE: <LOD = below detection limit

When comparing median heavy metal concentrations between loggerhead turtle groups and prey samples (Table B-1), we found CCB turtles consistently to have one of the lowest concentrations for all heavy metals, whereas MAB was ranked in the middle for all heavy metals. The exception to this is zinc, where all loggerhead groups had higher concentrations than any prey samples.

We found six heavy metals in MAB scutes to have a TTF>1 (Table 3-5). They are chromium in crab; whelk shell and scallop shell; nickel in crab; iron and cobalt in whelk flesh, whelk shell and scallop shell; zinc in all prey samples; selenium in crab, whelk flesh and scallop flesh. We found nine heavy metals in NC loggerhead turtles to have a TTF>1 (Table 3-5). They are lead in whelk flesh, whelk shell and scallop shell; arsenic in whelk shell and scallop shell; aluminum in whelk flesh, whelk shell and scallop shell; chromium in crab, whelk operculum, whelk shell, scallop flesh and scallop shell; nickel in crab, whelk operculum and scallop flesh; iron in whelk flesh, whelk shell and scallop shell; cobalt in whelk shell; zinc and selenium in all prey components.

We found zinc to be the only heavy metal to have a TTF>1 across all prey samples and locations (Table 3-5). Selenium was the second most with TTF>1 in all prey samples in the NC turtles, and crab, whelk flesh and scallop flesh in MAB turtles. We found that arsenic only had a TTF>1 in whelk shell and scallop shell in NC turtles. On the other hand, we found manganese, silver, and cadmium to have a TTF<1 across all prey samples and locations. In MAB turtles, lead and aluminum had TTF<1 across all prey samples.

Table 3-5 Trophic transfer values for loggerheads from Cape Cod Bay, Massachusetts, Mid-Atlantic Bight and North Carolina.

Heavy Metals	Mid-Atlantic Bight						North Carolina					
	Crab	Scallop Flesh	Scallop Shell	Whelk Flesh	Whelk Shell	Whelk Operculu	Crab	Scallop Flesh	Scallop Shell	Whelk Flesh	Whelk Shell	Whelk Operculu
Silver	0.03	0.13	0.09	0.01	0.07	0.08	NA	NA	NA	NA	NA	NA
Aluminum	0.09	0.03	0.57	0.79	0.43	0.07	0.42	0.15	**2.61**	**3.60**	**1.98**	0.32
Arsenic	0.06	0.17	0.71	0.04	0.83	0.29	0.17	0.49	**2.04**	0.11	**2.39**	0.83
Cadmium	0.05	0.00	0.47	0.01	0.31	0.09	0.05	0.00	0.51	0.01	0.33	0.09
Lead	0.08	0.06	0.57	0.66	0.47	0.06	0.53	0.39	**3.65**	**4.24**	**3.03**	0.42
Cobalt	0.28	0.18	**1.18**	**1.07**	**3.81**	0.33	0.24	0.15	0.99	0.90	**3.19**	0.28
Chromium	**1.63**	0.62	**1.53**	NA	**4.55**	0.72	**3.35**	**1.27**	**3.13**	NA	**9.32**	**1.47**
Iron	0.25	0.11	**2.32**	**2.02**	**1.65**	0.30	0.40	0.17	**3.76**	**3.27**	**2.67**	0.48
Manganese	0.01	0.05	0.13	0.36	0.12	0.02	0.02	0.09	0.24	0.66	0.21	0.04
Nickel	**1.29**	0.78	NA	NA	NA	0.38	**4.12**	**2.48**	NA	NA	NA	**1.20**
Selenium	**1.06**	**1.73**	0.44	**5.55**	0.81	0.73	**10.43**	**17.07**	**4.30**	**54.60**	**7.95**	**7.19**
Zinc	**10.20**	**5.35**	**152.25**	**1.38**	**129.02**	**9.68**	**8.21**	**4.31**	**122.48**	**1.11**	**103.79**	**7.79**

Trophic transfer factors >1 are bolded, indicating potential risk of biomagnification.
NAs are heavy metal concentration ratios which were not calculated due to one or both values being <LOD.

3.5 Discussion

Our CCB and NC loggerhead turtles are still considered to be in their juvenile stages whereas some of our MAB loggerhead turtles are considered adults. While we can be quite certain that most of our MAB loggerhead turtles have recruited to neritic developmental waters and transitioned to a predominantly carnivorous diet, our CCB and NC turtles are probably undergoing or have just undergone this ontogenetic shift (Nelson 1988). Since scute samples reflect exposure from 4-6 years ago, it is likely that CCB and NC turtles' scute samples may have yet to reflect heavy metals from a predominantly carnivorous diet (Vander Zanden et al. 2013). Through analyzing heavy metal concentrations, we see shifts in loggerhead turtle diet through higher chromium, manganese, iron, and selenium concentrations in the MAB turtles, despite them having mean SCL measurements greater than CCB but smaller than NC turtles. We also see effects of environmental exposure in CCB turtles as they have the highest cadmium concentrations despite being the smallest sized turtles. As for comparing heavy metal concentrations in loggerhead turtle scute samples to their prey, zinc is the only heavy metal to show signs of biomagnification across all samples and prey, whereas silver, cadmium and manganese do not show any signs of biomagnification across all samples and prey.

3.5.1 Inter-site comparison of heavy metal concentrations – Environment

Despite CCB (0.256 ± 0.150 μg g^{-1}) loggerhead turtles being the smallest in size, they had the highest cadmium concentrations when compared to NC (0.103 ± 0.042 μg g^{-1}) and MAB (0.095 ± 0.040 μg g^{-1}) samples (

Table 3-2). We postulate that the higher cadmium concentrations in the CCB samples likely stem from Massachusetts smelting sites (Eckel et al. 2001) that releases cadmium as a by-product (Bradl 2005). As cadmium is a non-essential element, it is likely that the cadmium is accumulating in the loggerhead turtles' carapace as a detoxifying mechanism (Martín et al. 2021). This is supported by the fact that cadmium concentrations have a negative correlation with increasing carapace size, indicating that cadmium does not bioaccumulate in the loggerheads (Figure B-1). We also did not find MAB loggerhead turtles to have a TTF>1 when compared to any prey items, indicating that cadmium does not biomagnify up trophic levels (Table 3-5). Sun et al. (2020) also found cadmium to not biomagnify in higher trophic levels through marine food webs.

3.5.2 Inter-site comparison of heavy metal concentrations - Diet

Although NC loggerhead turtles are bigger than CCB turtles and smaller than MAB turtles, we found NC turtles to have a significantly higher concentration of chromium, manganese (CCB: 1.302 ± 1.480 μg g^{-1}; NC: 5.329 ± 4.589 μg g^{-1}; MAB: 2.962 ± 3.038 μg g^{-1}), iron (CCB: 73.89 ± 52.19 μg g^{-1}; NC: 425.17 ± 361.99 μg g^{-1}; MAB: 245.65 ± 271.21 μg g^{-1}), and selenium (CCB: 0.257 ± 0.047 μg g^{-1}; NC: 63.357 μg g^{-1}; MAB: 11.383 ± 11.066 μg g^{-1}) compared to the other two locations. We postulate that the higher heavy metal concentrations when comparing NC samples to the smaller CCB turtles is likely due to bioaccumulation in the turtles. This is supported by the weak but significant positive correlation in chromium (R=0.37, p-value=0.0058), iron (R=0.34, p-value=0.0079), and manganese (R=0.27, p-value=0.037) with increasing carapace size (Figure B-1). On the other hand, we found that MAB turtles that are bigger than NC turtles have lower chromium, manganese, iron, and selenium concentrations in their scute. This is likely due to the transition of loggerhead turtle diets from juveniles having an omnivorous diet to adults having a predominantly carnivorous diet (Shaver 1991). It is likely that NC loggerhead turtles might still be consuming algae and seagrass that have high manganese concentrations (Shaver 1991) and have the potential to bioaccumulate iron (Andreani et al. 2008). Even if the NC turtles have transitioned to a carnivorous diet, scute samples show longer term diet and environmental exposure (Vander Zanden et al. 2013). This means that scute samples from NC turtles might still reflect remnants of their omnivorous diet, whereas MAB scute samples could have begun reflecting elements of a carnivorous diet.

We found NC (201.79 ± 50.97 µg g^{-1}) and MAB (172.92 ± 52.16 µg g^{-1}) loggerhead turtles to have comparable zinc concentrations, although they were lower than CCB (184.66 ± 70.85 µg g^{-1}) samples (

Table 3-2). The difference in zinc concentrations is likely due to CCB loggerhead turtles being smaller than NC and MAB loggerhead turtles. According to Hatase and Tsukamoto (2008), smaller adult loggerhead female turtles had higher reproductive energy costs compared to larger turtles. This also meant that smaller turtles needed to consume more prey to meet this higher reproductive energy cost. While Hatase and Tsukamoto's (2008) study focused on reproductive energy cost, the theory of smaller loggerhead turtles having higher energy cost and requiring greater prey biomass could possibly be applied to other forms of energy consumption as well (i.e. growth). It is likely that our smaller CCB loggerhead turtles consume a greater quantity as part of their diet, contributing to higher zinc concentrations in their scute.

We found nickel concentrations in CCB (1.528 ± 1.691 μg g^{-1}) and MAB (1.097 ± 0.511 μg g^{-1}) turtles to be comparable, despite NC (3.258 μg g^{-1}) having a higher concentration outlier. Interestingly, we only detected nickel in one sample from NC loggerhead turtles, at higher concentrations than that of any CCB and MAB samples. Nickel being detected in fish samples in the Mid-Atlantic Bight (Balthis et al. 2009) and South Atlantic Bight (Cooksey et al. 2010) indicates that there is not a lack of nickel in these environments. While the reason behind the lack of nickel in the NC samples is unclear, this occurrence could possibly be related to their difference in behavior and habitat use. Although loggerhead turtles do forage in the MAB, the NC turtles behave slightly differently than those sampled directly in that foraging ground. Patel et al. (2022) found that turtles tagged in NC foraged more inshore and some travelled farther north than those that were tagged directly in offshore MAB.

3.5.3 Heavy metal concentrations in loggerhead turtles and their prey

Comparing prey samples to other studies conducted on the same species (Table 3-6), I found that whole crab samples had higher concentrations of cadmium, lead, and chromium but lower concentration of zinc when compared to leg muscle, leg and hepatopancreas on crab from the New England Coast in the 1980s (Pecci 1987). Our scallop flesh had slightly higher silver and lead concentrations, but much higher chromium and zinc concentrations when compared to scallop muscle collected from Eastern United States in the 1970s (Greig et al. 1978). These differences in concentration might be due to different crab and scallop parts having different concentrations of heavy metals, or the fact that heavy metal concentrations have drastically changed over the past 40-50 years. Comparing whelk flesh to a study on the same species collected in France, our whelk

flesh had higher concentrations of silver, cadmium, and zinc but lower lead levels (Amiard et al. 2008). Despite our samples being measured in dry weight and other studies being measured in wet weight, all heavy metals in all samples except for silver and lead in scallops are different by magnitudes big enough for the comparisons to remain true. The differences in heavy metal concentrations are therefore probably due to regional environmental differences.

Table 3-6 Heavy metal concentrations (μg g^{-1} wet weight) of same-species preys (crab leg muscle, crab leg, crab hepatopancreas, whelk flesh, scallop muscle, scallop male gonad and scallop female gonad) conducted in other studies.

	Crab (Pecci 1987)						Scallop (Greig et al. 1978)						Whelk (Amiard et al. 2008)	
	Leg Muscle		Leg		Hepatopan-creas		Muscle		Male Gonad		Female Gonad		Flesh	
Elements	*n*	mean ± SD	*n*	mean ± SD	*n*	mean ± SD	*n*	mean ± SD	*n*	mean ± SD	*n*	mean ± SD	*n*	mean ± SD
Non-essential elements														
Silver							*13*	0.15 ± 0.04	*24*	0.33 ± 0.15	*23*	0.30 ± 0.13	*6*	0.63 ± 0.05
Cadmium	*6*	0.11 ± 0.08	*5*	0.53 ± 0.39	*1*	17.3	*0*	<LOD	*26*	1.30 ± 0.75	*25*	1.56 ± 0.80	*6*	1.70 ± 1.00
Lead	*6*	0.09 ± 0.06	*5* *5*	0.35 ± 0.39	*1*	0.92	*2*	1.30 ± 0.57	*2*	1.15 ± 0.49	*4*	0.86 ± 0.21	*6*	0.37 ± 0.14
Essential elements														
Chromium	*6*	0.08 ± 0.06	*5*	0.35 ± 0.39	*1*	0.92	*10*	0.49 ± 0.12	*4*	1.51 ± 1.52	*2*	0.43 ± 0.08		
Nickel							*0*	<LOD	*9*	0.87 ± 0.67	*10*	0.55 ± 0.16		
Zinc	*6*	71.30 ± 7.62		89.42 ± 9.91	*1*	56.5	*40*	3.98 ± 1.63	*26*	15.83 ± 7.72	*25*	43.46 ± 15.96	*16*	61.00 ± 25.00

Zinc was the only heavy metal that had TTF>1 across loggerhead groups and the different prey samples. This indicates that there is a high potential for zinc to biomagnify up the loggerhead sea turtle food chain. Despite zinc being an essential element, it can be toxic at high concentrations (Wang 2005). Like cadmium, zinc is probably accumulating in the carapace as a detoxifying mechanism (Martín et al. 2021). In a global marine food web meta-analysis, Sun et al. (2020) found that zinc was transferred between trophic levels inconsistently. They suggested that this occurrence is likely due to different seasonal bioavailability of zinc and varying metabolic regulation mechanisms in different organisms.

The TTF<1 in silver, cadmium and manganese in all loggerhead groups indicate that these elements probably do not biomagnify in loggerhead turtles on east coast USA. While silver was detected in <12% of the loggerhead turtle samples, cadmium and manganese were detected in majority (65%) of the loggerhead turtle samples. In a heavy metal study on hawksbill, green, and loggerhead sea turtles, Mondragón et al. (2023) found manganese to biodilute with higher trophic levels. Wang (2005) also suggested that silver may not bioaccumulate in carapace tissue, which explains the lack of silver in our scute samples. This however does mean that scute samples are probably not useful indicators of silver concentrations in sea turtles.

3.5.4 Comparing heavy metal concentrations to other studies and their implications

Our NC loggerhead turtles had higher aluminum (246.67 ± 229.79 μg g^{-1}), arsenic (2.78 ± 1.52 μg g^{-1}), and lead (0.509 ± 0.350 μg g^{-1}) concentrations than CCB (Al: 43.70 ± 35.24 μg g^{-1}; As: 1.79 ± 0.85 μg g^{-1}; Pb: 0.197 ± 0.264 μg g^{-1}) and MAB (Al: 68.21 ± 68.79 μg g^{-1}; As: 0.83 ± 0.56 μg g^{-1}; Pb: 0.125 ± 0.100 μg g^{-1}) samples. As our arsenic concentrations did not increase with increasing carapace size, this indicates that arsenic does not bioaccumulate in sea turtles (Figure B-1). It is therefore likely that our smaller sized loggerhead turtles from CCB and NC had higher arsenic concentrations because of their more recent omnivorous diet which contains algae and sea grass (Shaver 1991). This is supported by the fact that our sub-adult MAB turtles had similar arsenic concentrations to Miguel et al.'s (2022) adult loggerhead turtles (Location 1: 0.96 ± 0.98 μg g^{-1}; Location 2: 1.01 ± 0.84 μg g^{-1}) (Table 3-7). Despite arsenic being a non-essential element that could result in negative physiological effects like reduced red and white blood cell count, as well as cancer (ATSDR 2007), our larger MAB loggerhead turtles having lower arsenic concentrations is reassuring as it shows that arsenic concentrations in loggerhead turtle scute

samples are likely to decrease as the turtles transition to a carnivorous diet. Although CCB and NC loggerhead turtles have higher arsenic concentrations than the safe levels for Lanzhou catfish is 1.288ppm (synonymous with μg g^{-1}), our MAB loggerhead turtles' levels are lower (Lian and Wu 2017).

Despite CCB loggerhead turtles being the smallest in size, we found scute samples collected from CCB loggerhead turtles (0.256± 0.150 μg g^{-1}) to have higher cadmium concentrations than NC (0.103 ± 0.042 μg g^{-1}) and MAB (0.095 ± 0.040 μg g^{-1}) samples. Across all loggerhead scute samples, we found that cadmium concentrations decreased with increasing carapace size. This indicates that cadmium is not likely to bioaccumulate in sea turtles (Figure B-1). Furthermore, we also found that none of the distinct loggerhead scute origins had a TTF>1 when compared to any sampled prey (Table 3-5). We postulate that cadmium does not biomagnify up trophic levels which is in agreement with Sun et al.'s (2020) finding that cadmium does not biomagnify up marine food webs. As scute samples collected from CCB also had higher cadmium concentrations compared to other similar studies in Japan (0.129 ± 0.034 μg g^{-1}; Sakai et al. 2000) and Brazil (Location 1: 0.004 ± 0.004 μg g^{-1}; Location 2: 0.008 ± 0.01 μg g^{-1}; Miguel et al. 2022), it is likely that these higher cadmium concentrations stem from Massachusetts smelting sites (Eckel et al. 2001) that releases cadmium as a by-product (Bradl 2005). As cadmium is a non-essential element, it is likely that the cadmium is accumulating in the loggerhead carapace as a detoxifying mechanism (Martín et al. 2021). While cadmium concentrations in our CCB loggerhead turtles were higher, this level is still lower than 2ppm, which is the level at which contamination is only considered in whole body tissue.

Table 3-7 Heavy metal concentrations found in loggerhead scute samples in the present study compared to other studies.

Elements	Cape Cod, MA		North Carolina		Mid-Atlantic Bight		References
	n	mean ± SD	*n*	mean ± SD	*n*	mean ± SD	
Non-essential elements							
Arsenic	*17*	1.79 ± 0.85	*9*	2.78 ± 1.52	*32*	0.83 ± 0.56	Present Study
			66	0.96 ± 0.98 [b]	*37*	1.01 ± 0.84 [c]	[b] Miguel et al. (2022)
							[c] Miguel et al. (2022)
Cadmium	*17*	0.256 ± 0.150	*4*	0.103 ± 0.042	*20*	0.095 ± 0.040	Present Study
	6	0.129 ± 0.034 [a]	*66*	0.004 ± 0.004 [b]	*37*	0.008 ± 0.01[c]	[a] Sakai et al. (2000)
							[b] Miguel et al. (2022)
							[c] Miguel et al. (2022)
Lead	*12*	0.197 ± 0.264	*7*	0.509 ± 0.350	*16*	0.125 ± 0.100	Present Study
	6	2.42 ± 0.52 [a]	*66*	0.05 ± 0.08 [b]	*37*	0.05 ± 0.08 [c]	[a] Sakai et al. (2000)
							[b] Miguel et al. (2022)
							[c] Miguel et al. (2022)
Essential elements							
Chromium	*16*	0.854 ± 0.968	*7*	4.187 ± 2.517	*35*	3.189 ± 3.977	Present Study
			66	0.5 ± 0.9 [b]	*37*	0.39 ± 0.46 [c]	[b] Miguel et al. (2022)
							[c] Miguel et al. (2022)
Iron	*17*	73.89 ± 52.19	*9*	425.17 ± 361.99	*37*	245.65 ± 271.21	Present Study
	6	26.2 ± 19.1[a]	*66*	358 ± 411[b]	*37*	247 ± 201[c]	[a] Sakai et al. (2000)
							[b] Miguel et al. (2022)
							[c] Miguel et al. (2022)
Manganese	*17*	1.302 ± 1.480	*9*	5.392 ± 4.589	*37*	2.962 ± 3.038	Present Study
	6	7.01 ± 3.49 [a]	*66*	8.44 ± 5.21[b]	*37*	7.16 ± 4.91[c]	[a] Sakai et al. (2000)
							[b] Miguel et al. (2022)
							[c] Miguel et al. (2022)
Nickel	*17*	1.528 ± 1.691	*1*	3.258	*37*	1.097 ± 0.511	Present Study
	6	0.0094 ± 0.022 [a]					[a] Sakai et al. (2000)
Zinc	*17*	201.79 ± 50.97	*9*	184.66 ± 70.85	*37*	172.92 ± 52.16	Present Study
	6	198 ± 37.2 [a]	*66*	33.7 ± 16.3 [b]	*37*	34.1 ± 25.7 [c]	[a] Sakai et al. (2000)
							[b] Miguel et al. (2022)
							[c] Miguel et al. (2022)

NOTE: Scute heavy metal concentration values are reported in µg g^{-1} wet weight

Zinc was the only heavy metal that had TTF>1 across loggerhead groups and the different prey samples. This indicates that there is a high potential for zinc to biomagnify up the loggerhead sea turtle food chain. Despite zinc being an essential element, it can be toxic at high concentrations (Wang 2005). Like cadmium, zinc is probably accumulating in the carapace as a detoxifying mechanism (Martín et al. 2021). In a global marine food web meta-analysis, Sun et al. (2020) found that zinc was transferred between trophic levels inconsistently. They suggested that this occurrence is likely due to different seasonal bioavailability of zinc and varying metabolic regulation mechanisms in different organisms.

The TTF<1 in silver, cadmium and manganese in all loggerhead groups indicate that these elements probably do not biomagnify in loggerheads on east coast USA. While silver was detected in <12% of the loggerhead samples, cadmium and manganese were detected in majority (65%) of the loggerhead samples. In a heavy metal study on hawksbill, green, and loggerhead sea turtles, Mondragón et al. (2023) found manganese to biodilute with higher trophic levels. Wang (2005) also suggested that silver may not bioaccumulate in carapace tissue, which explains the lack of silver in our scute samples. This however does mean that scute samples are probably not useful indicators of silver concentrations in sea turtles.

Despite heavy metals in crabs not correlating with any loggerhead groups, numerous heavy metals still had TTF>1 for the different loggerhead groups. MA loggerheads had TTF>1 for nickel and zinc, MAB and NC loggerheads had TTF>1 for chromium, nickel, zinc and selenium. This shows that loggerheads are possibly still consuming crabs but not all the heavy metals are being incorporated into the scute. It is also important to note that our crab sample size was rather small (n=5) and varied greatly in size (SD: 40.42g *d.w.*). A larger sample size is needed to determine if any heavy metals in crabs biomagnifies in loggerhead carapace.

3.6 Conclusion

We see that heavy metal concentrations between loggerhead turtles from different locations within the same region differ significantly. As we postulate that these differences are probably due to their different life stages, it is important to analyze the heavy metal concentrations in these populations as they make their ontogenetic shifts into adulthood. It is especially important to monitor zinc concentrations as this is the only heavy metal we found to biomagnify through trophic levels regardless of prey items and the sampling site of the loggerhead turtles. Knowing the

difference in heavy metal concentrations between juvenile and adult loggerhead turtles would help us better understand if the heavy metals are entering the turtles through diet or the environment. This is especially important as the east coast US continues to undergo more development.

The NW Atlantic, a known foraging ground for green, loggerhead, and Kemp's ridley sea turtles as they recruit to neritic habitats, is susceptible to even more pollution in years to come. The Atlantic Ocean is polluted by many non-point sources such as runoff from agriculture and farmland, roads, atmospheric deposition, and septic tank discharge (NRC 2000, Howarth et al. 2002, Valiela and Bowen 2002). The ever-increasing development in northeastern US will only cause more pollutants to enter the NW Atlantic. Furthermore, The Executive Office of Energy and Environmental Affairs (EEA) are working on initiatives to develop offshore wind projects in Massachusetts waters (Mass.gov). While these windmills are meant to decarbonize energy supply (Mass.gov), their galvanic anodes erode and release aluminum, zinc, and indium to the ocean. There is also a possibility that they might release cadmium, lead, and copper too (BSH and Hereon 2022). Establishing baseline heavy metal concentrations in scute samples of loggerhead turtles in the Mid-Atlantic Bay will facilitate future studies on pollution in the NW Atlantic.

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4. GENERAL CONCLUSIONS

Our study shows that heavy metal concentrations do indeed differ based on species, tissue, and location of turtle populations sampled. We found that turtle species believed to be associated with a heavier algae/ seagrass diet (i.e. green turtles skin samples, juvenile loggerhead turtles) tend to reflect higher concentrations of manganese, iron, cobalt, and selenium; whereas turtle populations associated with more polluted environments (i.e. Cape Cod Bay Massachusetts) tend to reflect higher concentrations of cadmium and arsenic, among other pollutants. Overall, we also saw higher heavy metal concentrations in scute samples compared to skin samples, which is likely due to scute samples having a slower turnover rate.

While we do not know the exact toxic concentration of these heavy metals in sea turtles, this study provides a baseline knowledge of heavy metal concentrations in skin and scute samples of sea turtles in the NW Atlantic Ocean. Such information is especially important with plans for offshore windfarm development soon and projected increase in thermal windows in the NW Atlantic. Collecting skin and scute samples from turtles is a relatively non-invasive process. Therefore, future studies would be able to collect skin and scute samples from turtles in the area to monitor local environmental pollution as well as the health of the turtle populations.

APPENDIX A. CAPE COD TURTLES

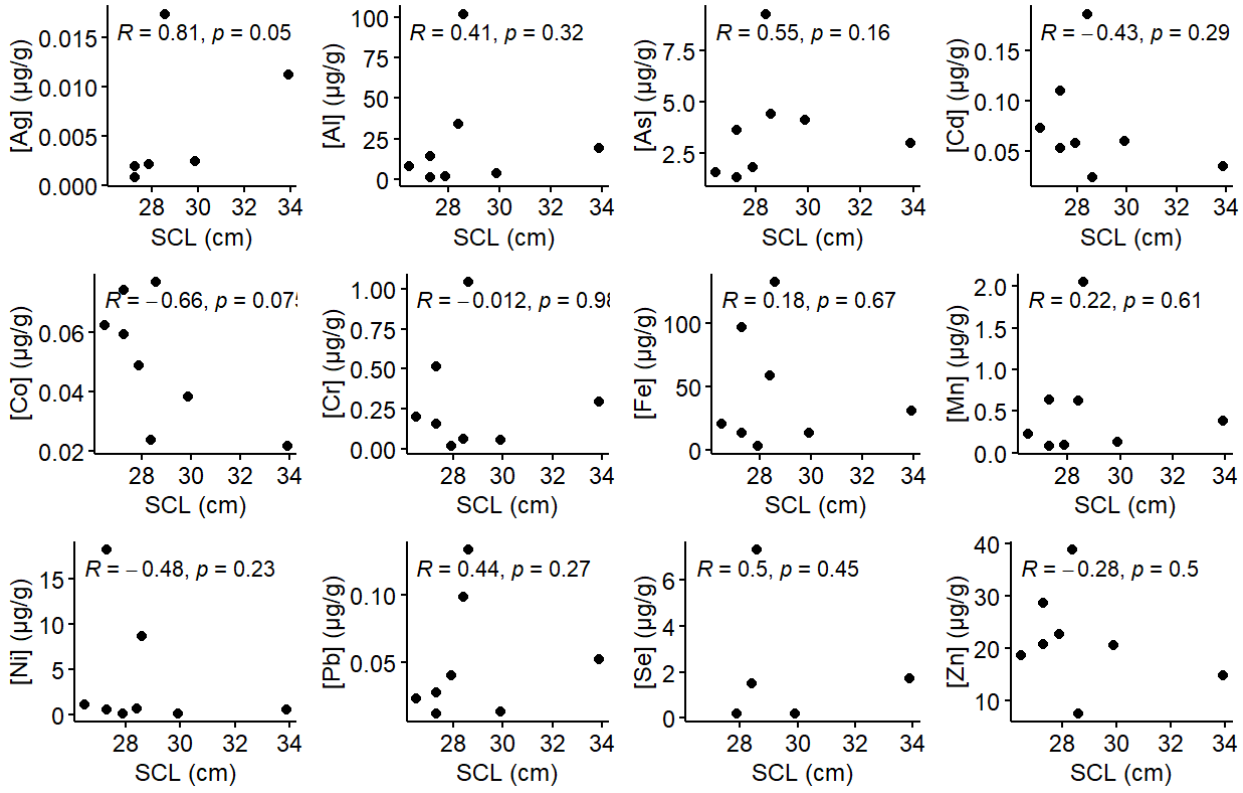

Figure A-1. Relationship between heavy metal concentrations in green sea turtle skin samples with increasing carapace size.

NOTE: SCL = Straight Carapace Length, Ag = silver, Al = aluminum, As = arsenic, Cd = cadmium, Co = cobalt, Cr = chromium, Fe = iron, Mn = manganese, Ni = nickel, Pb = lead, Se = selenium, Zn = zinc.

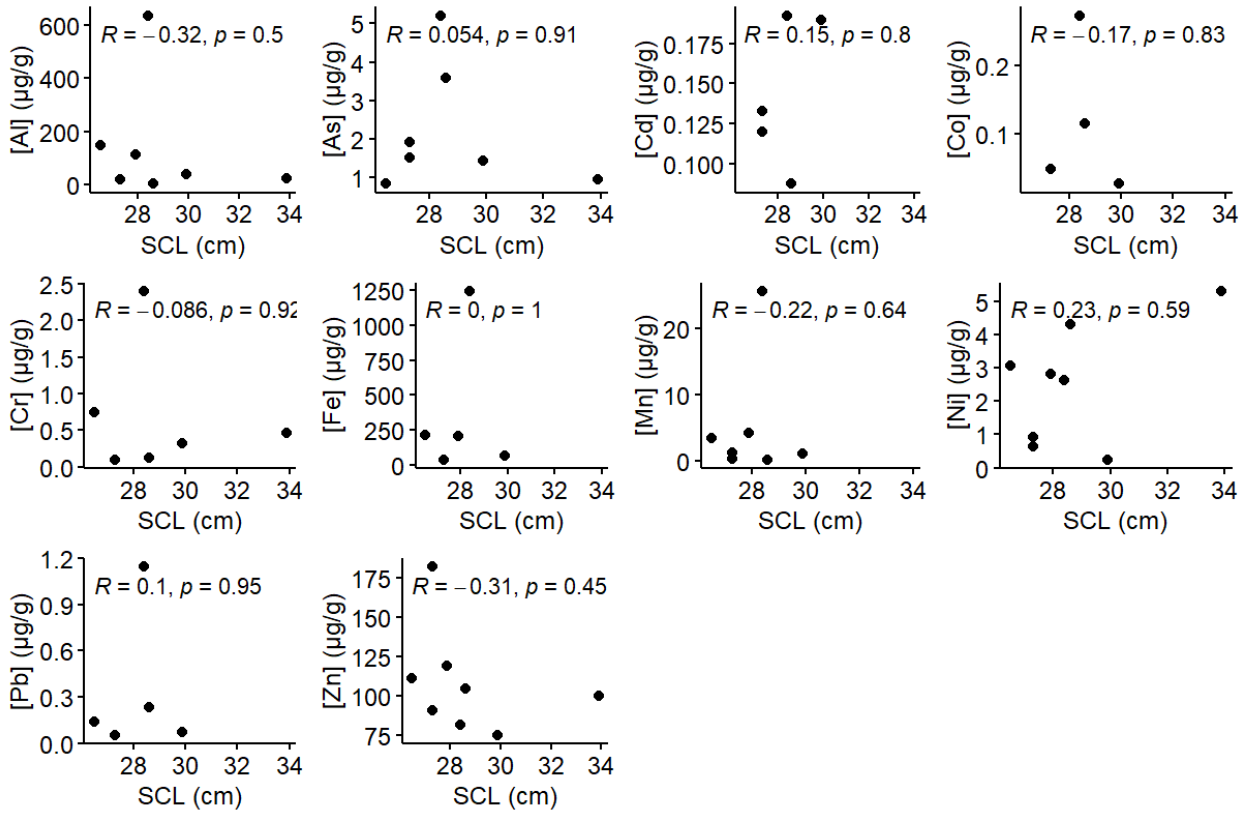

Figure A-2. Relationship between heavy metal concentrations in green sea turtle scute samples with increasing carapace size.

NOTE: SCL = Straight Carapace Length, Al = aluminum, As = arsenic, Cd = cadmium, Co = cobalt, Cr = chromium, Fe = iron, Mn = manganese, Ni = nickel, Pb = lead, Zn = zinc.

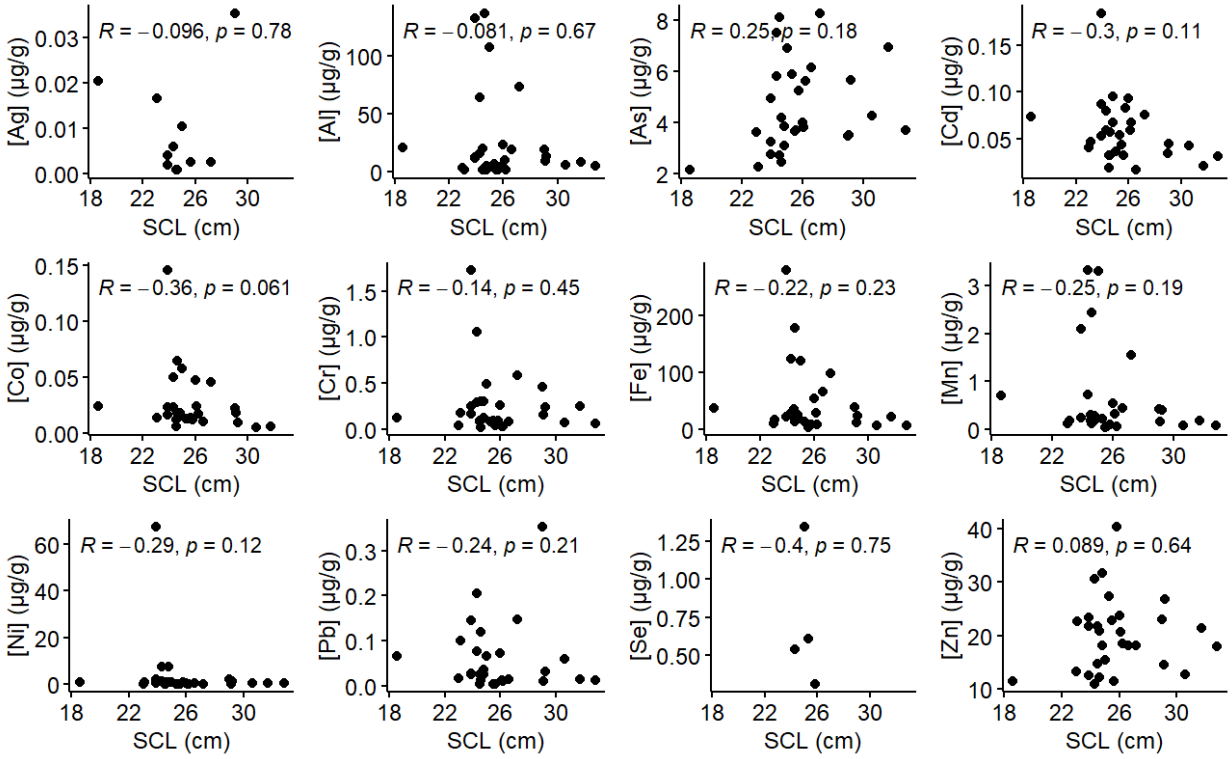

Figure A-3. Relationship between heavy metal concentrations in Kemp's ridley sea turtle skin samples with increasing carapace size.

NOTE: SCL = Straight Carapace Length, Ag = silver, Al = aluminum, As = arsenic, Cd = cadmium, Co = cobalt, Cr = chromium, Fe = iron, Mn = manganese, Ni = nickel, Pb = lead, Se = selenium, Zn = zinc.

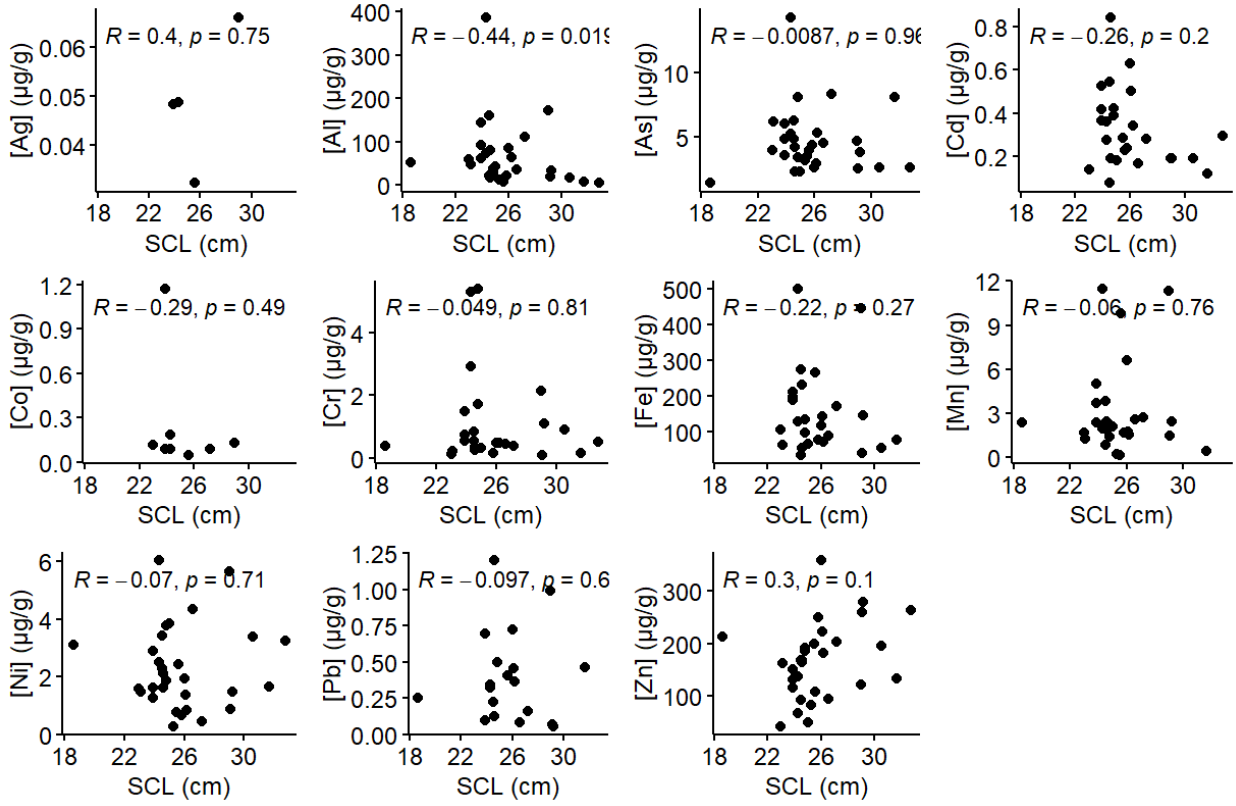

Figure A-4. Relationship between heavy metal concentrations in Kemp's ridley sea turtle scute samples with increasing carapace size.

NOTE: SCL = Straight Carapace Length, Ag = silver, Al = aluminum, As = arsenic, Cd = cadmium, Co = cobalt, Cr = chromium, Fe = iron, Mn = manganese, Ni = nickel, Pb = lead, Zn = zinc.

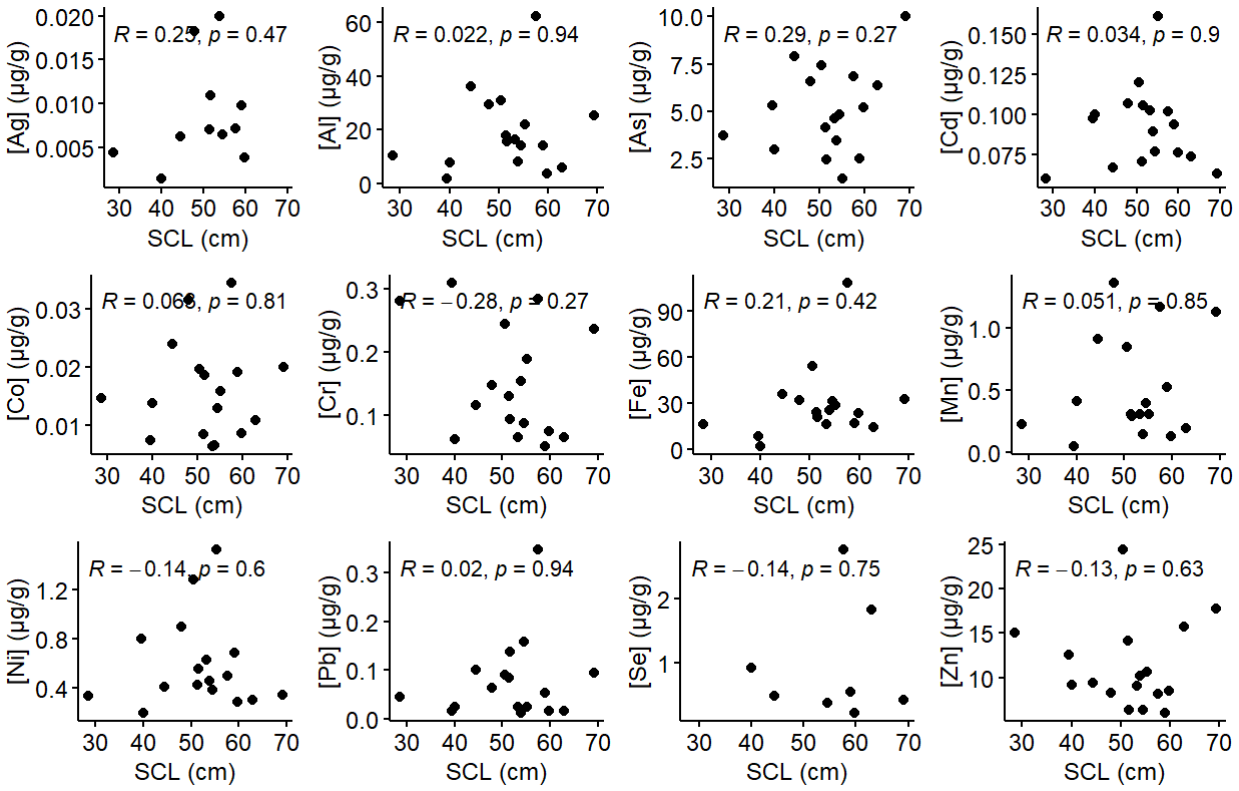

Figure A-5. Relationship between heavy metal concentrations in loggerhead sea turtle skin samples with increasing carapace size.

NOTE: SCL = Straight Carapace Length, Ag = silver, Al = aluminum, As = arsenic, Cd = cadmium, Co = cobalt, Cr = chromium, Fe = iron, Mn = manganese, Ni = nickel, Pb = lead, Se = selenium, Zn = zinc.

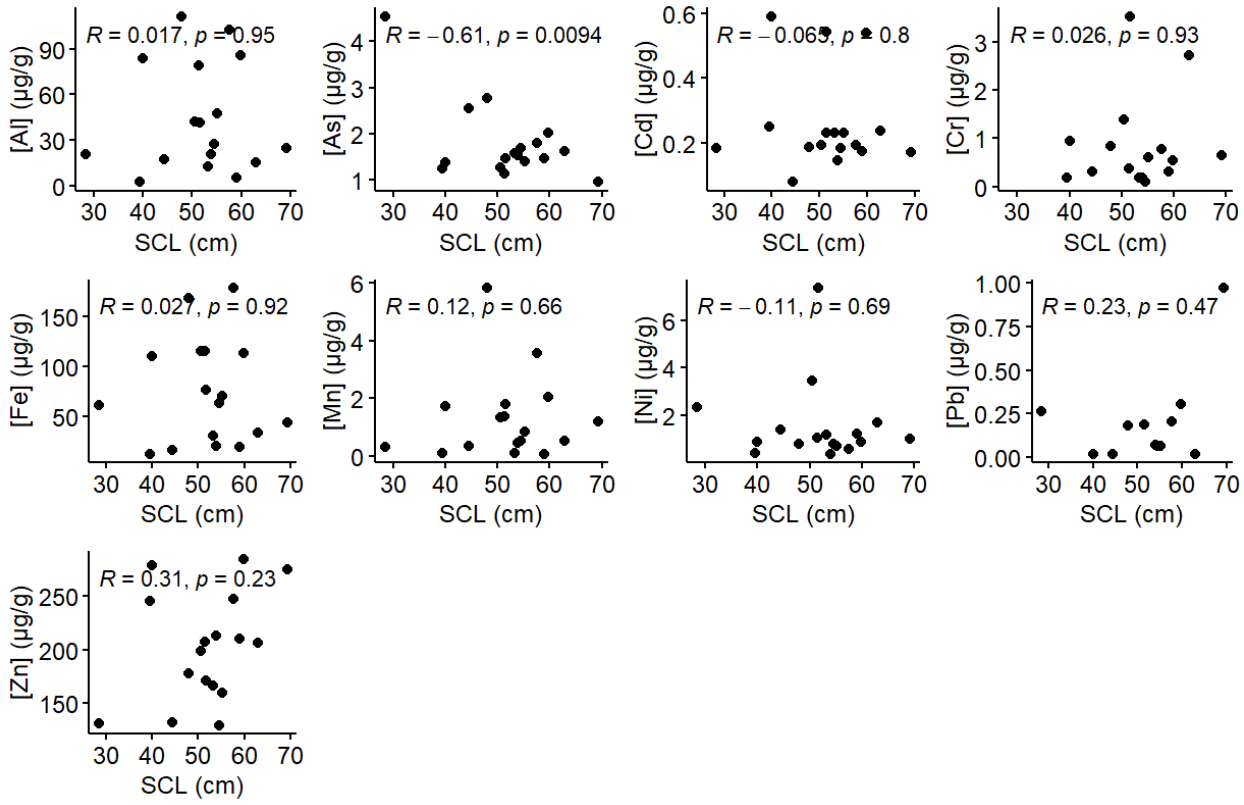

Figure A-6. Relationship between heavy metal concentrations in loggerhead sea turtle scute samples with increasing carapace size.

NOTE: SCL = Straight Carapace Length, Al = aluminum, As = arsenic, Cd = cadmium, Cr = chromium, Fe = iron, Mn = manganese, Ni = nickel, Pb = lead, Zn = zinc.

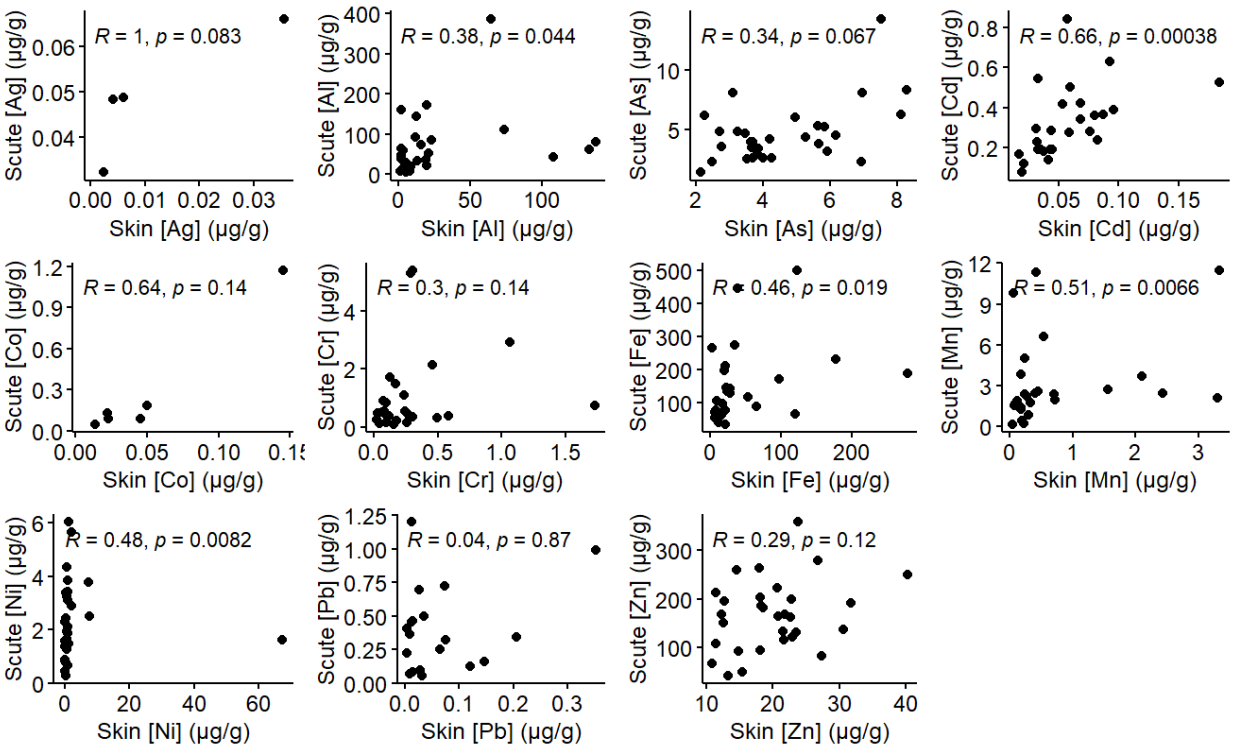

Figure A-7. Relationship between heavy metal concentrations in skin and scute samples of Kemp's ridley sea turtles.

NOTE: Ag = silver, Al = aluminum, As = arsenic, Cd = cadmium, Co = cobalt, Cr = chromium, Fe = iron, Mn = manganese, Ni = nickel, Pb = lead, Zn = zinc.

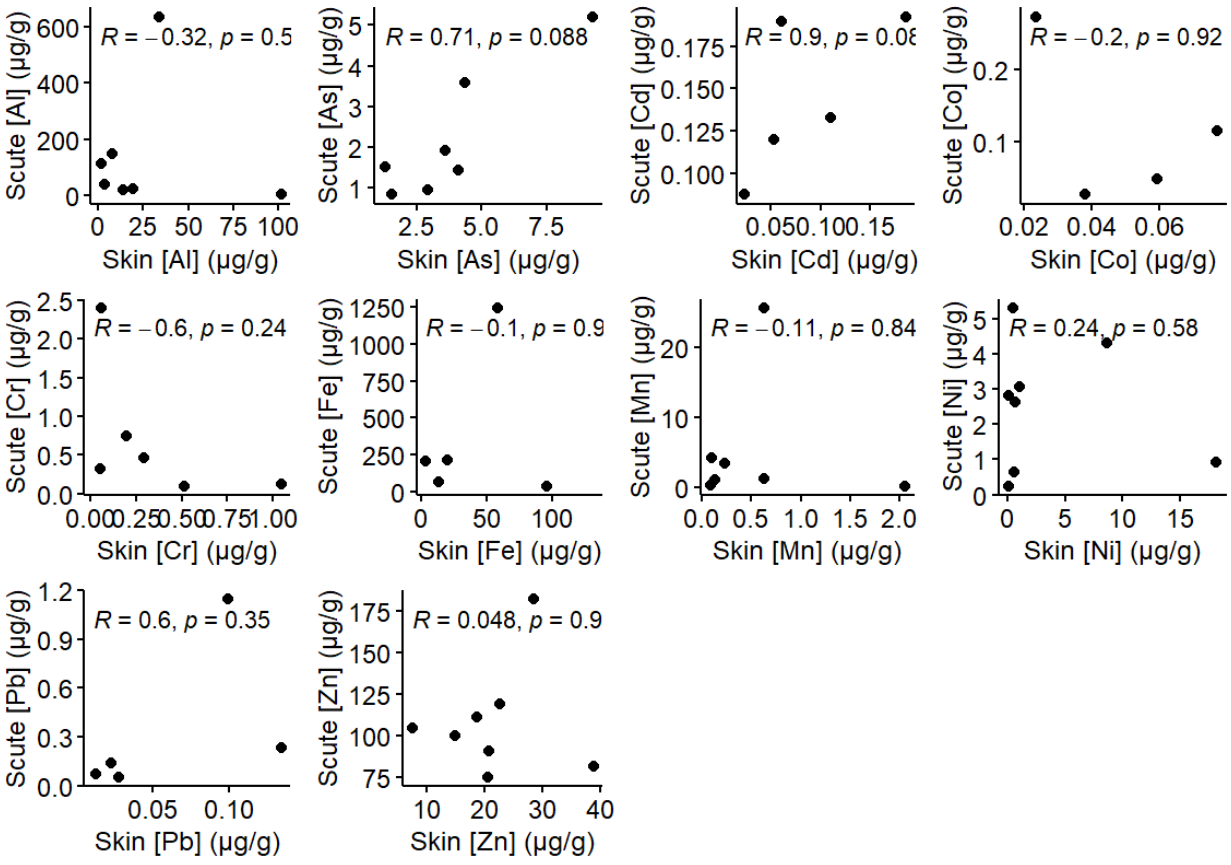

Figure A-8. Relationship between heavy metal concentrations in skin and scute samples of green sea turtles.

NOTE: Al = aluminum, As = arsenic, Cd = cadmium, Co = cobalt, Cr = chromium, Fe = iron, Mn = manganese, Ni = nickel, Pb = lead, Zn = zinc.

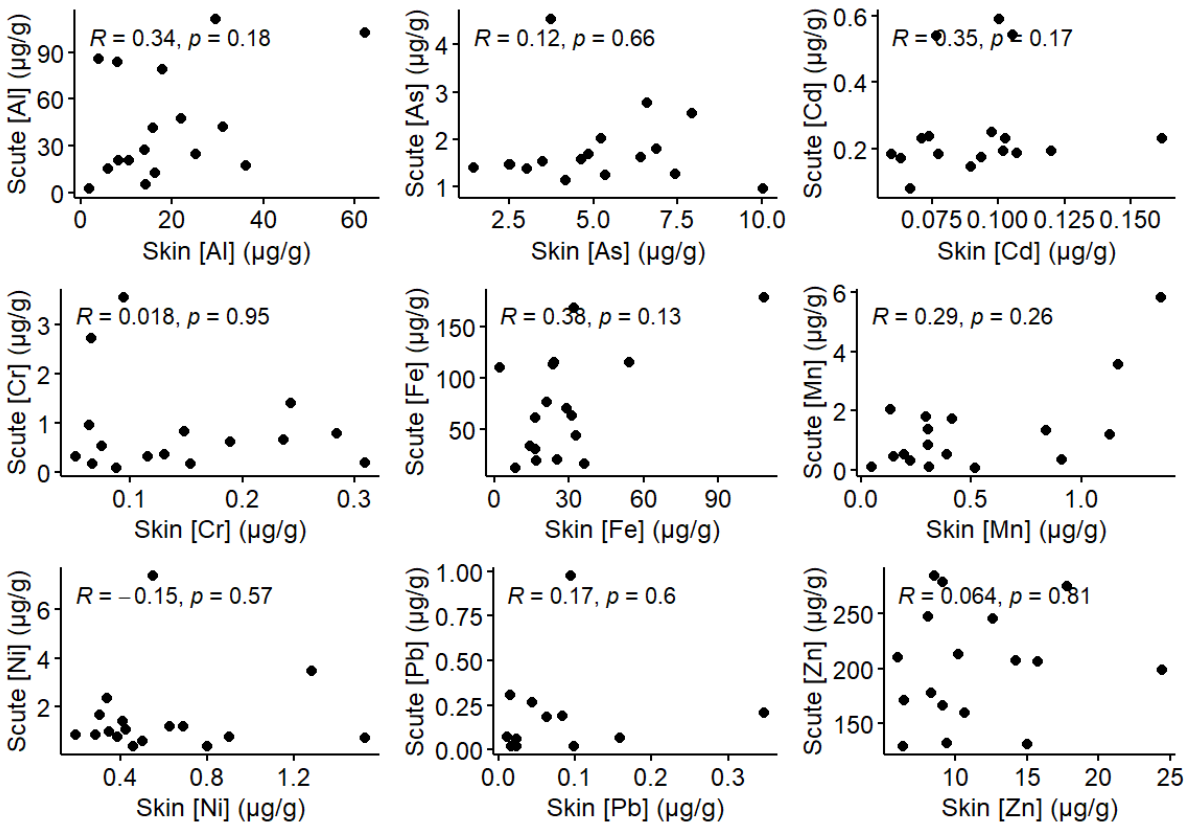

Figure A-9. Relationship between heavy metal concentrations in skin and scute samples of loggerhead sea turtles.

NOTE: Al = aluminum, As = arsenic, Cd = cadmium, Cr = chromium, Fe = iron, Mn = manganese, Ni = nickel, Pb = lead, Zn = zinc.

APPENDIX B. LOGGERHEADS AND PREY

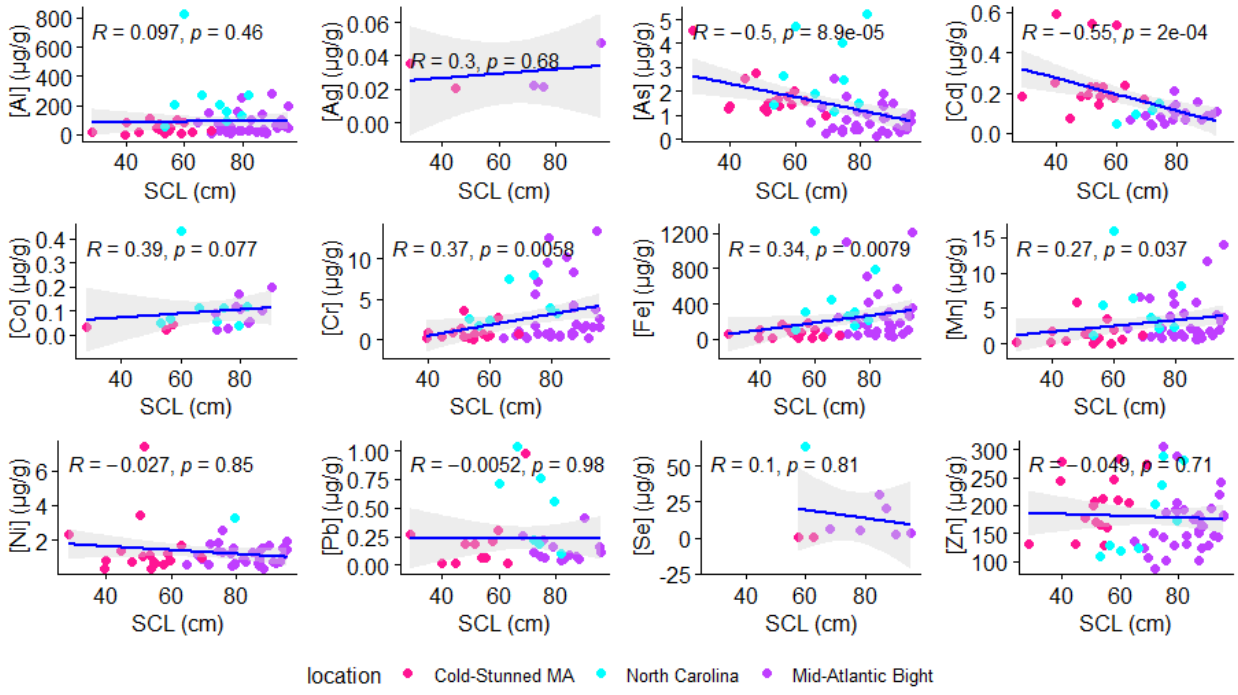

Figure B-1. Relationship between heavy metal concentrations in loggerhead sea turtle scute samples with increasing carapace size.

NOTE: SCL = Straight Carapace Length, Grey area = 95% Confidence Interval, Ag = silver, Al = aluminum, As = arsenic, Cd = cadmium, Co = cobalt, Cr = chromium, Fe = iron, Mn = manganese, Ni = nickel, Pb = lead, Se = selenium, Zn = zinc.

Table B-1 Median heavy metal concentrations (µg g^{-1} dry weight) of all loggerhead groups (MA, NC, MAB) and different prey samples, ranked in order from lowest to highest concentration (left to right) for each heavy metal.

Lead	MAB	Whelk Flesh	Scallop Shell	Whelk Shell	NC	Crab	Whelk Operculum	Scallop Flesh
	0.123	0.186	0.216	0.26	0.789	1.481	1.897	2.007
Arsenic	MAB	Scallop Shell	MA	NC	Whelk Operculum	Scallop Flesh	Crab	Whelk Flesh
	1.029	1.452	1.985	2.965	3.588	6.11	17.413	27.394
Aluminum	MA	Whelk Flesh	Scallop Shell	Whelk Shell	NC	Crab	Whelk Operculum	Scallop Flesh
	35.999	65.449	90.498	119.275	235.8	561.496	741.492	1606.833
Chromium	Whelk Shell	Crab	Scallop Shell	MAB	Whelk Operculum	Scallop Flesh	NC	Whelk Flesh
	0.447	1.245	1.333	2.035	2.84	3.272	4.168	NA
Manganese	MA	NC	Whelk Flesh	Scallop Shell	Whelk Shell	Scallop Flesh	Whelk Operculum	Crab
	1.087	4.502	6.802	19.142	21.109	48.436	128.344	258.03
Nickel	Crab	MAB	Scallop Flesh	Whelk Operculum	NC	Whelk Flesh	Scallop Shell	Whelk Shell
	1.021	1.32	1.698	3.514	4.206	NA	NA	NA
Iron	MA	Whelk Flesh	Whelk Shell	MAB	NC	Whelk Operculum	Crab	Scallop Flesh
	87.615	112.54	137.582	227.216	367.986	767.812	909.366	2163.578
Cobalt	Whelk Shell	NC	Scallop Shell	Whelk Flesh	MAB	Whelk Operculum	Crab	Scallop Flesh
	0.036	0.115	0.116	0.128	0.137	0.413	0.482	0.779
Zinc	Scallop Shell	Crab	Whelk Operculum	Scallop Flesh	Whelk Flesh	NC	MA	MAB
	1.599	23.86	25.157	45.469	176.15	195.849	230.746	243.455
Selenium	MA	Scallop Flesh	Crab	MAB	Whelk Shell	Whelk Operculum	Scallop Shell	NC
	0.332	4.793	7.842	8.311	10.29	11.375	19.032	81.794
Silver	MAB	Scallop Flesh	Scallop Shell	Whelk Operculum	Whelk Shell	Crab	Whelk Flesh	NC
	0.032	0.252	0.358	0.395	0.435	0.946	2.644	NA
Cadmium	MAB	MA	Scallop Shell	Whelk Shell	Whelk Operculum	Crab	Whelk Flesh	Scallop Flesh
	0.126	0.258	0.266	0.41	1.451	2.556	15.595	29.716