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Quantifying Pre-Industrial to Mid-Late 20th Century Anthropogenic Lead, Mercury and Cadmium Pollution in Caribbean Marine Environments Using Skeletonized Sea Turtle Remains

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By

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Various lines of evidence indicate that levels of anthropogenic pollutants, such as lead, mercury and cadmium, have increased in terrestrial and atmospheric environments since the early 19th century and the advent of industrialization. While the exposure to these three trace elements is a global concern, this study focused primarily on marine environments located throughout the Caribbean. Using ICP-MS, this study aimed to detect and quantify anthropogenic pollutants, specifically lead (Pb), mercury (Hg) and cadmium (Cd), using skeletonized remains of sea turtles as biological proxies for environmental quality. Archaeologically derived (n=5) and mid-late 20th century (n=6) Hawksbill and Green turtles were used to create a chronology of pollution exposure in Caribbean marine environments and establish a pre-industrial baseline for pollution exposure, useful for precisely gauging how human activities in the Caribbean, namely industrialization and tourism, have changed the concentration of these elements over time. Results from this study revealed that the industrial, modern sea turtle sample and the archaeological sample exhibit similar distributions of lead and cadmium ppm levels. Whereas, the mercury datasets revealed that the two samples share differing distributions

of ppm levels, but that the archaeological sample yielded the higher mercury concentrations. Based on these results, this study was unable to verify whether skeletal sea turtle remains, specifically humeri, can be used as a biological proxy to reconstruct anthropogenic pollution in marine environments. Furthermore, it failed to quantify pre-industrial to mid-late 20th century anthropogenic lead, mercury, and cadmium pollution in Caribbean Marine Environments.

DEDICATION

This thesis is dedicated to my father, Ruben Peña, who has been a constant source of support and encouragement during the challenges of graduate school and life. You have always loved me unconditionally and taught me to work hard for the things that I aspire to achieve. I'm truly thankful for having you in my life. I love you very much.

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER I

INTRODUCTION

Various lines of evidence demonstrate that levels of anthropogenic, or human-generated, pollutants such as lead, mercury, and cadmium, have increased in terrestrial, atmospheric, and marine environments since the advent of industrialization in the 18th and 19th centuries (Briggs, 2003; Emanuel, 2010; Lorey, 1999; Pacyna et al., 2006; Selin et al., 2009). This increase has been linked to the overall global lack of legislation and regulation relating to the import, export, transport, production, emission, storage, and disposal of many toxicants, including lead, mercury, and cadmium (Briggs, 2003; Johnson, 1998; Paez-Osuna et al., 2010; Schmeltz et al., 2011; UNEP, 2002). As a result, assessing anthropogenic pollution in various environments, including marine environments, has become an important area of study within ecology and environmental toxicology (Briggs, 2003; Johnson, 1998; Paez-Osuna et al., 2010; Schmeltz et al., 2011; UNEP, 2002; UNEP, 2008).

1.1 Problem Statement

Few scientific studies have quantified various marine pollutants in the Caribbean Region,or the Wider Caribbean Region (WCR), prior to the modern era (Figure 1.1).

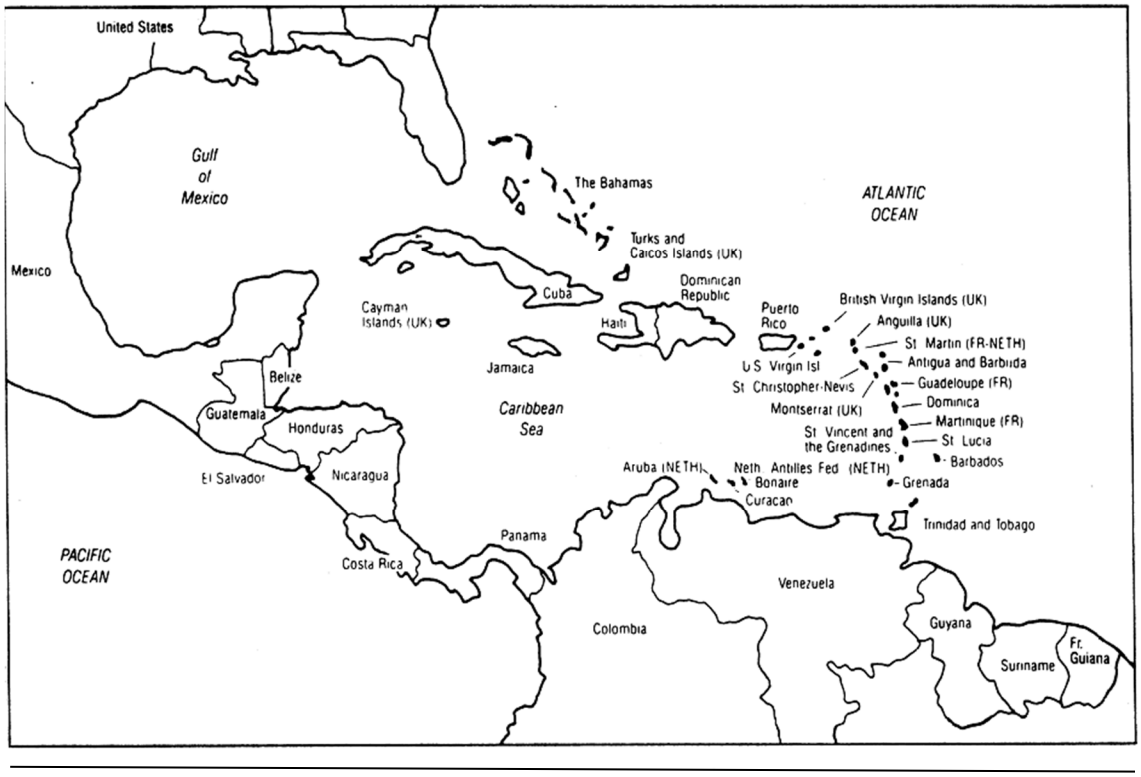

Figure 1.1 Map of the Wider Caribbean Regions (WCR).

Notes: Map of the Wider Caribbean Region (WCR). Source: UNEP: Guidelines for Integrated Planning and Management of Coastal and Marine Areas in the Wider Caribbean Region. UNEP Caribbean Environment Programme, Kingston, Jamaica, 1996.

More specifically, few have focused on quantifying marine pollutants within the WCR, beginning with the Industrial Revolution in the mid-18th century to the mid- 19th century and onwards (Siung-Chang, 1997; UNEP, 1984). This lack of long-term, longitudinal data on marine pollution in the WCR complicates estimations of how levels of pollutants have changed over time due to anthropogenic activity. Generally, it is understood that levels of pollutants in the WCR have increased over time from dramatic increases in global commerce and transport of goods and peoples, particularly in relation to tourism, beginning with the Industrial Revolution and continuing into the 20th and 21st centuries (Ellison and Farnsworth, 1996; Khan and Ghouri, 2011; Ortega, 2014). This study

attempts to address this void, in an attempt to provide quantified data on levels of environmental pollution, specifically from lead, mercury and cadmium, in the WCR prior to industrialization. By doing so, this study will provide an empirical backdrop for quantifying the degree to which anthropogenic, industrial pollution has altered marine environments in the WCR.

To address the overarching problem statement of this study, concentrations of lead, mercury, and cadmium were assessed and quantified in the skeletonized remains of sea turtles (N=11) recovered from St. Croix, in the United States Virgin Islands (USVI) in the Caribbean Sea. As discussed by the UNEP (2013) and Richir and Gobert (2016), aquatic animals that inhabit any particular environment, will accumulate and integrate the local trace elements over time. With this premise, this study followed this recommendation by employing skeletonized sea turtle remains to measure lead, mercury and cadmium concentrations found within the WCR. The sea turtle sample consists of two sub-samples: an archaeological sub-sample (n=5) which dates to the pre-industrial, pre-Columbian period, and a modern sub-sample (n= 6) dating from roughly 1975 to 2010, from the post-industrial period. Since these two sub-samples are associated with the pre-industrial and post-industrial periods, results from this study can be used to create a diachronic, quantified record of anthropogenic pollution in the WCR. Specifically, this study compared the preindustrial turtles' elemental concentrations to those from the modern, mid-late 20th century turtles in order to create a chronology of how pollution concentrations have changed over time, from the pre-industrial to industrial periods.

The geographic region for this study was chosen based on two factors. The first is coincidental: sea turtle remains from the Caribbean were made available to the author for

analysis. The second is that abundant data regarding modern-day pollution was available for the WCR as a result of wide-ranging concerns relating to the contamination of marine environments and degradation of reef ecosystems. Data on contemporary marine pollution has been published by numerous universities and research facilities, such as the University of the West Indies (de Astudillo et al., 2005), the Institute of Marine Affairs in Trindad and Tobago (Norville, 2005), the Caribbean Environmental Health Institute based in St. Lucia (Forde et al., 2011), the United Nations Environmental Programme (UNEP) (UNEP, 2006) and the Intergovernmental Oceanographic Commission (IOC) (Siung-Chang 1997). Studies from these institutions have documented that pollution from lead, mercury, and cadmium is extensive throughout the Caribbean (Siung-Chang, 1997). As a result, the demand for the development and governing of monitoring systems for marine pollution in the WCR has increased over the past twenty-five years (UNEP, 1999).

This study has two objectives. The first is to test whether the analysis of sea turtle remains, specifically trace element analysis of skeletal elements, represents an empirically valid approach for examining changes over time in marine environmental pollution. Various tissues from sea turtles, such as blood, muscle tissue, and shell, have been used to detect exposure to pollutants, such as lead, mercury and cadmium, in marine environments via trace element analysis (Fernandez et al., 2007; Garcia-Fernandez et al., 2009; Gardner et al., 2006; Guzman and Jimenez, 1992; Meyers-Schone et al., 1994; Paez-Osuna et al., 2010; Rojas de Astudillo et al., 2002; Smodis et al., 2004). However, a search of the literature reveals that none have used skeletal tissue. This is because published studies have focused on studying the scute and organs of recently dead turtles.

Additionally, the published literature does not include any studies that have analyzed archaeologically derived, pre-modern turtles for concentrations of environmental pollutants. The second objective is to quantify how levels of pollution have changed over time in the WCR in relation to human activity, specifically in relation to industrialization and tourism. Lead, mercury, and cadmium were chosen for this study as a result of their detectability in skeletal material through trace element analysis and the extensive body of scholarly literature focused on reconstructing their concentrations in biological material, excluding sea turtle bone, of numerous organisms (Costa et al., 2012; Dopierala et al., 2014; Gerhardsson et al., 2010; Lanocha et al., 2012; Sobota et al., 2011; Swanston et al., 2012). Furthermore, these three elements are relevant to studies of pollution in the WCR as they have been reported to be common overall marine pollutants, includedin the modern WCR, specifically (Alloway, 1997).

The skeletonized turtles analyzed in this study are from two species of sea turtle, hawksbill turtles (*Eretmochelys imbricata*) and green turtles (*Chelonia mydas*). Sea turtles were chosen for this research based on their migratory patterns and long life spans (Gibbons and Semlitch, 1982). While each species is capable of traveling long distances, they are philopatric; research has shown that returning to the island of their birth, which for the sample of turtles included in the proposed study is St. Croix, is common during the nesting stage of their life (Bass et al., 2006; Bolten, 2003; Carr, 1960; Lagueux, 2001; Reich et al., 2007). As a result of this pattern, it can be argued that the trace element levels obtained from the skeletons of these sea turtles broadly reflect environmental pollution in marine environments of the WCR. These species of sea turtle also live for

extended periods; studies have estimated that the life expectancy of green and hawksbill sea turtles ranges from 30 to 100 years (Anan et al., 2001; NMFS and USFWS, 2013). This extended life span is important for the purposes of this study, as the skeletons of each turtle potentially can therefore yield extended information on numerous years' worth of pollution levels in Caribbean marine environments. Additionally, the skeletons of sea turtles produce annual skeletal growth marks, the result of annual periodicity, which are located within the bone tissue (Ehret, 2004; Snover, 2002). This means that annual exposure to pollutants can be assessed for each individual.

The concentrations of lead, mercury, and cadmium in the humeri of both samples of sea turtles are assessed through trace element analysis, performed through Inductively Coupled Plasma-Mass Spectrometry (ICP-MS). Skeletochronology, an incremental growth technique used to provide an estimated age of an organism (Ehret, 2004; Snover, 2002), is employed to determine the age at death of each turtle. Fourier Transform Infrared Spectroscopy Attenuated Total Reflection (FTIR-ATR), an analytical technique used to determine the presence and degree of postmortem skeletal alteration (diagenesis), is performed to determine if the sea turtle remains underwent any diagenetic changes after the organisms' death and deposition, specifically postmortem exposure to lead, mercury, and cadmium that might affect the interpretation of antemortem exposure to these elements.

CHAPTER II

BACKGROUND LITERATURE

2.1 Environmental Pollution

Environmental pollution is a major source of degradation in modern ecosystems and a considerable source of morbidity and mortality for human and other animal populations. For instance, the World Health Organization (WHO) estimates that environmental pollution is responsible for a quarter of the diseases (e.g., chronic obstructive pulmonary disease, coronary disease) that currently plague many human populations around the globe (Pruss-Ustun and Corvalan, 2006; Valent et al., 2004; WHO, 2007). The United Nations Environmental Programme (UNEP) defines environmental pollution as "the presence of minerals, chemicals or physical properties at levels that exceed the values deemed to define a boundary between 'good or acceptable' and 'poor or unacceptable' quality, which is a function of the specific pollutants" in a given environment (UNEP, 2013). In environmental toxicology, it is understood that a link between environmental pollution and disease can be made as a result of a circular relationship between the introduction of pollutants into a given environment, the effect of the pollutants on organisms in the environment, the food chain, and public health (Alloway and Ayers, 1997). The majority of the harmful effects caused by environmental pollution are, however, subtle changes that typically do not become apparent until years later (Barouki et al., 2012). As a result, the development or refinement of environmental

monitoring strategies is essential (Holdgate, 1979; UNEP et al., 2006). However, before such strategies can be achieved, it is necessary to first determine where in a given environment pollutants are accumulating, the specific pollutants that are accumulating, and the sources from which they originated (Alloway and Ayers, 1997).

2.2 Anthropogenic environmental pollution

Anthropogenic environmental pollution is the introduction of pollutants into the environment as a result of both direct and indirect human activities (Halpern et al, 2008). According to Hong and colleagues (1996) this process has early roots; the earliest known instance of anthropogenic pollution is linked to metal smelting that occurred an estimated 2,000 years ago in the Northern Hemisphere, specifically in Europe and China. Anthropogenic pollution has greatly increased since then, with dramatic increases first produced by the Industrial Revolution in the mid-18th to mid-19th centuries (Boutron et al., 1994; Han et al., 2002; Hong et al., 1994; Ortega, 2014; UNEP, 2002). For instance, data published by Settle and Patterson (1980) indicate that there was a 200-fold increase in air-derived lead concentrations between the years of 1750 and 1989. These increases in pollution have led to increased concern among researchers determined to understand the long-term effects of pollution harmful to both human and on-human populations and the environments that they inhabit (Rhind, 2009).

While numerous developed, high income countries (HICs) have implemented monitoring programs to track modern-day levels of pollution, such programs have yet to be adopted or reinforced in developing, low to middle income countries (LMICs) (UNEP, 2002a). One way to increase awareness, further boost concern at the governmental level,

and shape policy to control pollution is to assess how pollution overall or specific pollutants have changed over time. To do so, it is important to demonstrate when shifts in pollution can be linked to human economic activities, such as industrialization, the globalization of commerce, and tourism (Ellison and Farnswortaimh, 1996; Khan and Ghouri, 2011; Rhind, 2009).

2.3 Anthropogenic environmental pollution from lead (Pb), mercury (Hg) and cadmium (Cd)

Lead (Pb), mercury (Hg) and cadmium (Cd) are heavy metals and inorganic pollutants. They can be detected in the tissues of biological organisms through trace element analysis. These three elements are present in geological strata in the natural world and are often released through natural processes, such as erosion, but are most commonly released into the environment via anthropogenic activities, such as fossil fuel combustion, mining, agriculture, and waste disposal (Alloway, 1997). The following three sections are dedicated to providing a more detailed description of each of these pollutants, as well as discussing their origins.

2.3.1 Lead (Pb)

Lead is a non-essential or inorganic element that naturally occurs in the earth's crust (Alloway, 1997; Garcia-Leston et al., 2010; Meyer et al., 2008). It is also poisonous if ingested, and accumulates in soft tissues as well as the skeleton, damaging the nervous system in animals and causing blood disorders in mammals (Talmage and Walton, 1991). Lead is a soft metal that deforms under stress, resists corrosion, conducts poorly, and is highly malleable (Garcia-Leston et al., 2010). These properties have made lead suitable for a variety of applications throughout history (Garcia-Leston et al., 2010; Meyer et al.,

2008). For example, lead has been used for household purposes, in cosmetics, as a sweetener, gasoline additive, for use in batteries, ammunition, pesticides, as an analytical reagent, an astringent, and as a water repellent (Alloway, 1997; Hu and MPH, 2002; Johnson, 1998; Meyer et al., 2008). Lead is still widely used for industrial purposes, such as the combustion of coals and oil, municipal solid waste combustion, and in mining and smelting (Garcia-Leston et al., 2010; Meyer et al., 2008; Nriagu, 1983). As of 1998, The United States utilized an estimated 1,400,000 metric tons of lead per year between the years of 1994 to 1995 (Johnson, 1998). The trend continues; globally, Hu (2002) estimates that the current annual production of lead is approximately 5.4 million tons, and increasing annually. This information supports the idea that the archaeological and modern sea turtle samples used in this study should exhibit a positive relationship when attempting to quantify the change in lead concentrations in the WCR since the advent of the Industrial Revolution.

2.3.2 Mercury (Hg)

Resembling lead, mercury (Hg) is classified as a non-essential heavy metal (Boyd et al, 2000; Gochfeld, 2003). It occurs in numerous chemical forms such as unreactive elemental mercury, vapor or liquid, inorganic mercurial salts and minerals, and organic mercurials (Bernhoft, 2011; Graeme et al., 1998; Neustadt and Pieczenik, 2007). Mercury has a low melting and boiling point and can easily convert between its various chemical forms. It can, therefore, be absorbed by various biological organisms (Hylander and Meili, 2002). Like lead, it is poisonous to animals, and can cause an array of functional disturbances, particularly affecting respiratory function and the nervous system (Talmage and Walton, 1991). Apart from being naturally released into the environment through

volcanic activity, erosion of geologic deposits, and volatilization from the ocean, mercury is also released by anthropogenic activities. The primary sources of mercury pollution are artisanal and small-scale gold mining (ASGM), coal burning, paper mills, runoff from deforestation, and the use of mercury in pesticides (Emanuel, 2010; Gochfeld, 2003; Hu, 2002). Additionally, mercury has been used in the manufacturing of medications, antiseptics, thermometers, dental amalgams, and batteries for several centuries (Emanuel, 2010; Gochfeld, 2003; Hu, 2002). Since mercury is used for industrial purposes and is also introduced into the environment via environmental disasters, it has been deemed a widespread contaminant (Hu, 2002; Koh et al., 2009).

According to the UNEP, global mercury assessment (2013), ASGM emissions have been reported to account for over 37 % of the annual amount of total anthropogenic emissions (727 tonnes a year to be exact). In regard to mercury in aquatic environments, it has been estimated "that anthropogenic emissions over the last 100 years have doubled the concentration of mercury in the surface layer of the ocean, and increased it by 25% in intermediate waters, and by 10% in deep waters" (UNEP, 2013; 26). According to the UNEP (2013) report, the total amount of mercury deposited into the oceans in 2008 was estimated to be 3700 tonnes. However, this information pertains to global anthropogenic mercury pollution data; it is not specific to the Caribbean (such information is discussed below). While pollution data for the Caribbean are available, information relating to the concentrations of the elements prior to the advent of industrialization is neglected. Therefore, the need for a study that quantifies the concentrations of mercury, lead and cadmium is necessary in order to provide estimation as to how levels of pollutants have changed over time due to anthropogenic activity.

2.3.3 Cadmium (Cd)

Similar to lead and mercury, cadmium (Cd) is characterized as a non-essential element (Chmielowska-Bak et al., 2014). It is found in the environment as a pure metal, but is also found in higher abundance as complex oxides, sulphides, and carbonates in zinc, lead, and copper ores (UNEP, 2010a). According to the Agency for Toxic Substances and Disease Registry (ATSDR), cadmium and its oxides are resistant to corrosion, have a melting point of 321 degrees C, and are nearly all insoluble in water. As a result of these chemical properties, cadmium is typically used in various human activities such as mining, metal production, and combustion of fossil fuels. The primary anthropogenic sources of cadmium pollution on land and in aquatic environments are from coal combustion, mine tailings, smelter slag and waste (UNEP, 2010b). Like mercury and lead, cadmium is poisonous. Exposure to cadmium as an environmental contaminant occurs primarily from fossil fuel combustion, phosphate fertilizers, iron and steel production, cement and nonferrous metals production, and municipal solid waste incineration (Morrow, 2010). Cadmium is classified as a carcinogen and has been associated with a variety of cancers in humans (IARC, 1993).

Currently, global cadmium emissions are estimated to be between 15,000 and 88,000 tons per year (UNEP, 2010). This is a significant increase from global cadmium emissions in 1989, which were estimated to be between 150 and 2,600 tons per year. These data have been supported by the Nordic Council of Ministers (2003), whose environmental report estimated the global amount of mobilized cadmium to have increased by a factor of four between the years of 1950 and 1990. As a result, researchers have exercised a great deal of energy studying the amount of cadmium pollution being

released into the environment and the adverse effects it has on both humans and animals (UNEP 2008). However, studies focusing on cadmium in the Caribbean are sparse. Therefore, this study focused on providing baseline information needed to measure the element in the WCR.

2.4 Lead, Mercury and Cadmium Pollution in the Caribbean

From the mid-1990s to present, an array of research has focused on studying the numerous forms of environmental media and pollutants that affect marine environments in the WCR (Allen, 1992; Ellison and Farnsworth, 1996; Fernandez et al., 2007; Rivera-Monroy et al., 2004; Siung-Chang, 1997). Environmental media refers to sources such as agricultural processes, electricity generation, mining and smelting, derelict gas works sites, metallurgical industries, chemical and electronic industries, general urban sources, water disposal, transport (long and short range), and incidental sources that are responsible for introducing contaminants into the environment (Alloway, 1997). For the WCR, agricultural sources, mineral extraction, sewage disposal, tourism, and incidental sources (e.g., oil spills) are the environmental media of greatest concern (Fernandez et al., 2007). For instance, the mineral extraction of bauxite, which is an aluminum ore and the world's main source of aluminum, has been a large concern in relation to environmental pollution in the Caribbean. Bauxite extraction is responsible for the introduction of several pollutants into the environment, namely lead, mercury and cadmium (Fernandez et al., 2007). The UNEP (1999) reported that the extraction of bauxite in the Caribbean accounts for at least 29.3% of the mineral's global production.

Oil production is another anthropogenic activity responsible for releasing large quantities of pollutants including lead, mercury, and cadmium into the WCR. According

to Clark and colleagues (2001), Venezuela, Trinidad, Tobago, and the Gulf of Mexico are leading locations for oil production; Venezuela, Trinidad, and Tobago are part of the WCR. Accidents such as overflows, blowouts, and pipeline fractures are not uncommon in these locations (Clark, 2001). For example, from 1979 to 1980, 350,000 tonnes of crude oil entered into the sea. When such events are coupled with sewage pollution caused by tourism and industrial processes, marine environments throughout the WCR are burdened by extensive amounts of pollution, including that from lead, mercury, and cadmium (Clark, 2001).

2.5 The Advent of Industrialization

The term 'Anthropocene' was coined by Zalasiewicz and colleagues (2010) to describe the impact that humans have had on the environment as early as the 18th century. While a precise date cannot be provided for the Anthropocene, it has been suggested that the greatest impact that humans have had on the environment began with the Industrial Revolution in the mid-18th century (Galuszka1 and Migaszewki1, 2011). Importantly, the Industrial Revolution was a driving force in anthropogenic pollution from heavy metals throughout global environments (Galuszka1 and Migaszewki1, 2011). Industrialization also initiated a vicious cycle; as rapid economic growth has continued, the demand for increased industrial production has led to increased pollution emissions worldwide (Galuszka1 and Migaszewki1, 2011). For instance, Ashton (1948) found that coal production increased from an estimated 4.5 million tons in 1750 to 16 million tons by the mid-1800s. As of 2013, total global coal production has been estimated to be 7,823 Mt (megatonnes). According to the U.S. Energy Information Administration (EIA) International Energy Statistics (2014), the amount of global CO_2 emissions produced in

2012 from the consumption of coal was estimated to be 14,228.954 million metric tons. This demonstrates a positive correlation between variables such as time, population size, consumer demand, and pollution. More specifically, as time proceeds, population size increases worldwide, which in turn increases the demand for products such as coal and essentially causes an increase in global anthropogenic pollution (Sherbinin et al, 2007).

2.6 Effects of the Industrial Revolution in the Caribbean

The Industrial Revolution, while specifically associated with Britain and the United States, had an indirect effect throughout all other regions of the world, including the Caribbean. The Industrial Revolution refers to "profound economic transformations resulting from the introduction of new technologies of production" (Foner and Garraty, 1991: 559). "This economic transformation led to transformative social effects within British society and thus leading to a large proportion of the population to be released from the land and absorbed into industry and the towns" (Daunton, 1995: 125). Population size increased, as did the demand for industrial and commercial products, such as coal and sugar. As Industrialization occurred globally throughout the 18th, 19th, and 20th centuries, this same process occurred in populations all around the world, with broad-ranging global effects occurring throughout.

The Caribbean was one region involved in this economic transformation. As Ortega (2014:1) states in regard to industrialization and the Caribbean, advances in technology associated with the Industrial Revolution "did not belong to a single empire or dominant economy". Instead, industrialization could be found throughout a variety of economies, especially those involved in the plantation-based production and processing of sugar cane in the Caribbean in the 18th and 19th centuries. For instance, with the

diffusion of vacuum pan technology and evaporation machinery (technology required for sugar cane production) within the West Indies and the Caribbean, increased European demands for sugar were met (Ortega, 2014). The mass production of sugar also paved the way for an increased production of additional commodities such as rum, molasses, and Falernum (sweet syrup). A brief discussion of these particular commodities is warranted, for it provides a link between agricultural production, environmental damage, and the moment in which industrialization and its effects increased and spread throughout the Caribbean.

Although the WCR was thriving from technological success in sugar cane productions in the 19th century, Ortega (2014) discusses how "the increased mechanization of the Caribbean plantation, especially with new sugar-cane-crushing technologies and the advent of the steam engine, led to systematic deforestation in the region" (Ortega, 2014: 10). This was the result of using forests as a natural source of fuel. As this natural resource became exhausted between 1812 and the 1850's, British coal was imported to the WCR as an additional form of fuel (Ortega, 2014). Knowledge of increasing deforestation and the import of coal within the WCR are important to this study, as it represents the moment in which the WCR likely experienced a tipping point and dramatic shift towards increased anthropogenic pollution, particularly in regard to lead, mercury and cadmium. Deforestation would have led to increased amounts of mercury being transported into streams and marine waters, while the burning of coal would have led to increased lead, mercury and cadmium emissions into the local atmosphere and marine environments (Ashton, 1948; Ortega, 2014).

2.7 Sea Turtles as Environmental Indicators

As previously discussed, the Industrial Revolution had a tremendous impact on industrialization throughout the Caribbean. With increasing population size and increased industrial services occurring throughout the Caribbean Region, it is no surprise that anthropogenic activities would have caused a temporal increase in the amount of contaminants introduced into the marine environments. To quantify such change, trace element analysis was performed on two sea turtle species: hawksbill (*Eretmochelys imbricata*) and the green turtle (*Chelonia mydas*).

2.7.1 Green turtle (*Chelonia mydas*)

There are seven different species of sea turtle located throughout the world. Of the seven, the green sea turtle (*Chelonia mydas*) is reported to be the second largest and has been reported to have a lifespan of between 80 and 100 years (Anan et al., 2001). This species is frequently sighted within tropical and subtropical waters, between the latitudes of 40^{o} N and 40^{o} S (Hirth, 1997; Lagueux, 2001). While green turtles are categorized as being circumglobal, numerous studies have found that they follow a cyclical migration pattern (Carr, 1960; Hirth, 1997; Lahanas, 1998; Luschi et al., 265). This migration pattern begins with the hatching process, which takes place at the nesting beach or internesting habitat. Upon hatching, the baby sea turtles proceed to the ocean, where they make their way to an epipelagic habitat. The epipelagic habitat or the upper zone of the ocean refers to the area just below the surface to approximately 100 meters in depth below the surface (Bjornadal, 1997; Frick, 1976). Within this epipelagic zone, the green sea turtle typically maintains an omnivorous diet. The epipelagic stage of a green turtle juvenile can last anywhere from 2 to 5 years, after which time they begin to migrate

towards near shore, or neritic, environments (Reich et al., 2014). Within these neritic environments, the green turtle shifts from an omnivorous diet to an herbivorous diet that consists of seagrass and algae (Bjorndal, 1985). Upon maturity, which can take up to 50 years, the adults begin to make their way back to the nesting beach on which they hatched, in order to begin their own nesting (Hirth, 1997; Lageuex, 2001).

2.7.2 Hawksbill Turtle (*Eretmochelys imbricata)*

The hawksbill sea turtle (*Eretmochelys imbricata*) is very similar to the green turtle, in that it exhibits a cyclical migration pattern, lives for an extended period of time, and experiences a habitat shift from an epipelagic habitat to a neritic habitat during the younger years of its life (Amorocho, 1999; Bjorndal and Bolten 2010). The hawksbill sea turtle has a life expectancy of 30 to 50 years, with maturity typically occurring between 20 to 40 years (NMFS and USFWS, 2013). Based on information gathered from sightings, strandings, and gut content analysis, hawksbill turtles have an omnivorous diet consisting primarily of sponges, corallimorpharians, and zoanthids (Bjorndal and Bolten, 2010). According to information obtained from tag returns, satellite telemetry, and genetic analyses, adult Caribbean hawksbills have been reported to travel anywhere from 314 km to 466 km when traveling between foraging and nesting grounds (Amorocho, 1999; Meylan, 1999; Bass, 1999; Troeng et al., 2005). It is not uncommon to observe hawksbill sea turtles travelling these distances every two to five years, as they are a philopatric to their breeding grounds during the reproductive stage of their life. This suggests that this species shares a strong fidelity to specific breeding grounds throughout the region (Amorocho, 1999). This is important, as it supports the use of sea turtle

remains collected in the Caribbean as good environmental indicators of the Wider Caribbean Region over time.

The life history, biology, behavior, and diet of green turtles and hawksbill turtles support the premise that sea turtles are good environmental indicators for assessing the degree at which anthropogenic activity has altered lead, mercury and cadmium concentrations within the WCR throughout time. With a life expectancy ranging from 30 to 100 years, turtles should yield heavy metal concentrations representing decades for analysis. This information is vital to this study, as it provides an opportunity to increase awareness regarding the contamination status for lead, mercury and cadmium throughout the WCR (Hayase et al., 2009). The assumption that trace elements obtained from the sea turtle remains will be a reflection of the Caribbean Region is based on both sea turtle species sharing a fidelity to particular nesting grounds within the Caribbean.

2.8 Diagenetic effects involving lead, mercury and cadmium from an archaeological context

The analysis of archaeological remains to gain insight on past populations, environmental changes, and catastrophic events has become a common occurrence in the field of anthropology (Uerpmann, 1973; Ericson, 1985; Mays, 1999). In regard to this study, chemical analysis was performed on Caribbean Sea turtle remains to assess the degree to which anthropogenic activity has altered Caribbean marine environments over time. However, to ensure that the interpretation of the analytical results was sound, an understanding of diagenesis, and the effects it can have on the chemical makeup of the archaeological sea turtle remains is imperative. In the following section, a brief discussion on the general structure of sea turtle bone and its formation process will be

provided. This will be followed by a discussion of diagenesis and the potential elemental changes it has on lead, mercury, and cadmium in archaeological sea turtle remains.

For anatomical purposes, the skeleton of sea turtles is typically divided into three main groups: the skull and hyoid apparatus, axial skeleton, and appendicular skeleton (Wyneken et al., 2013) (Figure). For this research, the appendicular skeleton was the primary focus, with a more specific focus on the humerus. The appendicular skeleton consists of the flippers, pelvic and pectoral girdle, and the hind limbs (Wyneken et al., 2001). As for the humerus, it is a long bone element that is subjected to bone remodeling throughout the entire life of the organism.

As bone is interred in sediment, it can become subjected to post-depositional changes, or diagenesis. These post-depositional changes have been described as destructive actions caused by biological processes such as root action, insects, and rodents that can be observed on the outside of the bone (Neilsen-Marsh and Hedges, 1999). However, post-depositional changes can also be microscopic, causing degradation to the interior of the bone in the form of microbial or chemical degradation (Turner-Walker, 2007). Chemical degradation has been defined as a contamination process that involves “the uptake and diffusion of chemical elements or other alteration processes like dissolution or erosion that change the elemental composition of bones"(Reiche et al., 1999: 657).

As discussed by Quanttropani et al. (1999), the effect diagenetic changes have on the chemical makeup of archaeological remains is dependent upon numerous factors within a given environment. One important factor is the preservation of bone, which is highly dependent upon the soil pH (Quanttropani et al, 1999). For instance, soils

that are classified as being neutral to slightly alkaline are advantageous to the preservation of bone. However, soils such as sand and gravel, which are more acidic, create a poor environment for bone preservation (Henderson et al, 1987). Addressing preservation of bone in an acidic environment is particularly important to this research, for the skeletonized sea turtle remains used for this study were previously interred in sand prior to being excavated and curated at the National Park Service's facility in Christiansted and the United States Fish and Wildlife Service Facility in Fredricksted, St. Croix. As stated by Muller et al. (2001), "heavy metals are among the quantitatively most important pollutant groups, and are highly persistent in the soil environment"(Muller et al., 2001: 11). Due to anthropogenic pollution such as sewage sludge, various industrial activities and the disposal of waste products, heavy metals have increasingly been introduced to the terrestrial environment (Muller et al., 2001). The presence of heavy metals in the terrestrial environment, specifically sand, is important to this study. It affirms the importance of understanding and accounting for any possible effects diagenesis may have on the concentrations levels of lead, mercury, and cadmium found within the sea turtle remains used in this study. A more detailed discussion on how to account for diagenesis in archaeological bone can be found in the methods section.

2.9 Theoretical Framework

An ecological approach was applied to analyze the social and cultural elements in the context of the total Caribbean environment, in which both the marine organisms and humans exist. According to Vaughn et al. (2014), "knowledge of the interaction between humans and the physical environment is an interdisciplinary field of study that, although naturally involving geography, also includes biology, environmental psychology, public

health, and anthropology". As defined by Dincauze (2004), “systems are bounded sets composed of entities and their relationships” (Dincauze, 2004:32). An open system or complex system, like that being studied in this research, is a system which receives matter, energy, or information from their environments (Dincauze, 2004: 32). These systems include living organisms which exhibit feedback responses from both inside and outside of their systems.

By employing an ecosystem ecology or ‘systems thinking’ approach it can be assumed that anthropogenic activity and pollution both on land and within global waters will, at one time or another, impact both local and global ecosystems. Therefore, it is not implausible to assume that chemical signatures from past anthropogenic activity throughout the Caribbean would demonstrate a change in elemental concentrations when analyzed in skeletonized sea turtle remains. With this reasoning, the use of an ecological approach was deemed appropriate for developing a framework that studies population dynamics and the creation of a hypothesis that focuses on addressing how past anthropogenic activity has altered concentrations of mercury, lead and cadmium in WCR marine environments.

CHAPTER III

MATERIALS AND METHODS

To create a chronology of anthropogenic pollutant exposure for Caribbean marine environments and establish a pre-industrial baseline of pollutant exposure, a sample (N=11) consisting of two sea turtle species, *Eretmochelys imbricata* and *Chelonia mydas*, were analyzed. The samples were recovered from St. Croix, USVI and are subdivided into two subsamples from two different time periods: a modern subsample of skeletonized sea turtle remains (n=6) and a prehistoric subsample (n=5) of sea turtle remains recovered from several archaeological sites. The modern turtle remains were recovered from the Sandy Point National Wildlife Refuge, USFWS, in Frederiksted. They represent the remains of deceased turtles that washed up onto the refuge between 1975 and 2010 and were buried in a 'sea turtle graveyard' on the beach at the refuge. The turtles were interred in sand to facilitate skeletonization, without any modifications to the remains. The archaeologically derived sea turtle remains are part of the Folmer Andersen Collection, a collection of pre-Columbian artifacts established in the early 20th century. These artifacts were excavated from various prehistoric archaeological sites on St. Croix and have been curated by the National Park Service in stainless steel cupboards for the past twenty years in a climate controlled curation facility at Fort Christianvaern, Christiansted, St. Croix.

Skeletochronology, Fourier Transform Infrared Spectroscopy (FTIR), and Inductively Coupled Plasma Mass Spectrometry (ICPMS) analyses were performed on the remains. These techniques were used to satisfy various objectives for each turtle individual in the sample. Skeletochronology was used to estimate the age at death, FTIR was used to assess whether diagenesis occurred within the remains and ICP-MS was used to characterize the concentrations of lead, mercury and cadmium within the remains.

3.1 Folmer Anderson Collection

Between the 1920s and1930s, Folmer Anderson, a manager of the Bethlehem Sugar Factory and several other sugar processing plantations on St. Croix, began conducting numerous excavations throughout the island (Hardy, 2008). From these excavations, the Folmer Anderson collection, "one of the largest collections of prehistoric Caribbean materials," was established (Hardy, 2008: 122). Archaeological analysis of ceramic material from the Folmer Anderson collection has generated a date between A.D. 400 and 1200 for artifacts within the collection (Hardy, 2008). This of course, is presuming that all of the sites Folmer Anderson worked at had ceramics and did not have pre-ceramic occupations. This collection is comprised of approximately 13,000 artifacts excavated from several prehistoric sites located throughout the island. These sites include: Salt River, St. George, Glynn, Windsor, Richmond, Krause (Prosperity) and Sprat Hall. However, there is little information available regarding the provenience of the artifacts within the collection; the site and approximate date of the turtle remains included in this study therefore are unknown (Hardy, 2008) (Figure 3.1).

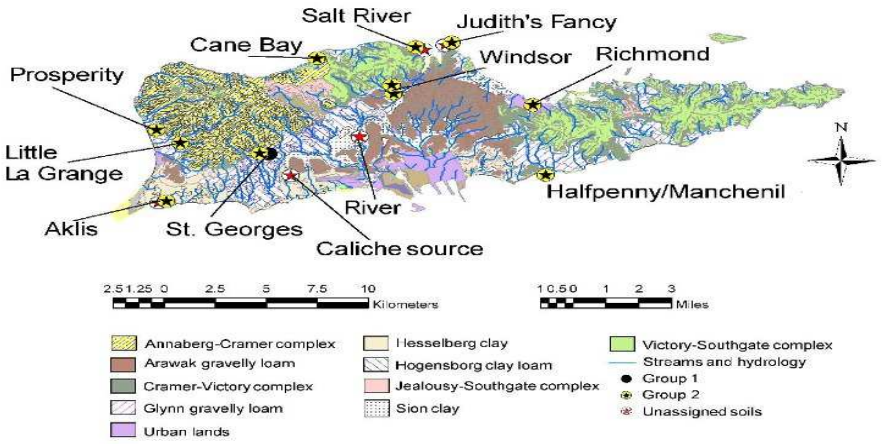

Figure 3.1 Map Showing the Archaeological Sites that Comprise The Folmer Anderson Collection. Source of photo: Hardy (2008)

3.1.1 Salt River

Salt River is a prehistoric site located on the north side of the island. Archaeologically, Salt River is considered to be "a major religious/cultural center, as well as a long-lived permanent settlement" for the Caribs, a Native American culture that inhabited the island of St. Croix prior to and shortly after the arrival of Columbus in the New World. Archaeological and historical evidence suggests that they took part in agricultural cultivation (Cissel, 1998). Salt River is referred to as the "Columbus Landing Site" and may represent the location where Columbus and his expedition made contact with the Caribs on their second voyage to the New World on November 14, 1493 (Cissel, 1988; Hardy, 2009). Analysis of human skeletal remains from this site yielded a radiometric date of 1050 +/-40 BP (Wentz, 2007). Salt River also featured in colonization of St. Croix by Europeans, acting as the focal point for several colonization attempts in the mid-17th century (Cissel (1988). Increased European presence in the 17th and early 18th centuries in the area led to the construction of plantations focused on growing cotton, indigo, tobacco, sugar, and various food

staples. By the mid-18th to 19th centuries, Salt River had become an integral site for "economic development" on St. Croix, with a primary focus on the export of sugar, rum, and molasses (Cissel, 1988).

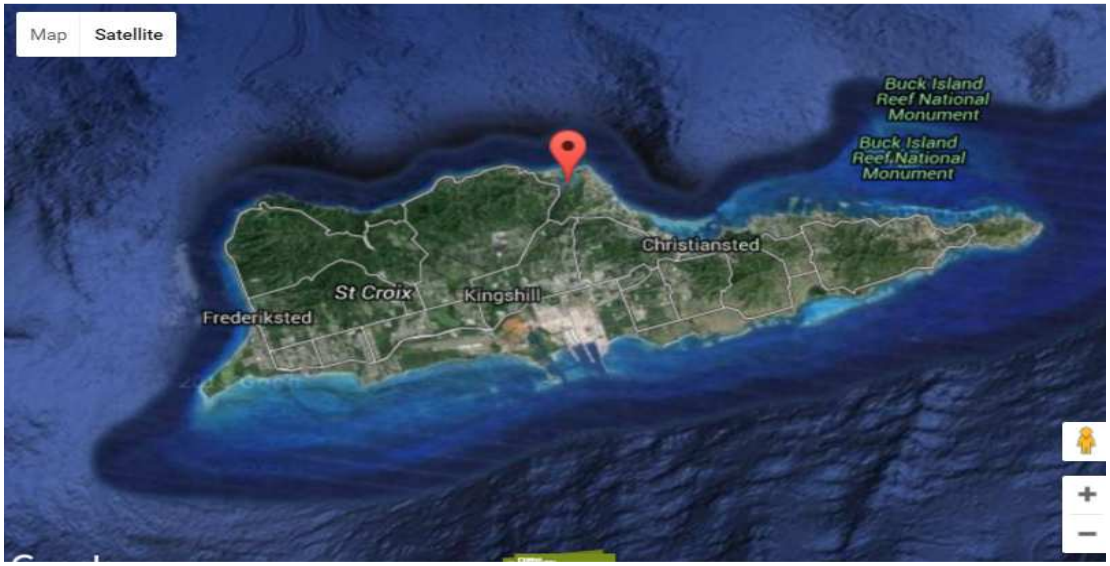

Figure 3.2 Salt River Bay; Provided by Google Earth

3.1.2 Richmond

Richmond was excavated in 1951 through joint efforts by Folmer Anderson, the Peabody Museum of Natural History at Yale University, and the St. Croix Museum Commission. The primary investigator for the Peabody Museum of Natural History survey was Gary Vescelius, who produced the only known archaeological publication on the site (Hardy (2009). In Vescelius's report, Richmond is described as a "cleared plaza surrounded by several middens". Excavation revealed that the middens had been disturbed after their initial accumulation construction, but gave no indication as to whether the disturbances were from previous excavations (Hardy, 2009). Recovered ceramics from the site indicate that possible settlements took place during the periods

known as Coral Bay-Longford (ca. A.D. 400-600), Magens Bay-Salt River I (ca. A.D. 600-900) and Magens Bay-Salt River II (A.D. 900-1200) (Hardy, 2009; Hardy 2007). The analysis of human skeletal remains from this site yielded a radiometric date of 1280 +/- 40 BP (Wentz, 2007).

Figure 3.3 Richmond, St. Croix; Image Provided by Google Earth.

3.1.3 Glynn & Windsor

The Glynn site is located "north of Concordia 'Gut' (i.e., drainage, creek) and northeast of Lebanon Gut, southwest of the Salt River site (Hardy, 2008: 132). The Windsor site is located near the Windsor Great House and is in close proximity to the Glynn site. Analysis of artifacts from the sites focused on ceramic attributes and decoration. Results from these analyses have linked these sites to the Prosperity-Coral Bay-Longford and Magen Bay-Salt River I periods (ca. A.D. 400-900). Despite the recovery of organic materials, such as bivalve shells identified as *Anomalocardia brasiliana* and *Crassostrea rhizophorae* (Carib pointed –venus and mangrove oyster) from these sites, no radiocarbon dates are available (Hardy, 2008).

Figure 3.4 Glynn & Windsor, St. Croix; Image Provided by Google Earth

3.1.4 Prosperity

The Prosperity site is located on the west side of the island, near Frederiksted. This site was investigated by Gudmond Hatt in 1923, Herbert Kreiger in 1937, Folmer Anderson in the 1920's to1930's, the 1950 St. Croix Archaeology Project, and Gary Vescelius from 1976 to 1979. Analysis of artifacts from the Prosperity site has led researchers to postulate that this site represents where prehistoric trade networks between St. Croix, Montserrat (represented by the Trants site), Vieques (represented by the La Hueca/Sorce site), and Trinidad (represented by the Pearls site), were established. Artifact analysis of ceramic attributes and styles has also led researchers to date this site to the Early Saladoid period (ca. 500-B.C. – A.D. 600) (Hardy, 2008). This information suggests that these sites may be associated with the earliest archaeological sites located on the island (Hardy, 2008). At this time, there are no known radiocarbon dates for this site.

Figure 3.5 Prosperity, St. Croix; Image Provided by Google Earth

3.1.6 St. George

The St. George site is located on the western side of the island and has been linked to the "Early Saladoid period communities in St. Croix" (Hardy, 2008:147). However, "no systematic archaeological survey or excavation has ever been conducted" for the St. George site (Hardy, 2008). Information on the site is scarce but Hardy (2008) has described it as consisting of at least six separate middens that are approximately two meters deep.

Figure 3.6 St. George, St. Croix; Image Provided by Google Earth

3.1.7 Sprat Hall

The Sprat Hall site is located approximately 1.2 km north of the Prosperity site, on the western portion of St. Croix (Hardy, 2008). This site was investigated by Folmer Anderson, Georg Nordby, Gary Vescelius, and Alfredo Figuerdo. Excavations and survey at the side indicate that it is a "multicomponent site, comprised of both prehistoric, Late Saladoid through Ostionoid (ca. 500 B.C. – A.D. 1492) and historic, Danish colonial plantation era remains" (Hardy, 2008). Several middens and burials have been excavated at the site, which has led researchers to hypothesize that this site represents a village. However, despite its close proximity to the coast, it appears to lack an abundance of shell when compared to other coastal sites within the area. Even though artifacts such as ceramic sherds, stone tools (celts), and conch have been recovered at this site, no radiometric dates are available (Hardy, 2008).

Figure 3.7 Sprat Hall, St. Croix; Image Provided by Google Earth.

3.2 Research Objectives

This study aimed to answer the following overarching research question: How have lead (Pb), mercury (Hg), and cadmium (Cd) concentrations in Caribbean marine environments been altered since the advent of industrialization to the 21st century? To address this overarching research question, two secondary research questions were created:

- Can sea turtle skeletal material, specifically humeri, be used as biological proxies to reconstruct anthropogenic pollution in Caribbean marine environments?
- Do the trace element concentrations of the anthropogenic pollutants lead, mercury, and cadmium recovered from skeletonized sea turtle remains change over time, between the prehistoric and modern eras?

To address these questions, trace element analysis was used to determine the concentrations of lead, mercury, and cadmium in the skeletal remains of sea turtles.

These concentrations were then meant to be used to generate a chronology of pollutant exposure in Caribbean marine environments and establish a pre-industrial baseline of pollutant exposure. To address the above research questions, the following hypothesis was tested:

Hypothesis 1 (H1): The industrial, modern sea turtle humeri and the archaeological sample do not exhibit a similar distribution of lead, mercury and cadmium ppm levels.

As pointed out by Phillips and Rainbow (1993: 10), anthropogenic pollution likely had an impact on aquatic environments as far back as the 'early nomadic groups of *Homo sapiens'*. But, it was not until the late 1700s that human impacts began to drastically alter local aquatic environments. According to Phillips and Rainbow (1993), the 1780s marked a period when industry, population size, and the production of organic wastes from sewage and agriculture began to increase in the developed, industrializing world, with impacts on global environments. Previous trace element studies have determined that global lead, mercury, and cadmium emissions have generally increased over time, reaching an all-time peak in the late 1880s, experiencing a decline between WWI and WWII, and beginning to increase again from the mid-1950s into the present. For results of the trace element analysis to be consistent with the above hypothesis, the two subsamples would need to provide trace element data that demonstrate a positive relationship. Specifically, the archaeological sample would exhibit small amounts of trace element contaminants, whereas the modern sample would exhibit higher concentrations of trace elements. In order to test this hypothesis, three sub-hypotheses were tested.

H_1a: The modern sea turtle sample will yield higher lead ppm levels and will therefore, exhibit a different ppm level distribution than that of the archaeological sample

Several studies conducted between 1995 and 2006 employing ice cores, fresh water sediments, and peat bogs have documented an increase in lead deposition since the advent of industrialization (Candelone and Hong, 1995; Coggins et al., 2006; Farmer et al., 1997; UNEP, 2006). For instance, ice core data obtained from the Greenland Summit glacier exhibited a 12-fold increase that could be linked to the 1970s (UNEP, 2006). Bruland and Franks (1983) published a study focusing on trace elements in ice cores that found evidence of a significant increase in lead concentrations since the beginning of the Industrial Revolution in the 19th century. Similar results were recorded in the Caribbean by Dodge and Gilbert (1984), who confirmed an increase in lead concentrations from 10.3 nmol Pb/mol Ca in 1954 to 24.5 nmol/ Pb/mol Ca in 1976. As of 2004, the UNEP estimated that anthropogenic activity was responsible for 3.15 million tons of mobilized lead, which is extracted lead from coal and lime (UNEP, 2006). This is an increase from 1983, when it was estimated that the total amount of mobilized lead disposed with waste from mining, base metal production, and from the use of coal was between to be .4 and 1.0 million tons (UNEP, 2006). These global data are the foundation for the hypothesis that time and lead concentrations are positively correlated. Results in support of this hypothesis would be that lead concentrations in the modern sample of sea turtles are higher than those in the archaeological sample of sea turtles. This finding would be consistent with those of other trace element studies in the published literature. Findings not in support of this hypothesis would include an inverse correlation between these

variables. Such data would be inconsistent with those of other studies in the published literature and would raise questions regarding the suitability of sea turtle humeri for reconstructing environmental pollution from heavy metals over time. Not to mention, such results may also suggest that sea turtles are merely not affected by the pollutants in this study.

H_1b: The modern sea turtle sample will yield higher mercury ppm levels and will therefore, exhibit a different ppm level distribution than that of the archaeological sample

As reported by the UNEP (2002b), global mercury concentrations have increased significantly since the advent of industrialization, with anthropogenic activities responsible for increasing the amount of atmospheric mercury by a factor of 3. Additionally, the UNEP has found that in close proximity to industrial areas, "the deposition rates of mercury have increased by 2 to 10 times during the last 200 years" (UNEP, 2002b: 90). The UNEP also predicts that with increasing fossil fuel combustion, the amount of mercury emissions globally will increase as well. Results from de la Cruz (2002) revealed that by-products produced from anthropogenic activities such as gold mining, nonmetal extraction, dental laboratories and hospitals, fish canning, municipal waste, mercury lamps, and batteries and electrical components have led to a substantial increase in mercury concentrations in the Caribbean Region. As a result, it is hypothesized that time and mercury concentrations in the Caribbean are positively correlated.

Results in support of this hypothesis would indicate that mercury concentrations in the modern subsample of sea turtles were higher than those in the archaeological

subsample of sea turtles. This finding would be consistent with those of other trace element studies in the published literature. As with H1a, findings not in support of this hypothesis include an inverse correlation between these variables. These would be inconsistent with those of other studies in the published literature. Such results would raise the same issues as produced by H1a.

H_1c: The modern sea turtle sample will yield higher cadmium ppm levels and will therefore, exhibit a different ppm level distribution than that of the archaeological sample.

Cadmium was discovered in 1817, began to be heavily mined in the 1940s (Hill, 2004), and escalated as a source of environmental pollution in the 1960s and 1970s. The UNEP (2008), for instance, documented that environmental deposition of cadmium in the 1960s and 1970s was eight times higher than that seen at the advent of industrialization. According to Gallego and colleagues (2012), the annual amount of cadmium introduced into the environment is estimated to be 13,000 tons. The UNEP has also released data that demonstrate a decrease in global cadmium deposition between the years of 1990 and 2003. However, it has also been stated that in some developing countries and countries that engage in negligent hazardous disposal practices of cadmium, cadmium concentrations have been reported to be increasing. In the Caribbean specifically, activities such as dredging in ports cause a disturbance in the already contaminated sediments, which are then introduced into the water column and ingested by marine organisms (GIWA 2006). Following these lines of evidence, an assumption is made here that environmental levels of cadmium and therefore concentrations of cadmium in the sea turtle sample will be higher in the industrial era than in the prehistoric period.

Results in support of this hypothesis would demonstrate that cadmium concentrations in the modern sample of sea turtles that are higher than those in the archaeological sample of sea turtles. This finding would be consistent with those of other trace element studies in the published literature.

3.3 Skeletochronology

Skeletochronology is an incremental growth technique used to provide an estimated age of an organism, which is determined through the analysis of the annual skeletal growth marks located within bone tissue (Ehret, 2004; Snover, 2002). These skeletal growth marks result from annual periodicity, defined as the moment in which bone formation stops completely or slows down dramatically. Associated with these growth marks are lines of arrested growth (LAG). Lines of arrested growth are the product of changes during bone formation that typically occur before new, accelerated bone growth commences and takes place within the primary periosteal compacta (Snover, 2002).

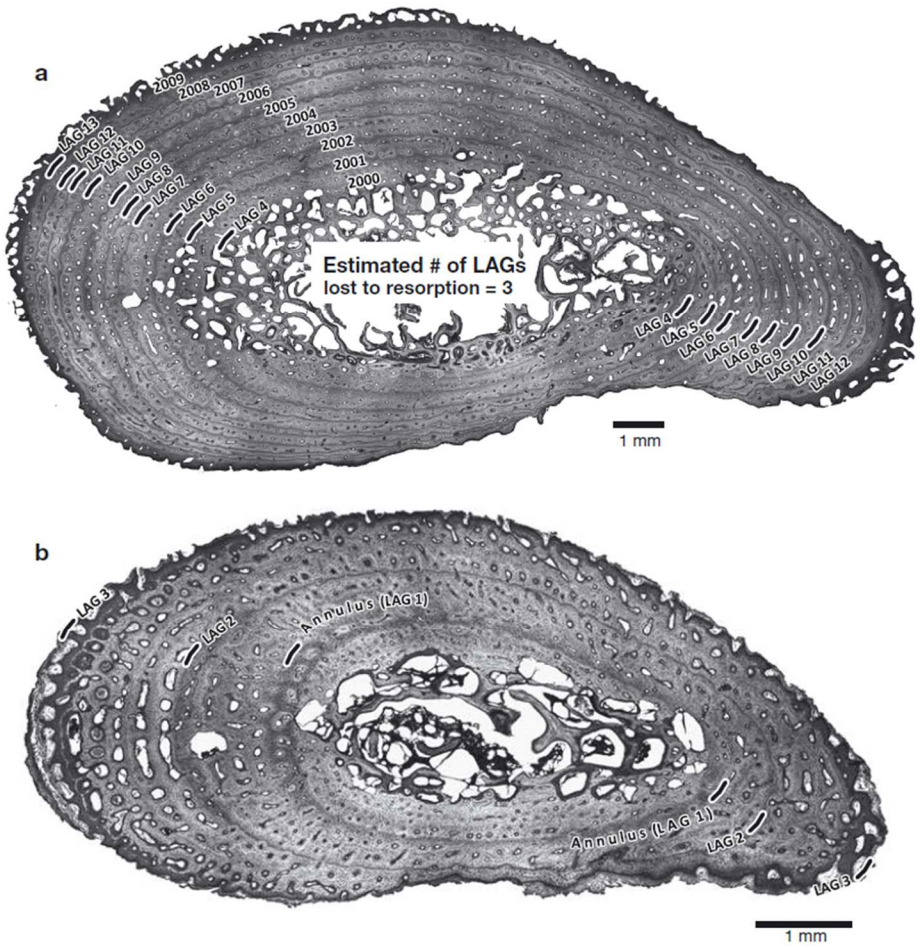

Figure 3.8 Sea Turtle Lines of Arrested Growth (LAG)

Notes: Photos provided by Avens and Goshe (2007).

In this study, skeletochronology was performed on three of the seven sea turtle humeri. The humerus was chosen for this study as it is the most commonly used element for the application of skeletochronology (Ehret, 2004; Snover and Rhoden, 2008; Zug et al., 2001). The bone exhibits muscle insertion scars capable of being used as landmarks for identifying reliable sectioning sites, making sample preparation much easier. Furthermore, the humerus has been characterized as having a slower and more reliable rate of remodeling and resorption, which allows for research focused on assessing change over time within sea turtle skeletal remains to take place (Ehret, 2004; Snover and Rhoden, 2008).

The skeletochronology sample preparation protocol for this study was previously established by Avens and Goshe (2007). Cross sections (2-3mm thick) were taken from the midshaft of the humerus, which is distal to the deltopectoral crest and is the narrowest diameter of the diaphysis (Figure 3). Per Zug et al. (2001), using cross sections from the midshaft of the humerus has been shown to generate a more reliable estimation of the number of growth cycles (periosteal layers) than does the use of other portions of the element. Once the cross sections were created, the slices of tissue were rinsed with distilled water and allowed to dry overnight. Following the soaking process, a Buehler IsoMet 1000 precision saw was used to obtain thin sections ranging between .95 mm and 1.07 mm. The thin sections were then ground down using a Buehler Metaserve 3000. The thin sections were stained using a 1:1 solution of Ehrlich's hematoxylin stain and distilled water. According to Snover and Rhoden (2008: 2), LAGs are found within the "histologic cross-sections of bone and are concentric thin layers that stain dark with hematoxylin." Once stained, the thin sections were mounted onto microscope slides using 100% glycerin to assist with the counting of LAGs (Avens and Goshe (2007). Once the LAGs were counted, width measurements (long-axis diameters) were taken at each successive growth cycle and resorption core of the humerus (Zug et al., 2001). By doing this, a minimum number of growth layers were obtained.

However, since sea turtle humeral bone resorbs and redeposits layers of bony tissue from the core of the bone outward, accurate age estimation was not obtained from the observable growth layers alone. In order to account for the unobservable component (growth layers in the remodeled core of the humerus), the correction-factor (CF) method was applied. The correction-factor method estimates the age of the turtle by adding together the number of growth layers observed in the outer region of the humeral section to the predicted number of resorbed growth layers present within the remodeled core of the humerus (Zug et al., 2001). The predicted number of resorbed growth layers was determined through the application of the following equation: C(R-Rh); where R denotes the radius of the absorption core, Rh denotes the radius of the turtle's humerus and C denotes the correction factor (Zug et al., 2001). Since the correction factor is a constant 'aging rate' (yr/mm), it can be applied to the resorption core and used to calculate the reciprocal of the mean growth layer width (Zug et al., 2001). By calculating the reciprocal of the mean growth layer width, an estimated age at death was determined and as a result, the interval of time in which each turtle was exposed to lead, mercury and cadmium throughout its lifetime was estimated.

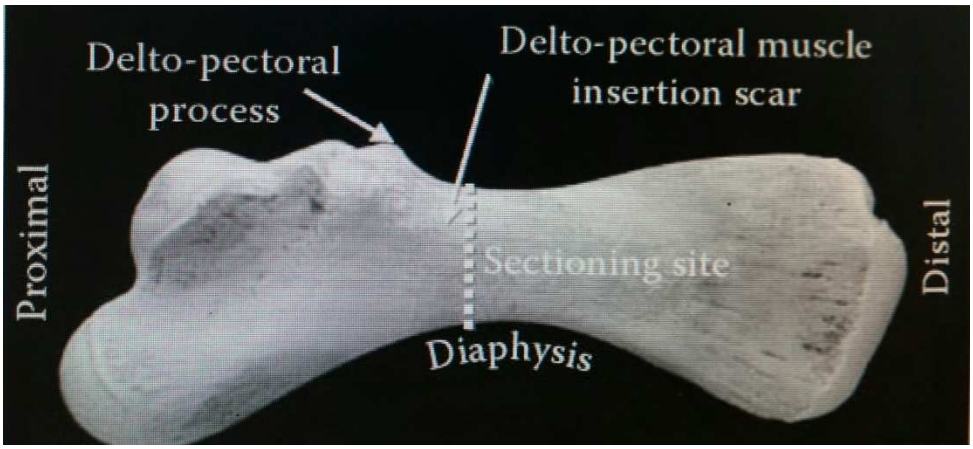

Figure 3.9 Ventral view of a sea turtle humerus.

Notes: Ventral view of a juvenile sea turtle humerus, displaying recommended site for cross-sectioning during Skeletochronology. Source: *The Biology of Sea Turtles*. Edited by: Lutz PL and Musick JA.

3.4 Radiocarbon Dating

Radiocarbon (^{14}C) is the product of cosmic rays interacting with nitrogen 14 (^{14}N) as it enters the Earth's atmosphere. The ^{14}C is oxidized to carbon dioxide and follows one of two pathways. The carbon dioxide can either enter the terrestrial biosphere via photosynthesis, where it continues along the food chain by way of herbivores and omnivores, or it will exchange into marine reservoirs, where it will eventually enter into the food chain again by photosynthesis (Brock & Cook, 2017). This process continues until the organism dies, at which time, the radiocarbon is no longer entering the body. The ^{14}C located within the body at time of death begin to decrease by radioactive decay, "at a rate measured by the ^{14}C half-life" (Taylor & Aitken, 1997: 65).

Radiocarbon analysis is an analytical technique used to measure the ratio of $^{14}C/^{12}C$ in archaeological and environmental samples (Brock & Cook, 2017). This process allows researchers to determine the radiocarbon age or calendar age of a sample

by measuring its residual ^{14}C content. This study employed this method to determine the age of the archaeological sea turtle remains, with the goal of providing a more precise as to when the remains were interred in sand. With a date of interment, this research could provide a more accurate timeline of what concentration levels for cadmium, lead, and mercury levels were prior to the advent of industrialization.

3.4.1 Analytical Sample preparation

Analytical sample preparation followed methods previously established by Hollund et al. (2013). These particular methods were chosen because they have previously been tested and validated as appropriate for 'establishing the type and extent of alteration' occurring in both fossil and archaeological bone (Hollund et al., 2013: 507). Prior to the analysis of the sea turtle subsamples, three transverse cross sections ranging from 3 to 7 mm thick were created. The cross sections were cleaned with deionized water and allowed to dry overnight. Following the drying process, one of the cross sections was wrapped in aluminum foil and placed in a sealed plastic bag. This process was performed for samples 1813-9 and 1813-10/11, which were then sent to the AMS laboratory at the University of Arizona for radiocarbon analysis. For the second cross section, a handheld Dremel tool with a tungsten-carbide drill attachment was used to create powdered samples from the bone cross-sections. The total amount of powder collected from the humeri sample was used for FTIR and ICP-MS analysis. The third cross section was not drilled, but marked with a permanent marker to denote where the trace element samples were collected from. The cross section was then wrapped in aluminum foil and sent to Georgia State University for stable isotope analysis.

Prior to drilling, the dremel tool was rinsed in distilled water and cleaned with hydrochloric acid (HCL) to rid the equipment of any dust particles, water-soluble salts, and any remaining mercury (Hg) that may be present. Once the dremel was cleaned with HCL, it was rinsed again with distilled water and allowed to dry. The entire preparation table was then covered with clean white paper and then covered with clean aluminum foil. The drill was used to remove 1 to 2 cm^2 of surface material and then gently brushed in order to remove any remaining bone dust. Once the initial surface material was removed, new aluminum foil was put into place and the drill was cleaned once more using the previously discussed method. Following this step, the drill was used to remove the compact, cortical bone. Where appropriate, samples were taken from outside the absorbed area, from the middle of the compact bone, and also from the outside of the bone. Upon removal, the powder was placed into pre-cleaned centrifuge vials.

Figure 3.10 Cross Section of Bone with Dremel Holes.

3.7 Carbon and Nitrogen Stable Isotope Analysis

Isotopes are chemical elements which have the same number of protons, but exhibit a different number of neutrons (Larsen, 1997). While there are several hundred stable isotopes, only 10 elements have been identified as having biological significance. Of those 10, carbon and nitrogen have been used to reconstruct and interpret the diets of organisms (Larsen, 1997).

Carbon has two stable isotopes, ^{12}C and ^{13}C. The relative abundance of isotopes between dietary resources is expressed in a percentage, which differentiates between the plant photosynthetic pathways (Larsen, 1997). These pathways include either C_3, C_4 or crassulacean acid metabolism (CAM). For marine plants, the ^{13}C values have been found to be between the values of C_3 and C_4 terrestrial plants (-22% to -38% and -9% to -21%) (Schoeninger and Moore, 1992). This means that marine organisms can exhibit values that are closer to C_3 plants or values that are significantly different and closer to C_4 plants (Larsen, 1997). Similar to carbon, nitrogen also has two stable isotopes, ^{14}N and ^{15}N. As discussed by Larsen 1997 (283), "nitrogen is bound up as N_2 in the atmosphere or ocean water. The ^{15}N values for terrestrial plants vary widely, but are generally between 4 to 20 percent lower than those found in marine organisms.

As dicussed by Avens et al., 2013:246, "when an animal moves among spatially discrete food webs that are isotopically distinct, the stable isotope values of its tissues can provide information about its previous environment" (Avens et al., 2013: 246). Hawksbill and Green Turtle juveniles have been documented to inhabit open ocean pelagic waters anywhere from 1-7 years after hatching (Goshe et al., 2010). During this time, these species exhibit an omnivorous foraging diet consisting of small ctenophores (comb

jellies) and *Janthina janthina* (pelagic snail) (Bjornadal, 1997; Frick, 1976). Following the 1 to 7 year period in pelagic waters, the turtles move to neritic waters, the relatively shallow part of the ocean above the drop-off of the continental shelf. This change in environment coincides with a shift in diet from an omnivorous diet, to a more herbivorous diet consisting of algae and seagrasses. In terms of isotopic signatures of diet, an omnivorous diet (consisting of benthic species) in sea turtles is expected to be associated with enriched $\delta^{13}C$. Whereas, a more herbivorous diet made up of algae and sea grasses is expected to exhibit enriched $\delta^{15}N$ signatures. This dietary shift is significant to understanding anthropogenic pollution in the Caribbean, as it can assist with trace element monitoring by understanding contamination status for lead, mercury and cadmium (Hayase et al., 2009).

3.5 Fourier Transform Infrared Spectroscopy (FTIR-ATR)

Bone consists of three major components: organic matter, primarily proteins, mineral in the form of calcium phosphates, and water. The organic matter in dry bone accounts for 22 to 23 % by weight (Turner-Walker, 2007; 5). The mineral component consists of a carbonate-containing hydroxyapatite (HAP) analogue, which is composed primarily of $Ca_{10}(PO_4)_6(OH)_{2)}$, commonly referred to as bioapatite (Turner-Walker, 2007). Other components of the mineral phase are traces of other anionic and cationic species that variously adsorb on crystal surfaces or substitute for $Ca^{2+,}$ $PO^{2-}{}_4$ and hydroxyl ions in the lattice.

According to Hollund et al. (2013:508), "analysis by FTIR-ATR gives information on both the chemical composition and the crystallinity of the material,

providing a rapid identification of the state of both the mineral and the organic phase". Specifically, the application of FTIR-ATR can provide frequency spectral information regarding the bone material properties such as mineral to matrix ratio (the ratio of integrated phosphate and amide bands), mineral maturity/crystallinity, and collagen maturity (conveyed as the ratio of two of the major Type I bone collagen crosslinks) (Paschalis et al., 2011). FTIR-ATR is an analytical technique applicable for this study, for it assisted in determining whether diagenesis may have occurred within the sea turtle remains following burial (Hollund et al., 2013). This is relevant to both the archaeological, prehistoric samples and the modern samples, as both were deposited in sediments after death and may have been affected by postmortem taphonomic processes including but not limited to the exposure to trace elements in groundwater or the burial context. Results obtained from FTIR-ATR were used to determine which sea turtle remains underwent the least amount of diagenesis (i.e. are the least contaminated) and were therefore most suitable for undergoing ICP-MS. Furthermore, by accounting for any diagenetic alterations that may have occurred within the sea turtle remains, interpretations of the trace element analysis made at the conclusion of this study are discussed with greater confidence.

The FTIR-ATR method was chosen over the traditional FTIR- KBr method, as it requires minimal sample preparation when compared to FTIR-KBr, reducing t he risk of contaminants that could potentially alter the chemical digniture of the sample. The FTIR-KBr method requires that the sample (bone in this case) be crushed into a fine powder and then mixed with potassium bromide (KBr). Following this step, the mixture is then pressed into a pellet and analyzed (Surovell and Stiner, 2001). In contrast, FTIR-ATR

preparation involves directly placing the sample onto the optically dense ATR prism. This decreases the sample preparation time and reduces the risk of the introduction of contaminants that could potentially alter the chemical signature of the sample (Stathopoulou et al, 2008; Thompson et al, 2009).

3.5.1 FTIR Analysis

FTIR-ATR analysis was performed at the Mississippi State University Chemical Engineering lab by this investigator, with the assistance of MSU Chemical Engineering staff. Prior to analysis, the ATR stage and diamond surface of a Nicolet 6700 spectrometer with a MIRacle Tm attachment were cleaned using a cotton ball covered with methanol. Once the equipment was sterilized, approximately .3mg of bone sample, enough to cover the ATR stage was put into place and pressed against the diamond surface. In order to obtain background spectra, the empty sample chamber and the ATR stage were used. The ATR unit measures the loss of intensity that takes place within the total internally reflected (TIR) infrared beam that meets the sample (Hollund et al., 2013). To achieve the spectral information, 128 scans in the 4000-400 cm^{-1} were taken, at a spectral resolution of 4 cm^{-1}. Since the depth of penetration of IR radiation and the path length are wavelength dependent, an automatic ATR data treatment for the ATR spectra was performed using OMNIC software (Hollund et al., 2013). The OMNIC software automatically handles the Fourier transform and the subtraction of the background, prior to providing a spectrum for the analysis.

To evaluate diagenesis in bone bioapatite, previously established methods by Weiner and Bar-Yosef (1990) were applied. Per the Weiner & Bar-Yosef (1990)

equation, which is the dominant crystallinity measure in archaeology, the calculation of the splitting factor (SF) (the crystallinity index as obtained by FTIR) was determined by summing the heights of absorbance values at the 563 and 603 cm^{-1} peaks and then dividing them by the height of the trough between the two. Per Weiner and Bar-Yosef, the unaltered modern bone SF value should range between 2.5 to 3.25, whereas; any altered archaeological remains should exhibit an IR-SF value above 3.4.

To calculate the carbonate to phosphate ratio (C/P ratio), methods published by Wright and Schwarcz (1996) were applied. Per Beasley et al., (2014: 19), the phosphate peak height at 1035 cm^{-1} "is the main phosphate absorbance peak that is not affected by the phosphate peak splitting at the 565 and 605 cm^{-1} peak". The absorbance value at this peak was used to calculate the C/P ratio. To determine the collagen content within the remains, the ratio of absorbance intensities of the amide peak at 1640 cm^{-1} was calculated (Trueman et al., 2008).

3.6 ICP-MS

ICP-MS is a highly sensitive analytical technique with the capability of performing multi-element analyses and providing high sample throughput (Arson and Secor, 2008). ICP-MS was performed in this study because it employs a quadrupole mass analyzer and single multiplier detector. These particular tools are fundamental for this research as they provide rapid analysis, have low detection limit, and have the ability to tolerate sample impurities (Chan, 2004). Additionally, ICP-MS was chosen based on its ability to provide trace element information in resolutions ranging from .7 to 1.0 amu (atomic mass units) (USGS, 2014). This method was chosen over laser ablation

inductively coupled plasma mass spectrometry (LA-ICP-MS) due to the fact that LA-ICP-MS frequently faces issues associated with calibration, ablation, fractionation, and sample transport (Arlan and Secor, 2008). Furthermore, since this study is not concerned with obtaining seasonal information, the need for a spot ablation feature of laser was unnecessary.

Trial 1:

For this study, two trials using two different acid digestion methods for ICP-MS analysis were performed. Trial 1 was performed by the Department of Chemistry at Mississippi State University. The Chemistry department was given 20 powdered bone samples for analysis. Each sample was made into an aqueous solution using acid digestion, which involved digesting 20 mg of the powder solution with 10 mL of hydrochloric acid (HCL). The solution was then diluted with 40mL of deionized water and sonicated in a Branson 1210 and/or 1510 ultrasonic cleaner for 2 minutes. Following sonication, the solution was placed in a fume hood and allowed to sit for 48 hours. After 48 hours had passed, it was discovered that chemical precipitation had occurred and left crystalline solids suspended throughout the solution. To separate the precipitate and supernate (remaining liquid) various methods, such as filtration, centrifuging, or decanting could have been performed. However, to avoid contamination of the sample, it was decided that a second trial would be performed using a different acid digestion method.

Trial 2:

Trial 2 was performed by the Mississippi State Chemical Laboratory at Mississippi State University. Due to limited sample availability, the laboratory was given a total of 17 samples. For the lead and cadmium analysis, .5 grams of the bone sample were digested with 3 mL of peroxide and 5 mL of nitric Acid (HNO3). Microwave digestion was then performed on the solution using a MARSX press vessel and applying the protocol set forth by the Mississippi State Laboratory. Following the digestion process, the sample was brought to 10 mL final volume due to small sample size availability. The solution was then placed in a fume hood and allowed to cool for approximately 24 hours. Cadmium and lead analysis was then performed using an Agilent 7900 ICP-MS using the EPA 200.8 analysis method.

The mercury samples were analyzed using a Milestone Direct Mercury Analyzer 80 (DMA80). The DMA80 "determines total mercury content in various matrices with detection limits as low as PPT levels" (MSU MSCL Procedure Guidelines; 1). By using the DMA80, no sample preparation was required. The powdered sample was directly introduced into the instrument and the analysis of mercury was performed using the integrated sequence of Thermal Decomposition, Catalyst conversion, Amalgamation, and Atomic Absorption Spectrophotometry as described in the EPA 7473 protocol.

3.6.1 Statistical Analysis

To determine whether the archaeological and modern datasets were from populations with the same distribution, non-parametric statistical hypothesis tests was performed. To summarize the key characteristics between the samples, box plots were

first created. To test for normal distribution within the data, a Shapiro-Wilk normality test was performed. This was followed by a Wilcoxon rank sum test, to determine whether the samples have population mean ranks that differ. Lastly, an Exact Wilcoxon-Mann-Whitney Test was performed to obtain a confidence interval for the data.

CHAPTER IV

RESULTS

4.1 Skeletochronology Analysis

The equation C(R-Rh) was used to determine the age at death for the sea turtle remains in this study. However, due to the limited sample size in this study, the correction factor was not calculated by this author. Instead, it was taken from a previously published study by Zug et.al (2001). In their study, the reciprocal of the mean growth layer width was calculated from 104 Hawaiian *Chelonia mydas* sea turtles. Based on their calculation, the correction factor was determined to be 1.14 yr/mm. Due to the fragility of the sea turtle remains in this study, and the unavailability of equipment required for this analysis, age at death was determined for only three of the eleven sea turtle remains. The samples used in this analysis were 2015-6, 2015-8, and 2015-10. The radii of the absorption cores and humeri, as well as their calculated age at death, are given in Table 4.1.

Table 4.1 Age Estimation for *Eretmochelys imbricata and Chelonia mydas* WCR Sea Turtles, Using the Correction Factor Method.

Sample	Observed LAG's	Radius of the Core (mm)	Radius of the Humerus (mm)	Predicted # of Resorbed Growth Layers	Calculated Age at Death (yrs)
2015-6	8	16.095	20.265	4.7538	13
2015-8	8	11.95	18.66	7.6494	14
2015-10	7	9.0115	13.275	4.86039	12

Using the radial values for the absorption cores and humeri listed in Table 4.1, the predicted number of resorbed growth layers was calculated to be between 4.7 and 7.6. Each value was rounded to the nearest whole number and added to the observed LAG's from each sample. This calculation revealed that the age at death for the sea turtles ranged between 12 and 14 years.

4.2 Radiocarbon Dating

Radiocarbon analysis was performed by the AMS laboratory at the University of Arizona. The analysis was unsuccessful due to the absence of bone collagen in the archaeological sea turtle remains.

4.3 Stable Isotope Analysis

Samples were sent to the Gerogia State University for stable isotope analysis. The research is ongoing and no preliminary information is available at this time.

4.3 FTIR Analysis

To assess diagenesis in the bone, the carbonate-phosphate ratio and Infrared Splitting Factor (IR-SF) were calculated for the 15 modern and archaeological sea turtle remains found in table. The splitting factor value was calculated using the following

equation: ([565_{ht}+605_{ht}]/590_{ht}). This calculation summed the absorbance values of the two phosphate peaks at wavenumbers 565 and 605 and divided the resultant value by the peak height at 590. The carbonate to phosphate ratio was calculated by dividing the peak heights at wavenumbers 1415 and 1035.

Table 4.2 FTIR-ATR Samples

Sample Type	Sample #	Weight (grams)
Modern	2015-6A	.34
Modern	2015-6B	.10
Modern	2015-7A	.30
Modern	2015-7B	.27
Modern	2015-7C	.07
Modern	2015-8A	.09
Modern	2015-8B	.08
Archaeological	1813-8A	.48
Archaeological	1813-8B	.10
Archaeological	1813-9A	.54
Archaeological	1813-9B	.32
Archaeological	1813-9C	.05
Archaeological	1813-10/11A	.57
Archaeological	1813-10/11B	.10

Modern Remains

For the modern remains, the SF value and C/P ratio were calculated for seven of the modern samples. Only two of the seven were shown to have both an altered splitting factor and a depleted carbonate-phosphate ratio. Of the seven modern samples 5 were found to have an unaltered splitting factor. Of those five, 4 were found to have carbonate-phosphate ratios that were depleted.

Table 4.3 Modern FTIR- Analysis Results

Modern Sample #	Splitting Factor	C/P Ratio	Diagenetically Altered
2015-6A	4 (Altered)	.235 (Depleted)	Yes
2015-6B	5 (Altered)	-1 (Depleted)	Yes
2015-7A	.5 (No Alteration)	0 (Depleted)	Yes
2015-7B	1 (No Alteration)	0 (Depleted)	Yes
2015-7C	3 (No Alteration)	0 (Depleted)	Yes
2015-8A	2 (No Alteration)	0 (Depleted)	Yes
2015-8B	2.3 (No Alteration)	1 (Elevated)	Yes

Archaeological remains

For the archeological remains, the SF value and C/P ratio was calculated for eight samples. Only two of the eight archaeological samples were calculated to have both a splitting factor and a carbonate-phosphate ratio that fell within the normal ranges. Of the eight archaeological samples, 75% of them were found to have an unaltered splitting factor. Four of the eight samples were calculated to have either a depleted C/P ratios or elevated C/P ratios.

Table 4.4 Archaeological FTIR- Analysis Results

Archaeological Sample #	Splitting Factor	C/P Ratio	Diagenetically Altered
1813-8A	2.714 (No Alteration)	.55 (Elevated)	Yes
1813-8B	1.48 (No Alteration)	.25 (No Alteration)	No
1813-9A	3.5 (Altered)	.25 (No Alteration)	Yes
1813-9B	2.71 (No Alteration)	.4 (Depleted)	Yes
1813-9C	3 (No Alteration)	.272 (No Alteration)	No
1813-10/11A	0 (No Alteration)	0 (Depleted)	Yes
1813-10/11B	3 (No Alteration)	.192 (Depleted)	Yes
1813-12	2.2 (No Alteration)	.714 (Elevated)	Yes

4.4 ICP-MS Analysis

ICP-MS analysis was performed on the sea turtle remains and the results were then used to test whether the archaeological and modern samples were from populations with the same distribution. The ICP-MS results for the archaeological and modern datasets are located in Tables 4.5 and 4.6.

Table 4.5 Trace Element Concentrations for Archaeological *Eretmochelys imbricata and Chelonia mydas* WCR sea turtles

Sample #	Element	Ppb	Ppm
1813-8	Cadmium	120.5	0.1205
	Lead	1021	1.021
	Mercury	216.8	0.2168
1813-9 (1 of 2)	Cadmium	49.14	0.4914
	Lead	772.1	0.7721
	Mercury	482.3	0.4823
1813-9 (2 of 2)	Cadmium	96.65	0.09665
	Lead	1076	1.076
	Mercury	531.3	0.5313
1813-10	Cadmium	143.8	0.1438
	Lead	3589	3.589
	Mercury	114.8	0.1148
1813-12	Cadmium	194.4	0.1944
	Lead	1784	1.784
	Mercury	1763.3	1.7633
1813-21	Cadmium	428.7	0.4287
	Lead	1265	1.265
	Mercury	25.7	0.0257

Table 4.6 Trace Element Concentrations for Modern *Eretmochelys imbricata and Chelonia mydas* WCR sea turtles

Sample #	Element	Ppb	Ppm
2015-6 (1 of 1)	Cadmium	50.55	0.05055
	Lead	2192	2.192
	Mercury	1.5	0.0257
2015-6 (2 of 2)	Cadmium	33.82	0.03382
	Lead	810.2	0.8102
	Mercury	17.5	0.0175
2015-7 A	Cadmium	110.2	0.1102
	Lead	4785	4.785
	Mercury	13.6	0.0136
2015-7B (1 of 2)	Cadmium	61.96	0.06196
	Lead	3280	3.28
	Mercury	2.7	0.0027
2015-7B (2 of 2)	Cadmium	108.7	0.1087
	Lead	5477	5.477
	Mercury	4.8	0.0048
2015-8 A	Cadmium	108.6	0.1086
	Lead	7327	7.327
	Mercury	4.6	0.0046
2015-8B	Cadmium	598.5	0.5985
	Lead	5271	5.271
	Mercury	7.8	0.0078
2015-9	Cadmium	91.21	0.09121
	Lead	3248	3.248
	Mercury	17.1	.0171
2015-10	Cadmium	24.56	.02456
	Lead	737.7	.7377
	Mercury	5.4	.0054
2015-11A	Cadmium	71.24	.07124
	Lead	6452	6.452
	Mercury	1.6	.0016
2015-11B	Cadmium	67.01	.06701
	Lead	4503	4.503
	Mercury	13.1	.0131

4.4.1 Lead

To study the distributional characteristics of the archaeological and modern lead datasets, box plots were created. Figure 4.1 demonstrates that the archaeological box plot is comparatively short, indicating that the overall archaeological ppm levels for lead are very similar within the sample. However, the modern data box plot is comparatively tall, indicating that the modern ppm levels are quite different within the sample. When the two box plots are compared, it appears that the medians and the distribution in ppm levels are quite different.

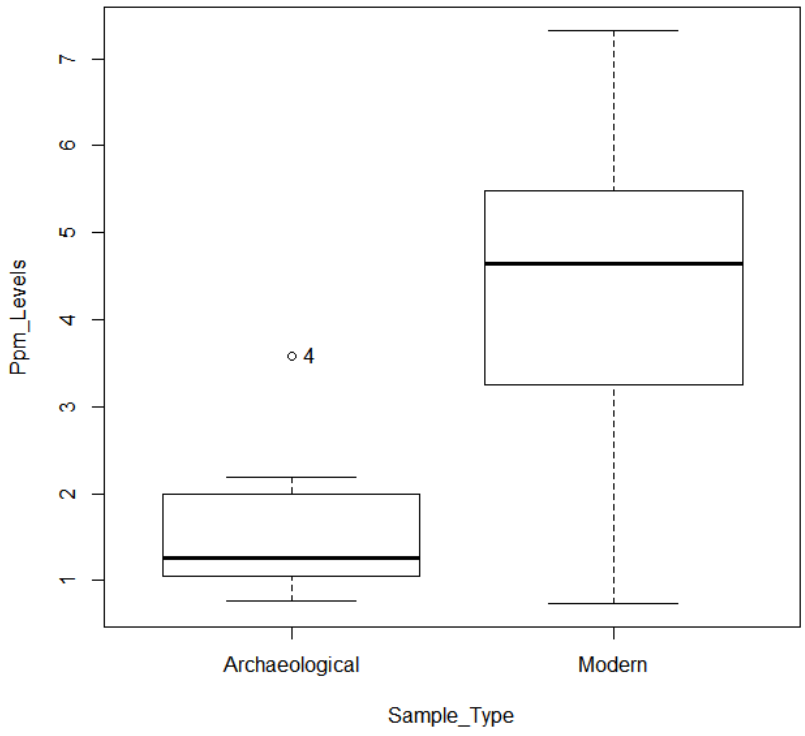

Figure 4.1 Box plot of the archaeological and modern lead datasets.

Prior to statistically determining whether the two samples were from populations with the same distribution, a Shapiro-Wilk normality test was first performed in order to test whether the datasets are normally distributed. The test produced a p-value for the archaeological sample was less than .05, suggesting that the sample does not fit a normal distribution. The p-value for the modern sample was greater than .05 and therefore exhibits a normal distribution. A Wilcoxon rank sum test calculated a p-value for both samples to be greater than .05. This implies that the samples are not significantly different at a .05 confidence interval. Despite the fact, that the difference in location was 2.4919, which suggests that the concentrations for the modern sample is approximately 2.5 times higher than the archaeological sample. The statistical results for the lead datasets can be seen in Table 4.7.

Table 4.7 **Lead Statistical Data (Archaeological vs. Modern)**

Shapiro-Wilk Normality Test (Archaeological & Modern)	Shapiro-Wilk Normality Test (Modern)	Shapiro-Wilk Normality Test (Archaeological	Wilcoxon Rank Sum Test	Exact Wilcoxon-Mann-Whitney Test	Difference in Location
p = .09169	p= .7229	p = .03299	p= .06157	.06157	p = 2.49

4.3.2 Cadmium

Figure 4.2 exhibits the archaeological and modern cadmium box plots. The archaeological box plot is comparatively short, which is characteristic of the lead archaeological ppm levels being very similar. The modern data box plot is slightly taller than the archaeological sample, indicating that the modern ppm levels are slightly higher.

When the two box plots are compared, it appears that the medians are different, but that the distribution in ppm levels is quite similar.

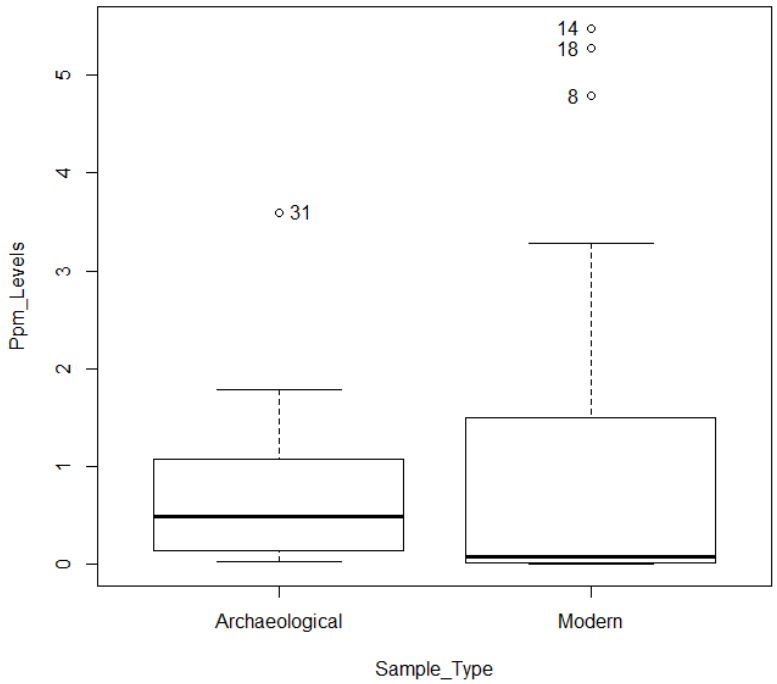

Figure 4.2 Cadmium Archeological and Modern Box Plots.

The Shapiro-Wilk normality test was performed and provided a p-value for the archaeological sample that was greater than .05, suggesting that with 95% confidence, the data fits a normal distribution. The p-value for the modern dataset was less than .05, which is not characteristic of a sample with a normal distribution. A Wilcoxon rank sum test was performed and provided a p-value that was greater than .05, implying that the samples are from identical populations. The difference in location was calculated to be .05304, which falls within the confidence interval of -.01282 to .13244. This suggests that

the difference between the two samples may be equal to zero. The statistical results for the cadmium samples are located in Table 4.8.

Table 4.8 Cadmium Statistical Results (Archaeological & Modern)

Shapiro-Wilk Normality Test (Archaeological & Modern)	Shapiro-Wilk Normality Test (Archaeological)	Shapiro-Wilk Normality Test (Modern)	Wilcoxon rank sum test	Difference in Location
p=.00000005825	p=.09888	p = .00003	p=.07939	.05304

4.3.3 Mercury

Due to the highly skewed distributions of the modern sample, a log transformation was performed. Figure 4.3 exhibits the archaeological and modern mercury box plots. The archaeological box plot exhibits a normal distribution. However, the modern sample box plot is comparatively short and has ppm levels which are lower than .05. When the two box plots are compared, it appears that the medians and the distribution in ppm levels are quite different.

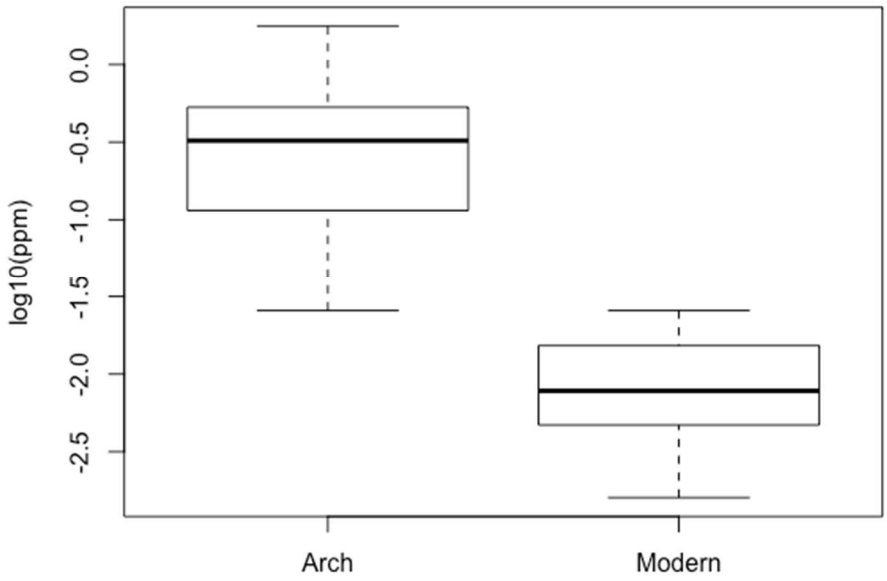

Figure 4.3 Cadmium Archeological and Modern Box Plots.

A Shapiro-Wilk normality test was performed and produced p-values for both samples that were greater than .05. Since the samples exhibited a normal distribution, a Welch two sample t-test was performed and produced a p- value that was less than .05. The statistical results for the cadmium samples are located in Table 4.9.

Table 4.9 Statistical Results for the Mercury (Archaeological & Modern).

Shapiro-Wilk Normality Test (Archaeological & Modern)	Shapiro-Wilk Normality Test (Archaeological)	Shapiro-Wilk Normality Test (Modern)	Wilcoxon Rank Sum Test	Difference in Location
p = 1.151e-06	p=.03264	p = .1398	p= .0001616	.34005

CHAPTER V

DISCUSSION

A statistical analysis of the lead and cadmium ICP-MS data revealed that the archaeological and modern samples were independent datasets from populations with similar trace element distributions. These results indicate that the cadmium and lead datasets reject the hypothesis that the industrial, modern sea turtle sample and the archaeological sample do not exhibit similar chemical levels. Whereas the statistical analysis for the mercury data revealed that the archaeological and modern samples were independent datasets from populations with different distributions. These results allow for the acceptance of the above overall hypothesis, but do not meet the expectation of the sub-hypothesis, which stated that the modern sea turtle sample would yield higher mercury levels than the archaeological sample. Meaning, that the modern datasets do not exhibit the same trend exhibited in previously published Caribbean trace element studies.

The Wilcoxon rank sum test for the lead datasets produced a p-value very close to the .05 confidence interval. Even though the p-value indicates that the samples are similar, the difference in location (2.49) is fairly high. In this instance, the difference in location is a value produced by the Exact Wilcoxon-Mann-Whitney Test, which quantifies the difference in levels between the archaeological and modern samples. Even though the samples were reported to share similar levels, the statistical analysis for the lead datasets may be the result of the small sample size. With such a small sample, the

statistical tests may have been rendered incapable of appropriately analyzing the data and providing sound conclusions about the population being studied. With that said, if the lead p-value is determined to be an anomaly, this would mean that the lead ppm values are two times higher than the archaeological samples. These results imply that the archaeological and modern samples follow the same trend exhibited by previously published Caribbean trace element studies and therefore, accept hypothesis 1 of this study.

Skeletochronology Analysis

The results from the skeletochronology analysis revealed that the age at death for the sea turtles remains ranged between eleven and fourteen years. This demonstrates that the trace element results accounted for 11 to 14 years of the anthropogenic pollution timeline. Since sea turtles live for several decades, the ages obtained in this study suggest that the analyzed remains represent three prematurely deceased turtles. This creates a major interpretive problem, due to the fact that the remains exhibit WCR trace element data for a short period of time, when compared to the 500 year timeline this study is attempting to address. Furthermore, since juvenile sea turtles spend their younger years in an the epipelagic zone, than the trace element data obtained in this study likely lacks data from the neritic environments. This suggests that the trace element concentrations obtained from their remains only reflect trace elements found in areas farther from the shoreline and therefore, do not provide any information pertaining to trace element concentrations closer to the shore.

FTIR-ATR & ICP-MS Analysis

The FTIR-ATR analysis was performed in this study to assist with determining whether the sea turtle remains were subjected to physical and chemical changes. Identifying which skeletal remains may have been subjected to diagenesis was an important factor for this study, for is assisted in a more accurate interpretation of the statistical analysis performed on the ICP-MS results and addressing the secondary research questions, hypothesis and sub hypotheses generated for this study.

The FTIR-ATR results revealed that eighty five percent of the modern and archaeological remains exhibited either an increased level of crystallinity or a reduced histological index. This means, that a majority of the samples exhibited a destruction of collagen and/or increased mineral crystal sizes. These results suggest that rapid diagenesis likely occurred and therefore distorted the chemical and physical structure of nearly all the modern and archaeological remains. With an altered chemical structure, all data produced by the trace element analyses was potentially skewed towards the elemental make up of the burial environment the sea turtles were interred in, rather than the makeup of the marine environments that they experienced during life. Such information may explain why the statistical analysis of the modern remains do not exhibit the same trend exhibited by previously published Caribbean trace element studies. If this supposition is correct, then one could surmise that the use of FTIR-ATR analysis was an appropriate method for determining whether diagenesis affected the chemical and physical structures of the sea turtle remains in this study.

Another factor that may have affected the chemical signature obtained in this study was the applicability of the reference sample for the ICP-MS analysis. As discussed by Chavagnac et al. (2007; 181) the accurate determination of trace element concentrations by ICP-MS requires the use of a direct matrix-matched standard with a similar major chemical composition and mineralogical form to the sample. More specifically, when performing ICP-MS on samples that have been subjected to seawater, a certified seawater reference material is required (Chavagnac et al., 2007; Sondergaard et al., 2015). The Mississippi State Laboratory employed the best standard available, which was the NIST 1643. This is a standard reference material (SRM), typically used in the determination of trace elements in fresh water. While this SRM is applicable for water samples, it is not typically used for the determination of trace elements in salt water Caribbean Sea turtle remains (National Institute of Standards and Technology (NIST) Material Database, 2003-2010). However, since ICP-MS analysis has never been performed on Caribbean Sea turtle remains, a reference standard of known elemental concentration has yet to be developed or identified. Suggestions for future reference standard of known elemental concentrations will briefly be discussed in the conclusion section of this study.

The ICP-MS and FTIR-ATR findings indicate that the use of sea turtle skeletal remains for assessing anthropogenic pollution in Caribbean marine environments may prove to be a valid approach. However, until further testing on sea turtle skeletal remains with little to no chemical or physical alterations can be performed, one cannot make any

further conclusions regarding the applicability of sea turtle remains in regards to the assessment in any marine environment.

CHAPTER VI

CONCLUSION

This is the first study of sea turtle humeri intended to quantify the degree to which anthropogenic pollution has altered marine environments in the WCR as the result of the advent of industrialization. When the results from the FTIR-ATR method were coupled with those obtained from the skeletochronology analysis, this research was able to ascertain that a large portion of data needed to truly quantify change in anthropogenic pollution overtime, was missing. The FTIR-ATR analysis provided results that identified this analytical technique as being a promising method for determining the presence of diagenesis in sea turtle remains. The skeletochronology analysis proved to be an applicable method for determining the age of sea turtles at time of death. However, the trace element analysis provided results that did not follow the same trend exhibited by previously published studies in the WCR. As a result, this study was unable to verify whether skeletal sea turtle remains, specifically humeri, can be used as a biological proxy to reconstruct anthropogenic pollution in marine environments. Furthermore, it was unable to robustly quantify pre-industrial to mid-late 20th century anthropogenic lead, mercury, and cadmium pollution in Caribbean Marine Environments.

Future researchers interested in using skeletal sea turtle remains to chart Caribbean anthropogenic pollution over time should consider performing stable isotope

analysis in conjunction with trace element analysis. The sea turtles species in this study exhibit a cyclical migration pattern, live for an extended period of time, and experience a habitat shift from an epipelagic habitat to a neritic habitat during their lifetime. By coupling stable isotope analysis and trace element analysis, it may be possible to identify the locations and levels in which lead, cadmium, and mercury concentrations are present in the marine environments of the WCR. This idea is based on the understanding that a majority of pollution accumulating in marine environments comes from large cities, specifically, from waste (industrial waste, sewage, and hydrocarbons) and agricultural runoff associated with large, concentrated human populations (Rezaee et al., 2010). As a result, it is presumed that the trace element concentrations with the sea turtle remains will be highest in neritic waters (waters located closest to the shore and to the contaminant sources).

To ensure accuracy in the interpretation of the analytical results, FTIR-ATR analysis should be performed in conjunction with an additional analytical test capable of identifying diagenetic alteration and essentially validating the FTIR-ATR results. One method that could potentially provide such assistance would be scanning electron microscopy (SEM). SEM would allow for the microstructures, such as lamellae and collagen fibers, to be compared to skeletal sea turtle remains with good histological preservation. Such an analysis would allow the researcher to ascertain whether the bone microstructure of the diagenetically altered samples was completely reorganized, which is typically indicated by the presence of irregular holes versus uniformed bone structure (Guarino et al., 2006). By employing this method in future studies and determining

whether the remains have been diagenetically altered, the results obtained from future ICP-MS and Radiocarbon analyses could be interpreted with more certainty.

Lastly, future research should address whether different sea turtle elements exhibit varying trace element concentrations. This would be achieved by performing trace elements analyses on several sea turtle elements, followed by the comparison of their ICP-MS results with an appropriate reference sample. For several years, the NOAA has been using satellite tracking to obtain the whereabouts of sea turtles in the Caribbean prior to their return to their island of birth. Once the tracked sea turtle are deceased, future research could focus on performing trace element analysis on several skeletal elements. This method may take several years to collect, but the data obtained from their skeletons could act as a reference standard of known elemental concentration in modern sea turtle remains prior to being subjected to the soil environment during the skeletonizaion process. While this method would not be a helpful comparison for archaeological remains, it may at least shed light on whether sea turtle remains would be a useful biological proxy for quantifying anthropogenic lead, mercury and cadmium pollution in Caribbean marine environments during modern times.

By performing the discussed future research, researchers could ascertain whether sea turtle skeletal elements are good biological proxies for assessing anthropogenic pollution in marine environments. The same data could then be compared to archaeological remains, to quantify the amount of change in lead, cadmium, and mercury ppm levels over time and geographically pinpoint the areas being affected the most. If such studies yield positive results, an alternative method for assisting governments will be available for refining and/or regulating policies for industrial waste, sewage,

hydrocarbons, and agricultural runoff entering the marine environments located not only in the WCR, but globally.

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