Trace metal content in Panamanian marine turtles, its potential to differentiate populations, and implications for human consumption

by

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August 2015

A thesis submitted to McGill University in partial fulfillment of the requirements of the degree of Master of Science

Dedication

To my family:

Joy, Glenn, Allyson, and Andrew

Table of Contents

Dedication ii

Table of Contents iii

List of Tables v

Chapter 1 v

Chapter 2 v

List of Figures vi

Chapter 1 vi

Chapter 2 vii

Abstract ix

Résumé x

Acknowledgements xii

Logical Order of the Thesis xiii

Contribution of the Authors xiii

Introduction 1

Chapter 1: Literature Review 8

Abstract 8

Introduction 9

Methods 12

Data Collection 12

Data Analysis 13

Question 1: Interspecies Differences 14

Question 2: Tissue-Specific Associations 14

Question 3: Geographic Variation 14

Question 4: Implications for Human Health 15

Results 15

Status of Current Knowledge 15

Identification of Overall Patterns 16

Question 1: Interspecies Differences 20

Question 2: Tissue-Specific Associations 21
Question 3: Geographic Variation 27
Question 4: Implications for Human Health 28
Discussion 29
Local vs. Global-Scale Observations 29
Interspecies Differences 29
Tissue-Specific Associations 30
Geographic Variation 33
Conclusions 34
Literature Cited 35
Linking Statement Between Chapters 1 and 2 44
Chapter 2 45
Abstract 45
Introduction 46
Materials and Methods 48
Sample Collection and Processing 48
Trace-Metal Analysis 50
Data Analysis 51
Results 54
Trace Element Concentrations 54
Interspecies and Geographic Variation 54
Implications for Human Health 60
Discussion 62
Trace Element Concentrations 62
Interspecies and Geographic Variation 65
Implications for Human Health 66
Conclusions 68
Acknowledgements 69
Literature Cited 69
General Conclusions and Contribution to Knowledge 79
Appendix 1: Supplementary Information for Chapter 1 83
Appendix 2: Supplementary Information for Chapter 2 118

List of Tables

Chapter 1

Table 1: Percent water per tissue, based on available percentages from the collected literature (Appendix A1.1), used to convert dry weight values to wet weight when not specified in the respective study. **13**

Table 2: Reported instances of sea turtle eggs with concentrations of either As, Cd, Hg, or Pb (mean ± SE, wet wt.) high enough to pose a health risk to humans consuming more than 75 g of egg per day. **32**

Chapter 2

Table 1: Detailed sample collection information. **49**

Table 2: Instrumental detection limits (measured in ng g^{-1}, wet wt); and percent relative deviation for each element from the certified value of the standard reference materials SRM 1577c (Bovine liver: National Institute of Standards and Technology, USA) and CRM DORM-4 (Fish protein homogenate: National Research Council, Canada). **53**

Table 3: Trace metal concentrations (median ± MAD, μg g^{-1} dry wt.) found in Green turtle (*Chelonia mydas*) and Olive Ridley (*Lepidochelys olivacea*) eggs from nesting sites along the Pacific coast of Panama. Values in parentheses indicate the number of samples above detection limit, if less than n. **56**

Table 4: Number of sea turtle eggs (median ± MAD, eggs day^{-1}) that a person must eat, using the average adult Panamanian body weight (69.9 kg; Walpole et al., 2012) before reaching the provisional tolerable daily intake (PTDI) of As (3 μg kg^{-1} day^{-1}), Cd (1 μg kg^{-1} day^{-1}) and Hg (0.1 μg kg^{-1} day^{-1}) through the consumption of sea turtle eggs alone; based on metal concentrations in eggs of Green turtles (*Chelonia mydas*) and Olive Ridleys (*Lepidochelys olivacea*) nesting in various sites along the Pacific coast of Panama. **61**

Table 5: The minimum body weight (median ± MAD, kg) required for a person to safely consume one sea turtle egg per day without exceeding the provisional tolerable daily intake (PTDI) of As (3 μg kg^{-1} day^{-1}), Cd (1 μg kg^{-1} day^{-1}) and Hg (0.1 μg kg^{-1} day^{-1}) through the

consumption of sea turtle eggs alone; based on metal concentrations in eggs of Green turtles (*Chelonia mydas*) and Olive Ridleys (*Lepidochelys olivacea*) nesting in various sites along the Pacific coast of Panama. **61**

List of Figures

Chapter 1

Figure 1: Distance biplot from an RDA of literature-collected sea turtle toxic metal data constrained by metal concentration, controlling for tissue and ocean. The metals (Cd, Hg, and Pb) are represented by vectors. Ellipses represent the 95% confidence limit, based on the standard error of the mean for each species. Only 2 Olive Ridley samples were included in the analysis, thus this species has no corresponding ellipse. Distance biplots may be interpreted as follows: the distance between objects reflects their Euclidean distances in multidimensional space; the angles between descriptor vectors, however, are meaningless; the projection of an object at right angle to a descriptor vector approximates its value along that vector. **18**

Figure 2: Distance biplot from an RDA of literature-collected sea turtle toxic metal data constrained by metal concentration, controlling for species and ocean. The metals (Cd, Hg, and Pb) are represented by vectors. Ellipses represent the 95% confidence limit, based on the standard error of the mean for each tissue. Tissues with only 1-2 samples are labeled in black (in the legend), and have no corresponding ellipse. Distance biplots may be interpreted as follows: the distance between objects reflects their Euclidean distances in multidimensional space; the angles between descriptor vectors, however, are meaningless; the projection of an object at right angle to a descriptor vector approximates its value along that vector. **19**

Figure 3: Distance biplot from an RDA of literature-collected sea turtle toxic metal data constrained by metal concentration, controlling for species and tissue. The metals (Cd, Hg, and Pb) are represented by vectors. Ellipses represent the 95% confidence limit, based on the standard error of the mean for each ocean. Only 1 sample was included in the analysis from the Indian Ocean, thus it has no corresponding ellipse. Distance biplots may be interpreted as follows: the distance between objects reflects their Euclidean distances in multidimensional

space; the angles between descriptor vectors, however, are meaningless; the projection of an object at right angle to a descriptor vector approximates its value along that vector. **20**

Figure 4: Mercury (Hg) concentration (μg g^{-1} wet weight) in the livers of Loggerhead and Green turtles..... **21**

Figure 5: Cadmium (Cd) concentration (μg g^{-1} wet weight) in separate tissues of all species combined..... **22**

Figure 6a: Cadmium (Cd) concentration (μg g^{-1} wet weight) in separate tissues of Loggerheads. **23**

Figure 6b: Cadmium (Cd) concentration (μg g^{-1} wet weight) in separate tissues of Green turtles. **24**

Figure 7a: Mercury (Hg) concentration (μg g^{-1} wet weight) in separate tissues of Loggerheads. **25**

Figure 7b: Mercury (Hg) concentration (μg g^{-1} wet weight) in separate tissues of Green turtles. **26**

Figure 7c: Mercury (Hg) concentration (μg g^{-1} wet weight) in separate tissues of Kemp's Ridleys. **27**

Figure 8: Cadmium (Cd) concentration (μg g^{-1} wet weight) in kidneys of Loggerhead and Green turtles from 3 broad sea turtle habitats..... **28**

Figure 9: The global distribution of trace metal data (As, Cd, Hg, and Pb) of sea turtle eggs, showing the number of reports of original data from each region. Individual reports are counted for as many different species or location data reported. **32**

Chapter 2

Figure 1: Map of Panama showing the nesting areas where eggs were collected. **50**

Figure 2: NMDS plot of toxic metal data (As, Cd, Hg, Pb) obtained from eggs of two sea turtle species that nest on the Pacific coast of Panama. Each point represents a single egg, and is labeled according to species. Ellipses represent the 95% confidence limit, based on the standard deviation for each species. **57**

Figure 3: NMDS plot of toxic metal data (As, Cd, Hg, Pb) obtained at four regions from Pacific-Panamanian sea turtle eggs. Each point represents a single egg, and is labeled according to the broad geographic location where it was collected. Ellipses represent the 95% confidence limit, based on the standard deviation for each area. **58**

Figure 4: NMDS plot of toxic metal data (As, Cd, Hg, Pb) obtained at four beaches from Pacific-Panamanian Green turtle eggs. Each point represents a single egg, and is labeled according to the nesting beach where it was collected. Ellipses represent the 95% confidence limit, based on the standard deviation for each collection site. **59**

Figure 5: NMDS plot of toxic metal data (As, Cd, Hg, Pb) obtained at three beaches from Pacific-Panamanian Olive Ridley eggs. Each point represents a single egg, and is labeled according to the nesting beach where it was collected. Ellipses represent the 95% confidence limit, based on the standard deviation for each collection site. **60**

Abstract

Anthropogenic input of chemical pollutants into the marine environment has led to substantial increases in the concentrations of naturally occurring trace metals, often with detrimental effects on the health of exposed marine animals and humans. Although they are a common food source in many tropical countries, quantitative toxicological assessments of marine turtles are generally limited in number of species investigated, number of data per species, and geographical distribution of samples. Chapter 1 presents a comprehensive review of sea turtle toxicology, and sea turtle eggs in particular given their widespread human consumption. From 70 collated studies reporting As, Cd, Hg, and/or Pb concentrations in edible sea turtle tissues, Loggerheads (*Caretta caretta*) and Green turtles (*Chelonia mydas*) were most frequently reported, with over half the data from liver, kidney, or muscle tissues. Eggs made up less than 10% of the data. Globally, no species was distinct for any metal; Cd and Hg showed tissue-specificity to kidney and liver, respectively; and Cd was significantly highest in the Pacific Ocean. Using a standard adult body weight (62.0 kg) and serving size (75 g), Cd was the most toxic metal. On average, eating $\leq$75 g week^{-1} would induce Cd poisoning from either Green or Loggerhead kidney and liver, and Hg poisoning from only Loggerhead liver. Chapter 2 presents measurements of 12 trace metals in Green turtle and Olive Ridley (*Lepidochelys olivacea*) eggs from Pacific Panama. Qualitatively, the metal profiles measured were similar to previous reports, with 8 of 12 falling in their previously reported ranges. In my samples, interspecific differences were found on a per-site basis, and geographic differences for each species, but the interaction of these patterns prevented overall significant differentiation. Cd was, again, the most toxic metal; however, consumption of these eggs at the reported average rates is unlikely to induce metal poisoning in adult consumers. However, males and females aged 16-30 and children up to ~3.4 yrs (~16.5 kg), consuming eggs at their maximum reported rates of 15 eggs day^{-1}, 30 eggs day^{-1}, and 5 eggs day^{-1}, respectively, may be putting themselves at risk of developing symptoms of long-term heavy-metal poisoning. The research presented here supports previous findings that Cd is highest in Green turtles, but that other toxic metals (Hg and Pb) are typically higher in more carnivorous species; we also show that, given the data available, patterns frequently observed in local comparisons are not always globally robust. Furthermore, we show that Cd is on average the most toxic metal in marine turtles concerning human health, but that additional analyses are needed to fully understand the health-risks of global sea turtle consumption.

Résumé

L'introduction anthropique de polluants chimiques dans l'environnement marin a conduit à une augmentation substantielle de concentrations naturelles de métaux traces, souvent avec des effets néfastes sur la santé des animaux marins exposés, et des humains. Les tortues marines sont une source de nourriture importante dans plusieurs pays tropicaux, mais leurs évaluations toxicologiques quantitatives ont peu d'espèces étudiées, peu de données par espèce, et la distribution géographique des échantillons demeure limitée. Le premier chapitre présente une revue compréhensive de la toxicologie des tortues marines - particulièrement sur leurs œufs - compte tenu de leur consommation humaine très répandue. Parmi 70 études qui ont inclue des concentrations de As, Cd, Hg et/ou Pb dans les tissus comestibles de tortues marines, la caouanne (*Caretta caretta*) et la tortue verte (*Chelonia mydas*) ont été le plus souvent documentées. Plus de 50% des données provenaient du foie, des reins ou de tissus musculaires, et moins de 10% des données provenaient des œufs. Globalement, aucune espèce n'était distincte pour n'importe quel métal. Le Cd était retrouvé spécifiquement dans le foie et le Hg, dans les reins; le niveau de Cd était significativement plus élevé dans l'océan Pacifique. Pour un poids standard d'adulte (62,0 kg) et une portion standard (75,0 g), le métal le plus toxique était le Cd. En moyenne, consommer ≤75 g sem-1 induit un empoisonnement de Cd par le foie et les reins de la caouanne et de la tortue verte, et un empoisonnement de Hg par le foie de la caouanne.
Le deuxième chapitre présente des mesures de 12 métaux traces retrouvées dans les œufs de la tortue verte et la tortue olivâtre (*Lepidochelys olivacea*) dans la côte pacifique du Panama. Qualitativement, les profils des métaux mesurés étaient similaires aux rapports précédents - 8 des 12 métaux démontraient des gammes similaires à celles reportées auparavant. Dans mon échantillonnage, des différences interspécifiques ont été trouvées par site, et des différences géographiques pour chaque espèce, mais l'interaction de ces patrons ont empêché une différenciation globale significative. Le Cd était encore une fois le métal le plus toxique, mais il est peu probable que la consommation moyenne de ces œufs puisse entraîner des intoxications aux métaux chez les consommateurs adultes. Cependant, chez les hommes de 16 à 30 ans, les femmes de 16 à 30 ans, et les enfants jusqu'à ~3,4 ans (~16,5 kg), où les taux de consommation d'œufs peuvent atteindre 15 œufs jour^{-1}, 30 œufs jour^{-1} et 5 œufs jour^{-1} respectivement, ils risquent de développer des symptômes à long termes d'intoxication aux métaux lourds. La recherche présentée ici confirme les résultats précédents que le Cd est le plus élevé chez les

tortues vertes, mais que d'autres métaux toxiques tels que le Hg et le Pb sont généralement plus élevés chez les espèces plus carnivores. Nous montrons aussi que compte tenu des données disponibles, les modèles fréquemment observées dans les comparaisons locales ne sont pas toujours robustes à l'échelle mondiale. De plus, nous montrons que le Cd est en moyenne le métal le plus toxique dans les tortues marines en ce qui concerne la santé humaine, mais des analyses supplémentaires sont nécessaires pour bien comprendre les risques de la consommation mondiale des tortues marines pour la santé.

Acknowledgements

I am extremely grateful to my advisory committee, Héctor M. Guzmán, Catherine Potvin, and Vincent van Hinsberg for their continuous support both academically and personally, their invaluable guidance and advice in the development of both manuscripts, and their comments and suggestions in editing the manuscripts included in this thesis.

Special thanks go to C. Guevara, H.M. Guzmán and M. Diaz for their assistance in collecting the samples that were fundamental to Chapter 2, and to C. Guevara for logistical assistance at STRI every step along the way, and for my inclusion in additional lab projects. Special thanks also go to A. Jung and I. Richer for their guidance through developing, testing, and implementing the analytical protocols used in Chapter 2; for providing the required lab space, major analytical equipment, and the instructions on using said equipment; and for their consistent reassurances that everything was running smoothly.

I would like to thank R. Urriola, B. Turner and the STRI Soils Lab, and the International Cooperative Biodiversity Groups Program for Panama for providing logistical assistance, bench space and the major lab equipment used at STRI to prepare my samples for transport and analysis.

Additional thanks go to my family, the members of the Potvin and Guzmán labs, the McGill-NEO program, and all the friends made along the way for their wisdom and support, without which I could not have realized the completion of this thesis.

Many thanks to the institutions that sponsored my studies and this research: the McGill-STRI NEO Program, McGill University, Dr. Richard Hart (Molson and Hilton Hart Fellowship - 00286SC), the Smithsonian Tropical Research Institute, and Secretaria Nacional de Ciencia y Tecnologia de Panama (SENACYT) through a grant to H.M. Guzmán.

Permits to collect, export, and import my samples and certified materials were provided by the Government of Panama (ANAM, MIDA), the Government of Canada (CFIA, CWS), and the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES).

Logical Order of the Thesis

This is a manuscript-based thesis composed of two chapters. Chapter 1 is a comprehensive review of the scientific and grey literature concerning metal contamination in sea turtles, setting the context for the work done in chapter 2. Chapter 2 reports novel data for trace metal contents in the eggs of two Panamanian-nesting sea turtle species, highlighting biological variation and toxicity.

Contribution of the Authors

I am the primary author of this thesis and both manuscripts within in. I collected and collated the data for Chapter 1. I researched and determined the best methods to use in processing and analyzing the samples for Chapter 2, obtained the necessary materials and equipment, and subsequently processed, analyzed, and re-analyzed the samples. I researched the best techniques to statistically analyze all the data, did the analyses, wrote both manuscripts, and compiled this thesis. My advisor Héctor M. Guzmán contributed to all phases of this project, including thesis proposal, sample collection, experimental design, permit application and sample transportation, sample analysis, data analysis, and writing both chapters in this thesis. My committee member Vincent van Hinsberg contributed to the data analysis and writing of Chapters 1 and 2; and to the specific details of experimental design, sample analysis, and data analysis and reporting for Chapter 2. My advisor Catherine Potvin contributed to the data analysis and writing of both chapters of this thesis.

Introduction

Trace metals, which naturally occur in all environments, have increased substantially in the marine environment as a result of increases in the anthropogenic use of chemical pollutants. This includes agricultural fertilizers and pesticides, burning of fossil fuels, mining, and various forms of waste disposal (Bradl 2005, Jakimska et al. 2011). A recent collaboration of 35 sea turtle researchers from 13 nations identified understanding the effects of pollution on sea turtles as a high-priority research area (Hamann et al. 2010). However, quantifying these effects in wild sea turtles, especially behavioral symptoms, is difficult. Thus toxic thresholds, and indeed baseline concentrations, for these metals are only recently beginning to be understood (Day et al. 2007, Deem et al. 2009, van de Merwe et al. 2009, Harris et al. 2011, Komoroske et al. 2011, Labrada-Martagon et al. 2011, Perrault et al. 2011).

Due to recent human activities, the six sea turtle species classified on the IUCN Red List are listed as either vulnerable - Leatherbacks (*Dermochelys coriacea* Vandelli, 1761) and Olive Ridleys (*Lepidochelys olivacea* Eschscholtz, 1829); endangered - Loggerheads (*Caretta caretta* Linnaeus, 1758) and Green turtles (*Chelonia mydas* Linnaeus, 1758); or critically endangered - (Hawksbill turtles (*Eretmochelys imbricata* Linnaeus, 1766) and Kemp's Ridleys (*Lepidochelys kempii* Garman, 1880); while the Flatback (*Natator depressus* Garman, 1880) is data deficient (Marine Turtle Specialist Group 1996a, b, Red List Standards & Petitions Subcommittee 1996, Seminoff 2004, Abreu-Grobois & Plotkin 2008, Mortimer & Donnelly 2008, Wallace et al. 2013). Directed poaching of nesting female turtles and/or their eggs, hunting of turtles at sea, by-catch and ghost nets in fishing industries associated with novel technology and methodologies, and increases in chemical and plastic-based pollution have been the predominant causes of declining turtle populations. In addition to these, nesting-beach degradation, associated to coastal development, and climate change related phenomena continue to hinder population recovery.

As with other frequently consumed marine animals, it is also important to understand the effects that consuming sea turtle products can have on humans, especially from chemical pollution. Mercury, cadmium, lead, and arsenic are considered to be the most harmful of these metals, owing to their high toxicity and widespread distribution (Kaplan et al. 2011). The effects of these metals, from both acute and chronic exposure, are well understood in humans. Mercury, especially in the organic compound methylmercury, primarily impacts the central and peripheral nervous system and may cause "tremors, insomnia, memory loss, neuromuscular effects,

headaches and cognitive and motor dysfunction" (WHO 2007). In the body, mercury binds to sulfhydryl (SH) groups, either blocking the activity of proteins containing these compounds, or inhibiting the oxidation of radical SH. Parkinson's, Alzheimer's, ALS, Lupus, Rheumatoid arthritis, and Autism have all been liked to cases of decreased sulfer oxidation (Zahir et al. 2005) It is particularly important to reduce mercury exposure in children and fetuses to prevent neurodevelopmental and motor system complications such as delayed walking, talking, and Autism (Zahir et al. 2005, Aguirre et al. 2006, WHO 2007, Warwick et al. 2013). Cadmium concentrates in, and is most damaging to kidney function, causing renal dysfunction (initially evidenced as renal tubular proteinuria), leading to renal failure, and kidney stones (Jarup 2003). High Cd burdens may also lead to osteoporosis due to interference in calcium metabolism, causing increases in calcium loss through urinary excretion (Jarup 2003, Satarug & Moore 2004, Aguirre et al. 2006, WHO 2010b). Hematological and neurological problems can occur with chronic exposure to lead. Increased levels of lead can inhibit hemoglobin synthesis, eventually leading to anemia. Additionally, chronic lead exposure can reduce nerve conductivity, causing slower reaction times, decreased memory retention, and reduced comprehension (Jarup 2003). As with mercury, unborn children and infants are at the highest risk of lead poisoning, where the greatest impact is on developing nervous systems, leading to reduced intellectual capacity (Jarup 2003, WHO 2010c). Arsenic exposure through seafood consumption is not a major concern: organoarsenic compounds, which are more prevalent in aquatic organisms, are thousand-fold less potent carcinogens than inorganic arsenic compounds (Roy & Saha 2002, WHO 2010a). Ingested inorganic arsenic is biomethylated in the liver, converting it into less toxic organic compounds. This methylation depletes the available methyl donors, resulting in DNA hypomethylation and subsequently aberrant gene expression and carcinogenesis (Roy & Saha 2002). Arsenic can also be damaging to various organ systems with heightened chronic exposure (WHO 2010a).

These toxic metals, to varying degrees, bioconcentrate in lower trophic level invertebrates (Rainbow 1990, Ansari et al. 2004), and through biomagnification may increase by orders of magnitude into large predatory fish, sharks and cetaceans (Jarup 2003, Ansari et al. 2004, Green et al. 2010), ultimately into humans. Long-lived animals, including marine turtles, may also accumulate increasing pollutant burdens simply through prolonged exposure. Marine turtles have been found with toxic concentrations of mercury and cadmium, predominantly in liver and kidney tissues from Loggerheads, Green Turtles, Leatherbacks, Hawksbills, and Olive

Ridleys. People who consume these contaminated tissues put themselves at risk of metal poisoning, and may become sick without understanding the cause (Grandjean et al. 1995, Dickman & Leung 1998, Iwegbue et al. 2009, Senko et al. 2009, Green et al. 2010, Kaplan et al. 2011). In some areas, especially isolated coastal communities, turtles and their eggs are an important source of food and income (Garcia-Martinez & Nichols 2000, Delgado & Nichols 2005, Aguirre et al. 2006, van de Merwe et al. 2009, Green et al. 2010, Pinzón Gómez 2012, Abd Mutalib et al. 2013). In Latin America, sea turtle is traditionally a delicacy served at weddings and other important holidays. Muscle is eaten directly, while soup is made from the organs, and eggs are considered as aphrodisiacs. Furthermore, where turtle is not considered red meat in Catholicism, turtle consumption increases during Lent (Aguirre et al. 2006, Senko et al. 2009, Warwick et al. 2013).

Marine turtle trace metal profiles have been reported worldwide, however there has been no assessment (known to the authors) that compares the results of more than a small subset of the available data (Bicho et al. 2005, Jerez et al. 2010, Ley-Quiñónez et al. 2013). Chapter 1 of this thesis is the first attempt at addressing this paucity of comprehensive reviews, by collecting all available data for toxic metal concentrations in edible sea turtle tissues. These data are used to quantitatively investigate previously established patterns in relative metal burdens across species, tissue, and location, at the global scale; and to determine how these data can be used to inform turtle consumers of the potential health risks. Turtle products, especially eggs, are widely consumed in Panama, both as a commodity sold along the highway and as an important food source in coastal communities. Chapter 2 presents first reported metal burdens in sea turtle eggs from Panamanian nesting sites, and is the first report for any sea turtle specimen from this country. These data are similarly analyzed for variation between species and site, but the main objective is to assess the risk of metal poisoning to people consuming the eggs.

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Chapter 1: Literature Review

Comprehensive review of toxic metal contamination in marine turtle tissues and its implications for human health

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KEY WORDS: Sea turtle; Trace metals; Pollution; Bioaccumulation; Global review; Human health

SHORT TITLE: Global sea turtle toxicology review

Abstract

Despite global awareness of trace-metal contamination in the ocean, its bioaccumulation through the food web, and resulting detrimental effects on health, the quantitative toxicology of marine turtles is still poorly known. Assessments are generally limited in number of species investigated, number of tissues analyzed, and geographical distribution of samples. We address these limitations by presenting a comprehensive review of sea turtle toxicology, and eggs in particular given their widespread human consumption. We collated 70 studies reporting As, Cd, Hg, and/or Pb concentrations in edible sea turtle tissues. The data were quantitatively analyzed for differences among species, tissues, and geographic locations. The majority (74%) of observations were from *Caretta caretta* or *Chelonia mydas*, with 52% of data from liver, kidney, or muscle; and only 7.3% from eggs. Using a confidence level of 0.050, we found no statistically significant difference among species. We observed a geographical effect, with Cd significantly highest in tissues from the Pacific Ocean, and tissue-selective distributions for Cd (kidney) and Hg (liver). Using the average adult body-weight (62.0 kg) and a standard serving size of 75g, Cd

contributed the most toxicity from turtles. When eating ≤75g per week, only kidney and liver were harmful; at this consumption, Hg was toxic only in *C. caretta* liver. These conclusions show that some, but not all reported local-scale trends are globally robust; and that Cd is most important when considering human health. Data are, however, sparse and more analyses are needed to fully understand the health-risks of global sea turtle consumption.

Introduction

Toxic heavy metals occur naturally in all environments. However, since the early 1900's, anthropogenic activities including agricultural fertilizer and pesticide use, industrial processes such as mining and smelting, fossil fuel combustion, and many forms of waste disposal have caused a dramatic increase in global oceanic levels of these inorganic pollutants. The accumulation of these metals to even low concentrations can pose a threat to the wildlife that interacts with these environments (Bradl 2005, Jakimska et al. 2011a). It is well known that through biomagnification, the increased concentrations of metals in the environment make it into the food web where animals that feed at higher trophic levels, notably large predatory fish, sharks, and cetaceans for the marine realm, often accumulate toxic concentrations of metals into their tissues (Green et al. 2010). With humans now the top trophic level in many environments, this heavy metal load is ultimately transferred to us, with potential detrimental effects on health (Grandjean et al. 1995, Dickman & Leung 1998, Iwegbue et al. 2009, Green et al. 2010, Kaplan et al. 2011).

Assessment of the toxicity of marine foods has mainly focused on high-trophic level fish and dolphins. However, most marine turtles also feed at high trophic levels, and their meat and/or eggs are an important food source in certain coastal communities (Garcia-Martinez & Nichols 2000, Delgado & Nichols 2005, Aguirre et al. 2006, van de Merwe et al. 2009, Green et al. 2010, Pinzón Gómez 2012, Abd Mutalib et al. 2013), both for historical reasons and as a result of cultural and religious beliefs and practices. In fact, it is the poaching, especially of nesting females and their eggs, in addition to fisheries by-catch, that has played a major role in the global decline of sea turtle populations, and at present, all sea turtles classified by the IUCN are listed as vulnerable to critically endangered (Marine Turtle Specialist Group 1996a, b, Red List Standards & Petitions Subcommittee 1996, Seminoff 2004, Abreu-Grobois & Plotkin 2008, Mortimer & Donnelly 2008, Wallace et al. 2013). Environmental inorganic pollutants are known

to be present in sea turtle tissues and eggs, and this consumption may thus be causing harm to those eating them (Aguirre et al. 2006, Senko et al. 2009, Warwick et al. 2013).

Many studies have looked at the heavy metal toxicity of sea turtles, focusing predominantly on liver, kidney and muscle tissue from stranded specimens often taken from more developed regions of the world (E.g. USA, Mexico, Europe, Japan and China; see Appendices A1.1; A1.3-A1.9). These studies provide a good baseline for an expected range of toxicity, but specific metal concentrations vary among species, tissues, and locations. It is moreover unlikely that stranded specimens are a representative sample of the population. It is also important to consider that these studies often do not come from regions where sea turtle consumption, due to limited alternative resources or cultural heritage, is common (Aguirre et al. 2006). Although inter-study comparative reviews have been made, these are all based on a subset of available data (Bicho et al. 2005, Jerez et al. 2010, Ley-Quiñónez et al. 2013). In contrast, in this study, we present a comprehensive review of all currently available data, where we include analyses for soft tissues and eggs of all marine turtles (database in Appendix).

Here, we focus on mercury, cadmium, lead, and arsenic, which are considered to be among the most harmful heavy metals to humans, via consumption, owing to their high toxicity and widespread distribution (Kaplan et al. 2011). Mercury has been found at toxic concentrations most often in liver, but also kidney, muscle, and various other tissues - predominantly from Loggerhead samples (Appendix A1.2). At these levels, ingestion of organic-mercury has been linked to complications in the nervous system, as well as the cardiovascular, hepatic, renal, and immune systems (Aguirre et al. 2006, WHO 2007, Warwick et al. 2013). Cadmium has been reported at toxic concentrations predominantly in kidney, and occasionally liver tissue from Green turtles, Loggerheads, Leatherbacks, Hawksbill turtles, and Olive Ridleys (Appendix A1.2). Additionally, given its lower trophic position, cadmium has been found at unexpectedly high levels in Green turtles, the highest of any species, although no clear mechanistic explanation has been described (Gladstone 1996, Anan et al. 2002, Andreani et al. 2008, Ley-Quiñónez et al. 2013). Cd is also higher than average in Leatherbacks, which has been linked to their diet of mainly gelatinous planktivores (cnidarians and ctenophores; Caurant et al. 1999). Cadmium toxicity is known to cause renal problems, and osteoporosis in high concentrations (Aguirre et al. 2006, WHO 2010b). Additionally, chronic exposure to lead commonly results in hematological and neurological problems. Pregnant women, infants and children are at the highest risk of lead

poisoning, where the greatest impact is on developing nervous systems (WHO 2010c). According to the World Health Organization, arsenic ingestion via seafood is unlikely to pose a threat. However, at sufficient concentrations, chronic arsenic exposure can affect multiple organs, and arsenic is also a known carcinogen (WHO 2010a).

Species, trophic level, and geographic location are all potentially important factors that impact the concentration of toxic metals. Based on their typical feeding ecology, herbivorous adult Green turtles (Storelli & Marcotrigiano 2003, Jones & Seminoff 2013) would be expected to have the lowest metal concentrations, while the carnivorous adult Loggerheads, Leatherbacks, Hawksbill turtles and Kemp's Ridleys (Eisenberg & Frazier 1983, Jones & Seminoff 2013) should have the highest. This relationship has been shown in several local-scale studies, especially between loggerhead and green turtles (Sakai et al. 1995, Caurant et al. 1999, Godley et al. 1999, Saeki et al. 2000, Sakai et al. 2000b, Anan et al. 2001, Kubota et al. 2003, Kaska et al. 2004, Gardner et al. 2006, Kampalath et al. 2006, Suzuki et al. 2012). Tissue specific metal accumulation has also been observed: As, Cd, Hg, and Pb concentrate in muscle, kidney, liver, and calcified tissues, respectively (Storelli & Marcotrigiano 2003, Jakimska et al. 2011b). Finally, Stoneburner et al. (1980) suggested that if geographic demes exist among female turtles nesting on different beaches, eggs collected from those beaches would have different heavy metal profiles: populations that utilize separate feeding grounds would obtain toxic metals from isolated sources, and therefore differences in environmental metal loads should be evident between isolated populations (Gardner et al. 2006).

Our review of the available data aims to address the following research questions: (1) Are reported local differences in toxic element concentration among species, supported by differences in feeding ecology and trophic position, robust to the global scale? (2) Are reported metal-tissue associations – that each toxic metal is sequestered into a specific tissue – evident at the global scale, either among all species or within each? (3) Is there a large-scale geographic pattern of metal accumulation amongst broad sea turtle habitats? (4) With the concern that consuming sea turtle products may be harmful, can the above findings be used, along with the respective empirical metal toxicity data, to inform global or local concern of health risks related to consuming sea turtle products in areas with no reported data?

Methods

Data Collection

Data collated for this review were extracted from all studies that reported numerical results of toxic metal loads (As, Cd, Hg, and Pb) in any of the seven sea turtle species: Loggerhead (*Caretta caretta* Linnaeus, 1758), Green turtle (*Chelonia mydas* Linnaeus, 1758), Leatherback (*Dermochelys coriacea* Vandelli, 1761), Hawksbill turtle (*Eretmochelys imbricata* Linnaeus, 1766), Kemp's Ridley (*Lepidochelys kempii* Garman, 1880), Olive Ridley (*Lepidochelys olivacea* Eschscholtz, 1829), and Flatback (*Natator depressus* Garman, 1880). To ensure the most inclusive compilation of data, the cited papers from each study were scanned for additional data, and this process was repeated until no further original reports could be located (Appendix A1.1).

To focus on the effects of metal loads in turtles on the humans consuming them, only results from soft (i.e. consumable) tissues and bone were included in the database. Juvenile-only data were excluded to reflect the higher likelihood of consuming an adult turtle, especially nesting females. The majority of toxic metals found in eggs are contained in the yolk (Sakai et al. 1995, Lam et al. 2006), in comparison with the albumin, therefore reports of yolk concentrations were considered as a proxy for eggs where no total egg concentration was given.

To assess broad-scale variation, the location of each data set was recorded at the level of oceanic region: Atlantic Ocean, Pacific Ocean, Indian Ocean, and Mediterranean Sea. Data collected from the Mediterranean were isolated from the Atlantic data for the analysis due to the relatively high number of publications and the narrow connection between the two bodies of water, resulting in significantly different ocean-water metal concentrations (Boyle et al. 1985, Davis 1993).

The data were converted into wet weight when necessary, using either the percent water reported in the study or the average of the values found in other papers when no conversion factors were reported. These values are presented in Table 1. If no conversion factor for a specific tissue was found, any dry weight concentrations of that tissue were left unchanged and were not included in the analysis.

Table 1: Percent water per tissue, based on available percentages from the collected literature (Appendix A1.1), used to convert dry weight values to wet weight when not specified in the respective study.

Tissue	% Water
Egg	73
Liver	72
Kidney	73
Muscle	80
Lung	73
Bone	20
Adipose	20
Blood	75

Data Analysis

Three partial redundancy analyses (RDA; Oksanen et al. 2013) were initially performed on the collected data to determine any overall patterns based on species, tissue, or location; these 3 factors were used as explanatory, or 'independent' variables. In brief, RDA identifies a series of axes based on the variation in this set of explanatory variables that best explain the variation in a second set of response data, or 'dependent variables'. For each RDA, the effects of 2 factors were statistically controlled for by specifying them as a separate conditioning matrix before plotting on a scaling-1 ordination so that observed groupings better reflected the factor of interest, not included in the conditioning matrix, and to account for imbalances in sample distribution. For example, in the RDA where species was the factor of interest, tissue and location data were specified as the conditioning matrix. Due to the low number of samples with recorded concentrations for all 4 toxic metals, only Cd, Hg, and Pb were used as response data. To account for the larger Cd concentrations than either Hg or Pb, the data were Hellinger-transformed before analysis (Borcard et al. 2011). This transformation reduces the importance of large values (in this case, the Cd concentrations) so that substantial differences between smaller values (Hg and Pb concentrations) will be reflected in the output. Ellipses were created using standard error, with a significance value of 0.050; and an analysis of similarities (ANOSIM;

Oksanen et al. 2013) was performed based on each grouping factor (species, tissue, and location) to determine the significance of any differences observed in the RDA biplots.

For each of questions 1-3, permutation-based 2-factor, unbalanced ANOVAs (Legendre 2008) were performed on each of the 4 metals, with the appropriate factors chosen to answer each question (see details below). Post-hoc analysis, using a modified Tukey's test based on the 'C procedure' described by Dunnett (1980) to account for unequal variance, was used for pairwise comparison where appropriate. When necessary, outliers and data without sufficient repetition across factors were removed. In cases where this resulted in reducing either factor to a single level, a permutation-based 1-factor, unbalanced ANOVA (Legendre 2007) was performed on the unaltered data set based on the factor of interest. A significance level of $p=0.050$ was used in all analyses, with data presented as mean ± SE.

Question 1: Interspecies Differences

To look at interspecies differences in toxic metal loads at a global scale, each of the metals was compared based on the concentrations in the tissue associated with its biologically highest levels: As in muscle, Cd in kidney, and Hg in liver (Storelli & Marcotrigiano 2003, Jakimska et al. 2011b). Since Pb is concentrated most highly in keratinous tissues, such as the carapace and bones (Storelli & Marcotrigiano 2003), it was first analyzed for differences among soft tissues, with species as the second analytical factor, to determine if any non-keratinous tissue has significantly higher Pb concentrations than the others. ANOVAs were then conducted on each single-tissue data set, using species and oceanic region as the analytical factors.

Question 2: Tissue-Specific Associations

To determine the robustness of reported metal-tissue associations, the data were analyzed at two levels: (a) across all species, where ANOVAs were performed using tissue and species as the analytical factors; and (b) within each species, where ANOVAs were performed on each species-specific data set using tissue and location as the analytical factors.

Question 3: Geographic Variation

Based on the results of Question 2, the analyses for geographic patterns were performed using only the tissue with the biologically highest concentration of each metal (as in Question 1), or on all tissues combined. To look for geographic patterns, ANOVAs were performed using location and species as the analytical factors.

Question 4: Implications for Human Health

To determine how the collected metal concentrations relate to human health, a standard measure of days serving^{-1} was established. This measure used the ng g^{-1} data for each sample and the most up to date provisional tolerable daily intake (PTDI) value for each metal (WHO 1995, 2010a, b, USEPA 2014); and the calculations were based on the recommended serving size of 75 g for meat products by Health Canada (HC-SC 2008) and the American Heart Association (AHA 2013), and the global average adult weight (Walpole et al. 2012). Mean ± SE days serving^{-1} based on species, tissue, and location was calculated for each metal. Using this measure, relative toxicity was calculated as the minimum time in which it is safe for an average-sized adult to regularly consume up to 75 g of turtle meat without exceeding the PTDI for the specified metal. Days serving^{-1} for the significant findings in Questions 1-3 were also calculated.

Results

Status of Current Knowledge

We identified 70 papers, with over 300 unique entries, encompassing all 7 species of sea turtle. Due to imbalances in the data based on species, it was impossible to include the Flatback in any analysis, and Indian Ocean data were included only in the RDAs and the analyses for Question 2a. Bias in data provenance (Appendix A1.3-A1.9) prevented statistical analysis based on sub-oceanic identification, where multiple regions would have been reduced to a sample size of ≤ 2 and thus omitted from the analyses.

Of the 7 sea turtle species, the Endangered Loggerhead and Green turtles (Marine Turtle Specialist Group 1996a, Seminoff 2004) are the most studied, making up 43% and 31% of the observations, respectively, while Leatherbacks and Olive Ridleys are underrepresented in the data set relative to their current conservation status of Vulnerable (Abreu-Grobois & Plotkin 2008, Wallace et al. 2013). The Critically Endangered Hawksbill and Kemp's Ridley (Marine Turtle Specialist Group 1996b, Mortimer & Donnelly 2008), and data deficient Flatback (Red List Standards & Petitions Subcommittee 1996) are the least studied.

Liver, kidney, and muscle are the most studied tissues at 21%, 16%, and 15%, respectively, agreeing with findings by Storelli & Marcotrigiano (2003) a decade ago. 39% of all the data were of liver, kidney, and muscle taken from either Loggerhead or Green turtles. Liver and kidney typically pose the highest health risk from consuming sea turtle meat; therefore the

bulk of the available data are indeed applicable to determining human health risks via consumption. Relative to their importance as a source of food and income around the globe (Chacon-Chaverri & Eckert 2007, Green et al. 2010, Abd Mutalib et al. 2013), especially in more remote areas for which toxicology data do not exist (Aguirre et al. 2006), eggs are disproportionately underrepresented in the data set. Eggs, and their components, make up just 7.3% of the tested tissues (yolk and albumin data were considered separately when whole egg data were unavailable). Similar to the whole data set, the majority of egg samples (78%) come from only Loggerhead and Green turtles (Appendix A1.1).

Most studies come from the northern Atlantic and Pacific Oceans and the Mediterranean Sea, with only a handful from the Indian Ocean. Samples from the Atlantic Ocean predominantly come from the Southeast US, the Gulf of Mexico, the Caribbean, and Western Europe (Appendix A1.3-A1.9), with one study each from French Guiana (Guirlet et al. 2008), Brazil (Barbieri 2009), and Gabon (Deem et al. 2006). Samples from the Pacific Ocean are predominantly from China, Japan, California, the Pacific coast of Mexico, and Northeast Australia (Appendix A1.3-A1.9), with two studies from Costa Rica (Harris et al. 2011, Roe et al. 2011), and one study each from Ecuador (Witkowski & Frazier 1982) and Hawaii (Aguirre et al. 1994).

Identification of Overall Patterns

Patterns observed from the RDA biplot ordinations are in line with the expected outcomes: that toxic metal concentrations differ among species, tissue and location. Similarly, ANOSIMs corresponding to the three RDA analyses showed significant differences among species ($R=0.0792$, $p=0.041$, 999 permutations), tissue ($R=0.420$, $p=0.001$, 999 permutations), and ocean ($R=0.0519$, $p=0.036$, 999 permutations). Distance biplots may be interpreted as follows: the distance between objects reflects their Euclidean distances in multidimensional space; the angles between descriptor vectors, however, are meaningless; the projection of an object at right angle to a descriptor vector approximates its value along that vector. Among species, Hawksbills had the highest Cd values, and Kemp's Ridleys had the highest Hg (Figure 1). Controlling for tissue and ocean, 97.2% of the variation among samples was explained by RDA axes 1 and 2 (69.9% and 27.3%), with respect to the concentration of the three metals. The loading factors on each axis, or interest correlations, of Cd and Pb were larger on RDA axis 1 (2.02, -1.69) compared with axis 2 (-0.33, 1.06), while those of Hg were similar on each axis (-

1.57, -1.56). Though all species clustered closely, the 95% confidence-limits (ellipses) for Loggerhead and Green turtles are separate from each other, both overlapping with Leatherbacks; Hawksbill and Kemp's Ridley turtles both appear distinct from the others, however it should be noted that the analysis included only 3 Hawksbill samples, and 5 of the 8 Kemp's Ridley samples were of blood. Among tissues, kidney and liver had the highest Cd values, while egg, muscle, and blood were highest in Pb, and egg and muscle were highest in Hg (Figure 2). Controlling for species and ocean, 97.3% of the variation among samples was explained by RDA axes 1 and 2 (70.6% and 26.7%). In this case, the loading factors of Cd and Pb were larger on RDA axis 1 (2.14, -1.95) than on axis 2 (0.19, -0.99), while that of Hg was larger on axis 2 (1.69) than axis 1 (-1.39). Tissues showed less clustering than species, with unique ellipses for liver and kidney samples, but an overlap of muscle, blood, and egg tissues. Among oceans, the Mediterranean and Atlantic had the highest values of Hg, and the Pacific had a slightly higher value of Cd (Figure 3). Controlling for species and tissue, 97.1% of the variation among samples was explained by RDA axes 1 and 2 (70.5% and 26.6%). The loading factors for Cd and Pb were, again, larger on RDA axis 1 (2.05, -1.72) than on axis 2 (-0.31, 1.02), while Hg had similar loading factors on each axis (-1.53, -1.56). There is a distinct separation of the Pacific from both the Mediterranean and Atlantic.

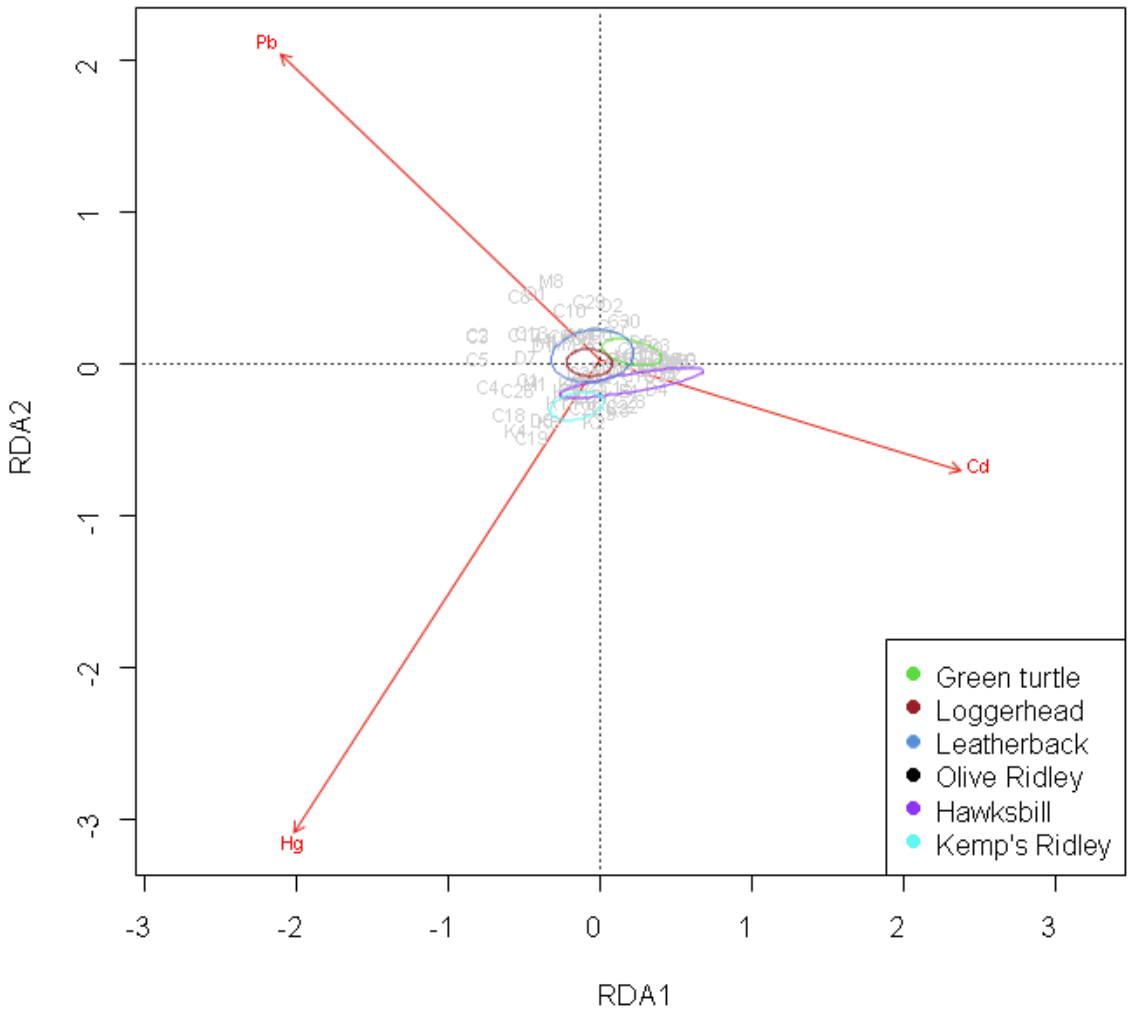

Figure 1: Distance biplot from an RDA of literature-collected sea turtle toxic metal data constrained by metal concentration, controlling for tissue and ocean. The metals (Cd, Hg, and Pb) are represented by vectors. Ellipses represent the 95% confidence limit, based on the standard error of the mean for each species. Only 2 Olive Ridley samples were included in the analysis, thus this species has no corresponding ellipse. Distance biplots may be interpreted as follows: the distance between objects reflects their Euclidean distances in multidimensional space; the angles between descriptor vectors, however, are meaningless; the projection of an object at right angle to a descriptor vector approximates its value along that vector.

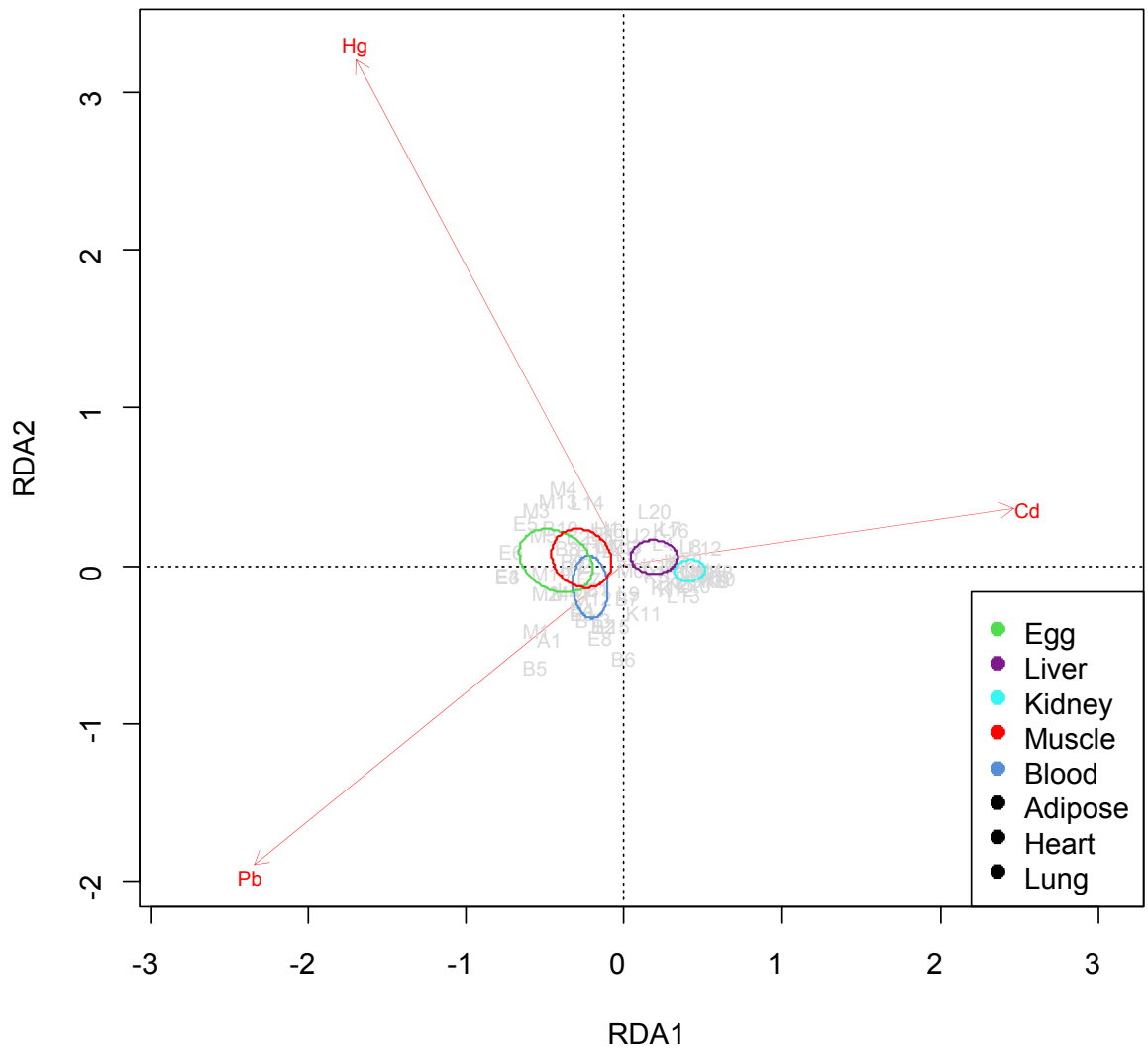

Figure 2: Distance biplot from an RDA of literature-collected sea turtle toxic metal data constrained by metal concentration, controlling for species and ocean. The metals (Cd, Hg, and Pb) are represented by vectors. Ellipses represent the 95% confidence limit, based on the standard error of the mean for each tissue. Tissues with only 1-2 samples are labeled in black (in the legend), and have no corresponding ellipse. Distance biplots may be interpreted as follows: the distance between objects reflects their Euclidean distances in multidimensional space; the angles between descriptor vectors, however, are meaningless; the projection of an object at right angle to a descriptor vector approximates its value along that vector.

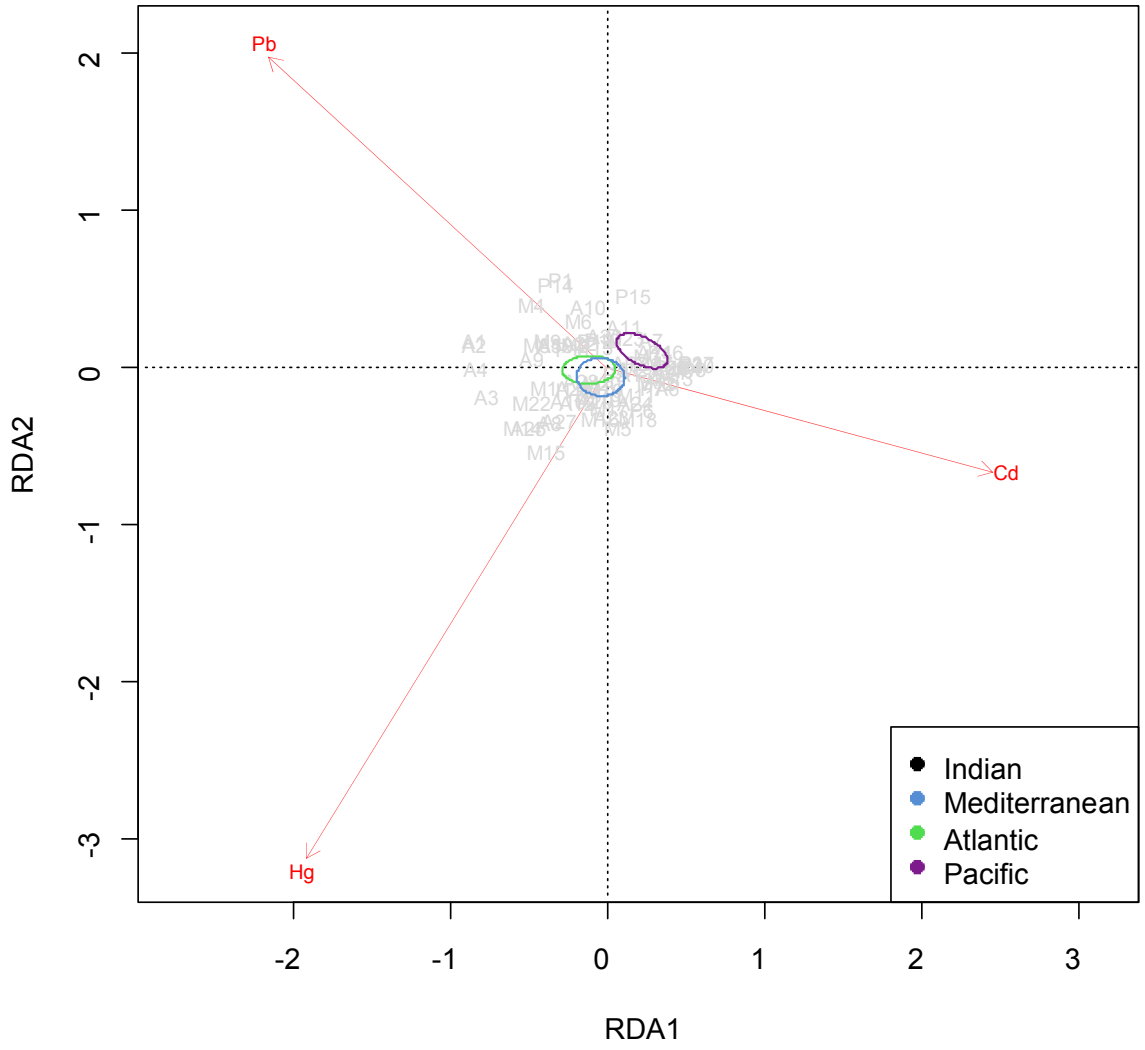

Figure 3: Distance biplot from an RDA of literature-collected sea turtle toxic metal data constrained by metal concentration, controlling for species and tissue. The metals (Cd, Hg, and Pb) are represented by vectors. Ellipses represent the 95% confidence limit, based on the standard error of the mean for each ocean. Only 1 sample was included in the analysis from the Indian Ocean, thus it has no corresponding ellipse. Distance biplots may be interpreted as follows: the distance between objects reflects their Euclidean distances in multidimensional space; the angles between descriptor vectors, however, are meaningless; the projection of an object at right angle to a descriptor vector approximates its value along that vector.

Question 1: Interspecies Differences

Although the ANOSIM results indicated significant differences among species in overall metal concentrations, species did not differ significantly in the concentrations of any specific

metal. While mercury appeared to be higher in Loggerheads (0.7 ± 0.2 μg g^{-1}) than in Green turtles (0.12 ± 0.03 μg g^{-1}; Figure 4), the large variation for the former species prevented the difference from being statistically significant. No significant difference among species was found for either As (df=3, F=0.787, p=0.524), Cd (df=1, F=1.079, p=0.294) or Hg (df=1, F=2.636, p=0.128). Pb does not concentrate into a specific non-edible tissue (see results for Question 2a) and therefore was not analyzed for interspecific differences.

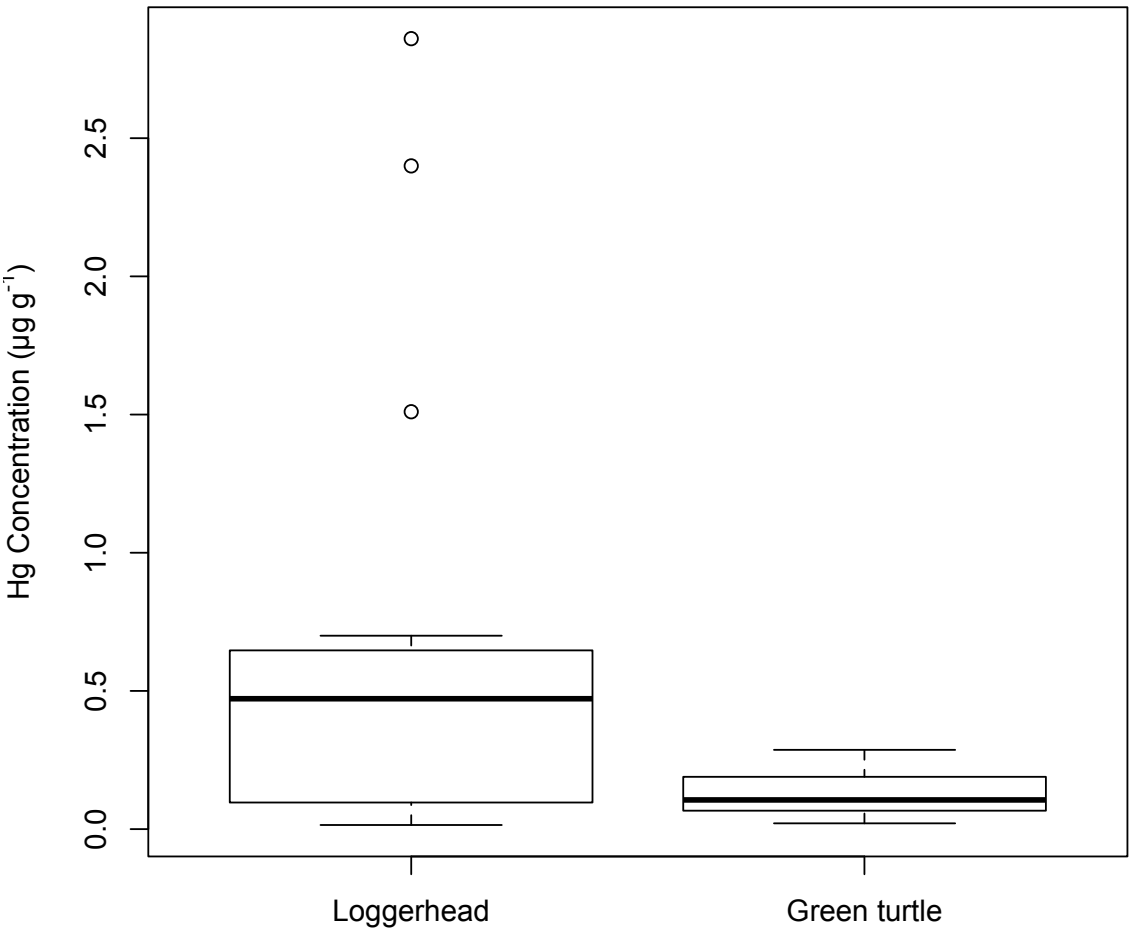

Figure 4: Mercury (Hg) concentration (μg g^{-1} wet weight) in the livers of Loggerhead and Green turtles.

Question 2: Tissue-Specific Associations

We found the expected tissue-specific associations for Cd and Hg in kidney and liver within species, whereas only Cd shows this relationship across all species. Cd was significantly highest in all kidney samples (16 ± 2 μg g^{-1}), and second highest in liver (5.1 ± 0.8 μg g^{-1}; df=2,

F=15.780, p=0.001; Figure 5); no difference was found among tissues for As, Hg, or Pb (0.127 < p < 0.347).

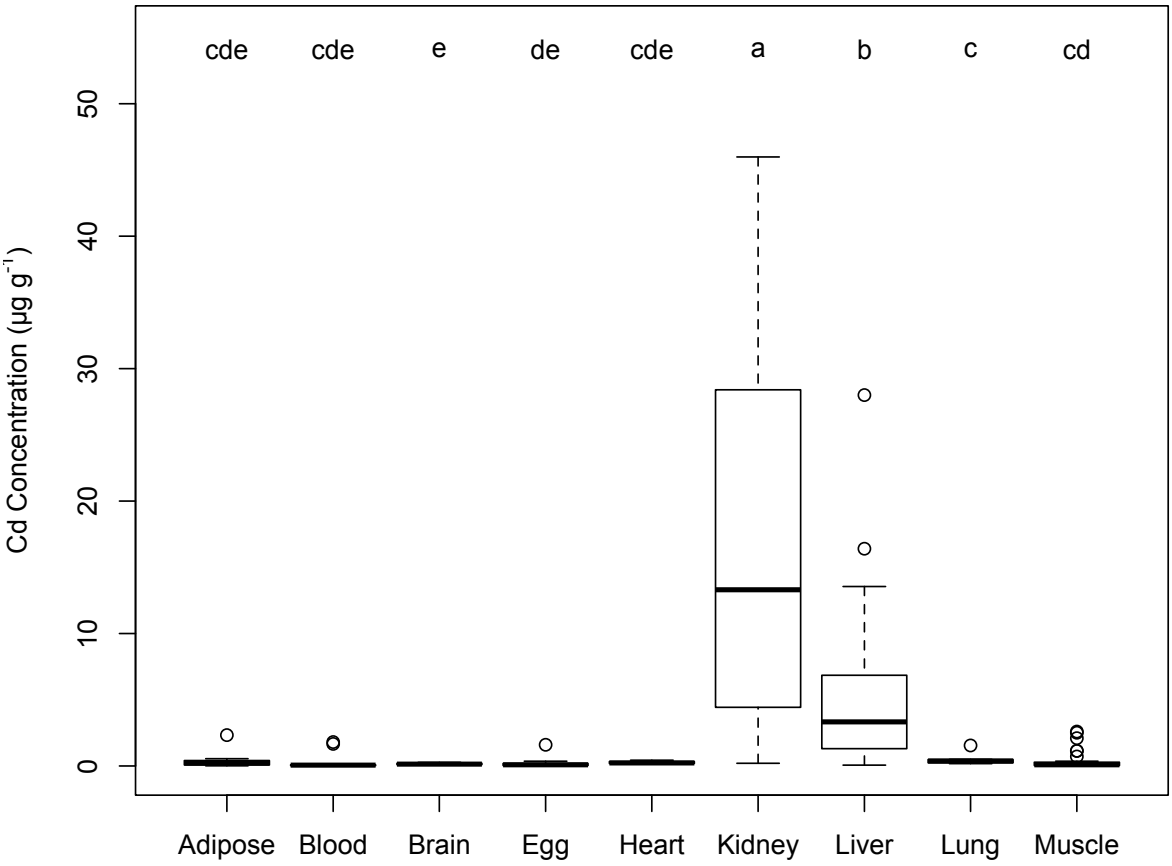

Figure 5: Cadmium (Cd) concentration (µg g^{-1} wet weight) in separate tissues of all species combined.

Within each species, Cd was significantly highest in the kidneys of Loggerhead (13 ± 3 µg g^{-1}; df=3, F=32.085, p=0.001; Figure 6a) and Green turtles (21 ± 4 µg g^{-1}; df=1, F=17.148, p=0.002; Figure 6b), although both had a significant interaction effect with ocean (df=11, F=3.592, p=0.022; df=3, F=14.536, p=0.010; respectively). Hg was significantly highest in the liver for Loggerheads (0.7 ± 0.2 µg g^{-1}; df=2, F=4.662, p=0.032; Figure 7a), Green turtles (0.12 ± 0.03 µg g^{-1}; df=4, F=6.189, p=0.001; Figure 7b), and Kemp's Ridleys (0.2 ± 0.1 µg g^{-1}; df=1, F=6.842, p=0.040; Figure 7c).

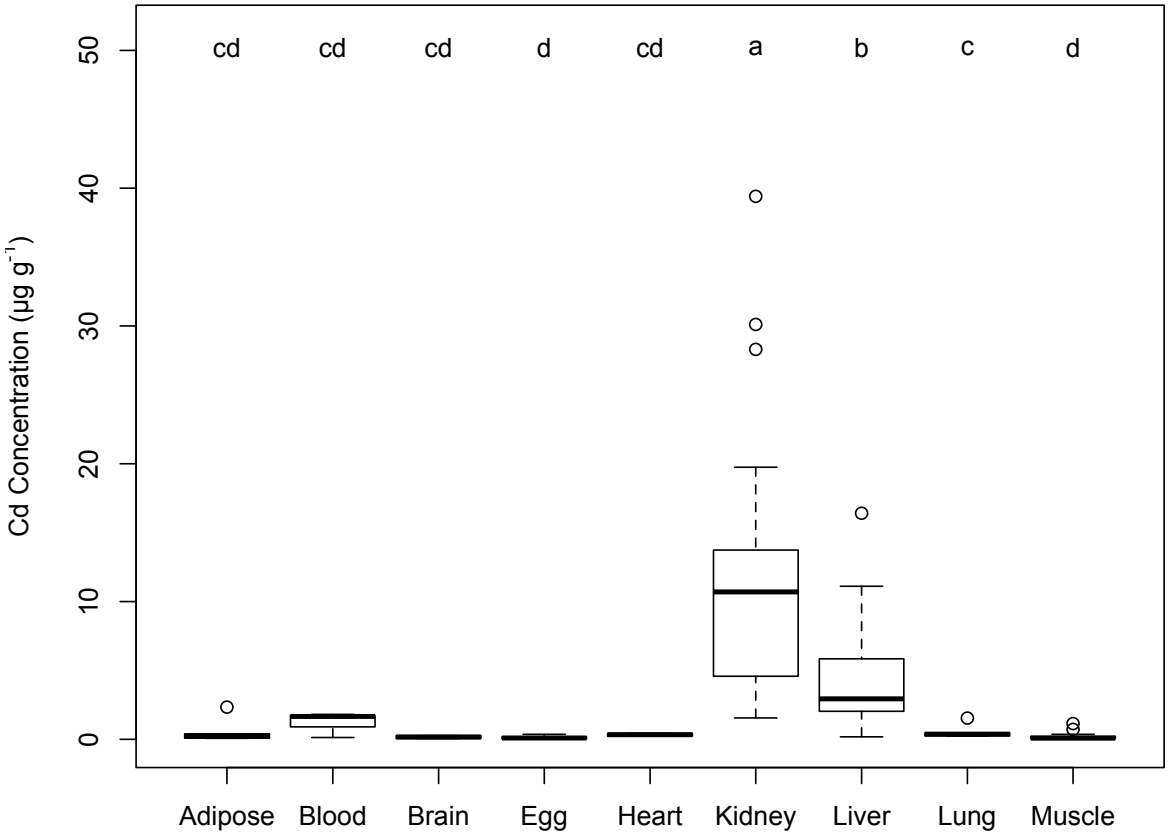

Figure 6a: Cadmium (Cd) concentration (µg g^{-1} wet weight) in separate tissues of Loggerheads.

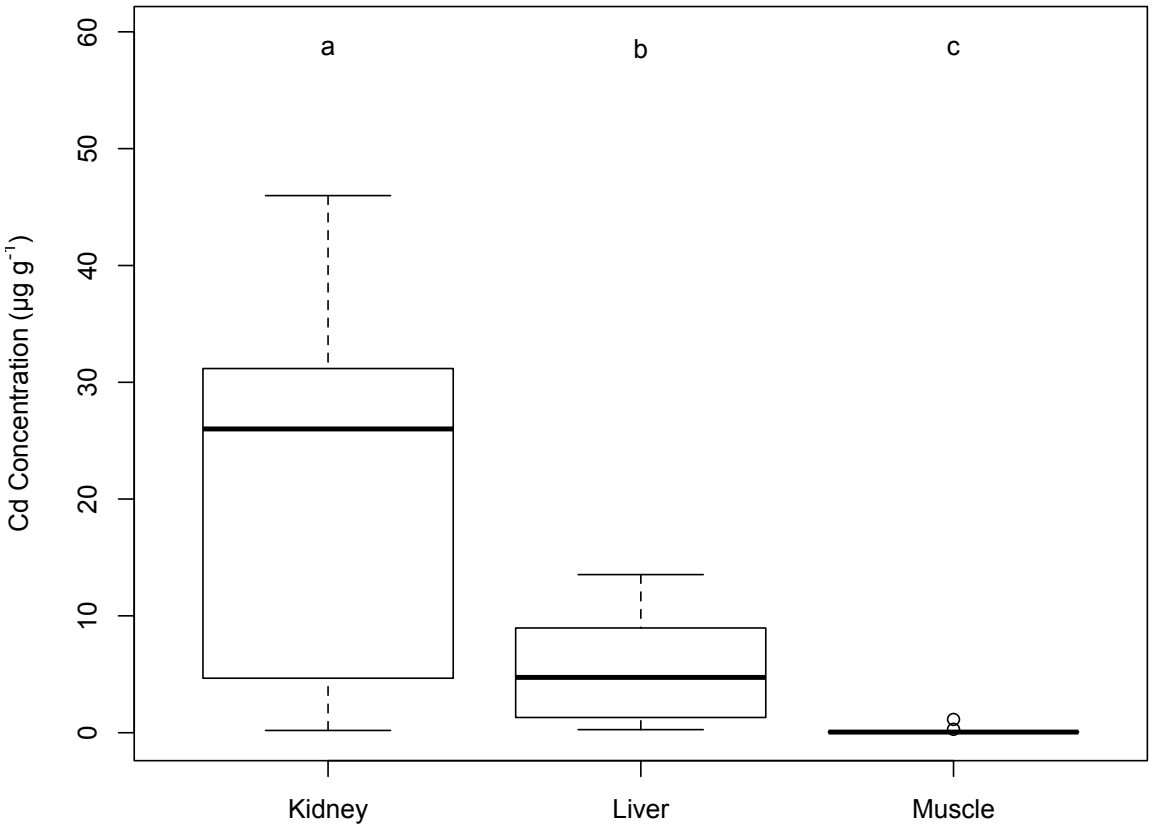

Figure 6b: Cadmium (Cd) concentration (µg g^{-1} wet weight) in separate tissues of Green turtles.

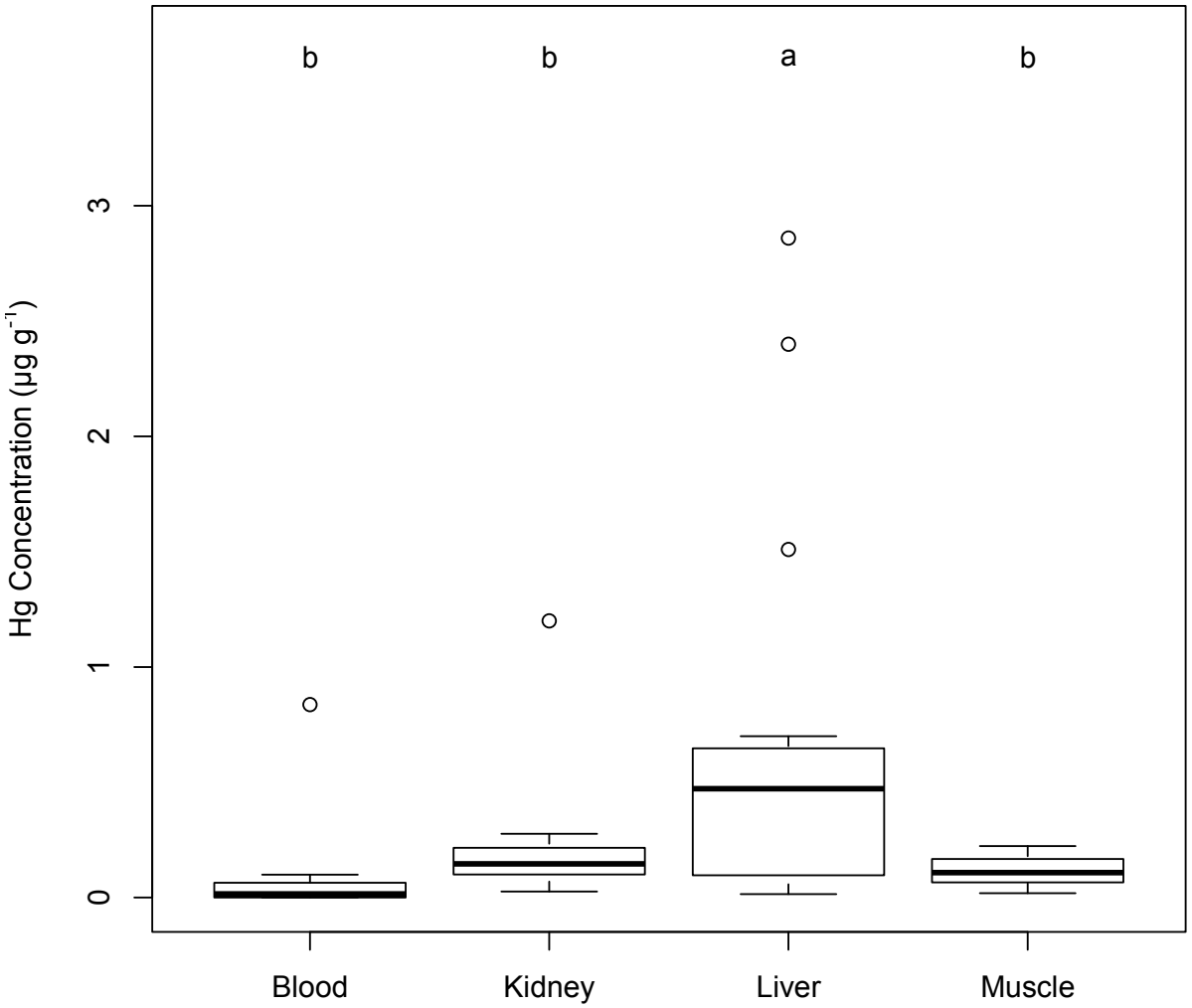

Figure 7a: Mercury (Hg) concentration (µg g^{-1} wet weight) in separate tissues of Loggerheads.

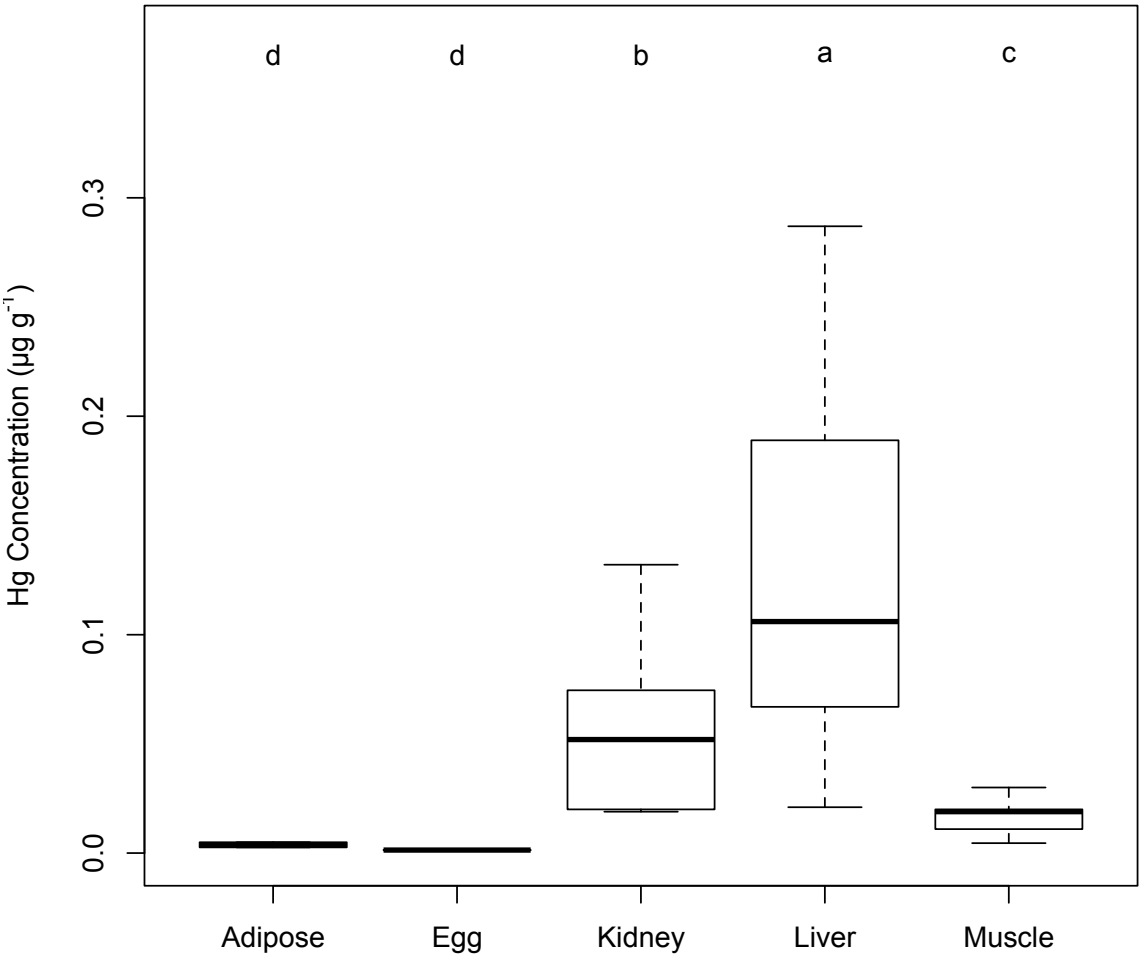

Figure 7b: Mercury (Hg) concentration (µg g^{-1} wet weight) in separate tissues of Green turtles.

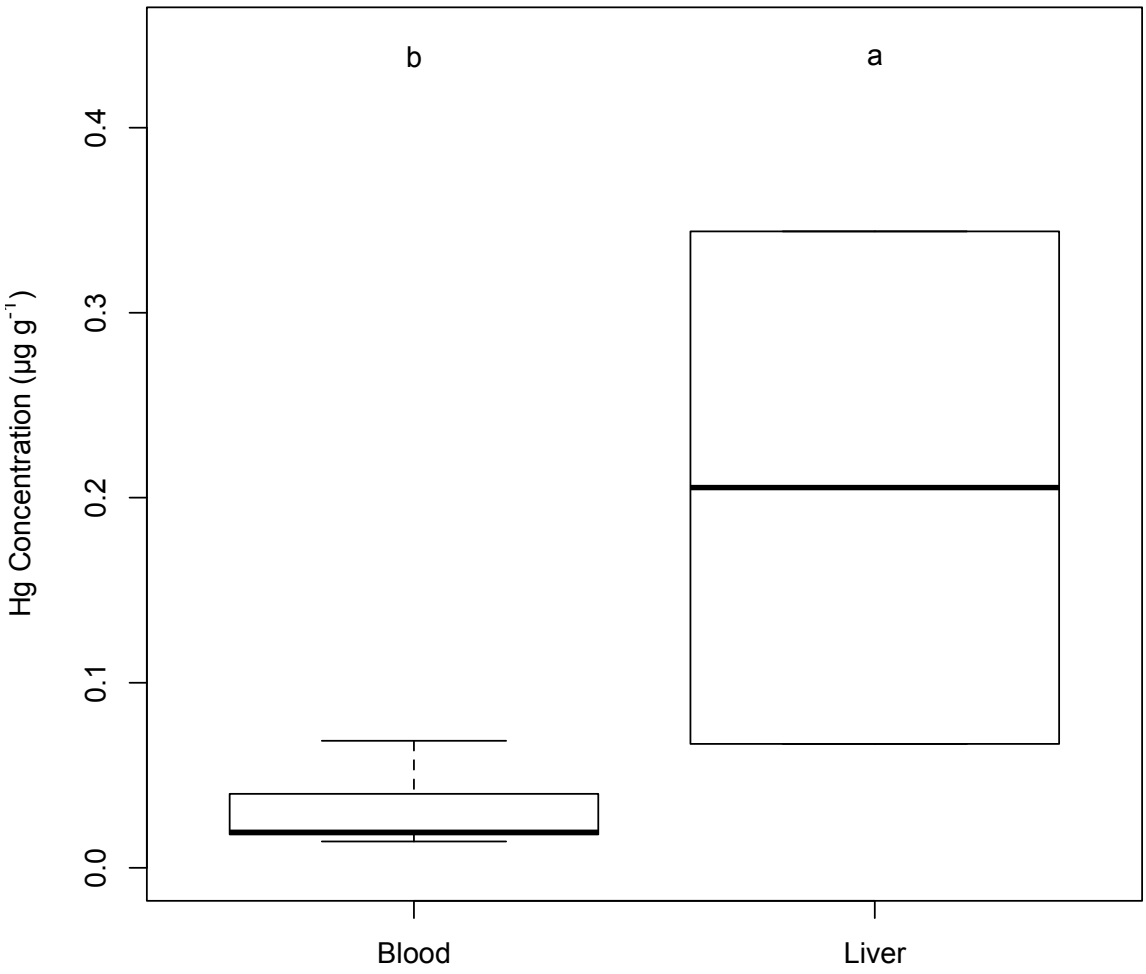

Figure 7c: Mercury (Hg) concentration (µg g^{-1} wet weight) in separate tissues of Kemp's Ridleys.

In addition, significant differences were found within Green turtles among oceans for Cd (df=1, F=25.158, p=0.001) and Pb (df=2, F=16.150, p=0.002). Cd was highest in the Pacific Ocean (14 ± 3 µg g^{-1}); while Pb was highest in the Mediterranean Sea (0.50 ± 0.08 µg g^{-1}), and higher in the Pacific (0.12 ± 0.04 µg g^{-1}) than in the Atlantic Ocean (0.06 ± 0.03 µg g^{-1}).

Question 3: Geographic Variation

Only Cd was found to be significantly different among oceans. Samples from the Pacific Ocean (31 ± 3 µg g^{-1}) contained significantly more Cd than from either the Mediterranean Sea (6 ± 1 µg g^{-1}) or Atlantic Ocean (9 ± 4 µg g^{-1}; df=2, F=26.982, p=0.001; Figure 8). Because we observed significant differences among tissues, Hg and Cd were analyzed for geographic

patterns using only liver and kidney samples. Conversely, As and Pb were tested including all tissues.

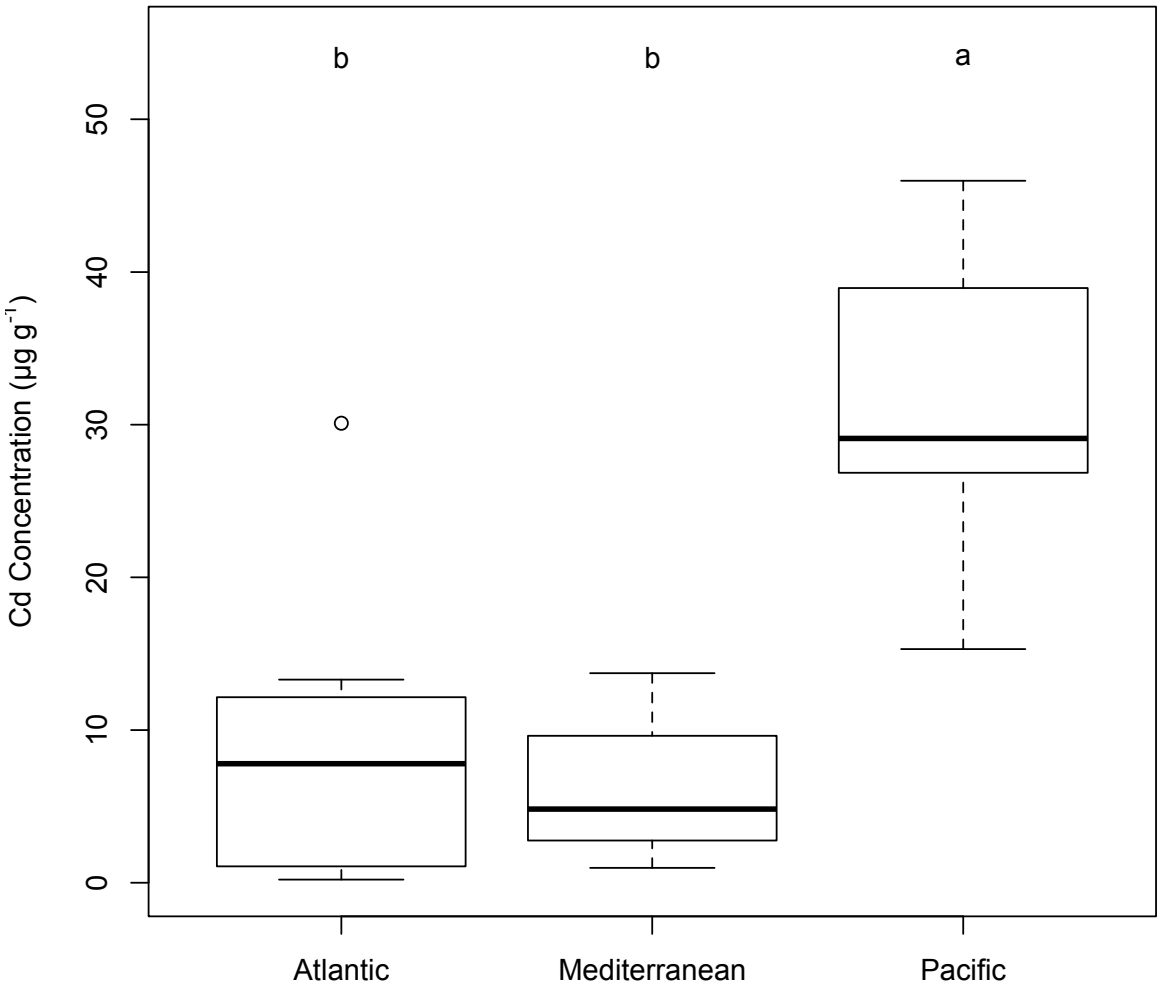

Figure 8: Cadmium (Cd) concentration (µg g^{-1} wet weight) in kidneys of Loggerhead and Green turtles from 3 broad sea turtle habitats.

Question 4: Implications for Human Health

Overall calculations found that qualitatively, Cd poses the highest health risk from eating sea turtle. Using the entire data set, looking at each factor individually regardless of the other 2, Cd was the most toxic of the 4 metals in the Pacific Ocean (9.6 ± 1.7 days $serving^{-1}$; 8 ± 1 µg g^{-1}), Atlantic Ocean (4.8 ± 1.4 days $serving^{-1}$; 4 ± 1 µg g^{-1}), and Mediterranean Sea (2.8 ± 0.6 days $serving^{-1}$; 2.3 ± 0.5 µg g^{-1}); among species it was the most toxic metal in Green (8.7 ± 1.9 days $serving^{-1}$; 7 ± 2 µg g^{-1}), Olive Ridley (7.6 ± 3.3 days $serving^{-1}$; 6 ± 3 µg g^{-1}), Leatherback (6.1 ± 3.0 days $serving^{-1}$; 5 ± 2 µg g^{-1}), and Loggerhead turtles (4.9 ± 1.0 days $serving^{-1}$; 4.1 ± 0.8 µg g^{-}

^{1}); and among tissues it was the most toxic metal in kidney (19.3 ± 2.6 days serving^{-1}; 16 ± 2 μg g^{-1}) and liver (6.2 ± 0.9 days serving^{-1}; 5.1 ± 0.8 μg g^{-1}). Hg was the most toxic metal only in Kemp's Ridley turtles (1.1 ± 0.5 days serving^{-1}; 0.09 ± 0.04 μg g^{-1}); while As was the most toxic metal in Hawksbill turtles (4.7 ± 2.6 days serving^{-1}; 5 ± 1 μg g^{-1}), as well as muscle (2.6 ± 0.6 days serving^{-1}; 6 ± 2 μg g^{-1}) and lungs (1.6 ± 0.7 days serving^{-1}; 4 ± 2 μg g^{-1}). Mercury and arsenic, when present at more toxic levels than cadmium in a given species or tissue, was not found to be harmful to those eating less than one 75 g serving of that species or tissue per week. Lead was found to be of no concern without eating more than one serving per day.

Discussion

Local vs. Global-Scale Observations

Interspecies Differences

Animals have the ability to regulate their internal concentration of essential metals to ensure optimal functioning of physiological and metabolic processes (Chapman et al. 1996): species with similar physiological needs should have similar levels of essential metals, regardless of their trophic level (Stoneburner et al. 1980). Conversely, toxic metals tend to accumulate in higher trophic-level organisms through biomagnification. The six sea turtle species included in the current analysis represent multiple trophic levels and a variety of diets, and thus allow these hypotheses to be tested. Indeed several studies have found that carnivorous Loggerheads contain higher toxic metal concentrations than herbivorous Green turtles (Sakai et al. 1995, Caurant et al. 1999, Godley et al. 1999, Saeki et al. 2000, Sakai et al. 2000b, Anan et al. 2001, Kubota et al. 2003, Kaska et al. 2004, Gardner et al. 2006, Kampalath et al. 2006, Suzuki et al. 2012); however, some have concluded that Green turtles may naturally accumulate high levels of cadmium (Gladstone 1996, Gordon et al. 1998, Anan et al. 2001, Anan et al. 2002, Gardner et al. 2006, Andreani et al. 2008, Ley-Quiñónez et al. 2013). While this phenomenon has been attributed to their feeding ecology, including alga with metal concentrations several orders of magnitude above ambient (Caurant et al. 1999, Sakai et al. 2000a, Franzellitti et al. 2004, Andreani et al. 2008); or a physiologically higher degree of bioaccumulation in Green turtles (Ley-Quiñónez et al. 2013); no conclusive mechanism has yet been described.

In this study, we found significant variation in overall metal profiles among species. While this suggests that there are differences in how each species accumulates non-essential

metals, no significant difference was found for any metal individually, possibly due to local variation that could not be accounted for in the analysis. Substantial variation among feeding grounds, along with unbalanced sampling, could obscure any broad-scale differences. Although estimates were not available from each study, age is important to consider as well. Negative correlations with body size and Cd have been found in Green turtles (Gordon et al. 1998, Sakai et al. 2000a), which has been attributed to their change from omnivory as a juvenile to herbivory as an adult (Jones & Seminoff 2013). Finally, the preferential diets reported in the literature for each species are generalizations only. For example, although adult Green turtles are considered herbivores (Seminoff 2004), they do consume animals such as sponges and other benthic invertebrates (Jones & Seminoff 2013). In this way, interspecific differences may be less than expected, especially at a global scale.

We could not identify global-scale interspecific differences in metal-specific toxicity, therefore no global-scale human-health related recommendations based on different species can be made from the current data. Rather, comparisons should be made based on any available regional data when considering the toxicity of different species. This would ensure that regional variation, especially for turtle populations around higher oceanic pollutant inputs, is minimized.

Tissue-Specific Associations

Different metals act differently in the body, and many studies have shown that each of As, Cd, Hg, and Pb ultimately concentrate in muscle, kidney, liver, and calcified tissues, respectively (Storelli & Marcotrigiano 2003, Jakimska et al. 2011b). Globally, including all species, this trend was found with significance only for Cd (Figure 5); while not significant, Hg was also highest in the liver for each species (for those with replicate liver samples). On a per-species basis, the expected trend was found, with significance, for Cd and Hg in Loggerheads and Green turtles, and also for Hg in Kemp's Ridleys. With the exception of Loggerheads and Green turtles, the other species often did not have sufficient data for the respective tissue to assess these relationships. While not novel, these results reinforce the reported metal-tissue associations for both Cd and Hg.

This pattern of tissue toxicity, coupled with the empirical data, indicates that turtle kidneys pose the highest health risk to humans. On average, eating kidney more than once every 2-3 weeks (based on a 75 g serving size; HC-SC 2008, AHA 2013) could lead to Cd toxicity; or specifically, eating kidney from a Loggerhead once every 2 weeks or from a Green turtle every

3.5 weeks would be considered toxic. Liver was the second most toxic tissue overall, from Hg and/or Cd concentrations, if eaten more than once every 1-2 weeks. Specifically, eating Loggerhead liver could lead to Hg toxicity if eaten more than once every 9 days, or Cd toxicity every 5 days; while eating liver from Green turtles could cause Cd toxicity if eaten more than once every week. No other tissue, from any species, was found to be toxic when eating ≤ 75 g per week.

Aguirre et al. (2006), Senko et al. (2009), Warwick et al. (2013) explain that sea turtle kidneys and livers are typically not eaten directly, but are often incorporated into a soup along with other internal organs and bones; while the less toxic muscle and eggs are eaten directly. Sea turtle is served most frequently during holidays, and especially during Lent as it is not considered a red meat under the Catholic restrictions for this period (Nichols et al. 2003). Oil and blood are traditionally considered to be remedies for respiratory problems and anemia, respectively. From this, we can suggest that adults in regions with no available toxicology information may safely consume ≤ 75 g sea turtle eggs and/or meat on a weekly basis. Consumption of kidneys should be limited to no more than twice per month, once for Green turtle kidneys, and liver no more than 2-4 times per month. Species ID, age, and location are important factors in toxic metal contamination, but until toxicology data becomes readily available for more areas of the world, these suggestions could be a good baseline for remote communities that rely on turtle meat. However, these results assume equal year-round consumption of turtles. While sea turtles may be available year-round in some areas, there are many regions with seasonal availability of turtles, especially for communities that rely on nesting females for turtle meat (Hamann et al. 2003). Because of this, safe seasonal consumption rates are higher than those reported here, relative to the length of the availability of the turtles.

As indicated earlier, eggs comprised a small proportion of the available data. Consumption of eggs is declining in some coastal nations, but poaching continues both for personal or economic purposes in countries around the globe (Garcia-Martinez & Nichols 2000, Delgado & Nichols 2005, Aguirre et al. 2006, van de Merwe et al. 2009, Green et al. 2010, Pinzón Gómez 2012, Abd Mutalib et al. 2013), many of which have no reports measuring the toxic metal loads of their eggs (Figure 9). We found that, on average, metals in eggs do not pose a risk to humans who eat 75 g (approximately 2-3 eggs) per week. However, Table 2 shows the individual egg data that were found to pose health concerns when eating more than 2-3 eggs (~75

g) per day. This dearth of information concerning a highly consumed sea turtle product remains a detrimental gap in understanding to what degree residents of coastal nations who consume sea turtle products are at risk from inorganic pollution.

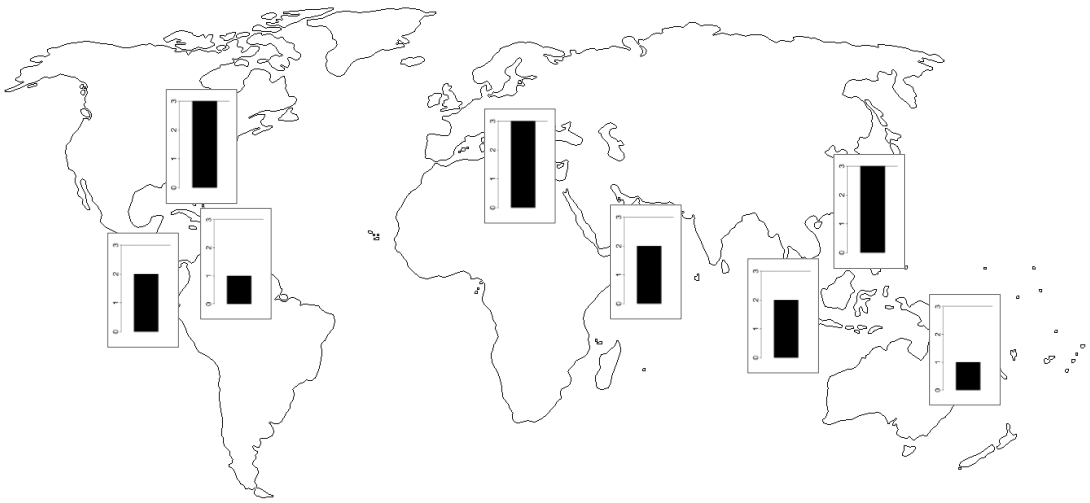

Figure 9: The global distribution of trace metal data (As, Cd, Hg, and Pb) of sea turtle eggs, showing the number of reports of original data from each region. Individual reports are counted for as many different species or location data reported.

Table 2: Reported instances of sea turtle eggs with concentrations of either As, Cd, Hg, or Pb (mean ± SE, wet wt.) high enough to pose a health risk to humans consuming more than 75 g of egg per day.

Species	Location	n	Metal	Concentration ($\mu g\ g^{-1}$)	Edible Frequency (days $75g^{-1}$)	Study
C. caretta	NW Atlantic	27	Hg	1.3595 ± 0.0394	16.4 ± 0.5	Stoneburner et al. 1980
C. caretta	NW Atlantic	33	Hg	1.3912 ± 0.1063	16.8 ± 1.3	Stoneburner et al. 1980
C. caretta	NW Atlantic	15	Hg	0.6352 ± 0.0141	7.7 ± 0.2	Stoneburner et al. 1980
C. caretta	NW Atlantic	21	Hg	0.4123 ± 0.0136	5.0 ± 0.2	Stoneburner et al. 1980
C. mydas	W Indian	75	Hg	0.10 ± 0.01	1.2 ± 0.1	Al-Rawahy et al. 2007
D. coriacea	E Central Pacific	26	Cd	1.6 ± 0.3	1.9 ± 0.4	Roe et al. 2011
L. olivacea	E Indian	24	Pb	3.6 ± 1.1	1.2 ± 0.4	Sahoo et al. 1996[1]
C. mydas	NW Pacific	30	As	2.500 ± 0.370	1.0 ± 0.1	Lam et al. 2006

1: Variance expressed as SD

Geographic Variation

To date, studies have typically compared only a fraction of the available data, often limited to 2-3 species from only a handful of areas. Most of these studies have used specimens from the northern hemisphere, and along with bias in the species sampled it was realistic to only look at geographic patterns based on ocean basin: the Atlantic, Pacific, and Indian Ocean; as well as the Mediterranean Sea, an enclosed sea turtle habitat surrounded by highly industrialized countries (Garcia-Fernandez et al. 2009).

While Cd has been found at considerably higher concentrations in the 'older' waters of the deep North Pacific compared with the North Atlantic (Bradl 2005), this difference is less extreme at the surface. In the open ocean, surface waters do not differ substantially among ocean basins, whereas coastal contamination varies both within and among oceans (Chester & Stoner 1974, Bruland et al. 1979, Bruland 1980, Danielsson 1980, Boyle et al. 1981, Boyle et al. 1985, Danielsson et al. 1985, Davis 1993, Bruland et al. 1994, Collado-Sanchez et al. 1996, Bruland 2013, Lamborg et al. 2014). It is difficult to know precisely where a specific turtle has fed without satellite tracking or direct observation, more so for stranded specimens - a major source of data (Storelli & Marcotrigiano 2003). However, with the exception of Leatherbacks, sea turtles predominantly forage in shallow, coastal waters (Plotkin 2003). Based on the data from Davis (1993), we should expect to see the highest Cd concentrations in the Atlantic and Pacific Oceans. Instead, no difference among oceans was found for Hg; and the significantly higher Cd burdens in the Pacific Ocean samples suggest that Cd contamination in turtles is more closely associated with sub-surface concentrations.

Based on these results, Cd levels from the Pacific Ocean were sufficiently high in kidneys to be toxic if eaten more than once per month, and every week from either the Atlantic Ocean or Mediterranean Sea. Green turtle samples followed this same trend across all tissues, but with shorter intervals - once per 2 weeks from the Pacific Ocean, and less than 1 week from either the Atlantic Ocean or Mediterranean Sea; as did Loggerheads, although not significantly – once per 1.5 weeks from the Pacific Ocean and less than 1 week from the others. These results support those from the previous sections, suggesting that Cd is the most toxic metal to humans consuming sea turtle products.

Anthropogenic cadmium emissions come from products whose manufacture, use, and/or disposal intentionally contain Cd, or that contain Cd as a natural but non-functional impurity

(ICdA). Common intentional uses for Cd and its compounds include, but are not limited to: Ni-Cd batteries, electronic components, a pigment in plastics and paints, alloys, electroplating, and a rust-prevention coating more resistant than Zn galvanizing (ICdA , Friberg et al. 1979, Bradl 2005). Products that contain or produce Cd as a non-functional byproduct include: fossil fuels, cement, phosphate fertilizers, and Zn mining, smelting, and refining (ICdA , Bradl 2005). Erosion, atmospheric deposition, direct discharge, and incidental seepage from contaminated soils and agriculture into rivers and coastal areas are all potential pathways for Cd to enter the ocean (ICdA). In the ocean, cadmium levels strongly correlate to phosphate, indicating a nutrient-like behaviour and an affinity for biogenous particulate matter (Bradl 2005). With these attributes, we might expect that high-productivity regions (i.e. high nutrient content) will also be high-Cd regions, suggesting that average oceanic concentrations may underestimate the contamination levels entering the food web at the lowest trophic levels. This nutrient-like behaviour may partially explain why Cd was found to be the most toxic. Additionally, Caurant et al. (1999) concluded that sea turtles in general bioaccumulate higher levels of Cd compared with other marine organisms, which may partially explain why Cd was found to pose a higher risk to humans than Hg, which is the most toxic metal in other seafood (Green et al. 2010). Cadmium primarily affects the kidneys, causing renal dysfunction and kidney stones. Because it interferes with calcium metabolism, high levels of cadmium can also lead to osteoporosis (Aguirre et al. 2006, WHO 2010b). In contrast, ingested organic-mercury is particularly harmful to the central and peripheral nervous system, causing “tremors, insomnia, memory loss, neuromuscular effects, headaches and cognitive and motor dysfunction” (WHO 2007). Mercury is especially harmful to children and fetuses, where it can cause neurodevelopmental issues (WHO 2007).

Conclusions

The objectives of this review were to determine the robustness of three common themes in sea turtle toxicology literature: differences among species, tissues and locations. The strongest conclusions that could be made from the available data relate to mercury and cadmium in liver, kidney and muscle tissues from Loggerhead and Green turtles. No interspecies difference was found for any metal. Although carnivorous species would be expected to have higher contaminant loads than herbivorous ones via biomagnification, sea turtles’ diets often vary

within a species according to age and population. Thus whereas interspecies variations can be apparent at a specific site, they are not universal.

The relationship among tissues showed the expected Cd and Hg distributions: concentrated in kidney and liver, respectively. Cd was the most apparent, significantly higher in kidney across all species; and Hg highest in liver for each species, though not significantly. Except for Loggerhead and Green turtles, there is a dearth of information on metals from the other sea turtle species, making quantitative comparisons difficult. Increasing this databank would provide valuable information for further comparisons, especially for the less threatened Olive Ridley and Leatherbacks. Geographically, Cd was unexpectedly higher in the Pacific Ocean specimens for both Loggerheads and Green turtles, suggesting that Cd burdens in sea turtles are likely associated with deeper water concentrations, and not with the surface waters where they often feed.

Concerning the human health impacts of consuming turtle meat, kidney and liver are the two most toxic tissues, predominantly from Cd but also Hg toxicity, and the only two that could be harmful when consuming less than 75 g per week. While no overall differentiation could be made among species, Cd was highest in the Pacific Ocean specimens of Loggerheads and Green turtles, thus consuming sea turtle kidney or liver in a Pacific country would pose the highest risk of metal toxicity.

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Linking Statement Between Chapters 1 and 2

The standardized measure of toxicity used in Chapter 1 shows that Cd is the most harmful metal in edible turtle tissues, specifically in kidney and liver of Green and Loggerhead turtles, while Hg is also harmful in the liver of Loggerheads. At a consumption rate of 2-3 eggs per day, the eggs of 4 species reported in 5 studies were found to be poisonous from either As, Cd, Hg, or Pb. Chapter 2 presents original trace metal data, including As, Cd, Hg, and Pb, measured from Green Turtle and Olive Ridley eggs collected on the Pacific Coast of Panama – the first reliable report for any turtle tissues from this area. This chapter looks at biological variation based on species and nesting site, and if known consumption rates of these eggs pose a health risk to those who eat them.

Chapter 2

Metal contents of marine turtle eggs (*Chelonia mydas*; *Lepidochelys olivacea*) from the Pacific coast of Panama and the implications for human health

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KEY WORDS: Green turtle; Olive Ridley; Eggs; Trace metals; Panama; Human health

SHORT TITLE: Panamanian sea turtle egg metal-toxicology

Abstract

Concentrations of 13 elements were measured in *Chelonia mydas* and *Lepidochelys olivacea* eggs collected along Panama's Pacific coast. Mn, Fe, Cu, Zn, As, Se, Cd and Hg concentrations were similar to previous studies, while Co, Ni, Mo, and Pb were lower than previously reported. We found no overall differences among species or sites; however there were significant interspecific differences at individual sites, and geographic differences within each species. Cd had the highest toxicity, with consumption rates of ≥21±4 eggs day^{-1} required for adult consumers (~69.9 kg) to attain the PTDI, which was lower than the maximum reported consumption rates from a coastal Panamanian community. While newborns (3.2 kg) typically weigh more than or equal to the minimum weight to safely consume 1 egg day^{-1} (≤3.3±0.6 kg), maximum consumption reported by those aged 0-15 would be toxic up to the age of ~3.4 yrs. Our conclusions support that interspecific differences are more complex than just trophic level; geographic demes might be found within Panama; and current turtle egg consumption on Panama's Pacific coast may pose a health risk to consumers. Furthermore, our calculations do

not account for metal intake from additional food sources and therefore inevitably overestimate the threshold number of eggs that may safely be consumed.

Introduction

Of the 7 sea turtle species found around the globe, 6 are currently listed as either vulnerable: Leatherbacks (*Dermochelys coriacea* Vandelli, 1761) and Olive Ridleys (*Lepidochelys olivacea* Eschscholtz, 1829); endangered: Loggerheads (*Caretta caretta* Linnaeus, 1758) and Green turtles (*Chelonia mydas* Linnaeus, 1758); or critically endangered: Hawksbill turtles (*Eretmochelys imbricata* Linnaeus, 1766) and Kemp's Ridleys (*Lepidochelys kempii* Garman, 1880); while the Flatback (*Natator depressus* Garman, 1880) is data deficient (Marine Turtle Specialist Group 1996a, b, Red List Standards & Petitions Subcommittee 1996, Seminoff 2004, Abreu-Grobois & Plotkin 2008, Mortimer & Donnelly 2008, Wallace et al. 2013). Centuries of poaching, both of nesting females and their eggs; combined with by-catch and ghost nets from large scale fisheries, and increasing chemical and plastic based pollution are the major sources of the decline in marine turtle populations. These activities, along with increased coastal development and climate change, continue to threaten turtles worldwide.

All marine turtles are long-lived, and known, for at least a part of their lives, to feed at relatively high trophic levels (Jones & Seminoff 2013). Of the 2 focal species in this study, female Green turtles may spend between 15-50 years as juveniles (Avens & Snover 2013), feeding on crustaceans, gastropods, tunicates, and jellyfish as juveniles and later switching mainly to seagrasses and algae (Jones & Seminoff 2013) before reaching the age of sexual maturity (ASM). As adults, they can remain in nearshore foraging grounds for over 5 years between migrations to their natal beach (remigration intervals), where they lay around 115 eggs per nest in an average of 5 nests per season (Hamann et al. 2003). They then migrate back to their foraging grounds, continuing this cycle for an average of 19 years (Chaloupka & Limpus 2005). Olive Ridleys mature around 13 years old (Avens & Snover 2013), foraging mainly on gastropods, crustaceans, small fish, and tunicates (Jones & Seminoff 2013). Throughout their lives, they remain in oceanic habitats, where issues in locating sufficient aggregations of juveniles for mark-recapture studies (Avens & Snover 2013) has limited ASM estimation to skeletochronology methods (Zug et al. 2006). Adult females typically remain in the foraging grounds for 2 years before remigrating to lay over 3 nests per season with on average more than

100 eggs per nest (Hirth et al. 1980). Due to the difficulties in age estimates, the maximum age range of olive ridleys is not known.

Trace metals, which have increased markedly in the marine environment owing to release of anthropogenic chemical pollutants (Bradl 2005, Jakimska et al. 2011), have repeatedly been shown to bioconcentrate in lower trophic level invertebrates (Ansari et al. 2004), and then biomagnify by orders of magnitude up the marine food web into large predatory fish, sharks and cetaceans (Jarup 2003, Ansari et al. 2004, Green et al. 2010), ultimately into humans. Furthermore, sea turtles, especially females, have a high daily rate of intake at foraging grounds during remigration intervals (Hamann et al. 2003), therefore turtles may be expected to show significant trace-metal bioaccumulation, with potentially detrimental effects to both turtles and to people who eat them and their eggs. Up to a year before breeding, female turtles begin producing eggs, beginning with vitellogenesis (Hamann et al. 2003, Guirlet et al. 2008). Trace metals accumulated while feeding are transferred into the developing eggs through the deposition of lipids and proteins, including non-essential metals. While the transfer of non-essential metals into eggs is not a substantial route of excretion for female turtles (Sakai et al. 2000b, Storelli and Marcotrigiano 2003, Paez-Osuna et al. 2010a), relationships between the metal concentration in eggs and mothers have been found (Sakai et al 1995, Guirlet et al. 2008, Paez-Osuna et al. 2010a), with Hg reported to be the most efficiently transferred non-essential metal (Storelli & Marcotrigiano 2003).

Yet, many of the regions in which coastal communities rely on sea turtle meat and/or eggs as an important part of the local diet (Garcia-Martinez & Nichols 2000, Delgado & Nichols 2005, Aguirre et al. 2006, van de Merwe et al. 2009, Green et al. 2010, Pinzón Gómez 2012, Abd Mutalib et al. 2013) have no toxicological data to inform them whether or not this food source is harming them (Ross et al., in review). One of these regions, which is the focus of this study, is the Eastern Pacific Ocean, specifically the southern coast of Panama. Consumption of turtle meat and eggs is common in Panama, especially in remote communities. Eggs, especially, are an important source of easy-access protein, as well as a source of income from their sale in markets and at stalls alongside the main highway.

Mercury (Hg), cadmium (Cd), lead (Pb) and arsenic (As) are considered to be the metals of most concern for humans, due to their widespread distribution and high toxicity. The detrimental effects of these metals to human development, growth and overall health have been

well-established (Grandjean et al. 1995, Dickman & Leung 1998, Iwegbue et al. 2009, Green et al. 2010, Kaplan et al. 2011). Indeed, sea turtle tissues with heavy metal concentrations at levels toxic to humans have been reported (Aguirre et al. 2006, Senko et al. 2009, Warwick et al. 2013); including 2 studies where the eggs of Leatherbacks from Western Costa Rica (Roe et al. 2011) and Loggerheads from Florida, USA (Stoneburner et al. 1980) contained sufficiently high concentrations of cadmium and mercury, respectively, to surpass the provisional tolerable daily intake (PTDI). However, there is significant variation in bioaccumulation in marine turtles among species (Sakai et al. 1995, Caurant et al. 1999, Godley et al. 1999, Saeki et al. 2000, Sakai et al. 2000b, Anan et al. 2001, Kubota et al. 2003, Kaska et al. 2004, Gardner et al. 2006, Kampalath et al. 2006, Suzuki et al. 2012), and among locations, even at a small geographical scale (Stoneburner et al. 1980). Therefore we cannot assume that marine turtle eggs from Panama have similarly toxic Cd concentrations as those from Costa Rica, or even that concentrations of any metal will be similar within a given species along just the Panamanian coast.

The primary aims of this study were to determine the concentrations of selected metals in eggs of Green and Olive Ridley sea turtles collected from along the Pacific coast of Panama; and to determine if known consumption rates within coastal communities may present a risk of heavy metal poisoning. The secondary aims were to examine how the heavy metal profiles of eggs collected in Panama compare with similar profiles from around the globe; to determine if any species-specific metal profiles could be identified, supporting that marine turtles that feed at different trophic levels will have respective differences in toxic metals; and to determine if any variation in toxic metal contamination exists among nesting sites, which would support that geographic demes might exist at such a small scale.

Materials and Methods

Sample Collection and Processing

During the 2012-2013 nesting season, 31 Green turtle eggs and 30 Olive Ridley eggs were collected from beaches along the Western Pacific Coast of Panama and the Pearl Islands Archipelago in the Gulf of Panama (Table 1, Figure 1). Eggs were collected within 12 hr of oviposition, rinsed with distilled water, and placed in a plastic bag; they were kept frozen at -4°C until processing at the Smithsonian Tropical Research Institute (STRI) in Panama City.

Table 1: Detailed sample collection information.

Species	Area	Beach	Coordinates		# Eggs	Date Collected
			Lat	Lon		
Chelonia mydas	Mariatos	Malena	7º 34.455	80º 57.864	10	17-Jul-12
	Coclé	Juan Hombrón	8º 18.998	80º 12.252	7	25-Oct-12
	Tonosí	Isla Cañas	7º 24.704	80º 18.548	10	26-Oct-12
	Pearl Islands	Galera	8º 22.958	79º 05.790	4	13-Nov-12
Lepidochelys olivacea	Tonosí	Ostional	7º 17.979	80º 23.746	10	26-Oct-12
	Pedasí	Lagarto	7º 29.689	80º 0.004	10	26-Oct-12
	Pearl Islands	Galera	8º 22.958	79º 05.790	10	12-Nov-12

Eggs were lyophilized in a Labconco® FreeZone® 4.5L Freeze Dry System, Model 77510. Fast-Freeze™ flasks were attached individually to the System, allowing it to recover the necessary temperature and vacuum before attaching the next flask. The flasks were removed after approximately 48 hr and weighed to determine dry weight of the egg. The dried yolks were homogenized in an acid-washed glass mortar and pestle, which was cleaned using 95% ethanol, nanopure water and again with ethanol between samples. Each egg was split into 2 polyethylene vials (1 egg in 10 was split into 3 to test for homogeneity and analytical precision) for transport to McGill University in Montreal, Canada for trace-metal analysis.

To prevent contamination, all sample manipulation at STRI was done in a Labconco® Purifier® Class II bio-safety cabinet using acid-washed tools and glassware. Equipment was detergent cleaned and rinsed with reverse-osmosis deionized water before being placed in a 5% analytical grade nitric acid bath for a minimum 8 hr, or overnight. Equipment was then rinsed three times with 18 mΩ UV filtered (nanopure) water before use. The eggs were weighed and sized to the nearest 0.001 g and 0.1 cm^3, respectively, before the shell was removed. Frozen eggs were placed into individual pre-weighed Labconco® Fast-Freeze™ Flasks, which were then reweighed to calculate wet weight. The flasks were sealed and kept at -4°C until lyophilization.

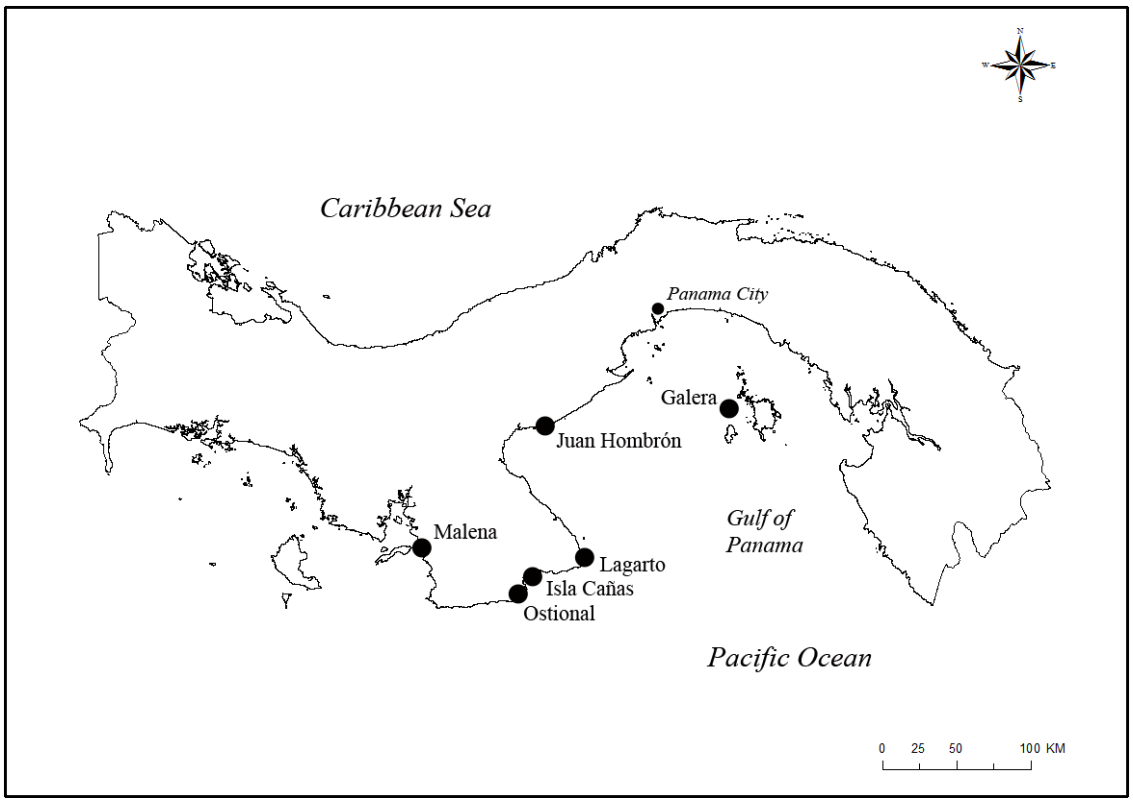

Figure 1: Map of Panama showing the nesting areas where eggs were collected.

Trace-Metal Analysis

To prepare the eggs for trace-metal analysis, 0.1 g of each was weighed and placed in an acid-washed Teflon® vessel with 4.00 ml Optima™ grade 70% nitric acid and 1.00 ml *Trace*SELECT ® Ultra 30% hydrogen peroxide. The vessels were sealed and digested in a CEM® MARS 6™ Microwave Accelerated Reaction System, based on US EPA method 3052 (USEPA 1996). This method uses a ramp time of 5 min 30 sec to reach a maximum temperature of 180°C and pressure of 650 psi, held for 9 min 30 sec, with a maximum power of 1200W. Digestion leads to complete oxidation of the organic molecules, transferring all metals to an inorganic solvation. This ensures equivalent behavior of all samples in the analytical quantification, quantification based on inorganic standards, and removes organic interferences. The digests were left to cool for 20 min before being removed from the System, and for another 30 min before they were transferred to 15 ml Sarstedt® polypropylene centrifuge tubes.

Eight elements were analyzed with a Thermo Scientific™ iCAP™ Qc inductively coupled plasma-mass spectrometer (ICP-MS): Chromium (isotope ^{53}Cr), Cobalt (^{59}Co), Nickel

(^{60}Ni), Arsenic (^{75}As), Molybdenum (^{98}Mo), Cadmium ($^{112, 114}$Cd), Mercury ($^{200, 202}$Hg), and Lead (^{208}Pb). 0.44 g of each digest was diluted to 10 g using nanopure water, spiked with a 10 ng g^{-1} Yttrium internal standard. This internal standard allowed for monitoring of, and correction for, any changes in plasma ionization efficiency, suppression or enhancement. None were found. Five elements were analyzed with a Thermo Fisher Scientific™ iCAP™ 6000 series inductively coupled plasma-optical emission spectrometer (ICP-OES): Manganese (at wavelength $Mn^{2576nm, 2605nm}$), Iron ($Fe^{2382nm, 2599nm}$), Copper ($Cu^{2247nm, 3247nm}$), Zinc ($Zn^{2025nm, 2138nm}$) and Selenium (Se^{1960nm}). 1.5 g of each digest was diluted to 4.5 g using nanopure water.

Instrumental detection limits ranged from 0.0003 - 0.3 ng g^{-1} for ICP-MS, and 0.07 - 4.5 ng g^{-1} for ICP-OES (Table 2). Data quality was verified by analyzing blanks to check for procedural contamination (none was found), calibration standards every 24 samples to check and correct for drift, sample triplicates to quantify precision, and certified reference materials to determine accuracy. Precision, as the relative standard deviation (RSD) of the triplicate samples, was found to have a median absolute deviation (MAD) of 21.3% for Cr (with 3 of 6 triplicates above the detection limit), 3.3% for Mn, 1.8% for Fe, 10.1% for Co, 26.1% for Ni, 2.8% for Cu, 1.7% for Zn, 2.9% for As, 0.8% for Se, 4.1% for Mo, 6.9% for Cd, 10.8% for Hg, and 17.9% for Pb (2 of 6 triplicates above detection), and is correlated with concentration (higher concentrations have lower replicate variance, as expected). Accuracy was determined to have a MAD of 57.9% for Cr, 2.3% for Mn, 5.1% for Fe, 21.8% for Co, 19.3% for Ni, 4.7% for Cu, 6.5% for Zn, 8.6% for As, 12.2% for Se, 19.1% for Mo, 10.2% for Cd, 22.5% for Hg, and 14.3% for Pb, from the certified values (Table 2, Appendix A2.2) based on the standard reference materials SRM 1577c (Bovine liver: National Institute of Standards and Technology, USA) and CRM DORM-4 (Fish protein homogenate: National Research Council, Canada).

Data Analysis

All statistical analyses were performed in the open access statistical software R (R Core Team 2014), using functions found in the packages ‘vegan’ (Oksanen et al. 2014) and ‘Hotelling’ (Curran 2013). Non-metric multidimensional scaling (NMDS) analyses were performed to identify potential differences in non-essential metal accumulation among species and location, as well as among nesting sites within each species. NMDS ordination, using a Bray-Curtis dissimilarity matrix, graphically represents the relationships, or dissimilarities,

among objects in a specified number of dimensions, therefore differences will be displayed as separate clusters of data points (Borcard et al. 2011). This rank-order technique was used due to relatively small sample sizes (≤10 eggs per species per site) and its ability to analyze data that do not meet metric statistical assumptions. Only the toxic metals As, Cd, Hg and Pb were included in the analysis, since physiological regulation of essential elements increases their similarity across trophic levels and geographic locations (Stoneburner et al. 1980, Sakai et al. 1995, Kaska & Furness 2001, Gardner et al. 2006). To account for larger concentrations of As and Cd, compared with Hg and Pb, the data were Hellinger-transformed before analysis (Borcard et al. 2011). This transformation reduces the importance of large values (in this case, As and Cd) so that substantial differences between smaller values (Hg and Pb) will be equally reflected in the output. Ellipses were created using standard deviation, with a significance value of 0.050; and analyses of similarities (ANOSIM; Oksanen et al. 2014) were performed to determine the significance of any differences among groups observed in the NMDS plots. All instances of BDL were replaced with DL/√2 for statistical analysis (Croghan & Egeghy 2003). For samples from the southern Azuero Peninsula and from Galera – the two regions from where both species were collected, permutation-based two-sample Hotelling's t-squared tests were used to determine the difference between each species.

Table 2: Instrumental detection limits (measured in ng g^{-1}, wet wt); and percent relative deviation for each element from the certified value of the standard reference materials SRM 1577c (Bovine liver: National Institute of Standards and Technology, USA) and CRM DORM-4 (Fish protein homogenate: National Research Council, Canada).

Element	Detection Limit (ng g^{-1})	% Relative deviation in SRM 1577c	% Relative deviation in CRM DORM-4
^{53}Cr	0.303	BDL	-45.8 – 88.0
Mn^{2576}	0.0795	-3.9 – 3.6	NA
Mn^{2605}	0.0744		
Fe^{2382}	0.944	-3.0 – 9.6	-9.1 – 18.1
Fe^{2599}	1.19		
^{59}Co	0.00311	-26.7 – 19.0	NA
^{60}Ni	0.0173	BDL	-24.3 – 24.7
Cu^{2247}	0.287	-3.1 – 5.7	-15.1 – 2.4
Cu^{3247}	1.70		
Zn^{2025}	1.36	-2.6 – 9.4	-13.2 – 8.6
Zn^{2138}	2.22		
^{75}As	0.0354	BDL	-7.3 – 18.8
Se^{1960}	4.51	-8.5 – 31.8	-7.5 – 15.8
^{98}Mo	0.00244	-24.3 – 15.5	NA
^{112}Cd	0.000436	-6.4 – 40.5	-7.3 – 6.1
^{114}Cd	0.000338		
^{200}Hg	0.0117	NA	-9.9 – 61.0
^{202}Hg	0.0117		
^{208}Pb	0.0149	-20.9 – 39.8	-13.3 – 46.7

To look at how our results relate to the health of people who might regularly consume these sea turtle eggs, the concentrations of As, Cd, Hg and Pb were converted to a standard measure of eggs day^{-1}. This measure used the ug g^{-1} data, the dry weight of the respective eggs,

the most up-to-date PTDI value for each metal (WHO 1995, 2010a, b, USEPA 2014), and the average adult body weight for Panama as reported by Walpole et al. (2012). The median ± MAD of eggs day^{-1} for each species, separated by collection site, was used along with information collected by Pinzón Gómez (2012) who compared consumption rates from a high and a low-consumption community on the Pacific coast of Panama, to determine the degree to which heavy metal contamination in the East Pacific may be adversely affecting those consuming sea turtle eggs. Based on the findings of Pinzón Gómez (2012), we also calculated the minimum weight required to safely consume 1 turtle egg per day, to determine at what point a person may safely consume eggs at this rate.

Results

Trace Element Concentrations

The relative concentrations (median ± MAD, μg g^{-1}; wet wt.) of elements measured in this study, for both Green (n=31) and Olive Ridley (n=30) eggs, respectively, were as follows: Zn (14 ± 3 μg g^{-1}; 16 ± 2 μg g^{-1}) > Fe (11.7 ± 0.9 μg g^{-1}; 11 ± 1 μg g^{-1}) > Se (1.6 ± 0.2 μg g^{-1}; 1.4 ± 0.3 μg g^{-1}) > Cu (0.5 ± 0.1 μg g^{-1}; 0.6 ± 0.2 μg g^{-1}) > Mn (0.31 ± 0.07 μg g^{-1}; 0.35 ± 0.09 μg g^{-1}) > As (0.12 ± 0.04 μg g^{-1}; 0.12 ± 0.06 μg g^{-1}) > Cd (0.09 ± 0.04 μg g^{-1}; 0.07 ± 0.02 μg g^{-1}) > Ni (0.02 ± 0.01 μg g^{-1}; 0.016 ± 0.007 μg g^{-1}) > Mo (0.011 ± 0.003 μg g^{-1}; 0.015 ± 0.004 μg g^{-1}) > Hg (0.006 ± 0.002 μg g^{-1}; 0.009 ± 0.005 μg g^{-1}) > Pb (0.003 ± 0.002 μg g^{-1}; 0.004 ± 0.002 μg g^{-1}) > Co (0.0027 ± 0.0007 μg g^{-1}; 0.003 ± 0.001 μg g^{-1}) (Appendix A2.1). Dry weight concentrations (median ± MAD, μg g^{-1}) of each metal, based on collection site, are presented in Table 3. Mean water content of the eggs was 80.2% for Green turtles and 81.6% for Olive Ridleys.

Interspecies and Geographic Variation

Observations from the NMDS plots suggest that small-scale geographic variation is stronger than potential interspecies differences. Based on species ID alone, NMDS analysis showed no distinction between Green turtle and Olive Ridley eggs (Figure 2). Additionally, no distinction was found based on collection region within 95% confidence limits (Figure 3). However, with the exception of a cluster of eggs from the southern Azuero Peninsula, there does appear to be slight but non-significant geographic variation. Careful comparison of Figures 2 and 3 revealed that this overlap is predominantly between Green turtle eggs from Isla Cañas in the southern Azuero, and Olive Ridley eggs from Galera in the Pearl Islands, suggesting that there

may be site-specific variation between species, and geographic variation within species. Indeed, Hotelling's t-squared tests revealed that Green turtle eggs were significantly different from Olive Ridley eggs in both the southern Azuero ($df=24$, $t=6.787$, $p<0.01$) and Galera ($df=8$, $t=29.219$, $p<0.01$). Looking at the metals individually: Cd was significantly higher ($W=198$, $p<0.01$) in Green turtles (0.7 ± 0.1 μg g^{-1}) than in Olive Ridleys (0.4 ± 0.1 μg g^{-1}) from the southern Azuero. From Galera, As (0.62 ± 0.01 μg g^{-1}; 0.43 ± 0.06 μg g^{-1}; $W=40$, $p<0.01$) and Cd (0.56 ± 0.06 μg g^{-1}; 0.42 ± 0.04 μg g^{-1}; $W=40$, $p<0.01$) were significantly higher in Green turtles, while Hg (0.03 ± 0.01 μg g^{-1}; 0.017 ± 0.003 μg g^{-1}; $W=3$, $p=0.02$) and Pb (0.014 ± 0.005 μg g^{-1}; 0.0089 μg g^{-1}; $W=0$, $p<0.01$) were significantly higher in Olive Ridleys.

Species-specific NMDS plots support that there is species-specific variation across nesting sites. In Green turtle eggs, within 95% confidence, toxic metal profiles are distinct between turtles nesting at Isla Cañas and those nesting at both Malena and Juan Hombrón; and between turtles nesting at Juan Hombrón and Galera (Figure 4). Meanwhile Olive Ridley eggs show a strong distinction between sea turtle toxic metal profiles from the Pearl Islands (Galera) and from the southern Azuero Peninsula (both Ostional and Lagarto) (Figure 5). ANOSIMs corresponding to both intraspecies NMDS analyses confirmed significant differences among nesting sites for Green turtles ($R=0.581$, $p=0.001$, 999 permutations) and Olive Ridleys ($R=0.4922$, $p=0.001$, 999 permutations).

Table 3: Trace metal concentrations (median ± MAD, μg g^{-1} dry wt.) found in Green turtle (*Chelonia mydas*) and Olive Ridley (*Lepidochelys olivacea*) eggs from nesting sites along the Pacific coast of Panama. Values in parentheses indicate the number of samples above detection limit, if less than n.

Species	Beach	n	Mn	Fe	Co	Ni
Chelonia mydas	Isla Cañas	10	1.55 ± 0.08	56 ± 3	0.011 ± 0.004	0.09 ± 0.05
	Malena	10	1.6 ± 0.1	69 ± 4	0.015 ± 0.003	0.2 ± 0.1
	Juan Hombrón	7	2.2 ± 0.2	57 ± 2	0.0137 ± 0.0006	0.07 ± 0.03
	Galera	4	1.7 ± 0.3	52 ± 4	0.021 ± 0.001	0.10 ± 0.02
Lepidochelys olivacea	Ostional	10	1.2 ± 0.1	57 ± 4	0.016 ± 0.006	0.07 ± 0.03
	Lagarto	10	2.0 ± 0.3	57 ± 5	0.014 ± 0.004	0.09 ± 0.05
	Galera	10	2.2 ± 0.1	57 ± 4	0.019 ± 0.003	0.11 ± 0.05
			Cu	Zn	Se	Mo
Chelonia mydas	Isla Cañas	10	2.30 ± 0.09	72 ± 2	8 ± 1	0.056 ± 0.009
	Malena	10	3.1 ± 0.3	74 ± 5	7.6 ± 0.8	0.062 ± 0.01
	Juan Hombrón	7	3.8 ± 0.2	80 ± 3	8.3 ± 0.3	0.24 ± 0.02
	Galera	4	3.7 ± 0.5	70 ± 10	7.2 ± 0.3	0.045 ± 0.002
Lepidochelys olivacea	Ostional	10	3.8 ± 0.1	84 ± 5	7.9 ± 0.7	0.09 ± 0.01
	Lagarto	10	3.3 ± 0.4	84 ± 3	7 ± 3	0.06 ± 0.02
	Galera	10	2.7 ± 0.2	99 ± 5	7.5 ± 0.6	0.11 ± 0.03
			As	Cd	Hg	Pb
Chelonia mydas	Isla Cañas	10	0.7 ± 0.1	0.7 ± 0.1	0.05 ± 0.02	0.012 ± 0.005 (7)
	Malena	10	0.53 ± 0.07	0.42 ± 0.06	0.03 ± 0.01	0.017 (1)
	Juan Hombrón	7	0.47 ± 0.06	0.32 ± 0.02	0.028 ± 0.004	0.024 ± 0.007 (2)
	Galera	4	0.62 ± 0.01	0.56 ± 0.06	0.017 ± 0.003 (2)	0.0089 (1)
Lepidochelys olivacea	Ostional	10	1.0 ± 0.1	0.49 ± 0.06	0.05 ± 0.03	0.04 ± 0.01 (6)
	Lagarto	10	0.59 ± 0.06	0.3 ± 0.1	0.05 ± 0.03	0.016 ± 0.006 (8)
	Galera	10	0.43 ± 0.06	0.42 ± 0.04	0.03 ± 0.01	0.014 ± 0.005 (8)

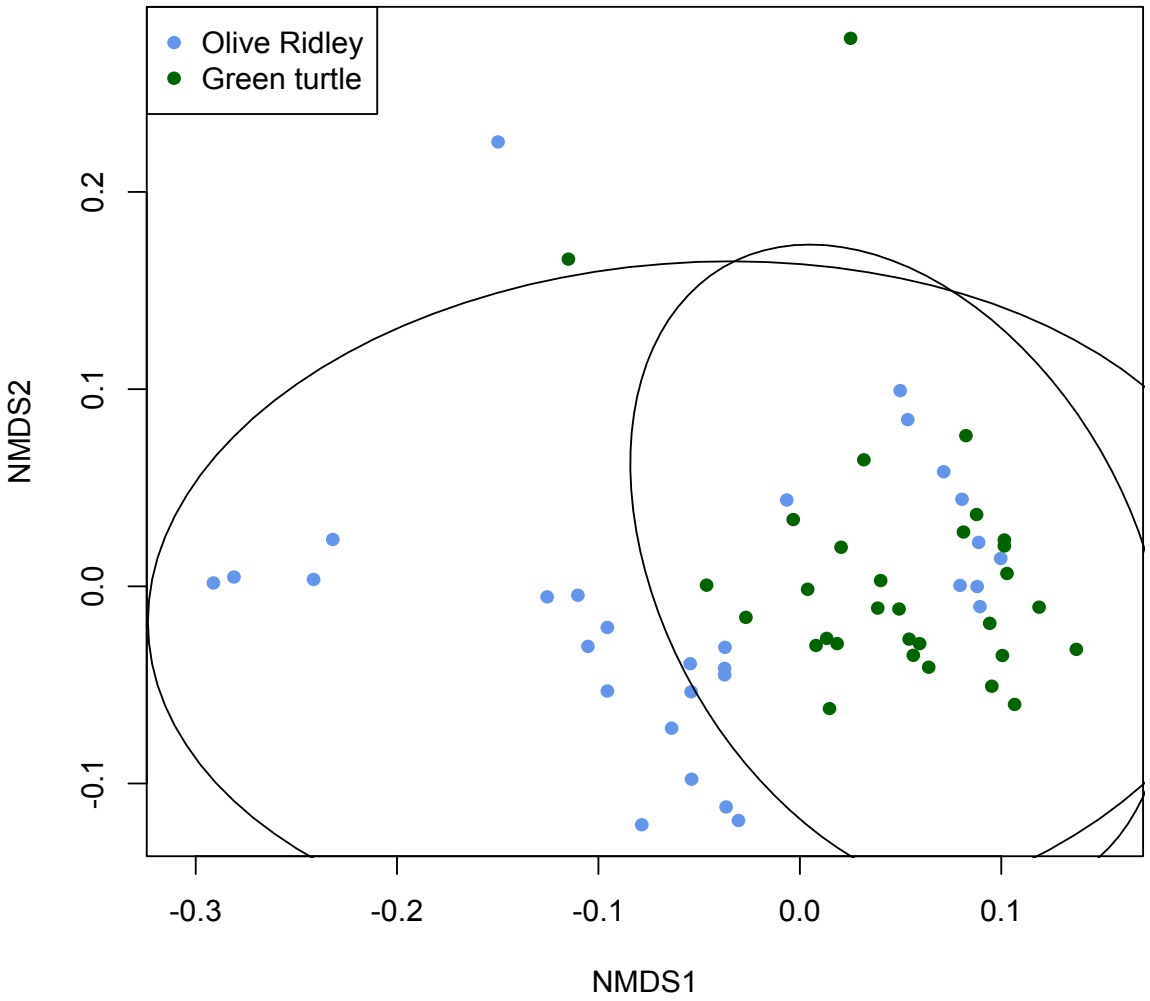

Figure 2: NMDS plot of toxic metal data (As, Cd, Hg, Pb) obtained from eggs of two sea turtle species that nest on the Pacific coast of Panama. Each point represents a single egg, and is labeled according to species. Ellipses represent the 95% confidence limit, based on the standard deviation for each species.

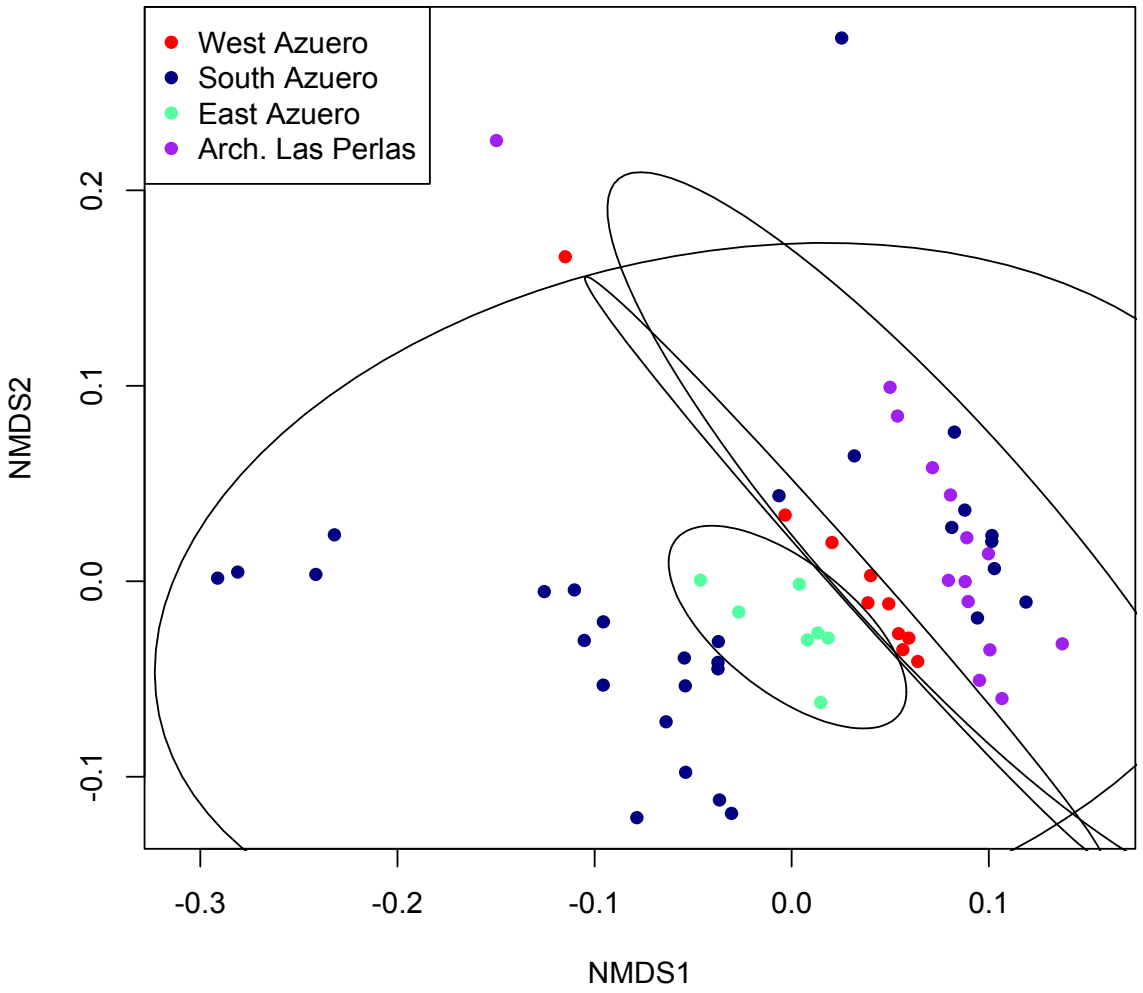

Figure 3: NMDS plot of toxic metal data (As, Cd, Hg, Pb) obtained at four regions from Pacific-Panamanian sea turtle eggs. Each point represents a single egg, and is labeled according to the broad geographic location where it was collected. Ellipses represent the 95% confidence limit, based on the standard deviation for each area.

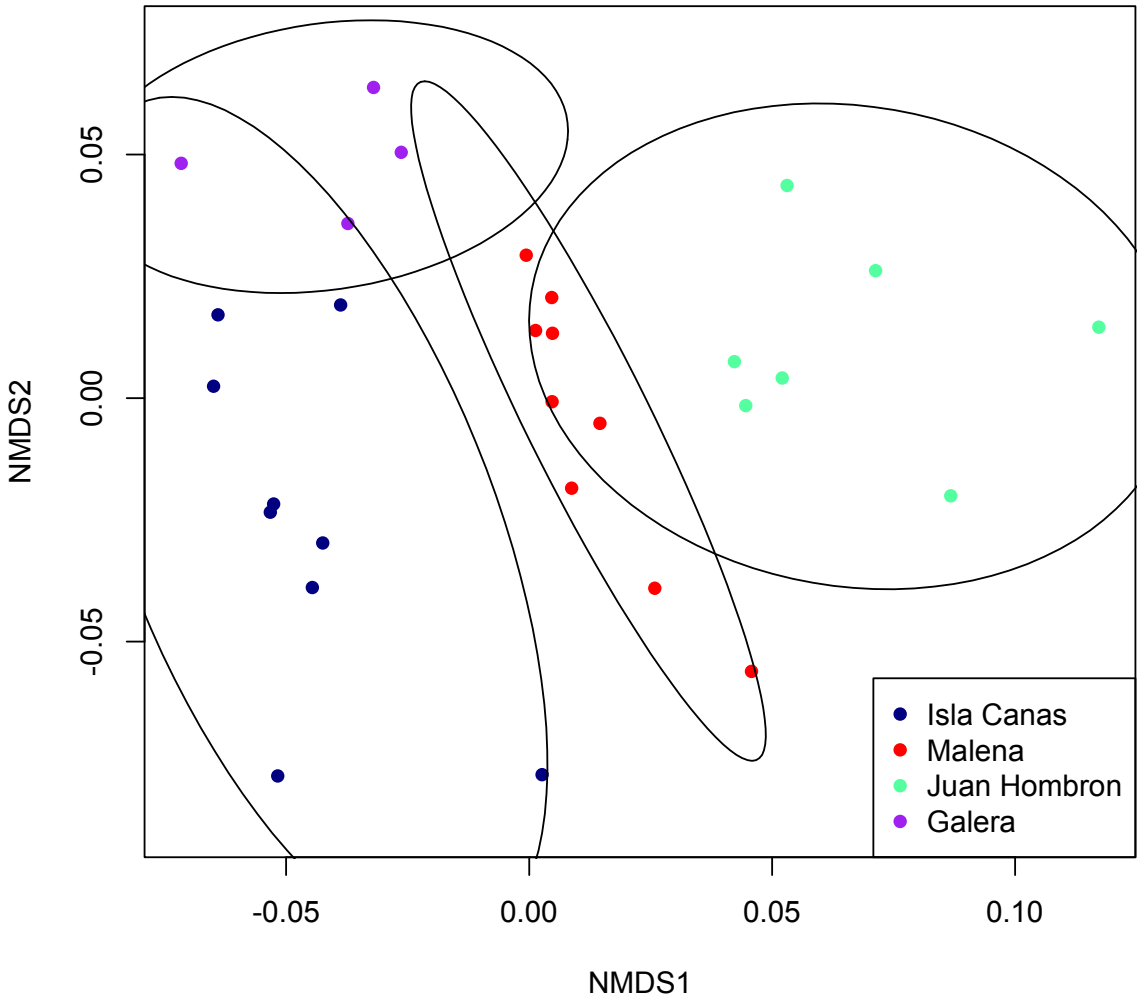

Figure 4: NMDS plot of toxic metal data (As, Cd, Hg, Pb) obtained at four beaches from Pacific-Panamanian Green turtle eggs. Each point represents a single egg, and is labeled according to the nesting beach where it was collected. Ellipses represent the 95% confidence limit, based on the standard deviation for each collection site.

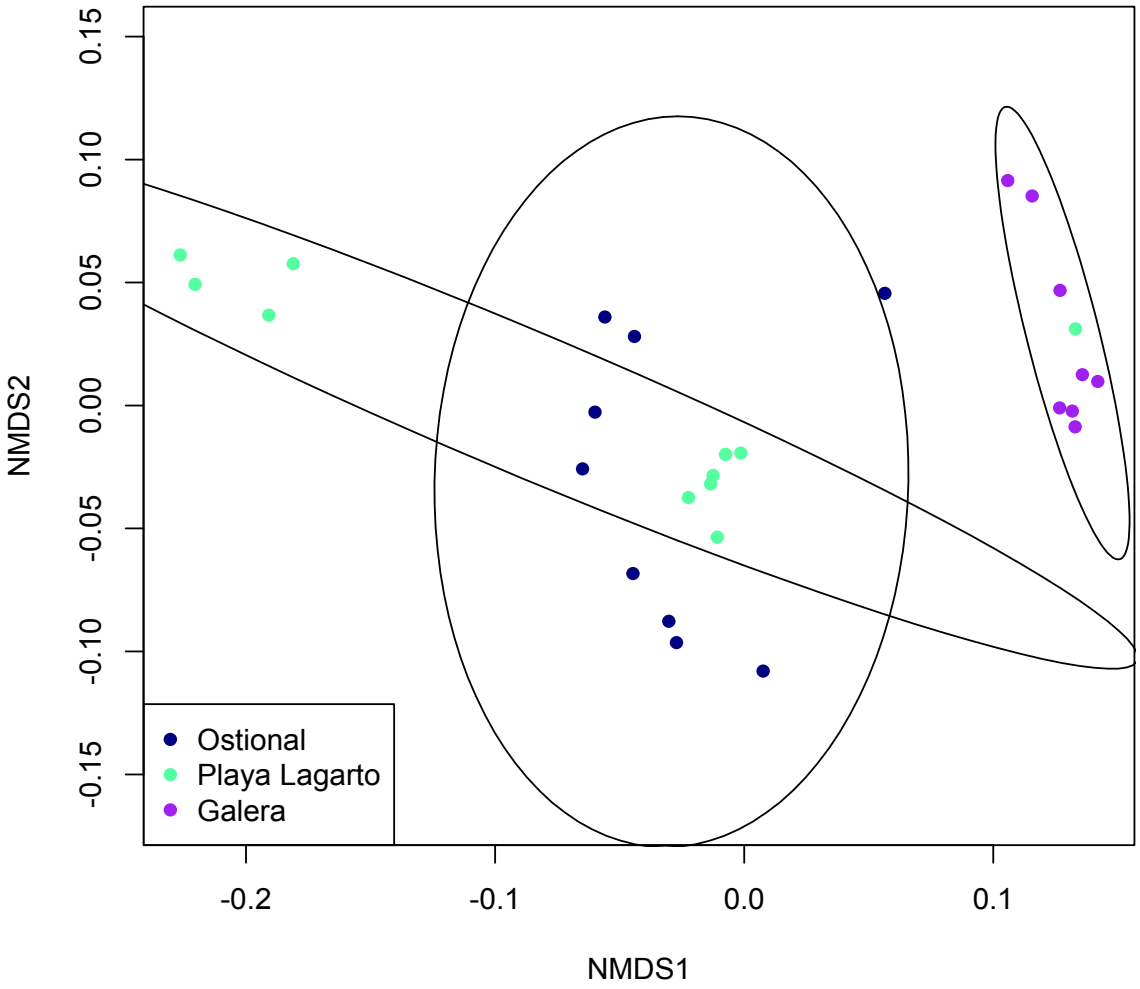

Figure 5: NMDS plot of toxic metal data (As, Cd, Hg, Pb) obtained at three beaches from Pacific-Panamanian Olive Ridley eggs. Each point represents a single egg, and is labeled according to the nesting beach where it was collected. Ellipses represent the 95% confidence limit, based on the standard deviation for each collection site.

Implications for Human Health

Overall, the relative toxicity of the metals was Cd > Hg > As > Pb (Table 4; values for Pb ranged from 191 – 6881 eggs day^{-1}). The most toxic eggs were Green turtle eggs from Isla Cañas, with a minimum toxic consumption rate of 21 ± 4 eggs day^{-1}, every day, based on their Cd concentrations. In fact, Cd was the most toxic metal for all Green turtle collection sites, as well as for Olive Ridley eggs from Galera in the Pearl Islands. For Olive Ridley eggs from Ostional and Lagarto, Hg was the most toxic. As was the least toxic of As, Cd and Hg concentrations in 6 of the 7 sites.

Table 4: Number of sea turtle eggs (median ± MAD, eggs day^{-1}) that a person must eat, using the average adult Panamanian body weight (69.9 kg; Walpole et al., 2012) before reaching the provisional tolerable daily intake (PTDI) of As (3 μg kg^{-1} day^{-1}), Cd (1 μg kg^{-1} day^{-1}) and Hg (0.1 μg kg^{-1} day^{-1}) through the consumption of sea turtle eggs alone; based on metal concentrations in eggs of Green turtles (*Chelonia mydas*) and Olive Ridleys (*Lepidochelys olivacea*) nesting in various sites along the Pacific coast of Panama.

Species	Beach	n	As	Cd	Hg
Chelonia mydas	Isla Cañas	10	63 ± 13	21 ± 4	30 ± 15
	Malena	10	87 ± 10	37 ± 6	48 ± 24
	Juan Hombrón	7	82 ± 9	41 ± 3	50 ± 11
	Galera	4	65 ± 4	24 ± 3	84 ± 16 (2)
Lepidochelys olivacea	Ostional	10	38 ± 7	27 ± 5	25 ± 14
	Lagarto	10	69 ± 17	50 ± 29	26 ± 22
	Galera	10	143 ± 26	49 ± 6	74 ± 51

Table 5: The minimum body weight (median ± MAD, kg) required for a person to safely consume one sea turtle egg per day without exceeding the provisional tolerable daily intake (PTDI) of As (3 μg kg^{-1} day^{-1}), Cd (1 μg kg^{-1} day^{-1}) and Hg (0.1 μg kg^{-1} day^{-1}) through the consumption of sea turtle eggs alone; based on metal concentrations in eggs of Green turtles (*Chelonia mydas*) and Olive Ridleys (*Lepidochelys olivacea*) nesting in various sites along the Pacific coast of Panama.

Species	Beach	n	As	Cd	Hg
Chelonia mydas	Isla Cañas	10	1.1 ± 0.2	3.3 ± 0.6	2.4 ± 1.2
	Malena	10	0.8 ± 0.1	1.9 ± 0.3	1.5 ± 0.6
	Juan Hombrón	7	0.8 ± 0.1	1.7 ± 0.1	1.4 ± 0.3
	Galera	4	1.1 ± 0.1	2.9 ± 0.4	0.9 ± 0.2 (2)
Lepidochelys olivacea	Ostional	10	1.8 ± 0.3	2.6 ± 0.5	2.8 ± 1.4
	Lagarto	10	1.0 ± 0.3	1.4 ± 0.9	2.7 ± 1.7
	Galera	10	0.5 ± 0.1	1.4 ± 0.2	1.0 ± 0.5

Similarly, Green turtle eggs from Isla Cañas required the highest minimum weight, 3.3 ± 0.6 kg, to safely consume 1 egg day^{-1} based on Cd concentrations. Cd was the highest threshold metal for all Green turtle sites, and for Olive Ridley eggs from Galera; and Hg was the highest threshold metal for Olive Ridleys from Ostional and Lagarto. Based on the 2011-2012 average birth weight for Panama (UNSD 2015), and given an approximate doubling of body weight in the first 6 months (CDC 2000), children at the recommended age to consume solid foods (6 months; WHO 2003) should be above the minimum weight required to safely eat Green turtle eggs from Isla Cañas at a nominal consumption rate of 1 egg day^{-1} (Pinzón Gómez 2012) (Table 5; values for Pb ranged from 0.01 to 0.37 kg).

Discussion

Trace Element Concentrations

Although there has been a considerable amount of research published on marine turtle toxicology from around the globe (see compilation in Ross et al., in review; current results not included), relatively little work has focused on the trace metal profiles of turtle eggs. Of that research, only one sample set comes from Central America (Roe et al. 2011), one set has been reported from Oaxaca, Mexico (Paez-Osuna et al. 2010a, b, 2011) and another from French Guiana (Guirlet et al. 2008). Here, we present the first heavy metal profiles of Panama-nesting sea turtles, including the first report for Green turtle eggs in the East Pacific, and the first report for Olive Ridley eggs outside of Mexico (Paez-Osuna et al. 2010a, b, 2011) and India (Sahoo et al. 1996) (see Appendix A2.1).

The overall pattern of relative metal concentration in both species analyzed here was Zn > Fe > Se > Cu > Mn > As > Cd > Ni > Mo > Hg > Pb > Co. Similar patterns have been observed in the eggs of Green turtles from Japan (Sakai et al. 2000b), Hong Kong (Lam et al. 2006), Malaysia (van de Merwe et al. 2009) and the Sultanate of Oman (Bicho et al. 2005, Al-Rawahy et al. 2007); in Loggerheads from Japan (Sakai et al. 1995, Sakai et al. 2000b) and Florida, USA (Alam & Brim 2000); Leatherbacks from French Guiana (Guirlet et al. 2008); and Flatbacks from Queensland, Australia (Ikonomopoulou et al. 2011). The similarity among patterns becomes even more evident when focusing only on essential elements, further supporting that these elements are regulated to similar physiological requirements across species and habitat.

Trace element concentrations in freshly laid eggs are a direct measure of maternal transfer. For nutritionally essential metals, this results in a relatively narrow range of concentrations across species and habitats due to similarities in physiological requirements, and the capacity to metabolically regulate these elements (Chapman et al. 1996). Toxic metals, however, tend to bioaccumulate in the long-lived species, and their concentrations are more influenced by environmental concentrations, trophic level, and maternal age (Maffucci et al. 2005, Barbieri 2009, Garcia-Fernandez et al. 2009). This relative inability to regulate toxic metals results in their transfer to developing eggs, whose concentrations reflect those of the mother (Paez-Osuna et al. 2010b, Ikonomopoulou et al. 2011).

The essential metals Mn, Fe, Cu, Zn and Se were present in our samples at concentrations within the range of concentrations reported in previous studies worldwide (Appendix A2.1). These trace elements are vital for growth and metabolism of many animals, including marine turtles, and typically have small optimal concentration ranges for similar species (Alam & Brim 2000). Mn, Fe and Cu are important elements in oxygen transport, energy production and enzyme-mediated processes. To avoid production of toxic compounds excess Fe and Cu is stored in the liver; in this way, the concentration of these elements is highly regulated within female turtles (Barceloux 1999b, Andreani et al. 2008), and maternal investment of these elements into eggs should be fairly consistent. Zn is essential to enzymatic structure and function (Cousins et al. 2006, Eisler 2006), and along with Cu has been demonstrated to show Cd-detoxifying properties in sea turtles, as well as other marine species. These metals induce production of metallothionein (MT) proteins, which can bind to non-essential metals such as Cd. The resulting Cd-MT then accumulates in the kidneys without toxic symptoms (Gardner et al. 2006, Storelli et al. 2008, Garcia-Fernandez et al. 2009). While the physico-chemical properties of Mn are similar to Fe and Cu (Frias-Espericueta et al. 2006), and correlations have been found in turtles between Mn and Cd (Gardner et al. 2006, Ley-Quiñónez et al. 2011), it remains unknown if Mn also plays a role in detoxifying Cd. Se does play an important role in reducing the toxic effects of Hg and Cd (Bryan 1984, Thompson 1990, Gordon et al. 1998, Storelli et al. 1998a, Khan & Wang 2009, Innis et al. 2010, Jerez et al. 2010). This connection may indicate that the relative ratios of Hg and/or Cd to Se could be similar when compared with these same ratios from previous studies. The essential element Mo, involved in the metabolic pathways of the carbon, sulfur and nitrogen cycles (Barceloux 1999c), was found at concentrations one quarter to one fifth of the previously

lowest reported yolk-albumen concentration (0.08 ± 0.17 µg g-1; Bicho et al. 2005); while Co, required for vitamin B_{12} production (Barceloux 1999a), was approximately one tenth of the previously lowest reported yolk-only concentrations (0.030 ± 0.0080 - 0.05 ± 0.00 µg g-1; Stoneburner et al. 1980, Lam et al. 2006, Al-Rawahy et al. 2007). Ni is considered essential for other taxa, but concerning turtles it is a non-essential element. Ni was found at approximately one half of the previously lowest reported yolk-only concentration (0.06 ± 0.00 µg g-1; Al-Rawahy et al. 2007), and it has been proposed that Ni does not concentrate in sea turtles due to low environmental burdens (see Goye & Clarkson 2001).

Toxic metals have been well documented to biomagnify to toxic concentrations in marine carnivores (Green et al. 2010), with particularly high concentrations in large, predatory fish (e.g. tuna, swordfish) (Jarup 2003, Ansari et al. 2004). For these animals, while they do take up metals passively from the environment, their primary source of contaminants is through their diet, with higher concentrations in higher trophic-level consumers. Marine turtle species vary in their preferential food sources, and therefore each species may be expected to have increased toxic metal concentrations relative to its respective diet. Indeed Loggerheads, which are predominantly carnivorous, are often found to have higher concentrations of toxic metals than preferentially herbivorous Green turtles (Sakai et al. 1995, Caurant et al. 1999, Godley et al. 1999, Saeki et al. 2000, Sakai et al. 2000b, Anan et al. 2001, Kubota et al. 2003, Kaska et al. 2004, Gardner et al. 2006, Kampalath et al. 2006, Suzuki et al. 2012). In contrast, however, Green turtles have been found with the highest reported Cd concentrations of any sea turtle, though no mechanistic explanation has yet been described (Gladstone 1996, Gordon et al. 1998, Caurant et al. 1999, Sakai et al. 2000a, Anan et al. 2001, Anan et al. 2002, Franzellitti et al. 2004, Gardner et al. 2006, Andreani et al. 2008, Ley-Quiñónez et al. 2013).

The toxic metals As, Cd and Hg were present in our samples at concentrations within the expected ranges based on previous sea turtle egg reports; however Pb was present at one quarter to one seventh of the previously lowest reported yolk-albumen concentrations (0.036 ± 0.001 µg g-1; Guirlet et al. 2008, 0.031 ± 0.003 µg g-1; van de Merwe et al. 2009). These results align with relative coastal contamination values reported by (Davis 1993), who showed that environmental Cd and Hg concentrations from the East Pacific are within the range of concentrations found where previous turtle egg concentrations have been reported. Coastal Pb

concentrations in the East Pacific, specifically SE Pacific, though, were lower than those reported in any other regions where previous turtle Pb levels are available.

Interspecies and Geographic Variation

As previously mentioned, trophic position and environmental contamination play important roles in determining heavy metal levels in marine animals. The two species included in our study, Green turtles and Olive Ridleys, are known to have predominantly different diets. Adult Green turtles are preferentially herbivorous, but are often documented eating benthic invertebrates; while the diet of Olive Ridleys consists mainly of soft and hard-bodied benthic invertebrates as well as small fishes (Jones & Seminoff 2013). From this, as we have only 1 previous report of whole Olive Ridley eggs for comparison (Sahoo et al. 1996), we might expect to find higher levels of As, Hg, and Pb in the carnivorous Olive Ridleys. In general, Green turtles are often found with the highest Cd levels (Gladstone 1996, Anan et al. 2002, Andreani et al. 2008, Ley-Quiñónez et al. 2013), but have the lowest previously reported egg concentrations of both Hg and Pb; previous reports of As burden in turtles eggs are insufficient to make interspecies comparisons (Appendix A2.1). Overall, across all locations, our samples showed no statistical difference between species. Although Green turtle eggs did have on average 43% higher levels of Cd than Olive Ridley eggs, this difference was less than the geographic variation within each species. Because we found significant site-based interspecific differences, this suggests that differences observed among nesting sites are stronger than between species. Indeed, in line with previous reports and tropic-level expectations, these site-specific differences show significantly higher Cd in Green turtles at the Southern Azuero and Galera, and higher Hg and Pb in Olive Ridleys at Galera. However, contrary to previous reports from adult turtle tissues (Saeki et al. 2000, Fujihara et al. 2003, Torrent et al. 2004), As was also higher in Green turtles at Galera. These studies have all found Green turtles to have lower concentrations of As than other turtle species, and are often found to have a negative correlation between carapace size and As concentration. Information on the mothers of the eggs used in this study is not available, thus their sizes, as proxies for age, are not known and this interaction could not be tested.

Stoneburner et al. (1980) suggested that even at small spatial scales, isolated feeding populations may exist, and that any environmental differences in feeding ground contamination should be reflected in the metal burdens of each population. Our results support the existence of

such demes, as we observed significantly different metal profiles among sites within each species, with a different pattern in each. In Green turtles, while there are multiple significant differences among sites, there is no clear geographic basis for these differences. Eggs from Malena were significantly different than those from the next closest site, Isla Cañas, but not from either Juan Hombrón or Galera on the other side of the Azuero Peninsula. Similarly, eggs from Galera were also different than those from the next closest site, Juan Hombrón, but not the other two sites. Whereas eggs from Olive Ridleys had a clear definition between eggs collected from the Pearl Islands Archipelago in the Gulf of Panama and those from the Southern Azuero Peninsula. As with the conclusion made by Stoneburner et al. (1980), the results presented here are insufficient on their own to conclude that demes do or do not exist across Pacific-Panamanian nesting sea turtles. They do suggest that there is, at least in Olive Ridleys, a degree of isolation among feeding grounds for female turtles nesting on separate beaches across Panama. In contrast, satellite-tracking data suggest that Olive Ridleys in this region are nomadic feeders, and in fact have no dedicated feeding sites, especially at the scale of the present study (Plotkin 2010). Genetic analysis, as well as additional trace metal work would provide evidence to support either case.

Implications for Human Health

In our data analysis, we chose to use the average body weight from Panama (69.9 kg) instead of the global average of 62.0 kg, as reported by Walpole et al. (2012), because sea turtle eggs from Panamanian beaches will likely be consumed or sold within the country; and because the metal concentrations found in our samples should not be relied on for precise metal-exposure estimations from these species' eggs outside the central East Pacific (e.g. Central America, Colombia, and Ecuador). We acknowledge that our calculations do not take into account differences in toxic thresholds based on age, gender, ethnicity, body weight, and other physiological, economic, or social attributes. Our results suggest that, based on the consumption rates reported by Pinzón Gómez (2012), average consumption of Green turtle or Olive Ridley eggs in Panama is unlikely to pose a threat to consumers; but that the maximum self-reported rates for men and women aged 16-30 and for children up to approximately 3 yrs old (Pinzón Gómez 2012) may put those consumers at risk of Cd and/or Hg poisoning, based on the concentrations found in Green turtle and Olive Ridley eggs at various sites.

Of the 19 previous studies reporting concentrations of toxic heavy metals in sea turtle eggs (Appendix A2.1), only two reported concentrations high enough to approach the PTDI while consuming 1 egg per day, including Leatherback eggs from Costa Rica (Stoneburner et al. 1980, Roe et al. 2011). In our samples, according to collection site, the minimum consumption rate of Green turtle and Olive Ridley eggs required for an adult to surpass the PTDI for any metal was at least 21 ± 4 eggs per day, every day. Although the average reported consumption rate for a coastal Panamanian community was just 1 egg day^{-1} (Pinzón Gómez 2012), maximum consumption rates for those aged 16-30 (15 eggs day^{-1} for men, 30 eggs day^{-1} for women) were, within error, above the PTDI for both Cd and Hg at multiple sites (Table 4), suggesting some individuals may currently be at risk of long-term Cd and/or Hg poisoning. Because additional sources of metals were not incorporated in the calculations, these reported values overestimate the threshold number of eggs that may safely be consumed on a daily basis. However, the report by Pinzón Gómez (2012) does not address the seasonal availability of sea turtle eggs in the area: responses were given on a daily, weekly, monthly, or yearly basis depending on the respondent. Therefore, especially in adults who have surpassed critical development stages, these self-reported consumption rates may not put consumers at risk if eggs are not eaten year-round.

The effects of these metals are known to be even more detrimental though, to developing fetuses and infants (Kurzel & Cetrulo 1981, Jarup 2003, Al-Saleh et al. 2011, Neeti & Prakash 2013), thus we also calculated the minimum body weight required to surpass the PTDI for each metal based on a consumption rate of 1 egg day^{-1} (Table 5; Pinzón Gómez 2012). These can then be scaled directly to determine the minimum body weight required to safely consume eggs at higher rates. In line with the initial toxicity table, Green turtle eggs from Isla Cañas required the highest body weight to safely eat one egg per day, every day, based on Cd; only 0.1 kg above the average birth weight in Panama (3.2 kg; UNSD 2015). At this weight, Green turtle eggs from Isla Cañas had sufficient Cd concentrations to pose a health risk at 1 egg day^{-1}. But direct egg consumption by newborn infants, especially one egg day per day, is unlikely. By the recommended time for children to start eating solid foods (6 months; WHO 2003), they should have surpassed the minimum weight at which this consumption would put them at risk. However, Pinzón Gómez (2012) reported that children may begin consuming eggs by the age of 1; that consumption rates by the youngest children are on average 0.4 - 2.5 eggs day^{-1}, up to a maximum of 5 eggs day^{-1}; and that consumption is higher in females than in males. At this maximum

reported consumption rate, children could be at risk of Cd poisoning below 16.5 kg, and Hg poisoning below 14 kg. Based on the weight-to-age equation proposed by Ali et al. (2012), this suggests that children aged ~3.4 yrs or younger (~2.4 yrs for Hg) may be consuming toxic amounts of turtle eggs.

The values reported in this study do not take into consideration additional sources of heavy metal intake, nor the seasonality of sea turtle egg availability. Due to the increased susceptibility of young children to developmental complications from toxic metals, we recommend that the values listed in this study be interpreted as overestimations of the realistic thresholds, and that young children avoid turtle egg consumption when possible.

Finally, although conservation is not the focus of this study, the potential implications for these threatened species from the results presented here should be addressed. For this we must consider both the consumption recommendations produced from this study, and current consumption rates: if a community does not eat eggs, toxicity reports cannot reduce their consumption. The study by Pinzón Gómez (2012) is the only known report on turtle egg consumption in Panama, therefore we will focus on the potential implications to turtles nesting in the Pearl Islands Archipelago. We found that, with the exception of adults aged 16-30 eating turtle eggs at the maximum reported rates (15 eggs day^{-1} for men, 30 eggs day^{-1} for women), children under the age of ~3.4 years were the only demographic at risk of metal poisoning based on the consumption rates reported by Pinzón Gómez (2012). Children under 4 years old account for only 9.7% of the district's population (INEC 2000), or roughly 41 (11%) of the 378 eggs eaten per day in La Esmeralda based on the average consumption from ages 0-15 reported by Pinzón Gómez (2012). In these isolated communities, whole nests are typically taken for consumption or sale, therefore if children under ~3.4 years stop eating turtle eggs, those 41 eggs may likely still be taken. Furthermore, Crouse et al. (1987) found that even a 50% increase in egg/hatchling survivorship would not produce a positive growth rate, or even have a substantial effect on intrinsic rate of growth, therefore even if the 11% of eggs eaten by children under ~3.4 years were left in the nest it would not have a substantial impact on conservation.

Conclusions

The primary objectives of this study were to provide the first reported heavy metal profiles for sea turtles nesting in Panama, using eggs of Green and Olive Ridley turtles collected

from the Pacific coast, and to determine the degree to which consumption of their eggs poses a health risk. We also used the data to look for differences in metal concentrations among species, and nesting sites. We found that the concentrations of Mn, Fe, Cu, Zn, Se, As, Cd and Hg were in line with previous reports for sea turtle eggs from around the globe, while Mo, Co, Ni and Pb were up to an order of magnitude lower than the previously lowest reported concentrations. Whereas no overall differences among species or nesting sites were present, we found evidence to suggest that interspecific differences in toxic metal concentrations are present at some sites; that even at this scale there are differences among sites in a given species; and that this geographic variation is greater than the differences between species. Finally, we found that previously reported average consumption rates should not pose a health risk to consumers, but that maximum consumption rates might be putting young adults and children at risk to develop symptoms of long-term Cd or Hg poisoning.

Acknowledgements

We would like to thank C. Guevara and M. Diaz for helping with sample collection. We would also like to acknowledge A. Jung and I. Richer from the Department of Earth and Planetary Sciences at McGill University, as well as R. Urriola and the lab of B. Turner for their invaluable support in sample management and analysis. This research was performed under Panamanian and Canadian regulations and laws under collection permit by ANAM (Autoridad Nacional del Ambiente) Scientific Permit No. SC/A-42-12; and by CITES (Convention on International Trade in Endangered Species) Export Permit No. SEX/A-70-2013 and Import Permit No. 13CA03456/CWHQ-1).

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General Conclusions and Contribution to Knowledge

There are currently over 70 studies worldwide, in the scientific and grey literature that report trace metal concentrations from any of the seven species of marine turtle. However, to date, each study has typically compared their own results to a small number of previous reports: no more than approximately 15. Additionally, cross-study comparisons are typically only qualitative, with little to no quantitative analysis used to investigate large-scale patterns of contaminant levels in these animals. Local-scale patterns frequently observed in marine turtle trace metal studies include variation among species, usually correlated to trophic level, most often between Loggerhead and Green turtles; tissue-specific distribution, where As in muscle, Cd in kidneys, Hg is highest in liver, and Pb in keratinous tissues such as bone and carapace; and finally geographic variation, where differences found among turtle tissues are attributed to respective differences in environmental contamination of their food sources. Chapter 1 is the first known review to compare more than 15 separate reports of sea turtle trace metal data, and to do so quantitatively. Looking at only the toxic metals As, Cd, Hg, and Pb, global-scale interspecific variation was not seen. There was no significant difference among species for any metal, analyzed using only data from their respective associated tissues and statistically controlling for geographic variation. Tissue-selective distribution, however, was robust to the global scale for both Cd and Hg, which were significantly highest in kidney and liver tissues, respectively. Overall, only Cd showed significant tissue-selective association regardless of geographic variation and statistically controlling for species. Looking within each species, and statistically controlling for geographic variation, Cd was significantly highest in kidney tissue for both Green and Loggerhead turtles; while Hg was significantly highest in liver tissue for Green, Loggerhead, and Kemp's Ridley turtles. Finally, although there was no significant variation within any ocean, geographic variation was still present at the global scale with Cd concentrations in Pacific Ocean turtles higher than those from the Atlantic Ocean, Indian Ocean, or Mediterranean Sea.

Chapter 1 also looked at the extent to which existing marine turtle toxicological data can be used to estimate the potential toxicological health risks from consuming turtle products where data do not exist. This could be either for areas with no reports at all, or for areas where some but not all species and/or tissues have been reported. Based on the metal concentrations, PTDIs, and average global body weight, only Cd and Hg were found at levels sufficient to induce metal poisoning at a nominal consumption rate of 75 g per week. Cd was the most toxic, at

concentrations sufficient to induce Cd poisoning at this consumption rate in kidneys and liver from Green and Loggerhead turtles. Hg was also present at toxic concentrations, based on this consumption rate, but only in the liver of Loggerheads. On average, consumption of eggs was unlikely to pose a health risk. However, 5 individual studies reported concentrations of either As, Cd, Hg or Pb in the eggs of 4 species at toxic concentrations, consuming 2-3 eggs per day. Based on the quantitative conclusions found above and the toxicity results, we can say that generally, consuming kidney or liver tissue from any species of turtle in the Pacific Ocean will pose the highest risk of metal poisoning, specifically from Cd.

Chapter 2 is complementary to Chapter 1, using original data measured in the eggs of Green and Olive Ridley turtles from Pacific Panama. These data are the first reported reliable trace metal concentrations for any turtle tissue or species from Panama. Chapter 2 is also the first report for Green turtle eggs in the East Pacific, and only the third report worldwide for Olive Ridley eggs. Mn, Fe, Cu, Zn, Se, As, Cd and Hg were found at concentrations similar to previous reports, while Mo, Co, Ni and Pb were up to an order of magnitude lower. No differences among overall species or nesting sites were present, although the data suggest that interspecific differences are present at a given site, that differences among sites exist within each species, and that it is the interaction of these patterns that obscures more general patterns.

Similarly, Chapter 2 also looked at how known rates of turtle egg consumption in a coastal Panamanian community is impacting those consumers with regards to toxic metal contamination. I acknowledge that, with regards to health and consumption limits, the values presented all overestimate the real values. Primarily, this is because PTDI thresholds include consumed metals from all sources, which were not taken into consideration due to the highly context-specific nature of total contamination exposure. Differences in body size (including sex, age, social status, ethnicity) and physiological condition, among other attributes, are also important in determining the health risks related to consuming turtle eggs. For simplicity, and to provide a clearly defined baseline that can be used to calculate individual risk, these attributes were not incorporated into the final report. Based on the average adult Panamanian body weight (69.9 kg), average egg consumption (1 egg day^{-1}) alone would be unlikely to cause harm; however, maximum reported consumption rates of 30 eggs per day for women aged 16-30 (15 egg per day for men) surpassed the PTDI, within error, for 6 of 7 sites based on Cd and/or Hg. Furthermore, the minimum weight required to consume 1 egg per day without surpassing the

PTDI, for any metal, is no larger than 3.3 ± 0.6 kg, only 0.1 kg higher than the national average birth weight. However, reported consumption rates of up to 5 eggs per day in the youngest consumers may be putting children 3 yrs old or younger at risk of metal poisoning from the most toxic eggs measured. I suggest that young children avoid consuming turtle eggs, due to the increased sensitivity during developmental years, and that adults reduce their egg consumption if they currently eat eggs near the maximum reported rates.

The conclusions formed from the combined results of Chapters 1 and 2 create a novel dimension for interpreting sea turtle toxicological data, and for designing future studies. The global-scale comparison in Chapter 1 was the first attempt, using basic statistics and existing data, to determine the plausibility of estimating trace-metal exposure from the consumption of previously unanalyzed sea turtle products. This produced significant results, but also revealed a considerable lack of data for many species, tissues, and regions. Future studies should, as much as possible, try to fill in these gaps. With more balanced data, it might be possible to create a mathematical model that incorporates expected metal concentration, human body weight, and additional information to give estimated maximum tolerable consumption rates based on geographic location, the species of turtle, and the tissue being consumed, for a range of body weights. Furthermore, while previous studies have addressed the potential harm to human consumers of turtle meat, few have based their conclusions on provisional tolerable daily intake values, measured body weights, and known consumption rates. Instead, they often rely on recommended maximum tolerable metal concentrations, which are designed around an estimated regular consumption rate and a specific food source; often fish. Using these values with other food sources can be misleading. As seen in Chapter 2, specific body weight, influenced by age and sex, and consumption rate are vital components of measuring toxic exposure, where the average consumption rate will inevitably underestimate the exposure for those consuming the most; and younger (i.e. smaller) consumers will surpass toxic consumption rates eating proportionately less than older consumers, based on relative body weight. Future studies that address human health from eating turtle meat or eggs must take this into account, using PTDI, and should ideally collect detailed local consumption.

Finally, it was found in Chapter 1 that turtle eggs have significantly lower toxic metal contents than adult tissues, and in Chapter 2 that current consumption of eggs may lead to symptoms of long-term Cd and Hg poisoning. Therefore the next steps in this research should be

to expand the study to Leatherbacks, Loggerheads, and Hawksbill turtles, including samples from both the Pacific and the Caribbean; and, where possible, to test tissue or blood samples from adult turtles and survey communities for consumption habits of adult turtles, to determine the health impacts not just from eating eggs, but from all turtle meat.

Appendix 1: Supplementary Information for Chapter 1

A1.1: Available data in the literature reporting concentrations for As, Cd, Hg and/or Pb in soft tissue or bone from sea turtles around the world, (Mean ± SD; μg g^{-1} wet wt.).

Species	Location	Tissue	n	Arsenic (As)	Cadmium (Cd)	Mercury (Hg)	Lead (Pb)	Reference
C. caretta	NW Atlantic	Yolk	Unkn	---	0.17†	0.02-0.09‡	2.87†	Hillestad et al. 1974
C. caretta	NW Atlantic	Albumen	Unkn	---	0.56†	0.01-0.03‡	12.0†	Hillestad et al. 1974
C. caretta	NW Atlantic	Yolk	27	---	0.1118 ± 0.0799	1.3595 ± 0.0394	2.1846 ± 1.1999	Stoneburner et al. 1980[1]
C. caretta	NW Atlantic	Yolk	33	---	0.1950 ± 0.0709	1.3912 ± 0.1063	1.1347 ± 0.8395	Stoneburner et al. 1980[1]
C. caretta	NW Atlantic	Yolk	15	---	0.0401 ± 0.0016	0.6352 ± 0.0141	1.7694 ± 1.1501	Stoneburner et al. 1980[1]
C. caretta	NW Atlantic	Yolk	21	---	0.0255 ± 0.0133	0.4123 ± 0.0136	1.2368 ± 1.0405	Stoneburner et al. 1980[1]
L. olivacea	E Central Pacific	Bone	1	---	---	---	41.5 ± 3.54	Witkowski & Frasier 1982
L. olivacea	E Central Pacific	Bone	1	---	---	---	86.6 ± 12.02	Witkowski & Frasier 1982
L. olivacea	E Central Pacific	Bone	1	---	---	---	97.2 ± 11.28	Witkowski & Frasier 1982
D. coriacea	NE Atlantic	Liver	1	0.16 ± 0.03	0.062 ± 0.006	0.11 ± 0.01	0.034 ± 0.006	Davenport & Wrench 1990[2]
D. coriacea	NE Atlantic	Muscle	1	0.04 ± 0.01	0.01 ± 0.002	0.02 ± 0.01	0.062 ± 0.006	Davenport & Wrench 1990[2]
D. coriacea	NE Atlantic	Adipose	1	1.02 ± 0.14	BDL	0.09 ± 0.02	0.03 ± 0.02	Davenport & Wrench 1990[2]
C. mydas	Central Pacific	Shell	3	BDL	0.2†	---	---	Aguirre et al. 1994
C. mydas	Central Pacific	Hatchling	3	BDL	BDL	---	---	Aguirre et al. 1994
C. mydas	Central Pacific	Liver	10	BDL	10.7 ± 9.2	---	---	Aguirre et al. 1994
C. mydas	Central Pacific	Kidney	10	BDL	28.5 ± 22.0	---	---	Aguirre et al. 1994
D. coriacea	E Indian	Muscle	1	4.4	---	---	---	Edmonds et al. 1994
D. coriacea	E Indian	Liver	1	1.2	---	---	---	Edmonds et al. 1994
D. coriacea	E Indian	Heart	1	0.70	---	---	---	Edmonds et al. 1994

C. caretta	NW Pacific	Egg	5	---	0.013 ± 0.004	0.00554 ± 0.00157	BDL	Sakai et al. 1995, 2000b
C. caretta	NW Pacific	Shell	5	---	BDL	0.00405 ± 0.00131	BDL	Sakai et al. 1995, 2000b
C. caretta	NW Pacific	Yolk	5	---	0.026 ± 0.007	0.0121 ± 0.00341	BDL	Sakai et al. 1995, 2000b
C. caretta	NW Pacific	Albumen	5	---	BDL	0.00049 ± 0.00024	BDL	Sakai et al. 1995, 2000b
C. caretta	NW Pacific	Liver	7	---	9.29 ± 3.30	1.51 ± 2.93	0.099 ± 0.067	Sakai et al. 1995, 2000b
C. caretta	NW Pacific	Kidney	7	---	39.4 ± 16.2	0.247 ± 0.130	BDL	Sakai et al. 1995, 2000b
C. caretta	NW Pacific	Muscle	7	---	0.062 ± 0.026	0.108 ± 0.049	BDL	Sakai et al. 1995, 2000b
C. mydas	SW Pacific	Muscle	7	0.018 ± 0.025	1.14 ± 2.935	0.02 ± 0.011	0.07 ± 0.105	Gladstone 1996
C. mydas	SW Pacific	Liver	7	0.019 ± 0.018	10.73 ± 3.437	0.08 ± 0.061	0.59 ± 0.461	Gladstone 1996
C. mydas	SW Pacific	Kidney	7	0.005 ± 0.005	26.0 ± 10.939	0.02 ± 0.01	0.07 ± 0.037	Gladstone 1996
C. mydas	SW Pacific	Intestine	7	0.095 ± 0.239	3.66 ± 6.994	0.03 ± 0.042	0.05 ± 0.016	Gladstone 1996
L. olivacea	E Indian	Shell (Fresh)	24	---	1.3 ± 0.5	---	11.0 ± 3.6	Sahoo et al. 1996
L. olivacea	E Indian	Egg	24	---	BDL	---	3.6 ± 1.1	Sahoo et al. 1996
L. olivacea	E Indian	Shell (Hatched)	24	---	BDL	---	15.6 ± 3.2	Sahoo et al. 1996
L. olivacea	E Indian	Hatchling	24	---	2.0 ± 1.0	---	20.0 ± 5.2	Sahoo et al. 1996
D. coriacea	E Central Pacific	Shell	5	---	0.43 (0.004-2.43)	---	5.70 (0.25-59.0)	Vazquez et al. 1997[3]
D. coriacea	E Central Pacific	Shell	5	---	0.29 (0.004-0.51)	---	3.11 (0.90-8.32)	Vazquez et al. 1997[3]
D. coriacea	E Central Pacific	Shell	5	---	0.21 (0.004-0.51)	---	2.54 (0.13-7.30)	Vazquez et al. 1997[3]
D. coriacea	E Central Pacific	Shell	Unkn	---	0.37 ± 0.25	---	4.76 ± 10.7	Vazquez et al. 1997[2]
D. coriacea	NE Atlantic	Liver	1	2.6	28	0.37	4.3	Godley et al. 1998
D. coriacea	NE Atlantic	Muscle	1	4.7	2.5	0.013	BDL	Godley et al. 1998
D. coriacea	NE Atlantic	Liver	2	---	8.5 ± 5.0	0.56 ± 0.38	0.03 ± 0.01	Godley et al. 1998
D. coriacea	NE Atlantic	Muscle	2	---	2.1 ± 1.0	0.21 ± 0.12	BDL	Godley et al. 1998

C. mydas	SW Pacific	Liver	38	0.26 ± 0.04	12.5 ± 2.0	0.021 ± 0.003	---	Gordon et al. 1998[1]
C. mydas	SW Pacific	Kidney	38	0.19 ± 0.05	15.3 ± 2.5	0.020 ± 0.004	---	Gordon et al. 1998[1]
C. caretta	SW Pacific	Liver	5	0.46 ± 0.24	16.4 ± 3.3	0.015 ± 0.006	---	Gordon et al. 1998[1]
C. caretta	SW Pacific	Kidney	5	0.71 ± 0.26	28.3 ± 5.7	0.045 ± 0.011	---	Gordon et al. 1998[1]
L. olivacea	SW Pacific	Liver	1	---	6.4	---	---	Gordon et al. 1998
L. olivacea	SW Pacific	Kidney	1	---	29.8	---	---	Gordon et al. 1998
E. imbricata	SW Pacific	Liver	3	1.02 ± 1.18	2.4-6.2‡	0.042 ± 0.008	---	Gordon et al. 1998[3]
E. imbricata	SW Pacific	Kidney	3	0.53 ± 0.57	3.6-12.7‡	0.036 ± 0.003	---	Gordon et al. 1998[3]
C. caretta	Mediterranean	Muscle	7	---	---	0.21 ± 0.13	---	Storelli et al. 1998a
C. caretta	Mediterranean	Liver	7	---	---	0.70 ± 0.32	---	Storelli et al. 1998a
C. caretta	Mediterranean	Liver	7	5.86 ± 6.06	2.04 ± 1.68	0.47 ± 0.27	0.43 ± 0.40	Storelli et al. 1998b
C. caretta	Mediterranean	Lung	7	7.42 ± 3.16	0.25 ± 0.14	0.09 ± 0.05	0.10 ± 0.05	Storelli et al. 1998b
C. caretta	Mediterranean	Kidney	7	12.29 ± 17.48	3.31 ± 2.05	0.17 ± 0.05	0.15 ± 0.03	Storelli et al. 1998b
C. caretta	Mediterranean	Muscle	7	22.90 ± 12.59	0.05 ± 0.03	0.22 ± 0.14	0.12 ± 0.04	Storelli et al. 1998b
C. caretta	NE Atlantic	Liver	7	---	2.58 ± 4.12	---	---	Caurant et al. 1999
C. caretta	NE Atlantic	Kidney	5	---	13.3 ± 13.6	---	---	Caurant et al. 1999
C. caretta	NE Atlantic	Muscle	21	---	0.08 ± 0.05	---	---	Caurant et al. 1999
D. coriacea	NE Atlantic	Liver	18	---	6.84 ± 3.66	---	---	Caurant et al. 1999
D. coriacea	NE Atlantic	Kidney	5	---	30.3 ± 28.1	---	---	Caurant et al. 1999
D. coriacea	NE Atlantic	Muscle	16	---	0.35 ± 0.20	---	---	Caurant et al. 1999
D. coriacea	NE Atlantic	Pancreas	2	---	68.8†	---	---	Caurant et al. 1999
L. kempii	NE Atlantic	Muscle	6	---	0.09 ± 0.09	---	---	Caurant et al. 1999
C. mydas	Mediterranean	Hatchling	29	---	0.063 (BDL-0.26)	BDL (BDL-0.066)	BDL (BDL-1.06)	Godley et al. 1999[3,4]
C. mydas	Mediterranean	Embryo	18	---	0.091 (BDL-0.26)	BDL (BDL-0.033)	0.18 (BDL-0.938)	Godley et al. 1999[3,4]

C. mydas	Mediterranean	Egg	24	---	0.074 (0.01-0.34)	BDL (BDL-0.052)	BDL (BDL-0.443)	Godley et al. 1999[3,4]
C. caretta	Mediterranean	Embryo	29	---	0.058 (BDL-0.300)	0.003 (BDL-0.061)	BDL (BDL-1.78)	Godley et al. 1999[3,4]
C. caretta	Mediterranean	Egg	3	---	0.063 (0.063-0.15)	0.052 (0.044-0.16)	0.052 (BDL-1.08)	Godley et al. 1999[3,4]
C. caretta	Mediterranean	Hatchling	16	---	0.094 (BDL-0.399)	0.006 (BDL-0.21)	0.036 (BDL-2.904)	Godley et al. 1999[3,4]
C. mydas	Mediterranean	Liver	6	---	1.30 (0.557-2.361)	0.12 (0.059-0.301)	BDL (BDL-0.405)	Godley et al. 1999[3,4]
C. mydas	Mediterranean	Kidney	1	---	0.969	BDL	0.507	Godley et al. 1999[3,4]
C. mydas	Mediterranean	Muscle	6	---	0.080 (0.026-0.17)	**0**.02 (BDL-0.080)	BDL (BDL-0.527)	Godley et al. 1999[3,4]
C. caretta	Mediterranean	Liver	5	---	1.90 (1.13-2.853)	0.530 (0.18-1.65)	BDL (BDL-1.08)	Godley et al. 1999[3,4]
C. caretta	Mediterranean	Kidney	2	---	8.540 (5.264-11.82)	0.13 (0.036-0.22)	0.686 (BDL-1.37)	Godley et al. 1999[3,4]
C. caretta	Mediterranean	Muscle	7	---	0.12 (0.065-0.307)	0.10 (BDL-0.383)	0.529 (BDL-1.19)	Godley et al. 1999[3,4]
C. caretta	NW Atlantic	Egg	20	0.468	---	0.033	0.19	Alam & Brim 2000[†]
C. mydas	NW Pacific	Liver	19	0.493 ± 0.27	---	---	---	Saeki et al. 2000
C. mydas	NW Pacific	Muscle	19	4.82 ± 2.62	---	---	---	Saeki et al. 2000
C. mydas	NW Pacific	Kidney	19	1.54 ± 0.807	---	---	---	Saeki et al. 2000
C. caretta	NW Pacific	Liver	4	1.77 ± 4.37	---	---	---	Saeki et al. 2000
C. caretta	NW Pacific	Muscle	4	4.12 ± 2.62	---	---	---	Saeki et al. 2000
C. caretta	NW Pacific	Kidney	4	2.56 ± 1.45	---	---	---	Saeki et al. 2000
E. imbricata	NW Pacific	Liver	4	4.28 ± 2.46	---	---	---	Saeki et al. 2000
E. imbricata	NW Pacific	Kidney	4	30.6 ± 13.0	---	---	---	Saeki et al. 2000
E. imbricata	NW Pacific	Muscle	4	7.64 ± 2.65	---	---	---	Saeki et al. 2000
C. mydas	NW Pacific	Liver	50	---	5.58 ± 4.05	0.287 ± 0.156	BDL	Sakai et al. 2000a
C. mydas	NW Pacific	Kidney	23	---	38.5 ± 21.3	0.132 ± 0.077	0.18 ± 0.07	Sakai et al. 2000a
C. mydas	NW Pacific	Muscle	47	---	0.05 ± 0.08	0.019 ± 0.030	BDL	Sakai et al. 2000a

C. mydas	NW Pacific	Egg	1	---	BDL	0.00135	BDL	Sakai et al. 2000b
C. mydas	NW Pacific	Shell	1	---	BDL	0.00120	BDL	Sakai et al. 2000b
C. mydas	NW Pacific	Yolk	1	---	BDL	0.00251	BDL	Sakai et al. 2000b
C. mydas	NW Pacific	Albumen	1	---	BDL	0.00005	BDL	Sakai et al. 2000b
C. mydas	NW Pacific	Liver	2	---	8.0 ± 5.8	0.189 ± 0.159	BDL-0.12‡	Sakai et al. 2000b
C. mydas	NW Pacific	Stomach	2	---	0.143 ± 0.076	---	BDL	Sakai et al. 2000b
C. mydas	NW Pacific	Pancreas	2	---	9.9 ± 3.9	0.0256*(1)	BDL-0.03‡	Sakai et al. 2000b
C. mydas	NW Pacific	Heart	2	---	0.150 ± 0.119	---	BDL	Sakai et al. 2000b
C. mydas	NW Pacific	Lung	2	---	0.178 ± 0.065	0.00248 ± 0.00033	BDL	Sakai et al. 2000b
C. mydas	NW Pacific	Kidney	2	---	41.3 ± 6.0	0.0450 ± 0.0040	BDL-0.14‡	Sakai et al. 2000b
C. mydas	NW Pacific	Brain	2	---	0.134 ± 0.043	---	BDL	Sakai et al. 2000b
C. mydas	NW Pacific	Adipose	2	---	0.062 ± 0.002	**0**.00262 ± 0.00028	BDL	Sakai et al. 2000b
C. mydas	NW Pacific	Muscle	2	---	0.023 ± 0.016	0.00454 ± 0.00340	BDL	Sakai et al. 2000b
C. mydas	NW Pacific	Bone	2	---	0.034 ± 0.012	0.00208 ± 0.00010	2.35 ± 0.07	Sakai et al. 2000b
C. mydas	NW Pacific	Gullet	2	---	0.277 ± 0.159	0.0274 ± 0.0306	BDL-1.20‡	Sakai et al. 2000b
C. mydas	NW Pacific	Intestine	2	---	4.38 ± 2.16	0.00747 ± 0.00011	BDL	Sakai et al. 2000b
C. mydas	NW Pacific	Trachea	2	---	0.133 ± 0.069	0.00214 ± 0.00045	BDL	Sakai et al. 2000b
C. mydas	NW Pacific	Bladder	2	---	0.126 ± 0.050	0.00397 ± 0.00028	BDL	Sakai et al. 2000b
C. mydas	NW Pacific	Spleen	2	---	0.704 ± 0.410	0.00637 ± 0.00074	0.06 ± 0.01	Sakai et al. 2000b
C. mydas	NW Pacific	Salt Gland	2	---	0.742 ± 0.228	0.00655 ± 0.00023	BDL	Sakai et al. 2000b
C. mydas	NW Pacific	Spermary	1	---	1.190	0.00950	BDL	Sakai et al. 2000b
C. mydas	NW Pacific	Oviduct	1	---	0.026	0.00461	BDL	Sakai et al. 2000b
C. mydas	NW Pacific	Ovary	1	---	BDL	0.00472	BDL	Sakai et al. 2000b

C. caretta	NW Pacific	Stomach	7	---	0.408 ± 0.231	0.0362 ± 0.0139 *(6)	BDL	Sakai et al. 2000b
C. caretta	NW Pacific	Pancreas	7	---	36.6 ± 19.2	0.770 ± 1.830	BDL	Sakai et al. 2000b
C. caretta	NW Pacific	Heart	7	---	0.426 ± 0.377	0.0922 ± 0.0126 *(6)	BDL	Sakai et al. 2000b
C. caretta	NW Pacific	Lung	7	---	0.354 ± 0.163	0.052 ± 0.043	BDL	Sakai et al. 2000b
C. caretta	NW Pacific	Brain	7	---	0.267 ± 0.073	0.0387 *(1)	BDL	Sakai et al. 2000b
C. caretta	NW Pacific	Adipose	7	---	0.070 ± 0.041	0.0101 ± 0.0146	BDL	Sakai et al. 2000b
C. caretta	NW Pacific	Bone	7	---	0.127 ± 0.042	0.00823 ± 0.00372	3.29 ± 1.91	Sakai et al. 2000b
C. caretta	NW Pacific	Gullet	7	---	0.275 ± 0.320	0.0697 ± 0.0682	BDL	Sakai et al. 2000b
C. caretta	NW Pacific	Intestine	7	---	1.31 ± 1.66	0.0567 ± 0.0381	BDL	Sakai et al. 2000b
C. caretta	NW Pacific	Trachea	7	---	0.052 ± 0.106	0.0136 ± 0.0109	BDL	Sakai et al. 2000b
C. caretta	NW Pacific	Bladder	7	---	0.269 ± 0.114	0.0434 ± 0.0262	BDL	Sakai et al. 2000b
C. caretta	NW Pacific	Spleen	7	---	1.09 ± 0.992	0.0890 ± 0.107	BDL	Sakai et al. 2000b
C. caretta	NW Pacific	Salt Gland	7	---	1.42 ± 0.470	0.0765 ± 0.0774	BDL	Sakai et al. 2000b
C. caretta	NW Pacific	Spermary	1	---	0.775	---	BDL	Sakai et al. 2000b
C. caretta	NW Pacific	Oviduct	6	---	0.061 ± 0.033	0.0220 ± 0.00883	BDL	Sakai et al. 2000b
C. caretta	NW Pacific	Ovary	6	---	0.037 ± 0.019	0.0175 ± 0.00567	BDL	Sakai et al. 2000b
C. caretta	Mediterranean	Muscle	7	15.47 ± 11.91	---	---	---	Storelli & Marcotrigiano 2000
C. caretta	Mediterranean	Liver	7	6.70 ± 4.49	---	---	---	Storelli & Marcotrigiano 2000
C. mydas	NW Pacific	Liver	26	---	5.6 ± 3.0	0.13 ± 0.06	0.157 ± 0.127	Anan et al. 2001
C. mydas	NW Pacific	Kidney	25	---	28 ± 12	0.06 ± 0.03	0.159 ± 0.109	Anan et al. 2001
C. mydas	NW Pacific	Muscle	12	---	0.046 ± 0.032	0.01 ± 0.01	0.018 ± 0.010	Anan et al. 2001
C. mydas	NW Pacific	Stomach	8	---	0.367 ± 0.399	0.03 ± 0.01	0.198 ± 0.054	Anan et al. 2001
E. imbricata	NW Pacific	Liver	22	---	2.18 ± 1.97	0.27 ± 0.58	0.052 ± 0.040	Anan et al. 2001
E. imbricata	NW Pacific	Kidney	19	---	18.3 ± 14.9	0.25 ± 0.23	0.053 ± 0.046	Anan et al. 2001

E. imbricata	NW Pacific	Muscle	9	---	0.013 ± 0.007	0.01 ± 0.01	0.008 ± 0.010	Anan et al. 2001
E. imbricata	NW Pacific	Stomach	6	---	0.591 ± 0.209	0.04 ± 0.03	0.212 ± 0.090	Anan et al. 2001
C. caretta	Mediterranean	Yolk	22	---	0.359 ± 0.135	BDL	1.307 ± 0.228	Kaska & Furness 2001
C. caretta	Mediterranean	Shell	22	---	0.649 ± 0.131	BDL	0.633 ± 0.162	Kaska & Furness 2001
C. caretta	Mediterranean	Liver (Embryo)	22	---	1.26 ± 0.43	0.51 ± 0.046	2.48 ± 0.46	Kaska & Furness 2001
L. kempii	NW Atlantic	Blood	106	---	---	0.0180 (0.0005-0.0673)	0.0110 (0.0-0.0343)	Kenyon et al. 2001[3]
C. mydas	NW Pacific	Liver	9	---	8.96 (3.21-21.6)	---	0.140 (0.031-0.361)	Anan et al. 2002[3]
E. imbricata	NW Pacific	Liver	7	---	1.23 (0.634-1.82)	---	0.033 (0.007-0.056)	Anan et al. 2002[3]
C. mydas	NW Pacific	Liver	5	1.2 ± 0.6	---	---	---	Fujihara et al. 2003
E. imbricata	NW Pacific	Liver	5	4.4 ± 2.5	---	---	---	Fujihara et al. 2003
C. mydas	NW Pacific	Liver	5	1.02 ± 0.57	---	---	---	Kubota et al. 2003
C. caretta	NW Pacific	Liver	5	3.14 ± 0.84	---	---	---	Kubota et al. 2003
C. mydas	NW Atlantic	Liver	13	---	0.871	0.067	0.157	Wang et al. 2003[†]
C. mydas	NW Atlantic	Kidney	13	---	0.197	0.019	0.031	Wang et al. 2003[†]
C. mydas	NW Atlantic	Muscle	13	---	0.023	0.012	0.061	Wang et al. 2003[†]
C. caretta	NW Atlantic	Liver	14	---	3.750	0.041	0.084	Wang et al. 2003[†]
C. caretta	NW Atlantic	Kidney	14	---	1.550	0.026	0.052	Wang et al. 2003[†]
C. caretta	NW Atlantic	Muscle	14	---	0.108	0.019	0.150	Wang et al. 2003[†]
C. caretta	Mediterranean	Liver	30	---	2.84 ± 0.72	---	---	Franzellitti et al. 2004
C. caretta	Mediterranean	Lung	13	---	0.47 ± 0.13	---	---	Franzellitti et al. 2004
C. caretta	Mediterranean	Muscle	17	---	0.36 ± 0.11	---	---	Franzellitti et al. 2004
C. caretta	Mediterranean	Adipose	7	---	2.33 ± 0.52	---	---	Franzellitti et al. 2004
C. mydas	Mediterranean	Liver	22	2.72 ± 1.41	2.07 ± 0.76	---	0.69 ± 0.70	Kaska et al. 2004
C. mydas	Mediterranean	Bladder	20	10.89 ± 5.00	9.34 ± 5.14	---	1.52 ± 0.95	Kaska et al. 2004

C. mydas	Mediterranean	Kidney	14	3.72 ± 1.46	4.27 ± 1.74	---	0.53 ± 0.41	Kaska et al. 2004
C. mydas	Mediterranean	Lung	15	2.03 ± 1.13	0.50 ± 0.54	---	0.52 ± 0.61	Kaska et al. 2004
C. mydas	Mediterranean	Muscle	22	3.09 ± 1.39	0.29 ± 0.11	---	0.29 ± 0.07	Kaska et al. 2004
C. caretta	Mediterranean	Liver	32	3.98 ± 2.13	3.04 ± 1.09	---	0.99 ± 0.37	Kaska et al. 2004
C. caretta	Mediterranean	Bladder	20	14.40 ± 10.72	11.16 ± 4.61	---	3.96 ± 2.16	Kaska et al. 2004
C. caretta	Mediterranean	Kidney	20	5.13 ± 2.77	4.58 ± 2.65	---	1.08 ± 0.57	Kaska et al. 2004
C. caretta	Mediterranean	Lung	10	2.34 ± 2.08	1.54 ± 0.96	---	1.07 ± 1.14	Kaska et al. 2004
C. caretta	Mediterranean	Muscle	32	4.16 ± 1.99	0.71 ± 1.17	---	0.48 ± 0.65	Kaska et al. 2004
C. mydas	NW Pacific	Adipose	2	1.018 ± 0.159	BDL	0.003821 ± 0.002314	0.068 ± 0.021	Lam et al. 2004
C. mydas	NW Pacific	Kidney	2	1.881 ± 0.015	0.672 ± 0.472	0.09226 ± 0.01016	0.084 ± 0.051	Lam et al. 2004
C. mydas	NW Pacific	Heart	2	4.826 ± 0.431	BDL-0.977‡	0.2164 ± 0.02118	0.209 ± 0.115	Lam et al. 2004
C. mydas	NW Pacific	Liver	2	1.303 ± 1.109	0.307 ± 0.279	0.2186 ± 0.0540	0.043 ± 0.012	Lam et al. 2004
C. mydas	NW Pacific	Lung	2	0.890 ± 0.123	BDL-0.003‡	0.05405 ± 0.00727	0.046 ± 0.006	Lam et al. 2004
C. mydas	NW Pacific	Muscle	2	2.89 ± 0.976	BDL-0.019‡	0.08512 ± 0.04302	0.016 ± 0.021	Lam et al. 2004
C. mydas	NW Pacific	Stomach	2	4.058 ± 2.082	175.5 ± 248.0	0.2990 ± 0.06381	0.600 ± 0.704	Lam et al. 2004
C. mydas	NW Pacific	Muscle	3	2.92 ± 1.495	0.034 ± 0.012	0.01050 ± 0.00720	0.053 ± 0.023	Lam et al. 2004
C. mydas	NW Pacific	Liver	1	5.48 ± 0.553	0.405 ± 0.171	0.03520 ± 0.02376	0.231 ± 0.025	Lam et al. 2004
C. caretta	NE Atlantic	Kidney	78	13.8 ± 2.40	5.01 ± 1.02	0.04 ± 0.01	2.44 ± 0.48	Torrent et al. 2004
C. caretta	NE Atlantic	Muscle	78	7.35 ± 1.37	1.14 ± 0.28	---	2.26 ± 0.51	Torrent et al. 2004
C. caretta	NE Atlantic	Bone	78	12.45 ± 2.53	1.36 ± 0.35	---	2.36 ± 0.50	Torrent et al. 2004
C. caretta	NE Atlantic	Liver	78	17.07 ± 2.96	2.53 ± 0.45	0.04 ± 0.01	2.94 ± 0.59	Torrent et al. 2004
C. mydas	W Indian	Egg	32	---	0.312 ± 0.27	---	0.74 ± 0.69	Bicho et al. 2005
C. mydas	W Indian	Liver	6	---	1408.150 ± 1.87	---	5.85 ± 4.46	Bicho et al. 2005
C. caretta	NW Atlantic	Blood	34	---	---	0.029 ± 0.008	---	Day et al. 2005

C. caretta	NW Atlantic	Blood	6	---	---	0.099 ± 0.042	---	Day et al. 2005
C. caretta	NW Atlantic	Muscle	6	---	---	0.155 ± 0.070	---	Day et al. 2005
C. caretta	NW Atlantic	Kidney	6	---	---	0.214 ± 0.046	---	Day et al. 2005
C. caretta	NW Atlantic	Liver	6	---	---	0.594 ± 0.155	---	Day et al. 2005
C. caretta	Mediterranean	Liver	22	---	6.18 ± 10.9	0.35 ± 0.54	---	Maffucci et al. 2005
C. caretta	Mediterranean	Kidney	20	---	13.7 ± 8.30	0.22 ± 0.17	---	Maffucci et al. 2005
C. caretta	Mediterranean	Muscle	26	---	0.042 ± 0.042	0.084 ± 0.063	---	Maffucci et al. 2005
C. caretta	Mediterranean	Liver	19	---	3.36 ± 1.94	0.43 ± 0.29	0.16 ± 0.05	Storelli et al. 2005
C. caretta	Mediterranean	Kidney	19	---	8.35 ± 4.83	0.16 ± 0.07	0.12 ± 0.07	Storelli et al. 2005
C. caretta	Mediterranean	Muscle	19	---	0.07 ± 0.03	0.18 ± 0.21	0.04 ± 0.03	Storelli et al. 2005
C. caretta	Mediterranean	Spleen	19	---	0.90 ± 0.40	0.11 ± 0.07	0.12 ± 0.07	Storelli et al. 2005
C. caretta	Mediterranean	Heart	19	---	0.23 ± 0.14	0.12 ± 0.08	0.07 ± 0.04	Storelli et al. 2005
C. caretta	Mediterranean	Lung	19	---	0.24 ± 0.13	0.06 ± 0.04	0.03 ± 0.02	Storelli et al. 2005
C. caretta	Mediterranean	Adipose	19	---	0.08 ± 0.06	0.04 ± 0.03	0.09 ± 0.03	Storelli et al. 2005
L. kempii	NW Atlantic	Blood	30	---	0.0107 ± 0.0058	0.0186 ± 0.0144	0.0303 ± 0.0319	Wang et al. 2005
L. kempii	NW Atlantic	Blood	58	---	0.0110 ± 0.0086	0.0142 ± 0.0233	0.0282 ± 0.0262	Wang et al. 2005
L. kempii	NW Atlantic	Blood	18	---	0.0079 ± 0.0059	0.0197 ± 0.0092	0.0233 ± 0.0356	Wang et al. 2005
L. kempii	NW Atlantic	Blood	18	---	0.0149 ± 0.0137	0.0686 ± 0.0395	0.0481 ± 0.0242	Wang et al. 2005
L. kempii	NW Atlantic	Liver	9	---	0.596 ± 0.428	0.344 ± 0.549	0.042 ± 0.041	Wang et al. 2005
L. kempii	NW Atlantic	Kidney	8	---	0.651 ± 1.100	0.201 ± 0.116	0.044 ± 0.040	Wang et al. 2005
L. kempii	NW Atlantic	Muscle	10	---	0.0110 ± 0.0088	0.086 ± 0.067	0.028 ± 0.036	Wang et al. 2005
L. kempii	NW Atlantic	Blood	1	---	0.0382	0.0400	0.0470	Wang et al. 2005
D. coriacea	SE Atlantic	Blood	9	BDL	---	0.200 ± 0.200	0.0872 ± 0.0309	Deem et al. 2006
L. olivacea	NE Pacific	Liver	7	---	3.28 ± 0.38	---	3.32 ± 0.52	Frias-Espericueta et al. 2006

L. olivacea	NE Pacific	Kidney	7	---	5.28 ± 0.40	---	4.46 ± 0.63	Frias-Espericueta et al. 2006
L. olivacea	NE Pacific	Muscle	7	---	2.60 ± 0.4	---	1.8 ± 0.2	Frias-Espericueta et al. 2006
L. olivacea	NE Pacific	Heart	7	---	---	---	10.1 ± 1.1	Frias-Espericueta et al. 2006
C. mydas	NE Pacific	Liver	11	---	0.924 (BDL-28.6)	---	BDL	Gardner et al. 2006[3]
C. mydas	NE Pacific	Kidney	11	---	32.7 (1.64-176)	---	0.003 (BDL-0.097)	Gardner et al. 2006[3]
C. mydas	NE Pacific	Muscle	11	---	0.002 (BDL-7.848)	---	0.002 (BDL-0.246)	Gardner et al. 2006[3]
C. mydas	NE Pacific	Adipose	11	---	0.002 (BDL-0.400)	---	0.02 (BDL-0.30)	Gardner et al. 2006[3]
C. caretta	NE Pacific	Liver	5	---	0.490 (BDL-8.574)	---	BDL	Gardner et al. 2006[3]
C. caretta	NE Pacific	Kidney	5	---	19.74 (3.704-37.8)	---	0.008 (BDL-18.87)	Gardner et al. 2006[3]
C. caretta	NE Pacific	Muscle	5	---	0.02 (BDL-0.290)	---	0.002 (BDL-0.314)	Gardner et al. 2006[3]
C. caretta	NE Pacific	Adipose	5	---	0.4 (0.2-1.10)	---	BDL	Gardner et al. 2006[3]
L. olivacea	NE Pacific	Liver	6	---	5.01 (1.39-41.4)	---	BDL	Gardner et al. 2006[3]
L. olivacea	NE Pacific	Kidney	6	---	16.21 (0.22-74.0)	---	0.008 (BDL-0.710)	Gardner et al. 2006[3]
L. olivacea	NE Pacific	Muscle	6	---	0.096 (BDL-1.77)	---	BDL	Gardner et al. 2006[3]
L. olivacea	NE Pacific	Adipose	6	---	0.55 (0.26-2.03)	---	BDL	Gardner et al. 2006[3]
E. imbricata	NE Pacific	Liver	1	---	0.14	---	BDL	Gardner et al. 2006
E. imbricata	NE Pacific	Kidney	1	---	1.13	---	BDL	Gardner et al. 2006
E. imbricata	NE Pacific	Muscle	1	---	0.204	---	0.076	Gardner et al. 2006
E. imbricata	NE Pacific	Adipose	1	---	0.34	---	BDL	Gardner et al. 2006
C. caretta	NW Atlantic	Liver	1	1.14	4.48	2.40	1.62	Jacobson et al. 2006
C. caretta	NW Atlantic	Liver	9	13.0 ± 10.7	11.1 ± 8.23	2.86 ± 2.50	3.19 ± 3.36 *(2)	Jacobson et al. 2006
C. caretta	NW Atlantic	Kidney	1	1.42	11.0	0.11	1.42	Jacobson et al. 2006
C. caretta	NW Atlantic	Kidney	9	16.1 ± 10.4	30.1 ± 13.9	1.20 ± 1.79	0.984 ± 0.290	Jacobson et al. 2006
C. mydas	NE Pacific	Adipose	11	---	---	0.005 ± 0.004	---	Kampalath et al. 2006

C. mydas	NE Pacific	Liver	11	---	---	0.091 ± 0.058	---	Kampalath et al. 2006
C. mydas	NE Pacific	Muscle	10	---	---	0.021 ± 0.022	---	Kampalath et al. 2006
C. mydas	NE Pacific	Kidney	10	---	---	0.089 ± 0.090	---	Kampalath et al. 2006
C. caretta	NE Pacific	Adipose	6	---	---	0.009 ± 0.011	---	Kampalath et al. 2006
C. caretta	NE Pacific	Liver	4	---	---	0.152 ± 0.029	---	Kampalath et al. 2006
C. caretta	NE Pacific	Muscle	4	---	---	0.026 ± 0.011	---	Kampalath et al. 2006
C. caretta	NE Pacific	Kidney	2	---	---	0.100 ± 0.050	---	Kampalath et al. 2006
L. olivacea	NE Pacific	Adipose	8	---	---	0.033 ± 0.061	---	Kampalath et al. 2006
L. olivacea	NE Pacific	Liver	6	---	---	0.213 ± 0.286	---	Kampalath et al. 2006
L. olivacea	NE Pacific	Muscle	6	---	---	0.050 ± 0.048	---	Kampalath et al. 2006
L. olivacea	NE Pacific	Kidney	3	---	---	0.143 ± 0.198	---	Kampalath et al. 2006
C. mydas	NW Pacific	Yolk	30	2.500 ± 0.370	BDL	0.0015 ± 0.00013	0.049 ± 0.0077	Lam et al. 2006[1]
C. mydas	NW Pacific	Albumen	30	0.170 ± 0.022	BDL	0.000090 ± 0.000030	0.0047 ± 0.0010	Lam et al. 2006[1]
C. mydas	NW Pacific	Shell	30	0.220 ± 0.021	0.01576 ± 0.01015	0.00061 ± 0.00016	0.110 ± 0.024	Lam et al. 2006[1]
C. mydas	W Indian	Yolk	75	---	0.16 ± 0.03	0.10 ± 0.01	0.08 ± 0.01	Al-Rawahy et al. 2007[1]
C. mydas	W Indian	Liver	50	---	0.21 ± 0.03	0.22 ± 0.03	0.27 ± 0.02	Al-Rawahy et al. 2007[1]
C. caretta	NW Atlantic	Blood	66	---	---	0.029 ± 0.002	---	Day et al. 2007[1]
C. mydas	NE Pacific	Kidney	8	---	29.7 (17.57-176)	---	0.01 (BDL-0.470)	Talavera-Saenz et al. 2007[3,4]
C. mydas	NE Pacific	Liver	8	---	4.740 (BDL-20.32)	---	0.00 (BDL-0.02)	Talavera-Saenz et al. 2007[3,4]
C. mydas	NW Pacific	Muscle	20	12.3 (2.24-33.0)	---	---	---	Agusa et al. 2008[3]
C. mydas	NW Pacific	Kidney	10	4.46 (1.2-12.0)	---	---	---	Agusa et al. 2008[3]
C. mydas	NW Pacific	Liver	20	1.5 (0.3-2.7)	---	---	---	Agusa et al. 2008[3]
C. mydas	Caribbean	Liver	34	---	2.97 ± 0.31	---	0.02 ± 0.004	Andreani et al. 2008[1]
C. mydas	Caribbean	Kidney	33	---	10.6 ± 0.84	---	0.012 ± 0.001	Andreani et al. 2008[1]

C. mydas	Caribbean	Adipose	28	---	0.090 ± 0.02	---	0.050 ± 0.025	Andreani et al. 2008[1]
C. caretta	Mediterranean	Liver	11	---	0.67 ± 0.1	---	0.03 ± 0.02	Andreani et al. 2008[1]
C. caretta	Mediterranean	Kidney	9	---	1.6 ± 0.30	---	0.03 ± 0.02	Andreani et al. 2008[1]
C. caretta	Mediterranean	Muscle	10	---	0.16 ± 0.008	---	BDL	Andreani et al. 2008[1]
C. caretta	Mediterranean	Gonads	3	---	1.3 ± 0.3	---	0.05 ± 0.01	Andreani et al. 2008[1]
C. caretta	Mediterranean	Lung	3	---	0.38 ± 0.03	---	BDL	Andreani et al. 2008[1]
C. caretta	Mediterranean	Heart	3	---	2.2 ± 0.2	---	BDL	Andreani et al. 2008[1]
C. caretta	Mediterranean	Pancreas	2	---	2.6 ± 1.9	---	BDL	Andreani et al. 2008[1]
D. coriacea	Caribbean	Egg	76	---	0.024 ± 0.001	0.012 ± 0.003	0.036 ± 0.001	Guirlet et al. 2008
D. coriacea	Caribbean	Blood	78	---	0.08 ± 0.03	0.011 ± 0.003	0.18 ± 0.05	Guirlet et al. 2008
L. kempii	NW Atlantic	Blood	29	---	---	0.024 ± 0.009	---	Innis et al. 2008
L. kempii	NW Atlantic	Liver	6	---	---	0.067 ± 0.070	---	Innis et al. 2008
C. mydas	Mediterranean	Liver	7	---	4.26 ± 3.02	---	---	Storelli et al. 2008
C. mydas	Mediterranean	Kidney	7	---	5.06 ± 2.23	---	---	Storelli et al. 2008
C. mydas	SW Atlantic	Kidney	15	---	0.589 ± 0.073	---	NS	Barbieri et al. 2009
C. mydas	SW Atlantic	Kidney	15	---	0.270 ± 0.086	---	NS	Barbieri et al. 2009
C. mydas	SW Atlantic	Liver	15	---	0.268 ± 0.087	---	NS	Barbieri et al. 2009
C. mydas	SW Atlantic	Liver	15	---	0.078 ± 0.039	---	NS	Barbieri et al. 2009
C. caretta	NW Atlantic	Blood	23	0.007917 ± 0.002682	---	0.0003 (0.00025-0.0008)	0.000085 ± 0.000167	Deem et al. 2009
C. caretta	NW Atlantic	Blood	12	0.001823 ± 0.001313	---	0.00025 (0.00025-0.0004)	0.000096 ± 0.000041	Deem et al. 2009
C. caretta	NW Atlantic	Blood	3	0.003833 ± 0.002182	---	0.00025 (0.00025-0.0100)	0.000050 ± 0.000000	Deem et al. 2009
C. caretta	Mediterranean	Liver	16	---	5.85 ± 13.42	---	0.69 ± 0.41	Garcia-Fernandez et al. 2009
C. caretta	Mediterranean	Kidney	19	---	10.49 ± 23.58	---	0.17 ± 0.16	Garcia-Fernandez et al. 2009
C. caretta	Mediterranean	Muscle	20	---	0.04 ± 0.03	---	0.05 ± 0.05	Garcia-Fernandez et al. 2009

C. caretta	Mediterranean	Bone	2	---	BDL	---	0.99 ± 1.40	Garcia-Fernandez et al. 2009
C. caretta	Mediterranean	Brain	3	---	0.06 ± 0.06	---	0.17 ± 0.12	Garcia-Fernandez et al. 2009
C. mydas	E Indian	Egg	55	0.097 ± 0.011	0.009 ± 0.001	BDL	0.031 ± 0.003	van de Merwe 2009[1]
D. coriacea	NW Atlantic	Blood	16	---	0.07 ± 0.02	0.01 ± 0.00	---	Innis et al. 2010
D. coriacea	NW Atlantic	Blood	10	---	---	---	0.11 ± 0.02	Innis et al. 2010
D. coriacea	NW Atlantic	Blood	6	---	---	---	0.14 ± 0.05	Innis et al. 2010
C. caretta	Mediterranean	Liver	13	3.56 ± 3.65	0.23 ± 0.13	0.11 ± 0.11	0.06 ± 0.03	Jerez et al. 2010
C. caretta	Mediterranean	Kidney	7	9.37 ± 8.58	1.86 ± 1.69	0.12 ± 0.14	0.33 ± 0.23	Jerez et al. 2010
C. caretta	Mediterranean	Muscle	13	8.19 ± 7.46	0.02 ± 0.01	0.03 ± 0.04	0.04 ± 0.05	Jerez et al. 2010
C. caretta	Mediterranean	Bone	12	1.17 ± 0.51	0.04 ± 0.02	0.04 ± 0.06	1.48 ± 0.67	Jerez et al. 2010
C. caretta	Mediterranean	Blood	5	1.75 ± 2.32	0.03 ± 0.05	0.005 ± 0.002	0.08 ± 0.08	Jerez et al. 2010
C. caretta	Mediterranean	CNS	3	37.05 ± 12.95	0.11 ± 0.01	0.11 ± 0.13	0.2 ± 0.2	Jerez et al. 2010
C. caretta	Mediterranean	Skin	2	52.13 ± 6.1	0.04 ± 0.02	0.08 ± 0.09	0.02 ± 0.03	Jerez et al. 2010
C. caretta	Mediterranean	Liver	3	4.59 ± 5.33	0.18 ± 0.14	0.04 ± 0.01	0.05 ± 0.04	Jerez et al. 2010
C. caretta	Mediterranean	Kidney	4	9.25 ± 11.57	2.22 ± 1.89	0.12 ± 0.16	0.34 ± 0.30	Jerez et al. 2010
C. caretta	Mediterranean	Muscle	6	7.55 ± 6.29	0.02 ± 0.01	0.05 ± 0.06	0.05 ± 0.05	Jerez et al. 2010
C. caretta	Mediterranean	Bone	3	1.31 ± 0.97	0.04 ± 0.01	0.02 ± 0.01	1.73 ± 0.34	Jerez et al. 2010
C. caretta	Mediterranean	Blood	1	5.88	0.13	0.003	0.16	Jerez et al. 2010
C. caretta	Mediterranean	CNS	2	43.13 ± 10.68	0.11 ± 0.01	0.14 ± 0.16	0.25 ± 0.24	Jerez et al. 2010
L. olivacea	E Central Pacific	Yolk	25	---	0.090 ± 0.038	0.011 ± 0.004	0.30 ± 0.038	Paez-Osuna et al. 2010a, b, 2011
L. olivacea	E Central Pacific	Albumen	25	---	0.006 ± 0.002	0.00003 ± 0.00002	0.029 ± 0.005	Paez-Osuna et al. 2010a, b, 2011
L. olivacea	E Central Pacific	Shell	25	---	0.19 ± 0.04	0.0036 ± 0.0001	0.431 ± 0.082	Paez-Osuna et al. 2010a, b, 2011
L. olivacea	E Central Pacific	Blood	25	---	0.11 ± 0.050	0.0002 ± 0.0001	0.24 ± 0.050	Paez-Osuna et al. 2010a, b, 2011
C. mydas	SW Pacific	Muscle	16	5.25 ± 1.47	0.08 ± 0.01	0.03 ± 0.004	BDL	van de Merwe 2010[1]

C. mydas	SW Pacific	Liver	16	3.19 ± 0.79	13.54 ± 2.40	0.19 ± 0.04	0.09 ± 0.02	van de Merwe 2010[1]
C. mydas	SW Pacific	Kidney	16	2.74 ± 0.89	45.98 ± 9.06	0.06 ± 0.02	0.09 ± 0.01	van de Merwe 2010[1]
C. mydas	SW Pacific	Blood	16	4.3618 ± 1.4149	0.03547 ± 0.00952	0.00251 ± 0.00050	0.02218 ± 0.00583	van de Merwe 2010[1]
C. caretta	Mediterranean	Muscle	8	0.075 ± 0.01	0.27 ± 0.15	0.13 ± 0.03	3.144 ± 0.588	Abdallah & Abd-Allah 2011
C. caretta	Mediterranean	Liver	8	0.41 ± 0.004	2.79 ± 1.71	0.501 ± 0.33	2.64 ± 0.527	Abdallah & Abd-Allah 2011
C. caretta	Mediterranean	Kidney	8	0.086 ± 0.004	13.38 ± 3.657	0.28 ± 0.11	2.05 ± 0.668	Abdallah & Abd-Allah 2011
C. caretta	Mediterranean	Adipose	8	0.078 ± 0.02	0.212 ± 0.07	0.03 ± 0.01	1.61 ± 0.34	Abdallah & Abd-Allah 2011
C. caretta	Mediterranean	Blood	8	0.859 ± 0.428	1.661 ± 0.638	0.8368 ± 0.17	0.615 ± 0.12	Abdallah & Abd-Allah 2011
D. coriacea	NE Pacific	Blood	3	---	0.077 (0.069-0.085)	0.019 (0.014-0.035)	0.200 (0.200-0.220)	Harris et al. 2011[3,4]
D. coriacea	NE Pacific	Blood	9	---	0.078 (0.043-0.182)	0.022 (0.007-0.048)	0.190 (0.090-0.310)	Harris et al. 2011[3,4]
D. coriacea	Caribbean	Blood	11	---	0.042 (0.014-0.063)	BDL (BDL-0.013)	0.150 (0.080-0.190)	Harris et al. 2011[3,4]
N. depressus	SW Pacific	Egg	60	0.670 ± 0.019	BDL	BDL	BDL	Ikonomopoulou et al. 2011[1]
N. depressus	SW Pacific	Blood	20	---	BDL	BDL	BDL	Ikonomopoulou et al. 2011[1]
C. mydas	NE Pacific	Blood	42	---	0.06 ± 0.00	---	---	Labrada-Martagon et al. 2011[1]
C. mydas	NE Pacific	Blood	14	---	0.03 ± 0.00	---	---	Labrada-Martagon et al. 2011[1]
C. caretta	NE Pacific	Blood	22	4.09 ± 2.56	1.8 ± 0.63	BDL	BDL	Ley-Quinonez et al. 2011
C. mydas	NE Pacific	Blood	30	0.157 ± 0.0259	0.0132 ± 0.00420	0.00101 ± 0.00016	1.260 ± 0.222	Komoroske et al. 2011[1]
D. coriacea	NW Atlantic	Shell	15	---	---	0.002 (0.0009-0.016)	---	Perrault et al. 2011[3,4]
D. coriacea	NW Atlantic	SAG Shell	13	---	---	0.001 (0.0004-0.002)	---	Perrault et al. 2011[3,4]
D. coriacea	NW Atlantic	SAG Albumen	15	---	---	0.0003 (BDL-0.002)	---	Perrault et al. 2011[3,4]
D. coriacea	NW Atlantic	Blood	52	---	---	0.003-0.088[‡]	---	Perrault et al. 2011
D. coriacea	NW Atlantic	Blood	16	---	---	0.0007 ± 0.0003	---	Perrault et al. 2011
D. coriacea	NW Atlantic	Liver	22	---	---	0.017 ± 0.007	---	Perrault et al. 2011

D. coriacea	NW Atlantic	Yolk Sac	7	---	---	0.048 ± 0.029	---	Perrault et al. 2011
D. coriacea	E Central Pacific	Egg	26	---	1.6 ± 0.3	---	BDL	Roe et al. 2011[1]
D. coriacea	E Central Pacific	Hatchling	19	---	BDL	---	BDL	Roe et al. 2011[1]
C. mydas	NW Pacific	Plasma	9	0.297 ± 0.170	---	0.103 ± 0.042	1.032 ± 0.585	Suzuki et al. 2012
C. mydas	NW Pacific	Plasma	5	0.049 ± 0.034	---	0.119 ± 0.144	0.215 ± 0.129	Suzuki et al. 2012
C. caretta	NW Pacific	Plasma	9	1.362 ± 0.953	---	0.169 ± 0.154	4.286 ± 2.825	Suzuki et al. 2012
C. caretta	NW Pacific	Plasma	3	0.297 ± 0.135	---	0.061 ± 0.086	0.957 ± 0.367	Suzuki et al. 2012
E. imbricata	NW Pacific	Plasma	6	0.443 ± 0.470	---	0.036 ± 0.036	1.798 ± 1.437	Suzuki et al. 2012
E. imbricata	NW Pacific	Plasma	25	0.041 ± 0.047	---	0.116 ± 0.057	0.265 ± 0.168	Suzuki et al. 2012

1: Variance expressed as SE
2: Variance units not specified
3: Variance expressed as range
4: Concentration expressed as median
†: Variance not specified
‡: Concentration expressed as range
*: n expressed in parentheses
---: Not analyzed
BDL: Below detection limit
NS: Concentration not specified
Unkn: Unknown sample size
SAG: Shelled albumin globule

A1.2: Contaminated sea turtle samples reported toxic for human consumption by Aguirre et al. (2006), and other samples in the literature with equal or higher concentrations of Cd and/or Hg (mean ± SD; μg g^{-1} wet wt.).

Tissue	Species	Location	n	Metal Concentration	Reference
Cadmium (Cd)					
Kidney	*C. caretta*	NW Atlantic	1	11.0	Jacobson et al. 2006
	C. caretta	NW Atlantic	9	30.1 ± 13.9	Jacobson et al. 2006
	C. caretta	NE Atlantic	5	13.3 ± 13.6	Caurant et al. 1999
	C. caretta	Mediterranean	2	8.540 (5.264-11.82)*	Godley et al. 1999[3,4]
	C. caretta	Mediterranean	19	10.49 ± 23.58	Garcia-Fernandez et al. 2009
	C. caretta	NW Pacific	7	39.4 ± 16.2*	Sakai et al. 1995, 2000b
	C. caretta	SW Pacific	5	28.3 ± 5.7*	Gordon et al. 1998[1]
	C. caretta	NE Pacific	5	19.74 (3.704-37.8)	Gardner et al. 2006[3]
	C. mydas	Caribbean	33	10.6 ± 0.84	Andreani et al. 2008[1]
	C. mydas	NW Pacific	23	38.5 ± 21.3	Sakai et al. 2000a
	C. mydas	NW Pacific	2	41.3 ± 6.0	Sakai et al. 2000b
	C. mydas	NW Pacific	25	28 ± 12*	Anan et al. 2001
	C. mydas	SW Pacific	7	26.0 ± 10.939	Gladstone 1996
	C. mydas	SW Pacific	38	15.3 ± 2.5	Gordon et al. 1998[1]
	C. mydas	SW Pacific	16	45.98 ± 9.06	van de Merwe 2010[1]
	C. mydas	NE Pacific	11	32.7 (1.64-176)*	Gardner et al. 2006[3]
	C. mydas	NE Pacific	8	29.7 (17.57-176)	Talavera-Saenz et al. 2007[3,4]
	C. mydas	Central N Pacific	10	28.5 ± 22.0	Aguirre et al. 1994

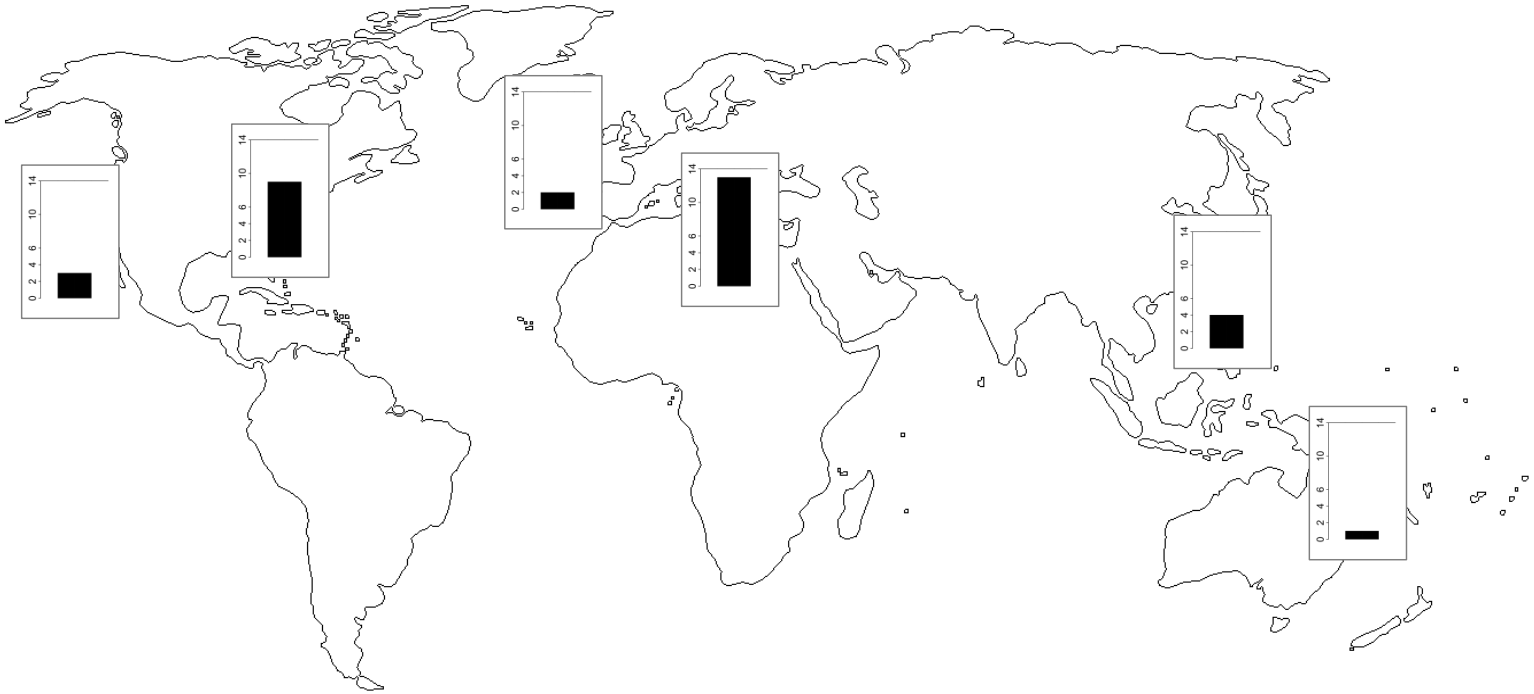
	D. coriacea	NE Atlantic	5	30.3 ± 28.1*	Caurant et al. 1999
	E. imbricata	NW Pacific	19	18.3 ± 14.9*	Anan et al. 2001
	L. olivacea	SW Pacific	1	29.8	Gordon et al. 1998
	L. olivacea	NE Pacific	6	16.21 (0.22-74.0)*	Gardner et al. 2006[3]
Liver	*C. caretta*	NW Atlantic	9	11.1 ± 8.23	Jacobson et al. 2006
	C. caretta	NW Pacific	7	9.29 ± 3.30	Sakai et al. 1995, 2000b
	C. caretta	SW Pacific	5	16.4 ± 3.3	Gordon et al. 1998[1]
	C. mydas	W Indian	6	1,408.150 ± 1.87*	Bicho et al. 2005
	C. mydas	NW Pacific	9	8.96 (3.21-21.6)	Anan et al. 2002[3]
	C. mydas	SW Pacific	7	10.73 ± 3.437	Gladstone 1996
	C. mydas	SW Pacific	38	12.5 ± 2.0	Gordon et al. 1998[1]
	C. mydas	Central N Pacific	10	10.7 ± 9.2	Aguirre et al. 1994
	D. coriacea	NE Atlantic	1	28	Godley et al. 1998
Pancreas	*C. caretta*	NW Pacific	7	36.6 ± 19.2	Sakai et al. 2000b
	C. mydas	NW Pacific	2	9.9 ± 3.9	Sakai et al. 2000b
	D. coriacea	NE Atlantic	2	68.8	Caurant et al. 1999[†]
Bladder	*C. caretta*	Mediterranean	20	11.16 ± 4.61	Kaska et al. 2004
	C. mydas	Mediterranean	20	9.34 ± 5.14	Kaska et al. 2004
Stomach	*C. mydas*	NW Pacific	2	175.5 ± 248.0	Lam et al. 2004
Mercury (Hg)					
Liver	*C. caretta*	NW Atlantic	6	0.594 ± 0.155*	Day et al. 2005
	C. caretta	NW Atlantic	1	2.4	Jacobson et al. 2006
	C. caretta	NW Atlantic	9	2.86 ± 2.50	Jacobson et al. 2006

	C. caretta	Mediterranean	7	0.70 ± 0.32*	Storelli et al. 1998a
	C. caretta	Mediterranean	7	0.47 ± 0.27*	Storelli et al. 1998b
	C. caretta	Mediterranean	5	0.530 (0.18-1.65)*	Godley et al. 1999[3,4]
	C. caretta	Mediterranean	22	0.51 ± 0.046	Kaska & Furness 2001
	C. caretta	Mediterranean	22	0.35 ± 0.54	Maffucci et al. 2005
	C. caretta	Mediterranean	19	0.43 ± 0.29	Storelli et al. 2005
	C. caretta	Mediterranean	8	0.501 ± 0.33	Abdallah & Abd-Allah 2011
	C. caretta	NW Pacific	7	1.51 ± 2.93*	Sakai et al. 1995, 2000b
	C. caretta	NE Pacific	4	0.152 ± 0.029*	Kampalath et al. 2006
	C. mydas	Mediterranean	6	0.121 (0.059-0.301)*	Godley et al. 1999[3,4]
	C. mydas	W Indian	50	0.22 ± 0.03	Al-Rawahy et al. 2007[1]
	C. mydas	NW Pacific	50	0.287 ± 0.156	Sakai et al. 2000a
	C. mydas	NW Pacific	2	0.189 ± 0.159	Sakai et al. 2000b
	C. mydas	NW Pacific	26	0.13 ± 0.06	Anan et al. 2001
	C. mydas	NW Pacific	2	0.2186 ± 0.0540	Lam et al. 2004
	D. coriacea	NE Atlantic	1	0.11 ± 0.01*	Davenport & Wrench 1990[2]
	D. coriacea	NE Atlantic	2	0.56 ± 0.38	Godley et al. 1998
	E. imbricata	NW Pacific	22	0.27 ± 0.58	Anan et al. 2001
	L. kempii	NW Atlantic	9	0.344 ± 0.549	Wang et al. 2005
	L. olivacea	NE Pacific	6	0.213 ± 0.286*	Kampalath et al. 2006
Kidney	*C. caretta*	NW Atlantic	6	0.214 ± 0.046	Day et al. 2005
	C. caretta	NW Atlantic	1	0.11	Jacobson et al. 2006
	C. caretta	NW Atlantic	9	1.20 ± 1.79	Jacobson et al. 2006
	C. caretta	Mediterranean	7	0.17 ± 0.05*	Storelli et al. 1998b

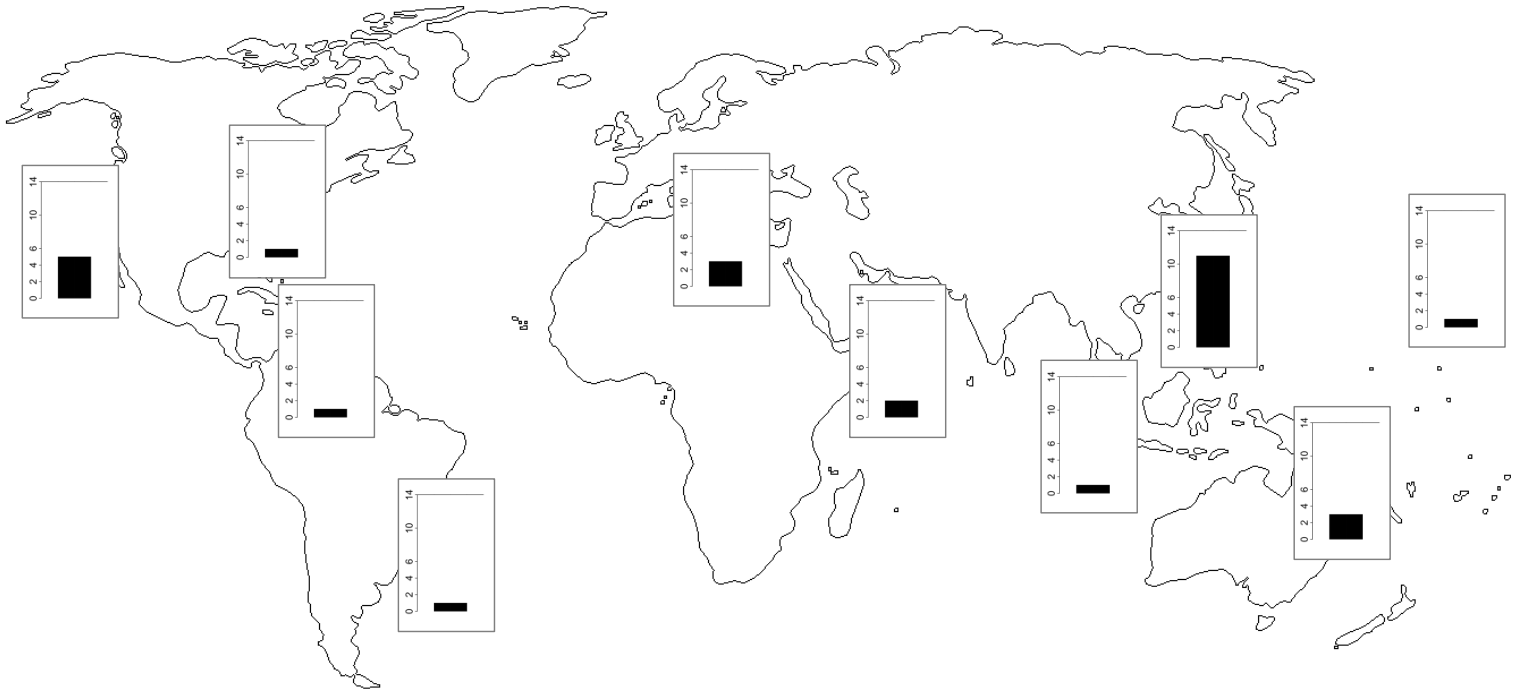
	C. caretta	Mediterranean	2	0.13 (0.036-0.22)*	Godley et al. 1999[3,4]
	C. caretta	Mediterranean	20	0.22 ± 0.17	Maffucci et al. 2005
	C. caretta	Mediterranean	19	0.160 ± 0.07	Storelli et al. 2005
	C. caretta	Mediterranean	8	0.28 ± 0.11	Abdallah & Abd-Allah 2011
	C. caretta	NW Pacific	7	0.247 ± 0.130	Sakai et al. 1995, 2000b
	C. mydas	NW Pacific	23	0.132 ± 0.077	Sakai et al. 2000a
	E. imbricata	NW Pacific	19	0.25 ± 0.23	Anan et al. 2001
	L. kempii	NW Atlantic	8	0.201 ± 0.116	Wang et al. 2005
	L. olivacea	NE Pacific	3	0.143 ± 0.198	Kampalath et al. 2006
Muscle	*C. caretta*	NW Atlantic	6	0.155 ± 0.070	Day et al. 2005
	C. caretta	Mediterranean	7	0.10 (BDL-0.383)*	Godley et al. 1999[3,4]
	C. caretta	Mediterranean	7	0.21 ± 0.13*	Storelli et al. 1998a
	C. caretta	Mediterranean	7	0.22 ± 0.14*	Storelli et al. 1998b
	C. caretta	Mediterranean	19	0.18 ± 0.21	Storelli et al. 2005
	C. caretta	NW Pacific	7	0.108 ± 0.049	Sakai et al. 1995, 2000b
	D. coriacea	NE Atlantic	2	0.21 ± 0.12	Godley et al. 1998
Egg	*C. caretta*	NW Atlantic	27	1.3595 ± 0.0394	Stoneburner et al. 1980[1]
	C. caretta	NW Atlantic	33	1.3912 ± 0.1063	Stoneburner et al. 1980[1]
	C. caretta	NW Atlantic	15	0.6352 ± 0.0141	Stoneburner et al. 1980[1]
	C. caretta	NW Atlantic	21	0.4123 ± 0.0136	Stoneburner et al. 1980[1]
Heart	*C. caretta*	Mediterranean	19	0.12 ± 0.08	Storelli et al. 2005
	C. mydas	NW Pacific	2	0.2164 ± 0.02118	Lam et al. 2004
Blood	*C. caretta*	Mediterranean	8	0.8368 ± 0.17	Abdallah & Abd-Allah 2011
Pancreas	*C. caretta*	NW Pacific	7	0.770 ± 1.830	Sakai et al. 2000b

Spleen	*C. caretta*	Mediterranean	19	0.11 ± 0.07	Storelli et al. 2005
Stomach	*C. mydas*	NW Pacific	2	0.2990 ± 0.06381	Lam et al. 2004
Blood	*D. coriacea*	SE Atlantic	9	0.200 ± 0.200	Deem et al. 2006

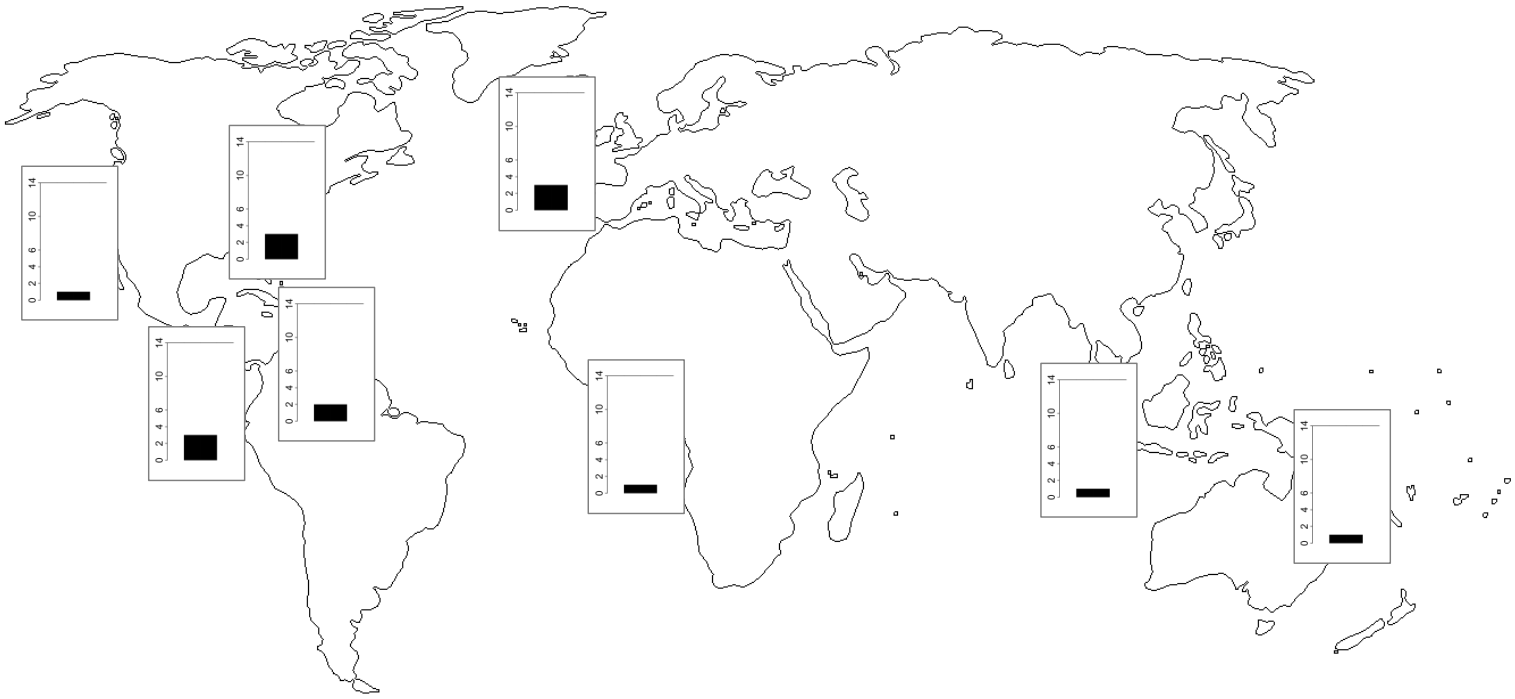
*: Reported by Aguirre et al. (2006)
1: Variance expressed as SE
2: Variance units not specified
3: Variance expressed as range
4: Concentration expressed as median
†: Variance not specified



A1.3: The global distribution of trace metal data (As, Cd, Hg, and Pb) from *Caretta caretta* soft tissues, showing the number of studies that present original data from each region.

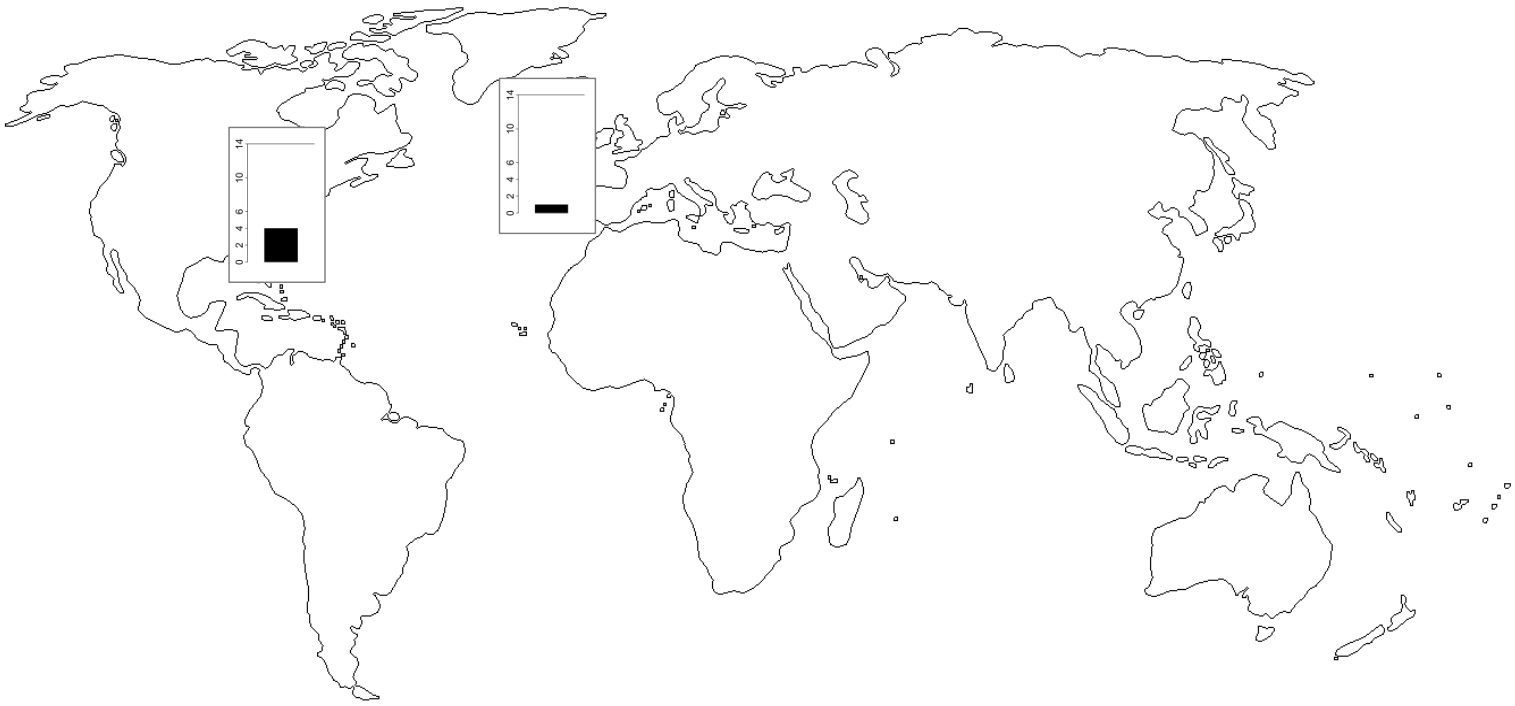


A1.4: The global distribution of trace metal data (As, Cd, Hg, and Pb) from *Chelonia mydas* soft tissues, showing the number of studies that present original data from each region.

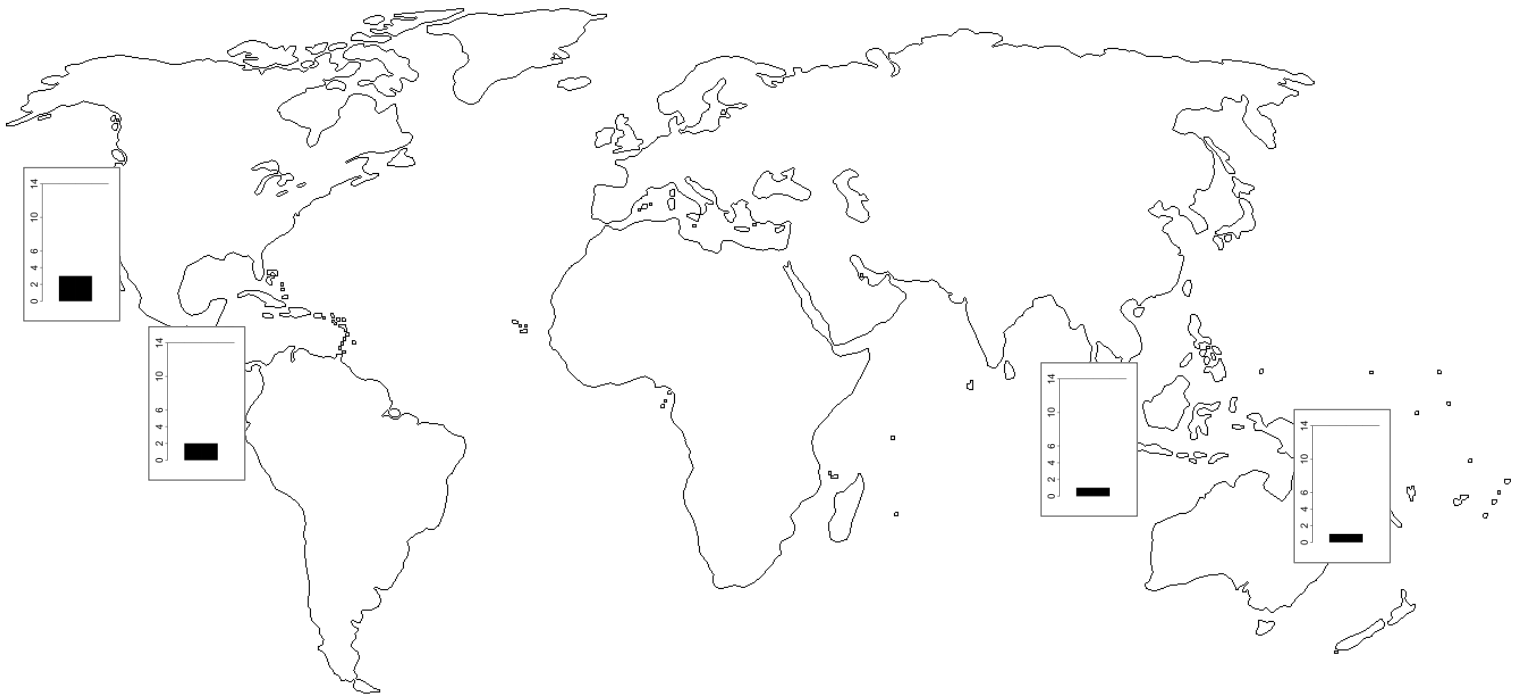


A1.5: The global distribution of trace metal data (As, Cd, Hg, and Pb) from *Dermochelys coriacea* soft tissues, showing the number of studies that present original data from each region.

A1.6: The global distribution of trace metal data (As, Cd, Hg, and Pb) from *Eretmochelys imbricata* soft tissues, showing the number of studies that present original data from each region.



A1.7: The global distribution of trace metal data (As, Cd, Hg, and Pb) from *Lepidochelys kempii* soft tissues, showing the number of studies that present original data from each region.



A1.8: The global distribution of trace metal data (As, Cd, Hg, and Pb) from *Lepidochelys olivacea* soft tissues, showing the number of studies that present original data from each region.

A1.9: The global distribution of trace metal data (As, Cd, Hg, and Pb) from *Natator depressus* soft tissues, showing the number of studies that present original data from each region.

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Appendix 2: Supplementary Information for Chapter 2

A2.1: Compilation of published worldwide trace metal concentrations in sea turtle eggs, including those reported in this study (Mean ± SD; µg g^{-1} wet wt.).

Tissue	Location	n	Manganese (Mn)	Iron (Fe)	Cobalt (Co)	Nickel (Ni)	Reference
C. caretta							
Yolk	NW Atlantic	Unkn	---	---	---	---	Hillestad et al. 1974[1]
Albumen	NW Atlantic	Unkn	---	---	---	---	Hillestad et al. 1974[1]
Yolk	NW Atlantic	27	---	71.6783 ± 13.1158	0.0484 ± 0.0093	BDL	Stoneburner et al. 1980[2]
Yolk	NW Atlantic	33	---	71.2745 ± 8.3766	0.0384 ± 0.0078	0.2527 ± 0.1739	Stoneburner et al. 1980[2]
Yolk	NW Atlantic	15	---	72.9812 ± 11.7213	0.0729 ± 0.0056	2.2791 ± 0.7981	Stoneburner et al. 1980[2]
Yolk	NW Atlantic	21	---	74.6737 ± 16.2950	BDL	BDL	Stoneburner et al. 1980[2]
Egg	NW Pacific	5	0.52 ± 0.26	11.5 ± 1.29	BDL	BDL	Sakai et al. 1995, 2000
Egg	Mediterranean	3	---	---	---	---	Godley et al. 1999[3,4]
Yolk	Mediterranean	22	---	15.79 ± 3.62	---	---	Kaska and Furness 2001
Egg	NW Atlantic	20	0.539	21.36	---	0.15	Alam and Brim 2000[1]
C. mydas							
Egg	Mediterranean	24	---	---	---	---	Godley et al. 1999[3,4]
Egg	NW Pacific	1	0.38	10.9	BDL	BDL	Sakai et al. 2000
Egg	W Indian	32	2.14 ± 1.14	---	0.26 ± 0.20	2.26 ± 2.34	Bicho et al. 2005
Yolk	NW Pacific	30	0.27 ± 0.040	45 ± 2.9	0.030 ± 0.0080	0.19 ± 0.025	Lam et al. 2006[2]
Albumen	NW Pacific	30	0.047 ± 0.0082	3.8 ± 0.75	0.0088 ± 0.0058	0.017 ± 0.0055	Lam et al. 2006[2]
Yolk	W Indian	75	0.30 ± 0.02	---	0.05 ± 0.00	0.06 ± 0.00	Al-Rawahy et al. 2007[2]
Egg	E Indian	55	---	---	BDL	---	van de Merwe 2009[2]
Egg	E Central Pacific	31	0.31 ± 0.07	11.7 ± 0.9	0.0027 ± 0.0007	0.02 ± 0.01	This study[3,5]
L. olivacea							
Egg	E Indian	24	4.3 ± 2.5	19.3 ± 3.2	2.3 ± 0.5	5.0 ± 2.0	Sahoo et al. 1996
Yolk	E Central Pacific	25	---	---	---	1.2 ± 0.2	Paez-Osuna et al. 2010a, b, 2011
Albumen	E Central Pacific	25	---	---	---	0.11 ± 0.11	Paez-Osuna et al. 2010a, b, 2011
Egg	E Central Pacific	30	0.35 ± 0.09	11 ± 1	0.003 ± 0.001	0.016 ± 0.007	This study[3,5]
D. coriacea							
Egg	Caribbean	76	---	---	---	---	Guirlet et al. 2008
Egg	E Central Pacific	26	0.9 ± 0.1	39.8 ± 6.5	---	1.9 ± 0.3	Roe et al. 2011[2]
E. imbricata							
Yolk	W Indian	48	---	---	---	---	Ehsanpour et al. 2014
Albumen	W Indian	48	---	---	---	---	Ehsanpour et al. 2014
N. depressus							
Egg	SW Pacific	60	0.17 ± 0.001	---	BDL	BDL	Ikonomopoulou et al. 2011

A2.1: (Cont.)

Tissue	Location	n	Copper (Cu)	Zinc (Zn)	Arsenic (As)	Selenium (Se)	Reference
C. caretta							
Yolk	NW Atlantic	Unkn	2.08	32.25	---	---	Hillestad et al. 1974[1]
Albumen	NW Atlantic	Unkn	6.0	26	---	---	Hillestad et al. 1974[1]
Yolk	NW Atlantic	27	5.9676 ± 0.7927	77.1009 ± 9.2881	---	---	Stoneburner et al. 1980[2]
Yolk	NW Atlantic	33	4.9659 ± 1.1180	73.5371 ± 3.6428	---	---	Stoneburner et al. 1980[2]
Yolk	NW Atlantic	15	5.4386 ± 1.1065	78.5057 ± 6.6992	---	---	Stoneburner et al. 1980[2]
Yolk	NW Atlantic	21	6.6065 ± 1.2890	80.5048 ± 5.5459	---	---	Stoneburner et al. 1980[2]
Egg	NW Pacific	5	1.05 ± 0.199	14.7 ± 1.44	---	---	Sakai et al. 1995, 2000
Egg	Mediterranean	3	---	---	---	---	Godley et al. 1999[3,4]
Yolk	Mediterranean	22	0.928 ± 0.102	57.21 ± 2.23	---	---	Kaska and Furness 2001
Egg	NW Atlantic	20	1.58	25.53	0.468	1.50	Alam and Brim 2000[1]
C. mydas							
Egg	Mediterranean	24	---	---	---	---	Godley et al. 1999[3,4]
Egg	NW Pacific	1	0.781	20.3	---	---	Sakai et al. 2000
Egg	W Indian	32	11.43 ± 11.68	---	---	---	Bicho et al. 2005
Yolk	NW Pacific	30	0.34 ± 0.036	45 ± 3.6	2.5 ± 0.37	3.5 ± 0.60	Lam et al. 2006[2]
Albumen	NW Pacific	30	0.063 ± 0.012	0.30 ± 0.059	0.17 ± 0.022	0.27 ± 0.058	Lam et al. 2006[2]
Yolk	W Indian	75	1.09 ± 0.09	8.58 ± 1.03	---	0.12 ± 0.02	Al-Rawahy et al. 2007[2]
Egg	E Indian	55	0.526 ± 0.023	15.34 ± 0.93	0.097 ± 0.011	0.464 ± 0.026	van de Merwe 2009[2]
Egg	E Central Pacific	31	0.5 ± 0.1	14 ± 3	0.12 ± 0.04	1.6 ± 0.2	This study[3,5]
L. olivacea							
Egg	E Indian	24	3.6 ± 0.5	4.3 ± 1.5	---	---	Sahoo et al. 1996
Yolk	E Central Pacific	25	0.825 ± 0.551	27.1 ± 4.09	---	---	Paez-Osuna et al. 2010a, b, 2011
Albumen	E Central Pacific	25	0.0953 ± 0.0775	0.907 ± 0.16	---	---	Paez-Osuna et al. 2010a, b, 2011
Egg	E Central Pacific	30	0.6 ± 0.2	16 ± 2	0.12 ± 0.06	1.4 ± 0.3	This study[3,5]
D. coriacea							
Egg	Caribbean	76	0.63 ± 0.10	14.16 ± 2.23	---	1.44 ± 0.38	Guirlet et al. 2008
Egg	E Central Pacific	26	25.9 ± 3.0	14.2 ± 0.7	---	---	Roe et al. 2011[2]
E. imbricata							
Yolk	W Indian	48	0.86 ± 0.13	12.80 ± 3.1	---	---	Ehsanpour et al. 2014
Albumen	W Indian	48	0.103 ± 0.031	0.0932 ± 0.0319	---	---	Ehsanpour et al. 2014
N. depressus							
Egg	SW Pacific	60	0.41 ± 0.003	10.40 ± 0.06	0.67 ± 0.019	0.30 ± 0.002	Ikonomopoulou et al. 2011

A2.1: (Cont.)

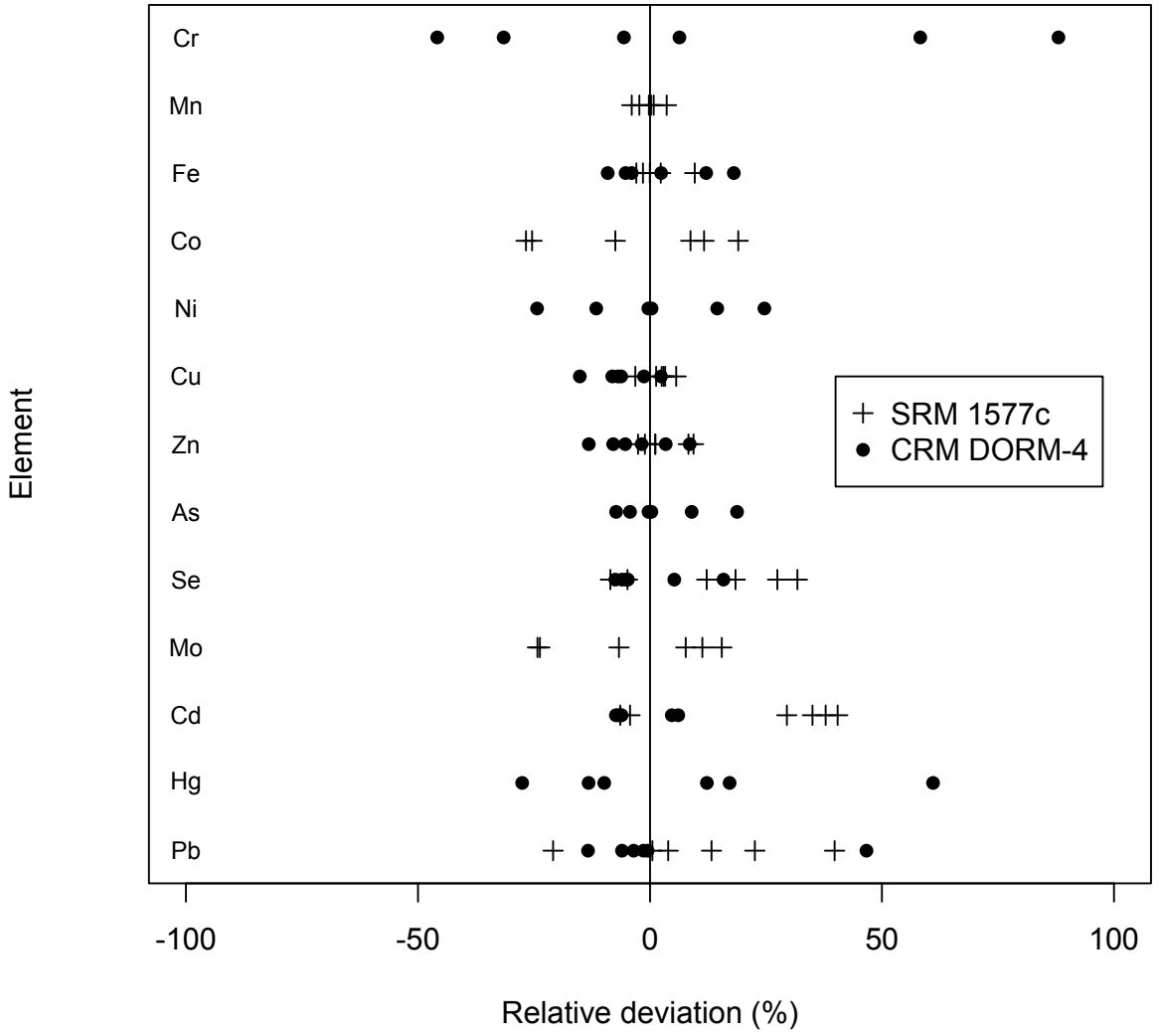
Tissue	Location	n	Molybdenum (Mo)	Cadmium (Cd)	Mercury (Hg)	Reference
C. caretta						
Yolk	NW Atlantic	Unkn	---	0.17	0.02-0.09‡	Hillestad et al. 1974[1]
Albumen	NW Atlantic	Unkn	---	0.56	0.01-0.03‡	Hillestad et al. 1974[1]
Yolk	NW Atlantic	27	17.9323 ± 1.8769	0.1118 ± 0.0799	1.3595 ± 0.0394	Stoneburner et al. 1980[2]
Yolk	NW Atlantic	33	2.6651 ± 1.0071	0.1950 ± 0.0709	1.3912 ± 0.1063	Stoneburner et al. 1980[2]
Yolk	NW Atlantic	15	3.1281 ± 1.4997	0.0401 ± 0.0016	0.6352 ± 0.0141	Stoneburner et al. 1980[2]
Yolk	NW Atlantic	21	3.8141 ± 1.5255	0.0255 ± 0.0133	0.4123 ± 0.0136	Stoneburner et al. 1980[2]
Egg	NW Pacific	5	---	0.013 ± 0.004	0.00554 ± 0.00157	Sakai et al. 1995, 2000
Egg	Mediterranean	3	---	0.063 (0.063-0.15)	0.052 (0.044-0.16)	Godley et al. 1999[3,4]
Yolk	Mediterranean	22	---	0.359 ± 0.135	BDL	Kaska and Furness 2001
Egg	NW Atlantic	20	---	---	0.033	Alam and Brim 2000[1]
C. mydas						
Egg	Mediterranean	24	---	0.074 (0.01-0.336)	BDL (BDL-0.052) *(17)	Godley et al. 1999[3,4]
Egg	NW Pacific	1	---	BDL	0.00135	Sakai et al. 2000
Egg	W Indian	32	0.08 ± 0.17	0.312 ± 0.27	---	Bicho et al. 2005
Yolk	NW Pacific	30	9.7 ± 1.3	BDL	0.0015 ± 0.00013	Lam et al. 2006[2]
Albumen	NW Pacific	30	5.2 ± 0.60	BDL	0.000090 ± 0.000030	Lam et al. 2006[2]
Yolk	W Indian	75	---	0.16 ± 0.03	0.10 ± 0.01	Al-Rawahy et al. 2007[2]
Egg	E Indian	55	---	0.009 ± 0.001	BDL	van de Merwe 2009[2]
Egg	E Central Pacific	31	0.011 ± 0.003	0.09 ± 0.04	0.006 ± 0.002 *(29)	This study[3,5]
L. olivacea						
Egg	E Indian	24	---	BDL	---	Sahoo et al. 1996
Yolk	E Central Pacific	25	---	0.090 ± 0.038	0.011 ± 0.0038	Paez-Osuna et al. 2010a, b, 2011
Albumen	E Central Pacific	25	---	0.0059 ± 0.002	0.000027 ± 0.00002	Paez-Osuna et al. 2010a, b, 2011
Egg	E Central Pacific	30	0.015 ± 0.004	0.07 ± 0.02	0.009 ± 0.005	This study[3,5]
D. coriacea						
Egg	Caribbean	76	---	0.024 ± 0.001	0.012 ± 0.003	Guirlet et al. 2008
Egg	E Central Pacific	26	---	1.6 ± 0.3	---	Roe et al. 2011[2]
E. imbricata						
Yolk	W Indian	48	---	0.16 ± 0.03	0.003 ± 0.001	Ehsanpour et al. 2014
Albumen	W Indian	48	---	0.016 ± 0.0095	0.0001 ± 0.0001	Ehsanpour et al. 2014
N. depressus						
Egg	SW Pacific	60	BDL	BDL	BDL	Ikonomopoulou et al. 2011

A2.1: (Cont.)

Tissue	Location	n	Lead (Pb)	Reference
C. caretta				
Yolk	NW Atlantic	Unkn	2.87	Hillestad et al. 1974[1]
Albumen	NW Atlantic	Unkn	12.0	Hillestad et al. 1974[1]
Yolk	NW Atlantic	27	2.1846 ± 1.1999	Stoneburner et al. 1980[2]
Yolk	NW Atlantic	33	1.1347 ± 0.8395	Stoneburner et al. 1980[2]
Yolk	NW Atlantic	15	1.7694 ± 1.1501	Stoneburner et al. 1980[2]
Yolk	NW Atlantic	21	1.2368 ± 1.0405	Stoneburner et al. 1980[2]
Egg	NW Pacific	5	BDL	Sakai et al. 1995, 2000
Egg	Mediterranean	3	0.052 (BDL-1.08)	Godley et al. 1999[3,4]
Yolk	Mediterranean	22	1.307 ± 0.228	Kaska and Furness 2001
Egg	NW Atlantic	20	0.19	Alam and Brim 2000[1]
C. mydas				
Egg	Mediterranean	24	BDL (BDL-0.443)	Godley et al. 1999[3,4]
Egg	NW Pacific	1	BDL	Sakai et al. 2000
Egg	W Indian	32	0.74 ± 0.69	Bicho et al. 2005
Yolk	NW Pacific	30	0.049 ± 0.0077	Lam et al. 2006[2]
Albumen	NW Pacific	30	0.0047 ± 0.0010	Lam et al. 2006[2]
Yolk	W Indian	75	0.08 ± 0.01	Al-Rawahy et al. 2007[2]
Egg	E Indian	55	0.031 ± 0.003	van de Merwe 2009[2]
Egg	E Central Pacific	31	0.003 ± 0.002 *(11)	This study[3,5]
L. olivacea				
Egg	E Indian	24	3.6 ± 1.1	Sahoo et al. 1996
Yolk	E Central Pacific	25	0.30 ± 0.038	Paez-Osuna et al. 2010a, b, 2011
Albumen	E Central Pacific	25	0.0292 ± 0.005	Paez-Osuna et al. 2010a, b, 2011
Egg	E Central Pacific	30	0.004 ± 0.002 *(22)	This study[3,5]
D. coriacea				
Egg	Caribbean	76	0.036 ± 0.001	Guirlet et al. 2008
Egg	E Central Pacific	26	BDL	Roe et al. 2011[2]
E. imbricata				
Yolk	W Indian	48	1.2 ± 0.35	Ehsanpour et al. 2014
Albumen	W Indian	48	0.075 ± 0.010	Ehsanpour et al. 2014
N. depressus				
Egg	SW Pacific	60	BDL	Ikonomopoulou et al. 2011

1: Variance not specified
2: Variance ecpressed as SE
3: Concentration expressed as median
4: Variance expressed as range
5: Variance expressed as MAD
Unkn: Unknown sample size
---: Not analyzed
‡: Concentration expressed as range
BDL: Below detection limit
*: n expressed in parentheses

A2.2: Percent relative deviation of each metal from 6 samples each of standard reference materials SRM 1577c (Bovine liver: National Institute of Standards and Technology, USA) and CRM DORM-4 (Fish protein homogenate: National Research Council, Canada), tested throughout the analysis to ensure the accuracy of the analytical procedure.



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