Environmental Toxicology and Chemistry—Volume 00, Number 00—pp. 1–15, 2023
Received: 7 October 2022 | Revised: 21 November 2022 | Accepted: 27 February 2023

Environmental Toxicology

Green Sea Turtles (*Chelonia mydas*) Accumulate Heavy Metals Near a Former Skeet Shooting Range in Kailua, O'ahu, Hawai'i

Katherine R. Shaw,[a,*] George H. Balazs,[b] T. Todd Jones,[c] Harry W. Lynch,[d] Jing Liu,[e] George P. Cobb,[f] David M. Klein,[g] and Jennifer M. Lynch[h,i]

[a]Department of Environmental Toxicology, Texas Tech University, Lubbock, Texas, USA
[b]Golden Honu Services of Oceania, Honolulu, Hawaii, USA
[c]Pacific Islands Fisheries Science Center, National Oceanic and Atmospheric Administration, Honolulu, Hawaii, USA
[d]Makanakai Marine Services, Honolulu, Hawaii, USA
[e]Environment Research Institute, Shandong University, Qingdao, China
[f]Department of Environmental Sciences, Baylor University, Waco, Texas, USA
[g]Department of Civil Engineering, Texas Tech University, Lubbock, Texas, USA
[h]Chemical Sciences Division, National Institute of Standards and Technology, Waimānalo, Hawaii, USA
[i]Center for Marine Debris Research, Hawai'i Pacific University, Waimānalo, Hawaii, USA
Present address: Katherine R. Shaw, Chemical Sciences Division, National Institute of Standards and Technology, Waimānalo, Hawaii, USA and Center for Marine Debris Research, Hawai'i Pacific University, Waimānalo, Hawaii, USA

Abstract: The present study determined if green sea turtles (*Chelonia mydas*) in Kailua Bay, Oahu, in the Hawaiian Islands have elevated blood and scute lead (Pb), arsenic (As), and antimony (Sb) concentrations resulting from lead deposition at a historic skeet shooting range. Blood and scute samples were collected and analyzed for Pb, As, and Sb via inductively coupled plasma-mass spectrometry. Prey, water, and sediment samples were also analyzed. Turtle samples in Kailua Bay (45) have blood Pb concentrations (328 ± 195 ng/g) greater than a reference population (Howick Group of Islands, 29.2 ± 17.1 ng/g). Compared with other green turtle populations, only turtles in Oman, Brazil, and San Diego, CA have blood Pb concentrations greater than turtles in Kailua Bay. The estimated daily exposure of Pb from algae sources in Kailua Bay (0.12 mg/kg/day) was significantly lower than the no observed adverse effect level (100 mg/kg) of red-eared slider turtles. However, the chronic effects of Pb on sea turtles is poorly understood and continued monitoring of this population will increase our understanding of the Pb and As loads of sea turtles in Kailua Bay. *Environ Toxicol Chem* 2023;00:1–15. © 2023 SETAC. This article has been contributed to by U.S. Government employees and their work is in the public domain in the USA.

Keywords: Marine turtle; Reptile; Lead; Scute; Hawai'i

INTRODUCTION

Lead (Pb) can adversely affect every organ and system in the body, with the main target being the nervous system (Agency for Toxic Substances and Disease Registry, 2020). Widespread use of Pb has led to its accumulation in the environment. The primary source of Pb in waterfowl and most bird species is Pb shot (Pain et al., 2019). The toxicity of Pb shot to waterfowl has been well established and due to these dangers Pb ammunition was phased out of use in the United States over a 5-year period, with a complete ban for hunting waterfowl since the 1991–1992 season. Lead shot is still legal for hunting other game and for target shooting.

Lead shot is primarily composed of three metals: Pb (98%), antimony (Sb, 1.75%), and arsenic (As, 0.5%; Potysz et al., 2023). In soil Pb shot will undergo oxidation, carbonation, and hydration, allowing the pellets to dissolve and release the elements into the environment (Cao et al., 2003). There are two stages in the breakdown of Pb shot pellets. First, corrosion products are formed during the initial weathering process, forming a crust around the pellet. This is followed by the interaction of those corrosion products with the soil colloids and soil solution (Rooney et al., 2007). The weathering rate of Pb shot pellets in soil is approximately 1% per year, while the dissolution rate in distilled water is 0.5%–6.6% per year (Jorgensen & Willems, 1987; Takamatsu et al., 2010). Weathering is enhanced by conditions such as high humidity,

This article includes online-only Supporting Information.
* Address correspondence to katherine.shaw@nist.gov
Published online 3 March 2023 in Wiley Online Library (wileyonlinelibrary.com).
DOI: 10.1002/etc.5601

temperature, and rainfall commonly found in tropical and subtropical locations. Shooting ranges accumulate high densities of pellets and due to the weathering of Pb shot, thus ranges contain highly contaminated soil (Cao et al., 2003). Shooting ranges with soil Pb concentrations up to 54 000 mg/kg excluding pellets have been recorded (Manninen & Tanskanen, 1993). This implies potential Pb accumulation in the nearshore sediments, water, and plants in the surrounding area.

The Honolulu Skeet Club was located in the Kaimalino neighborhood on the eastside of O'ahu, Hawai'i and was active for 23 years between 1933 and 1956. The club consisted of four shooting ranges, each with a 40-yard radius, stretching a total of 320 yards along the shoreline. More than 500 000 pounds of skeet shot is estimated to have been deposited over the years, with the majority in nearshore waters along the rocky coastline on the east shore of the Kaimalino neighborhood, wrapping a short distance around the south (Board of Land of Natural Resources, 2012). After the closure of the club, the area was filled with graded topsoil, paved with asphalt, and developed into 60 residential lots. Hawai'i Department of Health warning signs at public access points currently advise that Pb and As found in pellets may be harmful to children if swallowed. The Honolulu Skeet Club is listed as a site eligible for possible listing under the Comprehensive Environmental Response, Compensation, and Liability Act, commonly known as the Superfund. Pellets were removed from the sand and rocky tide pools using an updated sluice box in 2009 in a cleanup costing approximately $50 000 to prevent children from coming into contact with the Pb pellets (Aguiar, 2009). However, layers of Pb shot are still visible today, with more pellets being exposed after each storm event, causing additional pellets to accumulate in the sand, rocky tidepool, and ocean floor. In addition to the potential risk to human health, wildlife living and foraging in the area are at risk of Pb poisoning, including an important herbivorous species, the threatened Hawaiian green sea turtle (*Chelonia mydas*).

Hawaiian green turtles recruit from oceanic to neritic habitats at approximately 35 cm straight carapace length (SCL; Balazs & Chaloupka, 2004). The green turtles studied since 2000 along the shoreline of the Kaimalino neighborhood at the mouth of the Kawainui Marsh are highly resident. Between 40 and 100 juvenile turtles reside in the estuary of the Kawainui canal year-round, exhibiting strong site fidelity to the area (Asuncion, 2010; Francke et al., 2013; Jorgensen & Willems, 1987). Approximately 75% of turtles were recaptured during sampling events spanning 3 years, 2011–2013. This high site fidelity suggests that turtles may serve as good bioindicators of contaminants in Kailua Bay, having stayed in the area for a long period of time, potentially accumulating Pb from the area.

The present study aims to quantify Pb and As mass fractions (hereafter called concentrations) in the water, sediment, and algae in nearshore waters along the Kaimalino neighborhood in Kailua Bay to trace these elements through the ecosystem. Blood ($n = 35$) and scute ($n = 34$) samples were taken from green turtles from Kailua Bay to determine if Pb from the skeet shooting range is accumulating in fauna living in the bay. This is the first study to investigate the impacts of Pb shot on a threatened sea turtle species and evaluate if the accumulated Pb is contributing to health concerns such as fibropapillomatosis (FP), a tumor-causing disease that affects some sea turtles. The large sample set, range of sea turtle sizes, and repeat sampling of 10 recaptured turtles will provide a foundation for understanding the Pb exposure of this turtle aggregation.

METHODS

Sample collection

Samples were taken from live green sea turtles in Kailua Bay in 2011, 2012, and 2013 to be archived in the Biological and Environmental Monitoring and Archival of Sea Turtle Tissues (BEMAST) project of the National Institute of Standards and Technology Biorepository (Keller et al., 2014). Blood and scute samples were taken from 35 turtles captured once (2011–2013). Ten of these turtles were captured and sampled once more; nine turtles with 1 year between captures and one turtle with 2 years between captures. The health information for these turtles can be found in Supporting Information, Table S1, and in-water capture data can be found at https://www.fisheries.noaa.gov/inport/item/5449. Turtles were captured in either Region A, slightly closer to the historic skeet shooting range (tissue $n = 24$), or overlapping but slightly farther away Region B (tissue $n = 21$; Figure 1). Turtles captured in the overlapping region were assigned to one region dependent on the location the turtles were brought to on land that day, in either Region A or Region B. Turtles were brought ashore, and blood and scute samples were taken using standardized BEMAST protocols (Keller et al., 2014). Blood was drawn within 15 min of capture using double-ended needles into Vacutainer glass sodium heparin blood collection tubes (Becton Dickinson). Blood was kept on ice until it was brought back to the laboratory. Hematocrit (packed cell volume, PCV) was measured using centrifugation of capillary tubes containing whole blood; however, this method was only available in samples from 2012 to 2013. Whole-blood aliquots were transferred using glass pipettes cleaned with 3% HNO_3 to cryovials and stored in liquid nitrogen vapor (≤−150 °C) at the National Institute of Standards and Technology (NIST) Biorepository in the Hollings Marine Laboratory in Charleston, South Carolina, USA. A field blank was taken using Millipore high-purity deionized water (resistivity = 18 MΩ cm^{-1}, hereinafter referred to as deionized water) and processed in the same manner as the blood samples. Scute samples were taken from the fifth central scute (Keller et al., 2014; Shaw et al., 2021). The fifth central scute was cleaned with a plastic scrubbing pad to remove sloughing keratin and epibiotic organisms, and rinsed with isopropanol and deionized water. The top layer of keratin was shaved off and discarded. The next layers of keratin (<2.0 mm) were shaved off the entire scute with a knife and collected in a Teflon bag. Scute samples were homogenized with mortar and pestle at room temperature, split into aliquots, and stored in liquid nitrogen vapor by NIST. Blood and scute samples were shipped from the NIST Biorepository to Texas Tech University in

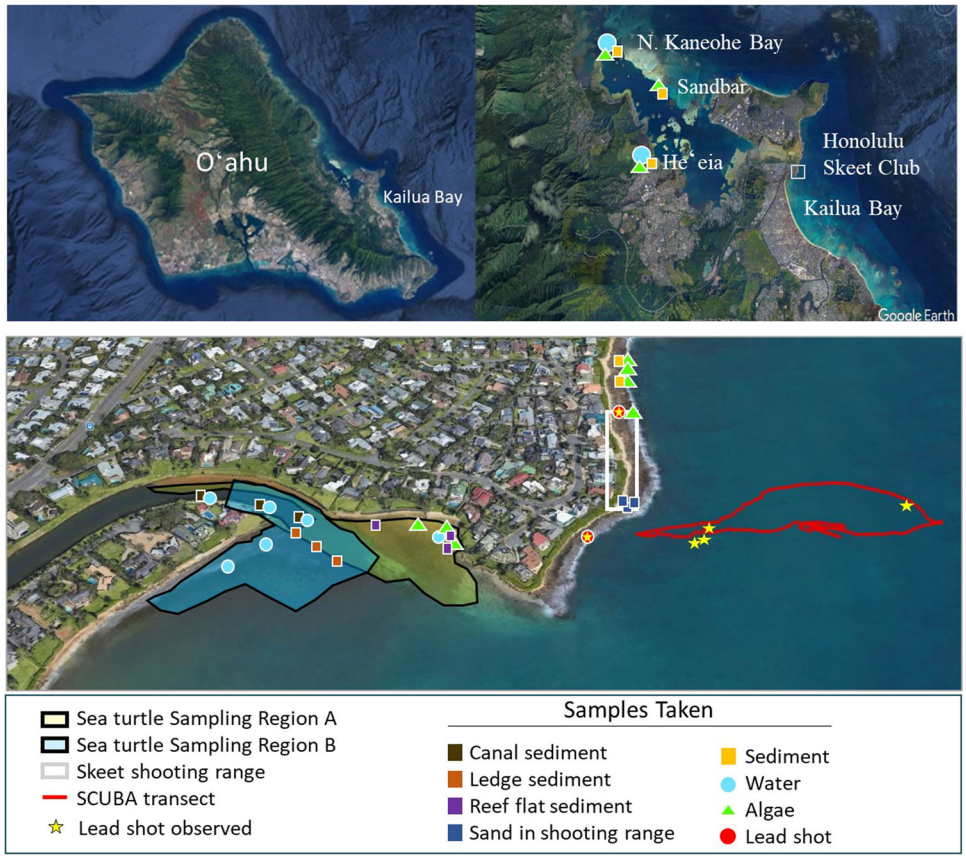

FIGURE 1: Sampling locations of environmental samples from East O'ahu and green turtles from Kailua Bay.

Lubbock, Texas, USA, for analysis with subsequent transfer to Baylor University in Waco, Texas, USA, for metals quantification.

Seawater, sediment, and algae samples were collected from Kailua Bay while snorkeling wearing gloves (Kimberly-Clark Professional™ Kimtech Pure™ G5 Co-Polymer Gloves) on October 9, 2017. Locations and additional information can be found in Supporting Information, Tables S2–S4. Seawater samples were collected by opening a sterile 50-ml centrifuge tube approximately 0.6 m underwater until filled, then capped underwater. Sediment and algae samples were taken in Ziploc bags. Sediment samples were collected from the top layers of the sediment by turning bags inside out and taking a handful of sediment. Bags were turned right side out and sealed while underwater and brought to the surface. Two known diet items (algae) of sea turtles inhabiting this site (*Acanthophora spicifera* and *Amansia* spp.) were collected (Arthur & Balazs, 2008). Algae samples were taken by turning the Ziploc bags inside out and taking a handful of algae, being careful to not remove the holdfast, and turning the bags right side out. Seawater samples were taken from the canal ($n = 3$) and reef flat ($n = 3$), sediment samples from the canal ($n = 3$), ledge ($n = 3$), and reef flat ($n = 3$), and algae samples solely from the reef flat ($n = 4$; Figure 1). Sediment and algae (*A. spicifera*) samples were collected from the Kaimalino rocky shoreline and tidepools (sediment $n = 7$, algae $n = 4$) in the same manner except by walking. Additional sediment, algae (*A. spicifera* and *Gracilliaria salicornia*), and water samples were collected from three other sites from East O'ahu to serve as comparison sites: Northern Kane'ohe Bay (sediment $n = 9$, algae $n = 3$, water $n = 5$), Kane'ohe Sandbar (sediment $n = 2$, algae $n = 1$), and He'eia (sediment $n = 4$, algae $n = 3$, water $n = 3$; Figure 1 and Supporting Information, Tables S2–S4).

In-water survey for Pb shot

The coastline directly offshore of the historic skeet shooting range was surveyed for Pb shot while scuba diving with scooters in June 2018. A track was captured using a floating Garmin GPS tethered to a diver (Figure 1). The depth of the area surveyed ranged from 3 to 8 m. The seafloor consists of rock inhabited by small patches of hard corals and algae with sand settled in low areas. A variety of fishes, eels, invertebrates, and sea turtles inhabit the area. Divers noted the GPS location of Pb shot piles when observed. These Pb shot piles are denoted by yellow stars in Figure 1.

Sample digestion and inductively coupled plasma-mass spectrometry analysis

Algae, scute, and blood samples were digested by a modified Small Mass, Affordable, Rapid, Transfer-less (SMART)

method (French et al., 2017; Shaw et al., 2021). Algae samples were oven dried overnight at 55 °C to complete dryness. The average moisture contents of *A. spicifera*, *G. salicornia*, and *Amansia* spp. were 63.1%, 76.1%, and 52.9%, respectively (Supporting Information, Table S2). Subsamples (0.2 g) were combined with trace metals grade nitric acid (HNO_3, 0.2 ml, 5.53 mol/L; Fisher Scientific), hydrochloric acid (HCl, 0.1 ml, 0.99 mol/L; Fisher Scientific), and 0.1 ml of deionized water in a 15-ml centrifuge tube and placed in a hot water bath (Precision Reciprocal Shaking Bath Model 66800) at 95 °C (±5 °C) for 1 h. The samples were cooled to room temperature, 0.1 ml of high-purity 30% hydrogen peroxide (H_2O_2; Fisher Scientific) was added, and the samples were placed back in the hot water bath for 30 min. After cooling, the samples were filtered through a 0.45-μm polytetrafluoroethylene (PTFE) filter and diluted to 10 ml with deionized water. An algae sample was used to create an in-house matrix control material. The alga was spiked with a custom multi-element standard containing aluminum (Al), Sb, As, cadmium (Cd), chromium (Cr), cobalt (Co), copper (Cu), Pb, mercury (Hg), nickel (Ni), selenium (Se), silver (Ag), strontium (Sr), tin (Sn), vanadium (V), and zinc (Zn; TTUNIV-1, Inorganic Ventures) at 100 ng/g. The trace element concentrations from the natural sample were subtracted from the spiked sample.

Whole-blood samples (0.2 ml weighed) were combined with 0.4 ml of HNO_3 (5.53 mol/L), 0.1 ml of HCl (0.99 mol/L), and 0.1 ml of deionized water in a 15-ml centrifuge tube. The samples were vortexed, sonicated for 10 min, then placed in a hot water bath at 95 °C (±5 °C) for 1 h. The samples were cooled to room temperature, 30% H_2O_2 (0.2 ml) was added, and the samples were vortexed and sonicated (10 min) again before being placed back in the hot water bath for 30 min. An additional 0.2 ml of 30% H_2O_2 was added, followed by vortexing and sonicating before the samples were placed in the hot water bath for an additional 30 min. After cooling, the samples were filtered through a 0.45-μm PTFE filter and diluted with deionized water to 10 ml.

Scute samples (0.1 g) were combined with HNO_3 (0.2 ml, 5.53 mol/L), HCl (0.1 ml, 0.99 mol/L), and 0.1 ml of deionized water in a 15-ml centrifuge tube, sonicated for 5 min, and placed in a hot water bath at 90 °C (±5 °C) for 1 h. The samples were cooled to room temperature, 30% H_2O_2 (0.1 ml) was added, then the samples were sonicated for 5 min and placed back in the hot water bath for 30 min. After cooling, the samples were filtered through a 0.45-μm PTFE filter and diluted with deionized water to 10 ml.

Sediment samples were digested according to a modified US Environmental Protection Agency (USEPA) method 3050B. Sediment samples were dried to completion at 55 °C. The average sediment moisture content was 38.3% (Supporting Information, Table S3). An aliquot of the sediment (1.0 g) was placed in a 150-ml beaker with 10 ml of Milli-Q water. Trace metals grade HNO_3 (10 ml, 5.53 mol/L) was added, and the beaker placed on a hot plate at 95 °C (±5 °C) and refluxed for 15 min. The samples were cooled, an additional 5 ml HNO_3 was added, and the samples were refluxed for 2 h. Deionized water (2.0 ml) and 30% H_2O_2 (3.0 ml) were added, and the samples heated until effervescence was minimal. An additional 4 ml of H_2O_2 was added in 1-ml aliquots until the resulting reaction was minimal. Samples were refluxed for an additional 2 h, cooled, and filtered through filter paper (Whatman No. 41 Ashless), then diluted with deionized water to 100 ml. Sediment samples were diluted a second time with deionized water to a 1:75 000 volume fraction for analysis.

Seawater samples were acidified based on USEPA method 6020A. Seawater samples (0.5 ml) were acidified with 1.4 ml of HNO_3 (5.53 mol/L) and subsequently diluted with deionized water to 1:100 volume fraction for analysis. An in-house matrix control material was made from a Kane'ohe Bay seawater sample spiked with a multi-element standard (TTUNIV-1, Inorganic Ventures). The trace element concentrations in the natural seawater were then subtracted from the spiked sample to account for the natural trace element concentrations in the seawater samples. The in-house matrix spike control materials for water and algae match the spiked concentration by ±18%.

Arsenic, Cd, Co, Cr, Ni, Pb, Sb, Se, Sr, and V were measured via inductively coupled plasma-mass spectrometry (ICP-MS) in helium collision mode (Agilent 7900 ICP-MS) equipped with an Agilent Technologies ASX-500 Series autosampler (Agilent). A seven-point multi-element calibration curve from 0.1 to 1000 ng/g using a custom multi-element calibration standard (TTUNIV-1, Inorganic Ventures) was analyzed at the beginning and end of every run with all r^2 values >0.9996. Additional check standards of 10 or 50 ng/g were run every 10 samples to ensure the calibration curve remained within 10% of the known concentration (Environmental Protection Agency, January 1998). Internal standards bismuth (Bi), germanium (Ge), indium (In), lutetium (Lu), rhodium (Rh), scandium (Sc), and terbium (Tb; Agilent Technologies) were added online and samples were reanalyzed if the recovery was outside the acceptable range of 80%–120%. Analytical methods were validated using certified reference materials (CRMs) of similar matrices. Seronorm™ Trace Elements Whole Blood L-3 ($n = 4$; ref 210313, lot 1509408, Sero AS) was digested with the same methods as blood samples, DOLT-5 Dogfish CRM for Trace Metals and Other Constituents ($n = 3$) was digested with the same methods as the scute samples, and SRM 2711a Montana II Soil Moderately Elevated Trace Element Concentrations ($n = 5$) was digested with the same methods as the sediment samples. Measured values of Pb and As in the Seronorm CRM were in agreement with the certified values and Sb overlapped with the certified value (Supporting Information, Table S5). In the Dolt 5 CRM, Pb was in agreement and As was within 20% of the certified values. Pb and As were in agreement with the values in the SRM 2711a Montana II Soil Table A1, leachable concentrations determined using USEPA methods 200.7 and 3050B. Lead isotope ratios were measured by ICP-MS (Supporting Information, Table S5). NIST SRM 981 Common Lead Isotopic Standard was used to determine the accuracy of the $^{206}Pb/^{207}Pb$ ratio to be 98.9%. Detection limits in this SRM were 0.30 ng/g for ^{206}Pb and 0.29 ng/g for ^{207}Pb. Measured concentrations for all elements in all CRMs can be found in Supporting Information, Table S5. The instrument detection limit (IDL) was determined by analyzing seven replicates of the

0.1 ng/g multi-element standard (Inorganic Ventures) and multiplying the standard deviation of these replicates by the Student *t*-test value (3.143), giving IDLs of 0.29 ng/g for Pb, 0.14 ng/g for Sb, and 0.17 ng/g for As (Supporting Information, Table S5; Creed et al., 1994). The limit of quantification (LOQ) was calculated by multiplying the lowest concentration of the calibration curve by the dilution factor. The LOQs were 10, 0.29, 2, 0.83, and 7500 ng/g for seawater, algae, scute, blood, and sediment, respectively. Laboratory (method) blanks and field blanks (produced from the same lot numbers of blood collection samples) were subtracted from the samples. All blood values are in ng/g wet mass (wm), scutes are in ng/g dry mass (dm; as received), algae in ng/g dm (oven dried), and sediment in mg/kg dm (oven dried).

Statistical analyses and data handling

All statistical analyses were performed using the statistical program JMP 14.1.0 (SAS Institute) or R (Ver 3.2.3, The R foundation for Statistical Computing). The Nondetects and Data Analysis for Environmental Data (NADA) package in R, which is recommended for left censored data, was used for any analyses containing samples below the LOQ (Helsel, 2005). The Shapiro–Wilks test was used to test for normality. Most data were not normally distributed so nonparametric tests were used. Spearman correlations were performed between turtle size (SCL or mass) and elemental concentrations, between turtle growth rate and elemental concentrations, between blood elemental concentrations and scute elemental concentrations, and between sediment and algae elemental concentrations. A list of all correlations performed can be found in Supporting Information, Table S6. Nonparametric group difference tests (empirical cumulative distribution function differences for left censored data using the R NADA function cendiff) were used to examine differences within blood/scute elemental concentrations by location or year and within sediment/algae elemental concentrations by location. A Wilcoxon Sum Rank Test was used to determine if there was a difference in blood elemental concentrations between size classes (<45 cm SCL or >45 cm SCL). Because Hawaiian green turtles recruit from a carnivorous pelagic stage to nearshore herbivores approximately 35 cm SCL, this size cutoff divided the turtle samples into suspected younger turtles ($n = 10$) that recruited a few years more recently than larger, potentially older, turtles ($n = 35$) that may have more years of residency in Kailua Bay (Suhring et al., 2021). A list of all group difference statistical tests performed (including *n*, *df*, test statistic, and exact *p* value) can be found in Supporting Information, Table S7. A repeated measures *t*-test was performed to determine changes in As or Pb concentrations in blood and scute samples taken from an individual over time. The *t*-score was calculated by dividing the mean of the differences between individuals over the estimated standard error of the mean of differences (Zar, 1996). The *t*-score was then compared with the *t*-value to determine if the null hypothesis that there is no difference between time points should be rejected. The body condition index (BCI) of each turtle was calculated as the body mass (kg) divided by the cube of the SCL (cm) and multiplied by 100 000 (Keller et al., 2004). Spearman correlations were performed between BCI and blood As or Pb concentrations. A screening level risk assessment was conducted by (1) assessing correlations between elemental concentrations in these turtles to indicators of their health (PCV, BCI, and growth rate), (2) comparing the concentrations measured in these turtles to other wild species to better understand how Kailua Bay green turtle exposure compares to other species or locations, (3) comparing these concentrations to concentrations known to cause toxicity in other taxa, and (4) comparing estimated daily exposure of these turtles to doses known to cause sublethal effects in a laboratory-exposed reptile model species. Estimated daily intake was calculated to estimate the daily exposure of turtles in Kailua Bay to Pb (Perrault, 2014; Shaw et al., 2021). Green sea turtles have a daily food intake of 127 g (dm) per day (Williams, 1988). The diet of turtles in Kane'ohe Bay is composed of *A. spicifera* (44%), *Amansia* spp. (30%), and other algae and seagrass (26%; Russell & Balazs, 2009). The weight of each diet item eaten per day (*Acanthaphora* 56 g, *Amansia* 38 g, other algae and seagrass 33 g) was multiplied by the concentration of Pb measured in each algal type. Pb concentrations in "other algae and seagrass" were estimated to be the average of the two algae Pb concentrations. These results were added together, giving the potential Pb exposure per day. The growth rate of the 10 recaptured turtles was determined by dividing the millimeters of growth by the number of days between measurements.

RESULTS AND DISCUSSION

Contaminant metal levels in turtles

Sea turtles ranged in size from 40.9 to 84.9 cm SCL (Supporting Information, Table S1). Additional sampling data, size, mass, and sex of individual turtles can be found in Supporting Information, Table S1. Based on the SCL, the turtles could be classified as juvenile to subadult. Two turtles had external FP tumors. Packed cell volume ranged from 11% to 41.5%. Healthy PCV range in green sea turtles is 28%–40% (Maier et al., 2004). The two turtles with the lowest PCV (11% and 22%) were also the only turtles to show signs of emaciation, and one of these had external FP tumors.

Each trace element concentration measured in individual turtle samples can be found in Supporting Information, Tables S8 and S9. Lead and As were found above the LOQ in all blood ($n = 45$) and scute ($n = 44$) samples. Antimony was greater than the LOQ in two blood samples and 11.4% of scute samples (Table 1). The mean and standard deviation could not be determined for Sb because <20% of the samples had detectable concentrations (Helsel, 2005). In humans, the highest concentration of Sb is found in hair, which is made of keratin similar to sea turtle scutes. A greater number of scute samples (11.4%) in the present study had detectable concentrations of Sb than blood samples ($n = 4.4\%$; Table 1). No significant relationships were found between blood As and blood Pb, scute As and scute Pb, scute Pb and blood Pb, or scute As and blood

TABLE 1: Arsenic (As), lead (Pb), and antimony (Sb) concentrations in blood (ng/g wet mass) and scute samples (ng/g dry mass; SD = 1 SD)

		Blood				Scute				
Location	Element	Median	Mean (SD)	Range	% Detected	Median	Mean (SD)	Range	% Detected	Reference
Kailua Bay	As	125	299 (399)	26.4–1950	100	330	512 (442)	114–1830	100	The present study
	Pb	301	328 (195)	15.6–923	100	189	201 (123)	23.6–585	100	
	Sb	—	—	<DL–1.04	4.4	—	—	<DL–24.3	11.4	
Kapoho Bay	As	28	35.6 (24.2)		100	138	144 (22.8)		100	Shaw et al. (2021)
	Pb	55.3	69.3 (30.5)		100	26.8	32.9 (12.0)		60	
	Sb					—	—	—	0	
Sea Life Park	As	22.8	28.8 (17.3)		100	9.2	30.3 (55.8)		50	Shaw et al. (2021)
	Pb	25.1	24.6 (12.7)		100	14.6	20.8 (16.8)		33.3	
	Sb	—	—	—	0	—	—	—	0	

Blood: $n = 45$ samples from $n = 35$ individual turtles, occasionally turtles were resampled on recapture. Scute: $n = 44$ samples from 35 individual turtles, occasionally turtles were resampled on recapture; one turtle was missing a scute sample. Kapoho Bay, HI, and Sea Life Park Hawaii are included as reference sites (Shaw et al., 2021). DL = detection limit.

As (Supporting Information, Table S6). Additional information on statistical tests performed can be found in Supporting Information, Tables S6 and S7. Because the focus of the present study is on the elements found in Pb shot (Pb, As, and Sb), only these three elements will be discussed further.

Lead has been measured in the blood of only one other wild green turtle population in the Hawaiian Islands. Turtles from Kapoho Bay on the Island of Hawai'i (now covered in new land from the 2018 Kīlauea eruption) had a mean blood Pb concentration of 69.3 ± 30.5 ng/g and scute Pb concentration of 32.9 ± 12.9 ng/g (Shaw et al., 2021). Kapoho Bay turtles had significantly lower blood and scute Pb concentrations than the turtles in the present study (p value $= 4 \times 10^{-4}$ and 0.003, respectively). Similarly, Kapoho Bay turtles had significantly lower concentrations of As in their blood (35.6 ± 24.2 ng/g) and scute (144 ± 22.8 ng/g) than the Kailua Bay turtles (p value $= 9 \times 10^{-4}$ and 0.008, respectively). This comparison suggests that Kailua Bay has elevated Pb and As compared with Kapoho Bay, which may originate from the Pb shot in this area.

Relationships between elemental concentrations of As or Pb and turtle size/age were explored. Sea turtles were then grouped based on SCL into those suspected to have lived in the area for less time (SCL ≤ 45 cm) and those with possibly longer residency (SCL > 45 cm). No differences were observed between turtle size groups in blood As or Pb (Wilcoxon p value = 0.2 and 0.8, respectively; Supporting Information, Figures S1 and S2) or in scute As (p value = 0.7; Supporting Information, Figure S3). Although not significantly different, larger turtles had slightly greater scute Pb concentrations (p value = 0.07; Supporting Information, Figure S4). We expected that larger turtles, having longer residence times in this area of Pb shot contamination, would have greater concentrations, especially in their scutes, which reflect longer-term accumulation than the blood. Taken together, these results suggest that scute tissue better reflects the long-term accumulation of elevated Pb from Kailua Bay. Turtles spending more years in Kailua Bay can reasonably be expected to accumulate higher cumulative levels in scutes than more recently recruited turtles. Furthermore, the turtles included in the present study ranged from 40.9 to 84.9 cm SCL. Had the present study included smaller turtles <40 cm SCL who would be more recent recruits to the area, a greater difference in scute Pb may have been seen. Because even the recently recruited turtles could have been in the bay for several years already, this comparison indicates that their blood concentrations reached higher concentrations quickly. Lack of difference in blood Pb concentrations between the two groups and the marginal difference in scutes between the groups helps illustrate the toxicokinetics of Pb in the blood and scute of sea turtles. Lead will only remain in the blood for a few weeks to months before it is distributed to other tissues. The lack of difference in blood Pb is therefore not indicative of residence time in Kailua Bay but an artifact of the time it takes Pb to disperse throughout the body. After incorporation into the scute, these elements likely become metabolically inactive and are not available for remobilization (Day et al., 2005). Additional research on the toxicokinetics of Pb in sea turtles would provide critical information on the distribution, storage, and excretion of Pb. No correlation was observed among concentrations in blood or scute and sea turtle SCL or mass (Supporting Information, Figures S5–S8).

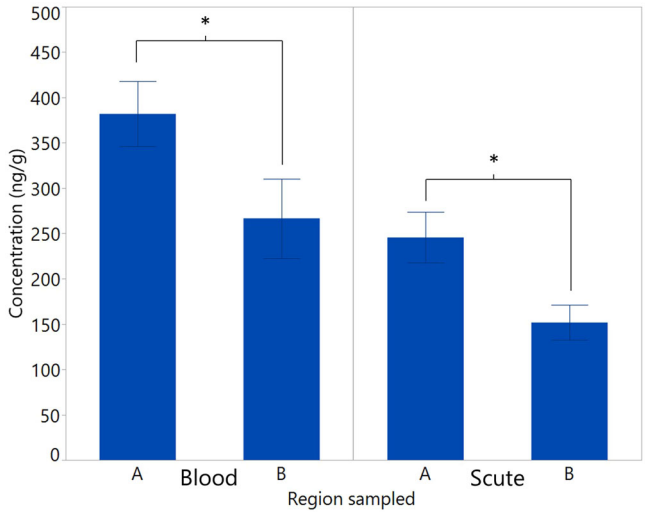

FIGURE 2: Lead concentrations in blood (ng/g wm) and scutes (ng/g dm) of green sea turtles sampled from two adjacent and slightly overlapping sites in Kailua Bay. Region A is closer to the skeet shooting range than Region B. The asterisks indicate a significant difference between regions (p value < 0.05).

Correlative trends across space and time

Turtles were sampled and brought ashore to one of two areas, Region A located to the north of the canal and Region B located to the south of the canal (Figure 2). Sampling Region A is located nearest to the skeet shooting range. There was no difference in As in scute or blood between the two groups (blood p value = 0.6, scute p value = 0.7; Supporting Information, Table S7) but Pb concentrations were significantly greater in blood (cendiff p value = 0.002) and scute (p value = 0.007) from turtles sampled in Region A than Region B (Figure 2). This finding supports the hypothesis that turtles in closer proximity to the accumulated Pb shot near the old shooting range are exposed to elevated Pb, either because the Pb from shot may transport into this region's habitat via currents or because these turtles may graze on algae in the area impacted by the shooting range. This difference in Pb concentrations between capture locations corroborates the finding that green turtles have high site fidelity to this region, as noted in Francke et al. (2013). In addition, eight of the 10 turtles captured twice were captured in the same sampling region (A or B). The present study confirms the high residency of sea turtles in Kailua Bay and emphasizes the site fidelity that makes sea turtles good bioindicators of contamination in the environment. Across time, blood Pb concentrations were significantly lower in turtles captured in 2011 than in 2012 (cendiff p value = 0.006) and 2013 (p value = 0.03), although blood As and scute Pb and As did not differ across time (Figure 3). This difference across years may have been influenced by a pellet cleanup event that occurred in 2009. Lead shot was removed from the sand and tide pools near the old shooting range in a joint project funded by the Hawai'i State Department of Health and the Department of Land and Natural Resources (Aguiar, 2009). However, more Pb shot is exposed after every storm event, washing additional Pb shot pellets into tide pools and nearshore waters, and the 2 years between the cleanup event and sampling in 2011 likely allowed more Pb shot into the area (Kailua resident, personal communication, October 2017). This increase in mean blood Pb concentrations of the turtles in Kailua Bay from 2011 to 2013 may be due to more Pb pellets being released into nearshore waters each year. While cleanup events such as the one held in 2009 removed Pb pellets from the shore, preventing children from being exposed to the pellets for the short term, cleanup events would have to be done each year to reduce sea turtle exposure to pellets as well as the subsequent leaching of Pb from the pellets. It is important to note that the difference across years could also be influenced by the confounding factor of region sampled. All turtles sampled in 2011 for the present study were only sampled in Region B, where concentrations were lesser, whereas turtles sampled in 2012 and 2013 were from both regions.

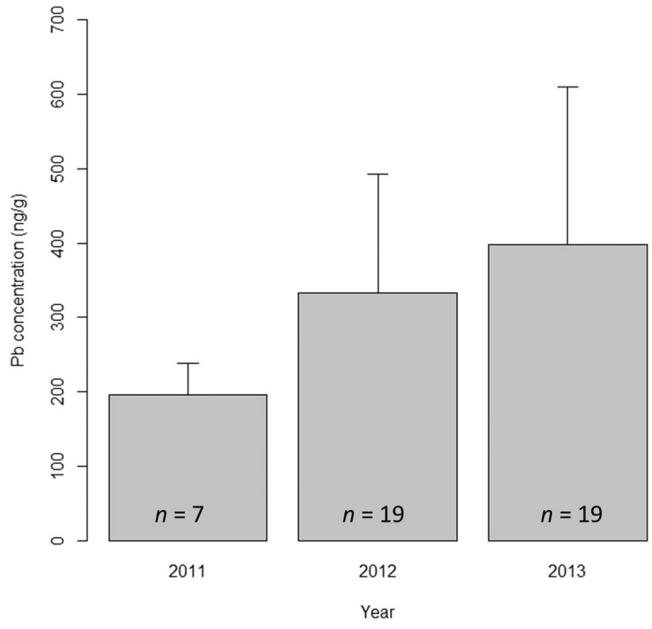

FIGURE 3: Blood lead (Pb) concentrations (ng/g wm) in Kailua Bay, O'ahu green sea turtles sampled in 2011, 2012, and 2013.

Metals concentrations in sediment, water, and algae

Sediment concentrations of Pb, As, and Sb did not differ between the ledge, channel, and reef in Kailua Bay (p value = 0.7, 0.4, and 0.3, respectively). Because these samples were not different, they were grouped together as "Kailua Bay Combined" in subsequent calculations (Table 2). North Kane'ohe Bay, Sandbar, and He'eia were chosen as comparative sites because they are geographically isolated from the historic skeet shooting range in Kailua Bay by the Mokapu Peninsula. Lead concentrations in Kailua Bay sediment were significantly greater than in sediment in North Kane'ohe (p value < 0.001) and He'eia (p-value = 0.03), but not in sediment within the shooting range (p value = 0.6). Sediment As concentrations did not differ significantly between locations. Sandbar sediment samples were not included in statistical analyses because of their low sample size, but the samples did not contain Pb, As, or Sb above the LOQ. Lead strongly adsorbs to organic matter in sediment, so it is no surprise that the sandbar, which consists almost completely of sand particles with little organic matter, had undetectable concentrations of Pb (Al-Abdali et al., 1996).

The highest concentrations of Pb in algae samples were seen in Kailua Bay (Table 2). The concentrations were significantly greater than algae from He'eia (p value = 0.03), North Kane'ohe (p value = 0.03), and the skeet shooting range (p value = 0.01). The algal species were not identical across the sampling sites (Supporting Information, Table S2), which may influence the concentration differences. However, when considering only *A. spicifera* which was sampled in Kailua Bay, Kailua Skeet, Kane'ohe Sandbar, and He'eia, the Pb concentrations were considerably greater in Kailua Bay. Algae samples in the skeet shooting range could only be accessed from tidepools along the rocky shoreline bench, where turtles do not feed due to dangerous surf. Just beyond the tidepools the shoreline descends like a cliff into the sea. Turtles inhabit this underwater cliff where algae could not be reached. Thus, Pb concentrations may differ between the sampled algae and the algae available for turtles to eat. Arsenic concentrations in

TABLE 2: Lead (Pb), arsenic (As), and antimony (Sb; ng/g) in sediment and algae samples in Kailua Bay, the Skeet shooting range, and the other sites

				As			Pb			Sb		
	Location	Sample type	*n*	Mean (SD)	Range	% Detected	Mean (SD)	Range	% Detected	Mean (SD)	Range	% Detected
Kailua Bay	Reef flat	Algae	4	5490 (1700)[ac]	<LOD–6890	75	26 000 (14 300)[a]	12 200–39 100	100	113 (39.3)[a]	63.5–153	100
	Reef flat	Sediment	3	<LOQ	—	0	53 200 (19 100)	33 300–71 400	100	<LOQ	—	0
	Channel	Sediment	3	<LOQ	—	0	61 700 (12 400)	48 700–73 300	100	<LOQ	—	0
	Canal	Sediment	3	—	<LOD–57 600	33.3	98 000 (89 800)	24 100–198 000	100	—	<LOQ–259 000	33.3
	Kailua Bay combined	Sediment	9	—	<LOD–57 600	11.2	71 000 (50 700)[B]	24 100–198 000	100	—	<LOQ–259 000	11.2
Skeet Shooting Range	Skeet shooting range	Algae	4	3060 (315)[c]	2710–3440	100	56.2 (16.6)[b]	<LOQ–76.0	50	86.6 (29.7)[a]	46.2–111	100
	Skeet shooting range	Sediment	5		<LOQ–22 600	20	2 500 000 (5 480 000)[AB]	19 100–12 300 000	100	—	<LOQ–494	20
	Lead shot 1	Lead pellets	1	—	2 720 000	100	—	388 000 000	100	—	182 000	100
	Lead shot 2	Lead pellets	1	—	3 120 000	100	—	653 000 000	100	—	318 000	100
Reference sites	N. Kane'ohe Bay	Algae	3	10 800 (6830)[ab]	4330–17 900	100	104 (110)[b]	<LOQ–225	66.7	1560 (2160)[b]	180–4050	100
	N. Kane'ohe Bay	Sediment	9	11 100 (1460)	<LOQ–13 600	33.3	<LOQ[C]	—	0	—	—	0
	Sandbar	Algae	1	—	16 000	100	<LOQ	—	0	—	198	100
	Sandbar	Sediment	2	<LOQ	—	0	<LOQ	—	0	—	—	0
	He'eia	Algae	3	37 700 (23 800)[b]	10 200–52 700	100	—[b]	<LOQ–74.6	33.3	91.7 (33.0)[a]	63.2–128	100
	He'eia	Sediment	4	18 800 (550)	<LOQ–19 500	75	21 000 (23 900)[A]	<LOQ–54 000	50	—	—	0

Statistically different concentrations are indicated by capital letters after the mean in sediment and lowercase letters in algae.
LOD = limit of detection; LOQ = limit of quantification.

algae were significantly different between sites, with the greatest concentrations seen in Kane'ohe Bay, Sandbar, and He'eia. Lead shot is not the only source of As in the ocean. Arsenic is ubiquitous and can be introduced from natural processes such as volcanic eruptions or anthropogenic sources such as As-based pesticides or smelters (Andreae, 1980).

Turtles in Kailua Bay are highly resident to the area and feed primarily in Kailua Bay. The high Pb concentrations in their blood and scutes is likely correlated to the high Pb concentrations measured in their food source (algae). In Kapoho Bay, *Amansia* spp. ($n = 1$, 1030 ng/g dm) had Pb concentrations 15-fold lower than in Kailua Bay ($n = 1$, 15 100 ng/g dm; Shaw et al., 2021). Likewise, different algae species from Kapoho Bay (*G. salicornia* $n = 1$, 388 ng/g dm) had much lower Pb concentrations than those from Kailua Bay (*A. spicifera* $n = 3$, 29 600 ± 15 100 ng/g dm).

Lead, As, and Sb were below the LOQ in all water samples. Most Pb found in the water column is bound to either small particles that stay suspended in the water column or larger particles that eventually precipitate to the ocean floor (Sparling, 2016).

Environmental samples (sediment, algae, and water) were collected 4–6 years after the sampling of sea turtles, which could confound relationships between measured Pb concentrations in environmental samples and turtle samples. Although Pb shot remains a problem in the area, concentrations of Pb in environmental samples collected in 2017 are potentially different to concentrations in 2011–2013 when the turtles were sampled. Storms and the resulting runoff that causes more Pb shot to be exposed potentially cause temporal variability in Pb concentrations of the surrounding environment and additional research should be done on these potential variations.

Metals in tissues of recaptured turtles

The changes in blood and scute Pb and As concentrations were determined for turtles recaptured and sampled twice over the 3-year period (Table 3). Turtles were recaptured an average of 420 days later. No significant changes ($p > 0.05$) were observed in blood As ($t = 1.59$), blood Pb ($t = 0.766$), scute As ($t = 1.76$) or scute Pb ($t = -0.091$) between sampling events (Supporting Information, Table S10). This suggests that either (1) the exposure of turtles in Kailua Bay over the study time period remains relatively stable, or (2) the variability between sampling events is so large that a trend could not be detected. The blood concentrations changed drastically within individual turtles between sampling events (the average [SD] was 215% [609%] increase for Pb and 740% [1350%] increase for As), but less so for scutes (24.3% [70.7%] increase in scute Pb and 47.5% [46.0%] increase in As). For example, the smallest turtle (470D01034B), potentially a recent recruit when sampled the first time, showed a 1932% increase in blood Pb over 1 year, while its scute Pb decreased by 33%. Blood is not an ideal tissue to track long-term changes in Pb or As exposure. Whole blood represents contaminant exposure in the previous weeks to months, not necessarily accumulation over years (Takeuchi et al., 2016; Villa et al., 2015). In contrast,

TABLE 3: Lead (Pb) and arsenic (As) concentrations in blood (ng/g wet mass) and scute (ng/g dry mass) of recaptured turtles

	Date			SCL (cm)		Blood Pb		Blood As		Scute Pb		Scute As	
Turtle ID	First capture	Second capture	Days between sampling	First capture	Second capture	First capture	Second capture	First capture	Second capture	First capture	Second capture	First capture	Secod capture
4601705479	7/10/12	7/9/13	364	55.2	56.7	193	236	224	686	99.3	135	397	890
4608021627	7/11/12	7/9/13	363	52.6	54.6	355	32.8	41.2	151	253	288	114	185
413522604F	3/30/11	7/8/13	831	61.4	63.0	168	234	202	26.4	352	223	148	233
443A197133	7/10/12	7/8/13	363	53.1	54.1	536	486	45.1	457	214	266	148	182
445258695E	7/10/12	7/8/13	363	55.0	55.8	376	101	163	454	335	369	328	627
45285D1A44	7/11/12	7/9/13	363	55.2	56.3	386	887	38.6	97.6	545	377	404	557
470D01034B	7/12/12	7/8/13	361	44.1	46.6	16	317	44.2	1950	246	166	498	654
483A2C017D	3/30/11	7/12/12	470	47.6	49.7	138	338	80.5	81.5	89.8	112	166	303
4A2D793C0A	7/10/12	7/9/13	364	52.3	54.7	369	224	62.4	1020	194	241	1280	1220
4A3973601B	7/10/12	7/8/13	363	44.8	46.9	344	682	759	178	59.3	184	852	594

SCL = straight carapace length.

scutes can provide information on long-term exposure to contaminants because concentrations in this tissue are expected to be more stable through time (Bezerra et al., 2013; Bryan, 2013; Day et al., 2005; Innis et al., 2008; Komoroske et al., 2011; Perrault et al., 2017; Sakai et al., 2000; van de Merwe, 2008). For this reason, the lack of differences in scutes between sampling events was not surprising because an animal resident to a particular area will deposit similar concentrations in each new layer of keratin. Older layers of scute could reflect a different contaminant exposure from a past life stage or foraging location, but those layers would need to be sampled before they are shed or scraped off naturally. The stability seen in the scutes in the present study suggests that keratin layers deposited during the pelagic life stage were gone before we sampled these turtles for the first time, or the sampling method homogenizes so many different layers that changes may be masked. It is important to mention that the entire surface area of the same scute was sampled both times, so the outer, older layers of the carapace were sampled the first time. The second sample would most certainly contain more recently deposited keratin. In addition, scutes grow continuously and older areas (posterior portion) become thicker over time while areas of new growth expansion (anterior portion) are thinner (Reich et al., 2007). By scraping the entire scute evenly, both older areas and newer areas are sampled, mixing the time periods together. For certain time order, future studies should compare scute Pb and As concentrations of pelagic immature green turtles captured as by-catch in the Hawaiian longline fishery (samples are available in the BEMAST) to determine if levels are lower than in resident turtles from Kailua Bay.

Pb isotopes

Lead isotope ratios $^{206}Pb/^{207}Pb$ were determined for blood, scute, and environmental samples in an effort to determine if the source of the Pb exposure was the Pb shot or another contamination source (Figure 4). All of the blood and scute samples fall within the known range of ratios for Pb shot. The wide range of ratios for Pb shot is due to multiple manufacturers using Pb from different ores as well as the mixing and recycling of Pb. The larger ranges of $^{206}Pb/^{207}Pb$ ratios in sediment samples, specifically the Kane'ohe Bay sediment, are due to various sources of Pb contamination. Multiple streams bring runoff into Kailua Bay and Marine Core Base Hawai'i is located in the bay. The canals and streams emptying into Kailua and Kane'ohe Bays also likely accumulate Pb from a combination of sources, including the Pb shot from the skeet shooting range and from runoff from inland areas that surround the marsh (Figure 1). This is seen in the range of isotope ratios in Kailua Bay sediment (Figure 4). All Pb isotopes seen in the blood and scute samples are within the range of Pb shot, indicating Pb shot may be a major source of Pb in the turtles. One of the scute samples was also within the isotope range of leaded gasoline, but this range is wholly within the larger Pb shot range, making it challenging to point to an additional source other than known Pb shot in the region.

Lead isotope ratios were further examined for turtles sampled twice in the present study. Two turtles (443A197133 and 4528D1A44) had almost identical blood isotope ratios between the first and second time points (Figure 5). To have the same isotope ratio the turtles would have to be exposed to the same source of Pb. These two turtles likely have a small foraging area within Kailua Bay.

Heavy metals and health

Comparisons of Pb and As concentrations to health indicators can provide circumstantial evidence for possible toxic effects and are worth exploring. Marginally significant negative correlations were observed between blood Pb or blood As and

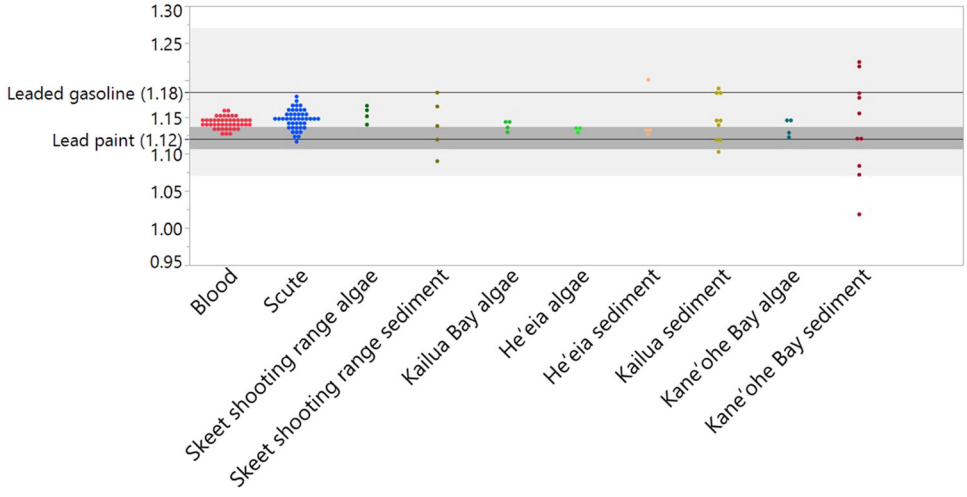

FIGURE 4: $^{206}Pb/^{207}Pb$ ratio in Kailua Bay green sea turtle blood and scute compared with other environmental samples collected from O'ahu. The light gray box is $^{206}Pb/^{207}Pb$ ratio in lead (Pb) shot pellets from multiple manufacturers (1.07–1.27) and the dark gray box is the $^{206}Pb/^{207}Pb$ ratio in Pb pellets collected from Kaimalino Beach (Svanberg et al., 2006). Leaded gasoline (1.184 ± 0.009) and Pb paint (1.12) are shown as horizontal lines (Sutherland et al., 2003; Svanberg et al., 2006). Additional anthropogenic values are illustrated by dashed horizontal lines: Pb in Pacific Marine Aerosols (green), Asian anthropogenic sources (orange) and the Ala Wai Canal (red) represent local anthropogenic Pb (Monastra et al., 2004).

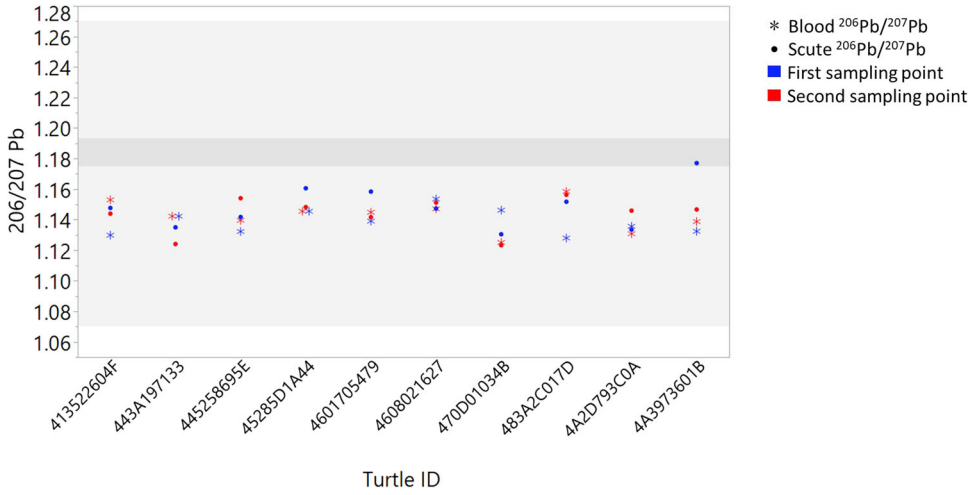

FIGURE 5: Blood $^{206}Pb/^{207}Pb$ isotope ratios for turtles sampled twice. Pb = lead.

PCV ($\rho = -0.294$, p value = 0.073, and $\rho = 0.311$, p value = 0.057, respectively; Supporting Information, Figures S9 and S10, Supporting Information, Table S6), although no significant relationship was seen between scute Pb or As and PCV. Similarly, American kestrels (*Falco sparverius*) fed a diet containing up to 448 ppm Pb showed no change in PCV (Custer et al., 1984; Franson et al., 1983). However, carp (*Cirrhinus mrigala*) exposed to sublethal amounts of Ni had significantly decreased PCV (Parthipan & Muniyan, 2013). This indicates some toxic heavy metals like Ni influence PCV while others like Pb may not.

It is interesting to note that the turtle with the highest blood Pb concentration (923 ng/g) was slightly emaciated with a low PCV (22%) and FP tumors. However, the other turtle in the present study with FP had a blood Pb concentration of 166 ng/g and a PCV of 34%. Because only two turtles with FP were included in the present study, no conclusions can be made about the relationship between FP and blood Pb concentration. Some studies have shown a correlation between elevated blood Pb and the occurrence of FP, while others have not (Bruno et al., 2021; da Silva et al., 2016). It thus remains unclear if Pb exposure is linked to FP.

The growth rate of the 10 recaptured turtles was compared with their initial blood and scute As and Pb concentrations using Kendal's tau correlation. Both blood As and Pb were significantly negatively correlated to growth rate (g/day p value = 0.032 and mm/day p value = 0.035, respectively; Supporting Information, Table S6). In addition, the BCI and blood Pb were negatively correlated (p value = 0.02; Supporting Information, Figure S11). Lead concentrations seen in Kailua Bay turtles may be affecting their health and thus slowing their growth. Kailua Bay turtles are mostly juveniles or subadults, thus possible effects of Pb on their reproductive success cannot be determined at this stage. No signs of Pb toxicosis have been reported in green turtles from San Diego Bay, which have much higher Pb concentrations, but the nesting and hatching success of these adult turtles is unknown. Many studies have predicted heavy metals, including Pb, affect hatching success, but more research is needed to substantiate this claim (Ehsanpour et al., 2014; Lam et al., 2006; Paez-Osuna et al., 2010; Sakai et al., 2000).

Risk assessment

Two species of algae were sampled in Kailua Bay: *A. spicifera* ($n = 3$) and *Amansia* spp. ($n = 1$). Lead concentrations in *A. spicifera* and *Amansia* spp. were 29 600 ± 15 100 ng/g and 15 100 ng/g, respectively. A potential daily Pb intake from food for Kailua Bay turtles was calculated at 3.08 mg Pb/day (Supporting Information, Table S11). The average mass of turtles sampled in Kailua Bay was 25.7 ± 14.1 kg. This gives an average dose of 0.12 mg/kg/day. Red-eared sliders in an acute exposure study injected once with Pb acetate indicated a no observed adverse effect level (NOAEL) of 100 mg/kg (Burger et al., 1998). Western fence lizards (*Sceloporus occidenalis*) exposed to Pb in a 14-day subacute study showed sublethal effects of weight loss and lowered food consumption at 62.5 mg/kg/day (Salice et al., 2009). A NOAEL for growth of 31.5 mg/kg/day was established for fence lizards exposed to Pb for 14 days. If the calculated daily intake of Pb for Kailua turtles is compared with the NOAEL of red-eared sliders, it could be assumed turtles in Kailua Bay are not at risk of Pb toxicity. However, the chronic exposure of wildlife to a pollutant is more problematic than acute exposure (Burger, 2008). The Western fence lizard had a much lower NOAEL for subacute exposure and although the NOAELs for turtles and lizards cannot be directly compared, chronic exposure will usually have a lower NOAEL than acute exposure. A precautionary approach would consider turtles spending years in Kailua Bay at risk of decreased body condition, lowered food consumption, or other changes in hematological parameters from the Pb contaminating this region.

The enzyme δ-aminolevulinic acid dehydratase (δ-ALAD) is inhibited by Pb, causing a decrease in heme group synthesis, and has been used to diagnose Pb exposure and effects (Martinez-Lopez et al., 2010). A significant negative correlation has been found between spur-thighed tortoise (*Testudo graeca*) blood Pb

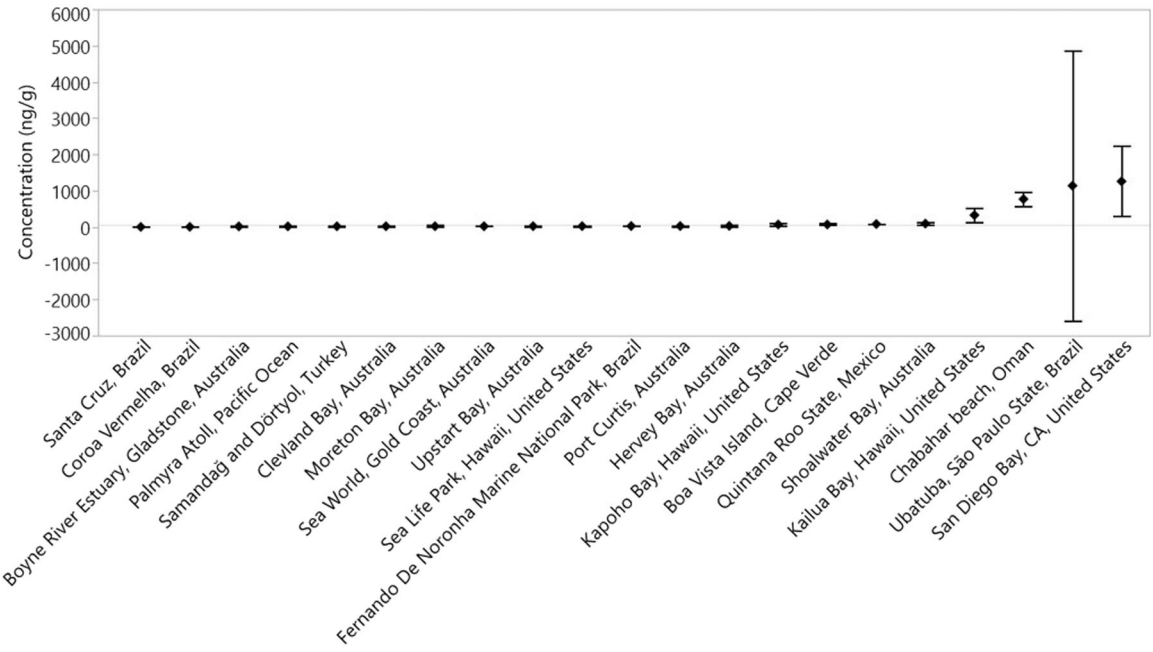

FIGURE 6: Blood lead concentrations (ng/g) in sea turtles around the world (Camacho et al., 2014; da Silva et al., 2016; Escobedo Mondragon et al., 2023; Finlayson et al., 2021; Gaus et al., 2012; Komoroske et al., 2011; McFadden et al., 2014; Miguel et al., 2022; Shaw et al., 2021; Sinaei & Bolouki, 2017; Villa et al., 2017; Yipel et al., 2017). Dots represent the mean and error bars are 1 SD. The gray shaded area is concentrations documented in green sea turtles from the Howick Islands, a relatively undisturbed region in Australia used as a reference population (Villa et al., 2017).

concentrations and δ-ALAD activity measured in whole blood (Martinez-Lopez et al., 2010). Tortoises with blood Pb concentrations >15.1 ng/g had a δ-ALAD activity level 30% below the mean value. The present study did not measure δ-ALAD activity, but all turtles in the present study were above this threshold, with the mean at 23 times higher. Future studies should include δ-ALAD activity to help determine if Pb toxicosis is occurring.

The scarcity of toxicological information on sea turtles makes it difficult to conduct a risk assessment, but Pb concentrations in green sea turtles from Kailua Bay can be compared with green sea turtle populations around the world for a better understanding of the risk Pb poses (Figure 6). Concentrations are not being compared amongst sea turtle species due to differences in feeding habits resulting in uneven exposure amongst species (Cortes-Gomez et al., 2017). Turtles foraging at the Howick Group of Islands were used as a reference population due to their distance from shore and potential anthropogenic contaminant sources (Villa et al., 2017). Sea turtles resident to Kailua Bay were found to have elevated blood Pb concentrations compared with the Howick Group of Islands turtles and turtles from most other locations around the world (Figure 6). Green turtles from Oman, Brazil, and San Diego had greater blood Pb concentrations than Kailua Bay, but it is unknown to the authors as to the health status of these turtles. The turtles in Kailua Bay, however, do not exhibit overt signs of Pb poisoning.

CONCLUSION

Kailua Bay is an important foraging ground for the Hawaiian green sea turtle and other animals living in the area. The present study demonstrates that Kailua Bay green turtles are exposed to elevated concentrations of Pb very likely caused by Pb shot from the historic Honolulu skeet shooting range. Levels of Pb found in all Kailua Bay turtles exceeded the threshold for δ-ALAD activity suppression in tortoises, but the estimated daily intake of Pb was significantly less than the acute concentrations that cause harm in red-eared slider turtles. Although Pb concentrations found in Kailua Bay turtles are greater than concentrations found in turtles in other locations in Hawai'i, concentrations are significantly lower than those found in turtles in San Diego Bay. Based on PCV values and emaciation score, most turtles in Kailua Bay are healthy and do not appear outwardly affected by their elevated blood Pb concentrations. Significant negative relationships were found between BCI or growth rate and blood Pb concentrations, indicating Pb may be reducing the BCI, leading to reduced growth. Additional research on hematological and physiological parameters should be done to determine the extent to which Pb is affecting this population. The daily intake of Pb by turtles in the region is a potential cause for concern and may have unforeseen consequences. Turtles in Kailua Bay have been exposed to elevated Pb concentrations for many years, and the effects of chronic Pb exposure on sea turtles is unknown. Continued monitoring of this population and remediation activities are warranted.

Supporting Information—The Supporting Information is available on the Wiley Online Library at https://doi.org/10.1002/etc.5601.

Acknowledgements—We thank the Hawai'i Department of Health for funding this project. We gratefully acknowledge the Department of Environmental Toxicology at Texas Tech University and the Department of Environmental Science at Baylor University for providing the facilities to perform the chemical analysis. We thank S. Hargrove, F. Nilsen, J. Jacob, A. Hansen, K. Garrido, K. VanHouten, and K. Clukey for sampling and processing assistance, and D. Ellisor and F. Grange for manuscript reviews. We thank the Sherman and Perry families for access to the sampling site and use of their properties. Finally, we thank the entire NIST Biorepository team for sample archival. Permits for the collection of samples are NMFS ESA Section 10(a)(1)(A) permits 1581 and 15685, and Annual Hawaii Division of Aquatic Resources Special Activity Permit to the NOAA PIFSC.

Disclaimer—The authors declare no competing financial interest. Certain commercial equipment, instruments, or materials are identified in the present study to specify adequately the experimental procedure. Such identification does not imply recommendation or endorsement by the National Institute of Standards and Technology, nor does it imply that the materials or equipment identified are necessarily the best available for the purpose.

Author Contributions Statement—**Katherine R. Shaw:** Conceptualization; Data curation; Formal analysis; Investigation; Methodology; Writing—original draft; Visualization; Writing—review & editing. **George Balazs:** Resources; Writing—review and editing. **T. Todd Jones:** Conceptualization; Resources. **Harry W. Lynch:** Methodology; Resources. **Jing Liu:** Data curation; Resources; Validation. **George P. Cobb:** Data curation; Formal analysis; Resources; Supervision; Writing—review and editing. **David M. Klein:** Formal analysis; Funding acquisition; Methodology; Project administration; Resources; Supervision; Validation; Writing—review & editing. **Jennifer M. Lynch:** Conceptualization; Data curation; Formal analysis; Funding acquisition; Resources; Software; Supervision; Writing—review and editing.

Data Availability Statement—Individual sample data is available in the Supporting Information. Additional data, associated metadata, and calculation tools are available from the corresponding author (katherine.shaw@nist.gov).

REFERENCES

Agency for Toxic Substances and Disease Registry. (2020). *Toxicological profile for Lead*. US Department of Health and Human Services, Public Health Service.

Aguiar, E. (2009). Hawaii to clean old lead pellets from parts of Kaimalino Beach. Honolulu Advertiser. http://the.honoluluadvertiser.com/article/2009/Feb/14/ln/hawaii902140327.html

Al-Abdali, F., Massoud, M. S., & Al-Ghadban, A. N. (1996). Bottom sediments of the Arabian Gulf- III. Trace metal contents as indicators of pollution and implications for the effect and fate of the Kuwait oil slick. *Environmental Pollution*, *93*(3), 285–301.

Andreae, M. O. (1980). Arsenic in rain and the atmospheric mass balance of arsenic. *Journal of Geophysical Research*, *85*(C8).

Arthur, K. E., & Balazs, G. H. (2008). A comparison of immature green turtle (*Chelonia mydas*) diets among seven sites in the main Hawaiian Islands. *Pacific Science*, *62*(2), 205–217. https://doi.org/10.2984/1534-6188(2008)62[205:Acoigt]2.0.Co;2

Asuncion, B. F. (2010). Characterizing juvenile green sea turtles (*Chelonia mydas*) habitat use in Kawainui, O'ahu: A multi-disciplinary approach [Master's thesis, Hawaii Pacific University].

Balazs, G. H., & Chaloupka, M. (2004). Spatial and temporal variability in somatic growth of green sea turtles (*Chelonia mydas*) resident in the Hawaiian Archipelago. *Marine Biology*, *145*(5), 1043–1059. https://doi.org/10.1007/s00227-004-1387-6

Bezerra, M. F., Lacerda, L. D., Lima, E. H., & Melo, M. T. (2013). Monitoring mercury in green sea turtles using keratinized carapace fragments (scutes). *Marine Pollution Bulletin*, *77*(1-2), 424–427. https://doi.org/10.1016/j.marpolbul.2013.09.020

Board of Land of Natural Resources. (2012, August 24). *Minutes for the meeting of the Board of Land and Natural Resources*. https://dlnr.hawaii.gov/meetings/blnr-meetings-2012/

Bruno, D. dA., Willmer, I. Q., Pereira, L. H. S. dS., Rocha, R. C. C., Saint'Pierre, T. D., Baldassin, P., Scarelli, A. C. S., Tadeu, A. D., Correia, F. V., Saggioro, E. M., Lemos, L. S., Siciliano, S., & Hauser-Davis, R. A. (2021). Metal and metalloid contamination in green sea turtles (*Chelonia mydas*) found stranded in Southeastern Brazil. *Frontiers in Marine Science*, *8*, 608253. https://doi.org/10.3389/fmars.2021.608253

Bryan, J. M. (2013). Concentrations of heavy metals in scute samples from nesting female olive ridley, Lepidochelys olivacea, and Eastern Pacific green, *Chelonia mydas agassizii*, sea turtles in Costa Rica [Master's thesis, Purdue University].

Burger, J. (2008). Assessment and management of risk to wildlife from cadmium. *Science of the Total Environment*, *389*(1), 37–45. https://doi.org/10.1016/j.scitotenv.2007.08.037

Burger, J., Carruth-Hinchey, C., Ondroff, J., McMahon, M., Gibbons, J. W., & Gochfeld, M. (1998). Effects of lead on behavior, growth, and survival of hatchling slider turtles. *Journal of Toxicology and Environmental Health. Part A*, *55*(7), 495–502. https://doi.org/10.1080/009841098158296

Camacho, M., Boada, L. D., Oros, J., Lopez, P., Zumbado, M., Almeida-Gonzalez, M., & Luzardo, O. P. (2014). Monitoring organic and inorganic pollutants in juvenile live sea turtles: Results from a study of *Chelonia mydas* and *Eretmochelys* imbricata in Cape Verde. *Science of the Total Environment*, *481*, 303–310. https://doi.org/10.1016/j.scitotenv.2014.02.051

Cao, X., Ma, L. Q., Chen, M., Hardison, D. W., Jr., & Harris, W. G. (2003). Weathering of lead bullets and their environmental effects at outdoor shooting ranges. *Journal of Environmental Quality*, *32*(2), 526–534.

Cortes-Gomez, A. A., Romero, D., & Girondot, M. (2017). The current situation of inorganic elements in marine turtles: A general review and meta-analysis. *Environmental Pollution*, *229*, 567–585. https://doi.org/10.1016/j.envpol.2017.06.077

Creed, J. T., Brockhoff, C. A., & Martin, T. D. (1994). *Determination of trace elements in water and waste by Inductively Coupled Plasma-Mass Spectrometry, Method 200.8, Revision 5.4*.

Custer, T. W., Franson, J. C., & Pattee, O. H. (1984). Tissue lead distribution and hematologic effects in American kestrels (*Falco sparverius L.*) fed biologically incorporated lead. *Journal of Wildlife Diseases*, *20*(1), 39–43. https://doi.org/10.7589/0090-3558-20.1.39

da Silva, C. C., Klein, R. D., Barcarolli, I. F., & Bianchini, A. (2016). Metal contamination as a possible etiology of fibropapillomatosis in juvenile female green sea turtles *Chelonia mydas* from the southern Atlantic Ocean. *Aquatic Toxicology*, *170*, 42–51. https://doi.org/10.1016/j.aquatox.2015.11.007

Day, R. D., Christopher, S. J., Becker, P. R., & Whitaker, D. W. (2005). Monitoring mercury in the loggerhead sea turtle, *Caretta caretta*. *Environmental Science and Technology*, *39*(2), 437–446. https://doi.org/10.1021/es049628q

Ehsanpour, M., Afkhami, M., Khoshnood, R., & Reich, K. J. (2014). Determination and maternal transfer of heavy metals (Cd, Cu, Zn, Pb and Hg) in the Hawksbill sea turtle (*Eretmochelys imbricata*) from a nesting colony of Qeshm Island, Iran. *Bulletin of Environmental Contamination and Toxicology*, *92*(6), 667–673. https://doi.org/10.1007/s00128-014-1244-3

Escobedo Mondragon, M., Perez Luzardo, O., Henriquez-Hernandez, L. A., Rodriguez-Hernandez, A., Zumbado, M., Rosiles Martinez, J. R.,

Gonzalez Farias, F., Suzan, G., & Gonzalez-Rebeles Islas, C. (2023). Trophic behavior of inorganic elements in nesting sea turtles (*Chelonia mydas, Eretmochelys imbricata, and Caretta caretta*) in Quintana Roo: Biomagnification and biodilution effect in blood and scute tissues. *Marine Pollution Bulletin*, *187*, 114582.

Finlayson, K. A., Leusch, F. D. L., Villa, C. A., Limpus, C. J., & van de Merwe, J. P. (2021). Combining analytical and in vitro techniques for comprehensive assessments of chemical exposure and effect in green sea turtles (*Chelonia mydas*). *Chemosphere*, *274*, 129752. https://doi.org/10.1016/j.chemosphere.2021.129752

Francke, D. L., Hargrove, S. A., Vetter, E. W., Winn, C. D., Balazs, G. H., & Hyrenbach, K. D. (2013). Behavior of juvenile green turtles in a coastal neritic habitat: Validating time–depth–temperature records using visual observations. *Journal of Experimental Marine Biology and Ecology*, *444*, 55–65. https://doi.org/10.1016/j.jembe.2013.03.011

Franson, J. C., Sileo, L., Pattee, O. H., & Moore, J. F. (1983). Effects of chronic dietary lead in American kestrels (*Falco sparverius*). *Journal of Wildlife Diseases*, *19*(2), 110–113. https://doi.org/10.7589/0090-3558-19.2.110

French, A. D., Ashbaugh, H. M., Steinmetz, G., Barnes, M., Conway, W. C., & Klein, D. M. (2017). The S.M.A.R.T. (small mass, affordable, rapid, transfer-less) digestion method for heavy metal determinations. *International Journal Environmental Analytical Chemistry*, *97*(6), 499–507. https://doi.org/10.1080/03067319.2017.1328060

Gaus, C., Grant, S., Jin, N. L., Goot, K., Chen, L., Villa, A., Neugebauer, F., Qi, L., & Limpus, C. (2012). *Investigation of contaminant levels in green turtles from Gladstone*. National Research Center for Environmental Toxicology.

Helsel, D. (2005). *Nondetects and data analysis: Statistics for censored environmental data*. John Wiley & Sons.

Innis, C., Tlusty, M., Perkins, C., Holladay, S., Merigo, C., & Weber, E. S. (2008). Trace metal and organochlorine pesticide concentrations in cold-stunned juvenile Kemp's Ridley turtles (*Lepidochelys kempii*) from Cape Cod, Massachusetts. *Chelonian Conservation and Biology*, *7*(2), 230–239. https://doi.org/10.2744/ccb-0707.1

Jorgensen, S. S., & Willems, M. (1987). The fate of lead in soils: The transformation of lead pellets in shooting-range soils. *Ambio*, *16(1)*, 11–15.

Keller, J. M., Kucklick, J. R., Stamper, M. A., Harms, C. A., & McClellan-Green, P. D. (2004). Associations between organochlorine contaminant concentrations and clinical health parameters in loggerhead sea turtles from North Carolina, USA. *Environmental Health Perspectives*, *112*(10), 1074–1079. https://doi.org/10.1289/ehp.6923

Keller, J. M., Pugh, R. S., & Becker, P. R. (2014). Biological and Environmental Monitoring and Archival of Sea Turtle Tissues (BEMAST): Rationale,c protocols, and initial ollections of banked sea turtle tissues.

Komoroske, L. M., Lewison, R. L., Seminoff, J. A., Deheyn, D. D., & Dutton, P. H. (2011). Pollutants and the health of green sea turtles resident to an urbanized estuary in San Diego, CA. *Chemosphere*, *84*(5), 544–552. https://doi.org/10.1016/j.chemosphere.2011.04.023

Lam, J. C., Tanabe, S., Chan, S. K., Lam, M. H., Martin, M., & Lam, P. K. (2006). Levels of trace elements in green turtle eggs collected from Hong Kong: Evidence of risks due to selenium and nickel. *Environmental Pollution*, *144*(3), 790–801. https://doi.org/10.1016/j.envpol.2006.02.016

Maier, P. P., Segars, A. L., Arendt, M. D., Whitaker, J. D., Stender, B. W., Parker, L., Vendetti, R., Owens, B. W., Quattro, J., & Murphy, S. R. (2004). Development of an index of sea turtle abundance based upon in water sampling with trawl gear. South Carolina Department of Natural Resources, Office of Fisheries and Management. https://dc.statelibrary.sc.gov/bitstream/handle/10827/11189/DNR_Development_of_an_Index_of_Sea_Turtle_2004-3-31.pdf?sequence=1&isAllowed=y

Manninen, S., & Tanskanen, N. (1993). Transfer of lead from shotgun pellets to humus and three plant species in a Finnish shooting range. *Archives of Environmental Contamination and Toxicology*, *24*(3), 410–414.

Martinez-Lopez, E., Sousa, A. R., Maria-Mojica, P., Gomez-Ramirez, P., Guilhermino, L., & Garcia-Fernandez, A. J. (2010). Blood delta-ALAD, lead and cadmium concentrations in spur-thighed tortoises (*Testudo graeca*) from Southeastern Spain and Northern Africa. *Ecotoxicology*, *19*(4), 670–677. https://doi.org/10.1007/s10646-009-0441-z

McFadden, K. W., Gomez, A., Sterling, E. J., & Naro-Maciel, E. (2014). Potential impacts of historical disturbance on green turtle health in the unique & protected marine ecosystem of Palmyra Atoll (Central Pacific). *Marine Pollution Bulletin*, *89*(1-2), 160–167. https://doi.org/10.1016/j.marpolbul.2014.10.012

Miguel, C., Costa, P. G., Bianchini, A., Luzardo, O. L. P., Vianna, M. R. M., & Santos, M. R. D. (2022). Health condition of *Chelonia mydas* from a foraging area affected by the tailings of a collapsed dam in southeast Brazil. *Science of the Total Environment*, *821*, 153353. https://doi.org/10.1016/j.scitotenv.2022.153353

Monastra, V., Derry, L. A, & Chadwick, O. A. (2004). Multiple sources of lead in soils from a Hawaiian chronosequence. *Chemical Geology*, *209*(3–4), 215–231. https://doi.org/10.1016/j.chemgeo.2004.04.027

Paez-Osuna, F., Calderon-Campuzano, M. F., Soto-Jimenez, M. F., & Ruelas-Inzunza, J. R. (2010). Lead in blood and eggs of the sea turtle, *Lepidochelys olivacea*, from the Eastern Pacific: Concentration, isotopic composition and maternal transfer. *Marine Pollution Bulletin*, *60*(3), 433–439. https://doi.org/10.1016/j.marpolbul.2009.10.004

Pain, D. J., Mateo, R., & Green, R. E. (2019). Effects of lead from ammunition on birds and other wildlife: A review and update. *Ambio*, *48*(9), 935–953. https://doi.org/10.1007/s13280-019-01159-0

Parthipan, P., & Muniyan, M. (2013). Effects of heavy metal nickel on hematological parameters of fresh water fish. Cirrhinus mrigala. *Journal of Enivronment and Current Life Science*, *1*, 46–55.

Perrault, J. R. (2014). Mercury and selenium ingestion rates of Atlantic leatherback sea turtles (*Dermochelys coriacea*): A cause for concern in this species? *Marine Environmental Research*, *99*, 160–169. https://doi.org/10.1016/j.marenvres.2014.04.011

Perrault, J. R., Stacy, N. I., Lehner, A. F., Poor, S. K., Buchweitz, J. P., & Walsh, C. J. (2017). Toxic elements and associations with hematology, plasma biochemistry, and protein electrophoresis in nesting loggerhead sea turtles (*Caretta caretta*) from Casey Key, Florida. *Environmental Pollution*, *231*, 1398–1411. https://doi.org/10.1016/j.envpol.2017.09.001

Potysz, A., Binkowski, L. J., Kierczak, J., & Rattner, B. A. (2023). Drivers of Pb, Sb and As release from spent gunshot in wetlands: Enhancement by organic matter and native microorganisms. *Science of the Total Environment*, *857*(Pt 1), 159121.

Reich, K. J., Bjorndal, K. A., & Bolten, A. B. (2007). The 'lost years' of green turtles: Using stable isotopes to study cryptic lifestages. *Biology Letters*, *3*(6), 712–714. https://doi.org/10.1098/rsbl.2007.0394

Rooney, C. P., McLaren, R. G., & Condron, L. M. (2007). Control of lead solubility in soil contaminated with lead shot: Effect of soil pH. *Environmental Pollution*, *149*(2), 149–157. https://doi.org/10.1016/j.envpol.2007.01.009

Russell, D. J., & Balazs, G. H. (2009). Dietary shifts by green turtles (*Chelonia mydas*) in the Kāne'ohe bay region of the Hawaiian islands: A 28-year study. *Pacific Science*, *63*(2), 181–192. https://doi.org/10.2984/049.063.0202

Sakai, H., Saeki, K., Ichihashi, H., Suganuma, H., Tanabe, S., & Tatsukawa, R. (2000). Species-specific distribution of heavy metals in tissues and organs of logerhead turtle (*Caretta caretta*) and green turtle (*Chelonia mydas*) from Japanese coastal waters. *Marine Pollution Bulletin*, *40*(8), 701–709.

Salice, C. J., Suski, J. G., Bazar, M. A., & Talent, L. G. (2009). Effects of inorganic lead on Western fence lizards (*Sceloporus occidentalis*). *Environmental Pollution*, *157*(12), 3457–3464. https://doi.org/10.1016/j.envpol.2009.06.013

Shaw, K. R., Lynch, J. M., Balazs, G. H., Jones, T. T., Pawloski, J., Rice, M. R., French, A. D., Liu, J., Cobb, G. P., & Klein, D. M. (2021). Trace element concentrations in blood and scute tissues from wild and captive Hawaiian green sea turtles (*Chelonia mydas*). *Environmental Toxicology and Chemistry*, *40*(1), 208–218. https://doi.org/10.1002/etc.4911

Sinaei, M. and Bolouki, M. (2017). Metals in blood and eggs of green sea turtles (*Chelonia mydas*) from nesting colonies of the Northern coast of the Sea of Oman. *Archives of Environmental Contamination and Toxicology*, *73*(4), 552–561. https://doi.org/10.1007/s00244-017-0421-x

Sparling, D. W. (2016). *Ecotoxicology essentials: Environmental contaminants and their biological effects on animals and plants*. Academic Press.

Suhring, R., Diamond, M. L., Bernstein, S., Adams, J. K., Schuster, J. K., Fernie, K., Elliott, K., Stern, G., & Jantunen, L. M. (2021). Organophosphate esters in the Canadian Arctic Ocean. *Environmental Science and Technology*, *55*(1), 304–312. https://doi.org/10.1021/acs.est.0c04422

Sutherland, R. A., Day, J. P., & Bussen, J. O. (2003). Lead concentrations, isotope ratios, and source apportionment in road deposited sediments,

Honolulu, Oahu, Hawaii. *Water, Air, & Soil Pollution*, *142*, 165–186. https://doi.org/10.1023/A:1022026612922

Svanberg, F., Mateo, R., Hillstrom, L., Green, A. J., Taggart, M. A., Raab, A., & Meharg, A. A. (2006). Lead isotopes and lead shot ingestion in the globally threatened marbled teal (*Marmaronetta angustirostris*) and white-headed duck (*Oxyura leucocephala*). *Science of the Total Environment*, *370*(2-3), 416–424. https://doi.org/10.1016/j.scitotenv.2006.07.006

Takamatsu, T., Murata, T., Koshikawa, M. K., & Watanabe, M. (2010). Weathering and dissolution rates among Pb shot pellets of differing elemental compositions exposed to various aqueous and soil conditions. *Archives of Environmental Contamination and Toxicology*, *59*(1), 91–99. https://doi.org/10.1007/s00244-009-9449-x

Takeuchi, N. Y., Walsh, M. T., Bonde, R. K., Powell, J. A., Bass, D. A., Gaspard Iii, J. C., & Barber, D. S. (2016). Baseline reference range for trace metal concentrations in whole blood of wild and managed West Indian Manatees (*Trichechus manatus*) in Florida and Belize. *Aquatic Mammals*, *42*(4), 440–453. https://doi.org/10.1578/am.42.4.2016.440

US Environmental Protection Agency. (January 1998). *Method 6020A: Inductively coupled plasma- mass spectrometry*.

van de Merwe, J. P. (2008). *Persistent organic pollutants and heavy metals in the green sea turtle, Chelonia mydas* [Doctoral dissertation, Griffith University].

Villa, C. A., Finlayson, S., Limpus, C., & Gaus, C. (2015). A multi-element screening method to identify metal targets for blood biomonitoring in green sea turtles (*Chelonia mydas*). *Science of the Total Environment*, *512–513*, 613–621. https://doi.org/10.1016/j.scitotenv.2014.11.100

Villa, C. A., Flint, M., Bell, I., Hof, C., Limpus, C. J., & Gaus, C. (2017). Trace element reference intervals in the blood of healthy green sea turtles to evaluate exposure of coastal populations. *Environmental Pollution*, *220*(Pt B), 1465–1476. https://doi.org/10.1016/j.envpol.2016.10.085

Williams, S. L. (1988). *Thalassia testudinum* productivity and grazing by green turtles in a highly disturbed seagrass bed. *Marine Biology*, *98*(3), 447–455.

Yipel, M., Tekeli, I. O., Isler, C. T., & Altug, M. E. (2017). Heavy metal distribution in blood, liver and kidneys of Loggerhead (*Caretta caretta*) and green (*Chelonia mydas*) sea turtles from the Northeast Mediterranean Sea. *Marine Pollution Bulletin*, *125*(1–2), 487–491. https://doi.org/10.1016/j.marpolbul.2017.08.011

Zar, J. H. (1996). *Biostatistical analysis* (3rd ed.). Prentice Hall.

