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Lead accumulation in green sea turtles from lead shot

Green sea turtles (*Chelonia mydas*) accumulate heavy metals near a former skeet shooting range in Kailua, O'ahu, Hawai'i

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

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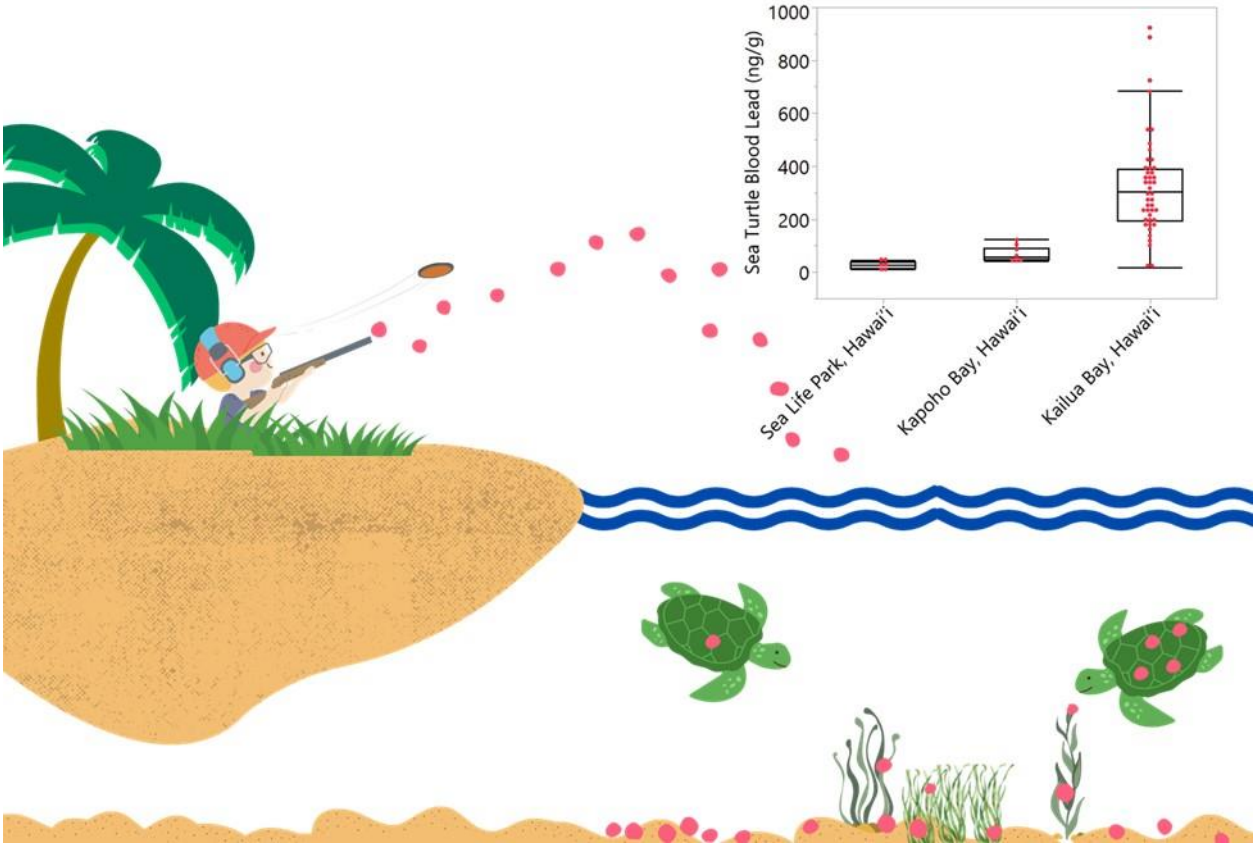
ABSTRACT

This study determined if green sea turtles (*Chelonia mydas*) in Kailua Bay, Oahu, in the Hawaiian Islands have elevated blood and scute Pb, As, and 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 (ICP-MS). 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 to 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 (NOAEL, 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.

Graphical Abstract



An estimated 500,000 pounds of lead (Pb) shot was deposited in the sand and surrounding waters by the Honolulu Skeet Club between 1933 and 1956 (Board of Land and Natural Resources, 2012). This has caused an increase in sediment, algae, and sea turtle blood and scute Pb concentrations in Kailua Bay, Hawaii.

Keywords: marine turtle, reptile, lead, scute, Hawai'i

INTRODUCTION

Lead can adversely affect every organ and system in the body, with the main target being the nervous system (ATSDR, 2020). Widespread use of lead (Pb) has led to its accumulation in the environment. The primary source of Pb in waterfowl and most bird species is lead shot (Pain et al., 2019). 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 compete 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 comprised of three metals: lead (98 %), antimony (Sb, 1.75 %), and arsenic (As, 0.5 %) (Potysz et al., 2023). In soil lead 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, 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, the 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. Greater 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 (CERCLA), commonly known as 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 about 35 cm straight carapace length (SCL) (Balazs & Chaloupka, 2004). 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 re-captured during sampling events spanning three 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.

This 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 lead shot on a threatened sea turtle species and evaluate if the accumulated Pb is attributing to health concerns such as fibropapillomatosis, 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 two years between captures Health information for these turtles can be found in 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 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, Franklin Lakes, NJ). Blood was kept on ice until it was brought back to the lab. 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 and 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 NIST Biorepository in the Hollings Marine Laboratory in Charleston, SC. 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 5th central scute (Keller et al., 2014; Shaw et al., 2021). The 5th 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 Lubbock, TX for analysis with subsequent transfer to Baylor University in Waco, TX 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, Roswell, GA) on October 9, 2017. Locations and additional information can be found in 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 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; (Fig. 1, Table S2 - S4).

In-water survey for lead shot

The coastline directly offshore of the historic skeet shooting range was surveyed for lead 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 m 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 ICP-MS Analysis

Algae, scute and blood samples were digested by a modified Small Mass, Affordable, Rapid, Transfer-less (S.M.A.R.T) method (French et al., 2017; Shaw et al., 2021). Algae samples were oven dried overnight at 55 °C to complete dryness. Average moisture content of *A. spicifera*, *G. salicornia*, and *Amansia* spp. were 63.1 %, 76.1 %, and 52.9 %, respectively (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 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 high purity 30 % hydrogen peroxide (H_2O_2, Fisher Scientific) was added, and the samples 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), Sn, vanadium (V) and zinc (Zn; TTUNIV-1, Inorganic Ventures, Christiansburg, VA USA) 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 HNO_3 (5.53 mol/L), 0.1 mL HCl (0.99 mol/L), and 0.1 mL 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 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, the samples 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 EPA method 3050B. Sediment samples were dried to completion at 55 °C. The average sediment moisture content was 38.3 % (Table S3). An aliquot of the sediment (1.0 g) was placed in a 150 mL beaker with 10 mL Milli-Q water. Trace metals grade HNO_3 (10 mL, 5.53 mol/L) was added, and the beakers 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 EPA method 6020A. Seawater samples (0.5 mL) were acidified with 1.4 mL 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, Santa Clara, CA 95051 USA). A 7-point multi-element calibration curve from 0.1 ng/g 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 ng/g 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, Santa Clara, CA USA) were added online and samples were reanalyzed if the recovery was outside the acceptable range of 80 % to 120 %. Analytical methods were validated using certified reference materials of similar matrices. Seronorm™ Trace Elements Whole Blood L-3 (REF 210313, LOT 1509408 Sero AS, Billingstad, Norway, n = 4) was digested with the same methods as blood samples, DOLT-5 Dogfish Liver Certified Reference Material (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 (Table S5). In the Dolt 5 CRM, Pb was in agreement and As was within 20 % of the certified values. Lead and As were in agreement with the values in 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 (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 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 an IDL of 0.29 ng/g for Pb, 0.14 ng/g for Sb and 0.17 ng/g for As (Table S5) (Creed et al., 1994). Limit of Quantification (LOQ) was calculated by multiplying the lowest concentration of the calibration curve by the dilution factor. The LOQs were 10 ng/g, 0.29 ng/g, 2 ng/g, 0.83 ng/g, and 7,500 ng/g for seawater, algae, scute, blood, and sediment, respectively. Lab (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; Cary, NC) or R (version 3.2.3, The R foundation for Statistical Computing, Vienna, Austria). 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 < LOQ (Helsel 2005). The Shapiro-Wilks test was used to test for normality. Most data were not normally distributed so non-parametric 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 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). Since Hawaiian green turtles recruit from a carnivorous pelagic stage to nearshore herbivores around 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 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 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 to 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 comprised of *Acanthophora spicifera* (44 %), *Amansia spp*. (30 %) and other algae and seagrass (26 %) (Russell & Balazs, 2009) The grams 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" was 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 mm 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 cm to 84.9 cm SCL (Table S1). Additional sampling data, size, mass, and sex of individual turtles can be found in Table S1. Based on the SCL, the turtles could be classified as juvenile to sub-adult. Two turtles had external fibropapillomatosis (FP) tumors. Packed cell volume ranged from 11 % to 41.5

%. Healthy PCV range in green sea turtles is 28 % to 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 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 less than 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 this 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 As (Table S6). Additional information on statistical tests performed can be found in Supplemental Data Tables S6 and S7. Because the focus of this 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 ng/g ± 30.5 ng/g and scute Pb concentration of 32.9 ng/g ± 12.9 ng/g (Shaw et al., 2021). Kapoho Bay turtles had significantly lower blood and scute Pb concentrations than the turtles in this study (p-value = $4x10^{-4}$ and 0.003, respectively). Similarly, Kapoho Bay turtles had significantly lower concentrations of As in their blood

(35.6 ng/g ± 24.2 ng/g) and scute (144 ng/g ± 22.8 ng/g) than the Kailua Bay turtles (p-value = $9x10^{-4}$ and 0.008, respectively). This comparison suggests that Kailua Bay has elevated Pb and As compared to Kapoho Bay, which may originate from the lead 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, Fig. S1, S2) or in scute As (p-value = 0.7, Fig. S3). Though not significantly different, larger turtles had slightly greater scute Pb concentrations (p-value = 0.07, Fig. S4). We expected that larger turtles, having longer residence times in this area of lead 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 this study ranged from 40.9 cm to 84.9 cm SCL. Had this 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. Since 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 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 lead 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 (Figures S5 – S8).

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, 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 lead shot near the old shooting range are exposed to elevated lead, either because the lead from shot may transport into this region's habitat via currents or 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 ten turtles captured twice were captured in the same sampling

region (A or B). This 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 2012 (cendiff; p-value = 0.006) and 2013 (p-value = 0.03), though 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 lead shot is exposed after every storm event, washing additional lead shot pellets into tide pools and nearshore waters and the two years between the cleanup event and sampling in 2011 likely allowed more Pb shot into the area (personal communication Kailua resident, October 2017). This increase in mean blood Pb concentrations of the turtles in Kailua Bay from 2011 to 2013 may be due to more lead 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 lead 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 this study were only sampled in region B, where concentrations were lesser, whereas turtles sampled in 2012 and 2013 were from both regions.

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 sediment in North Kane'ohe (p-value < 0.001) and He'eia (p-value = 0.03), but not from 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 and little organic matter, had undetectable concentrations of Pb (Al-Abdali et al., 1996).

The highest concentrations of Pb in algae samples was 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 (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 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 et al. 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; 1,030 ng/g dm) had Pb concentrations 15-fold lower than 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 Kailua Bay (*A. spicifera* n = 3; 29,600 ± 15,100 ng/g dm).

Lead, As, and Sb were <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 four to six years after the sampling of sea turtles which could confound relationships between measured Pb concentrations in environmental samples and turtle samples. Though Pb shot remains a problem in the area, concentrations of Pb in environmental samples collected in 2017 are potentially different than concentrations in 2011-2013 when the turtles were sampled. Storms and the resulting runoff that causes more Pb shot to be

exposed potentially causes 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 three-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 (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 one 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 scutes can provide information on long term exposure to contaminants, as 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 this 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 of scute) 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 resident turtles from Kailua Bay.

Lead 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, all 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 lead 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 this 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 lead. 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 PCV (rho = -0.294, p-value = 0.073; and rho = 0.311, p-value = 0.057, respectively; Figures S9 and S10, Table S6), though 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 this 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 this 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 to their initial blood and scute As and Pb concentrations using a Kendal's tau correlation. Both blood As and Pb were significantly negatively correlated to growth rate (Table S6; g/day p-value = 0.032, mm/day p-value = 0.035, respectively). In addition, the BCI and blood Pb were negatively correlated (p-value = 0.02, 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 sub-adults, thus possible effects of lead 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 ng/g ± 15,100 ng/g and 15,100 ng/g, respectively. A potential daily lead intake from food for Kailua Bay turtles was calculated at 3.08 mg Pb/day (Table S11). The average mass of turtles sampled in Kailua Bay was 25.7 kg ± 14.1 kg. This gives an average dose of 0.12 mg/kg/d. Red eared sliders in an acute exposure study injected once with lead 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 sub-acute study showed sublethal effects of weight loss and lowered food consumption at 62.5 mg/kg/d (Salice et al., 2009). A NOAEL for growth of 31.5 mg/kg/d was established for fence lizards exposed to Pb for 14 days. If the calculated daily intake of Pb for Kailua turtles is compared to 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 sub-acute exposure and though the NOAELs for turtles and lizards cannot be directly compared, chronic exposure will usually have a lower NOAEL than an 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 of the 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 tortoises (*Testudo graeca*) blood Pb 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 current study did not measure δ-ALAD activity, but all turtles in this study were above this threshold with the mean at 23 times higher. Future studies should include δ-ALAD activity to help determine if lead 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 to 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 (HWK) 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 lead concentrations compared to the HWK turtles and turtles from most other locations around the world (Figure 6). Green turtles from only 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. This study demonstrates that Kailua Bay green turtles are exposed to elevated concentrations of Pb very likely caused by lead 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, however the estimated daily intake of Pb was significantly less than acute concentrations that cause harm in red eared slider turtles. Though 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 PVC 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 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 are being 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.

SUPPLEMENTARY INFORMATION

Table S1: Information for sea turtles sampled. Samples coded with the same color were repeated samples taken from multiple captures of the same turtle. SCL = straight carapace length PCV = packed cell volume BCI = body condition index

Table S2: Elemental concentrations (ng/g dry mass) in algae samples.
Table S3: Elemental concentrations (ng/g dry mass) in sediment samples.
Table S4: Elemental concentrations (ng/g dry mass) in seawater samples.
Table S5: Detection limits, mean and standard deviation (range) of mass fractions measured in replicates of certified reference materials, and recoveries of spiked trace elements into seawater and algae samples.
Table S6: Correlations performed with results
Table S7: Group differences explored statistically with results.
Table S8: Elemental concentrations (ng/g dry mass as received) and Pb isotopic ratios in Kailua Bay, O'ahu, green sea turtle scute tissue.
Table S9: Elemental concentrations (ng/g wet mass) and Pb isotopic ratios in Kailua Bay, O'ahu, green sea turtle whole blood.
Table S10: Differences in elemental concentrations (ng/g) for turtles sampled twice.
Table S11: Calculations for potential daily lead intake from food for Kailua Bay turtles.
Figure S1: Blood As concentration (ng/g) in turtles < 45 cm SCL and > 45 cm SCL in Kailua Bay, HI (p-value = 0.2).
Figure S2: Blood Pb concentration (ng/g) in turtles < 45 cm SCL and > 45 cm SCL in Kailua Bay, HI (p-value = 0.8).
Figure S3: Scute As concentration (ng/g) in turtles <45 cm SCL and > 45 cm SCL in Kailua Bay, HI (p-value = 0.7).
Figure S4: Scute Pb concentration (ng/g) in turtles <45 cm SCL and > 45 cm SCL in Kailua Bay, HI p-value = 0.07).
Figure S5: No significant relationships noted between blood As (ng/g) and SCL (cm; rho = -0.17, p-value = 0.444) and scute As (ng/g) and SCL (cm; rho = -.077, p-value = 0.621)
Figure S6: No significant relationships noted between blood Pb (ng/g) and SCL (cm; rho = -0.036, p-value = 0.819) and scute Pb (ng/g) and SCL (cm; rho = 0.037, p-value = 0.810) in Kailua Bay green sea turtles.
Figure S7: No significant relationships noted between blood As (ng/g) and mass (kg, rho = -0.151, p-value = 0.323) and scute As (ng/g) and mass (kg, rho = -0.199, p-value = 0.196).
Figure S8: No significant relationships noted between blood Pb (ng/g) and mass (kg, rho = -0.102, p-value = 0.504) and scute Pb (ng/g) and mass (kg, rho = 0.150, p-value = 0.331).
Figure S9: Negative relationship between packed cell volume (PCV) and blood Pb concentrations (ng/g wm) in Kailua Bay turtles. Actual raw data points are plotted. A Spearman's rank correlation (rho = -0.294, p-value = 0.073) showed a moderately significant relationship.
Figure S10: Positive relationship between packed cell volume (PCV) and blood As concentrations (ng/g wm) in Kailua Bay turtles. Actual raw data points are plotted. A Spearman's rank correlation (rho = 0.311, p-value = 0.057) showed a moderately significant relationship.

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

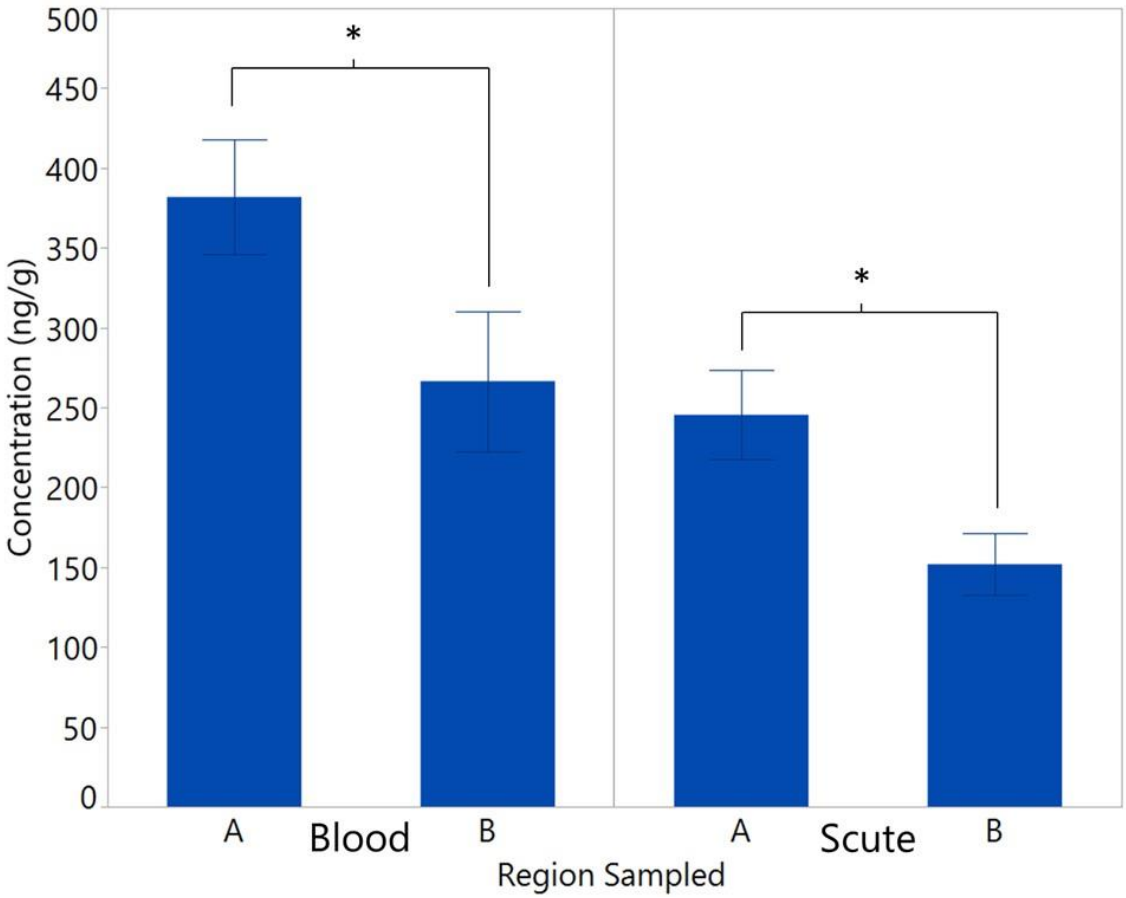

Figure 2: Pb concentrations in blood (ng/g wm) and scutes (ng/g dm) of green sea turtles sampled from two adjacent and slightly overlapping sites in Kailua Bay. Region A is closer to the skeet shooting range than region B. An asterisk indicates a significant difference between regions (p-value < 0.05).

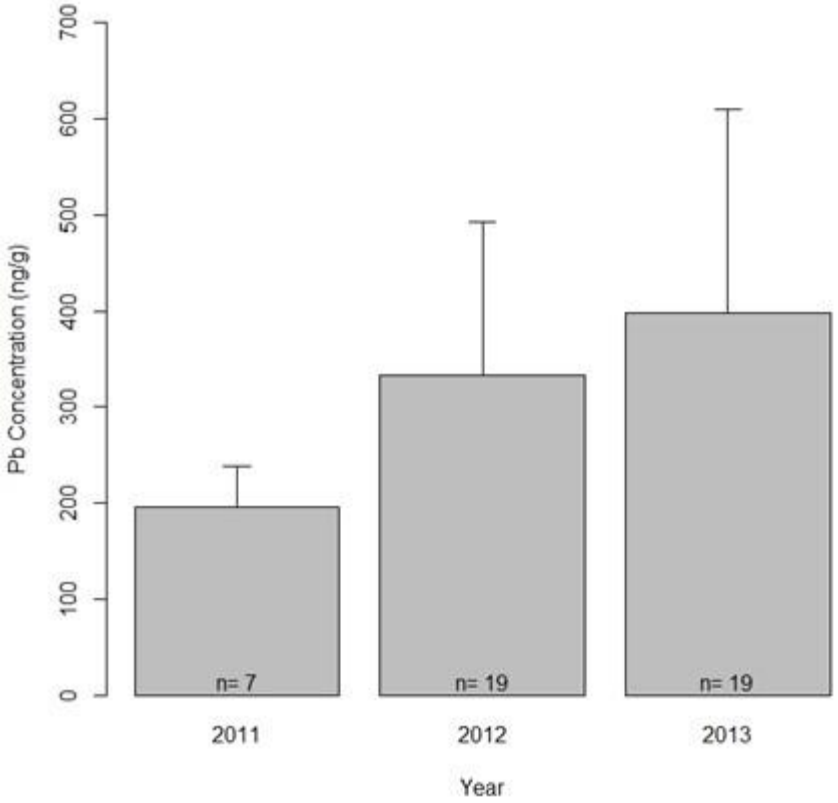

Figure 3: Blood Pb concentrations (ng/g wm) in Kailua Bay, O‘ahu green sea turtles sampled in 2011, 2012 and 2013.

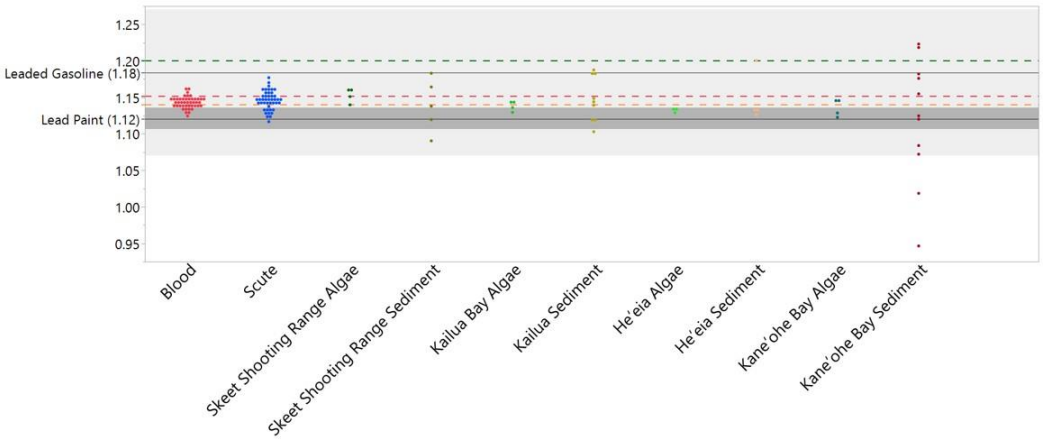

Figure 4: 206Pb/207Pb ratio in Kailua Bay green sea turtle blood and scute compared to other environmental samples collected from O‘ahu. The light grey box is 206Pb/207Pb ratio in lead shot pellets from multiple manufacturers (1.07 – 1.27) and the dark grey box is the 206Pb/207Pb ratio in Pb pellets collected from Kaimalino Beach (Svanberg et al., 2006). Leaded gasoline (1.184 ± 0.009) and Pb paint (1.12) are labelled horizontal

lines (Sutherland et al., 2003; Svanberg et al., 2006). Additional anthropogenic values are illustrated by dashed horizontal lines: lead in Pacific Marine Aerosols (green), Asian anthropogenic sources (orange) and the Ala Wai Canal (red) represents local anthropogenic lead (Monastra et al. 2004).

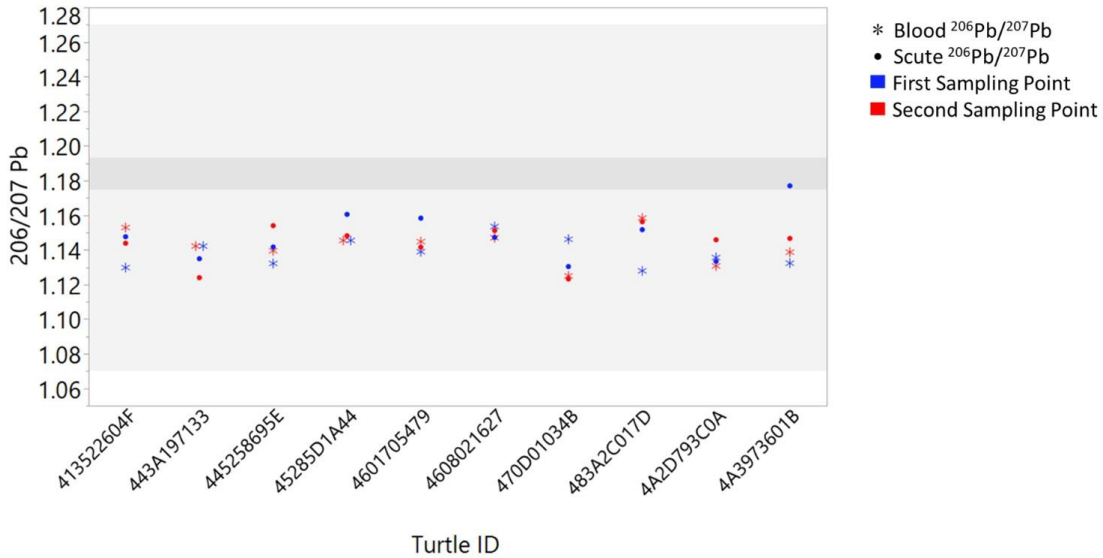

Figure 5: Blood 206Pb/207Pb isotope ratios for turtles sampled twice.

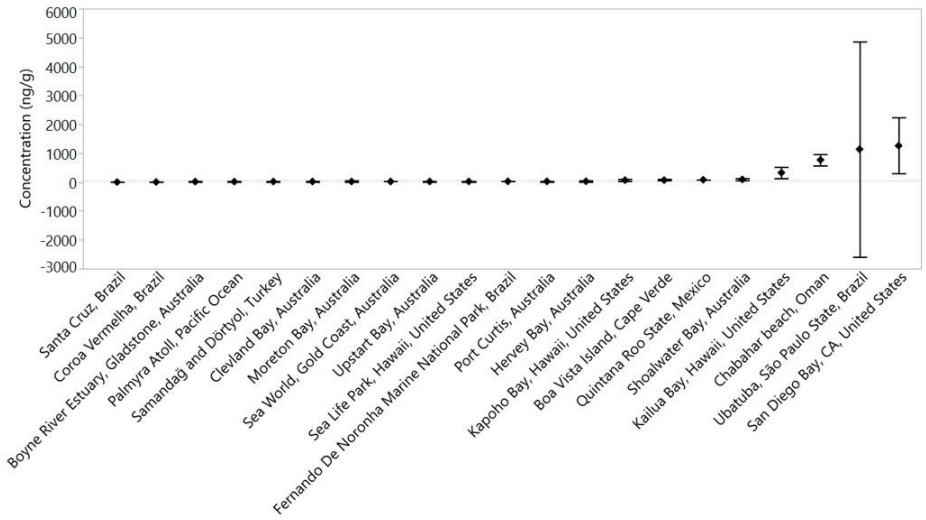

Figure 6: Blood Pb concentrations (ng/g) in sea turtles around the world (Camacho et al., 2014; da Silva et al., 2016; Escobedo Mondragon et al., 2023; Finlayson et al., 2021; Gaus et al., 2012; Komoroske et al., 2011; McFadden et al., 2014; Miguel et al., 2022; Shaw et al., 2021; Sinaei & Bolouki, 2017; Villa et al., 2017; Yipel et al., 2017). Dots represent the mean and error bars are one standard deviation. Grey 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).

Table 1: Arsenic, Pb and Sb concentrations in blood (ng/g wm) and scute samples (ng/g dm) (SD = one standard deviation). Blood: n = 45 samples from n = 35 individual turtles, occasionally turtles were resampled upon recapture. Scute: n = 44 samples from 35 individual turtles, occasionally turtles were resampled upon 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).

Location	Element	Blood				Scute				Reference
		median	mean (SD)	range	% detected	median	mean (SD)	range	% detected	
Kailua Bay	As	125	299 (399)	26.4 - 1950	100	330	512 (442)	114 - 1830	100	This 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	

Table 2: Lead, As, and Sb (ng/g) in sediment and algae samples in Kailua Bay, the Skeet Shooting Range, and the Other Sites. Statistically different concentrations are indicated by capital letters after the mean in sediment and lowercase letters in algae.

	Location	Sample Type	n	As			Pb			Sb		
				Mean (SD)	Range	% detected	Mean (SD)	Range	% detected	Mean (SD)	Range	% detected
Kailua Bay	Reef Flat	Algae	4	5,490 (1,700)[ac]	<LOD - 6,890	75	26,000 (14,300)[a]	12,200 - 39,100	100	113 (39.3)[a]	63.5 - 153	100
	Reef	Sedi	3	<LOQ	-	0	53,200	33,30	100	<LO	-	0

	Flat	ment					(19,100)	0 - 71,400		Q		
	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)	24100 - 198,000	100	-	<LOQ - 259,000	33.3
	Kailua Bay Combined	Sediment	9	-	<LOD - 57,600	11.2	71,000 (50,700)[B]	24100 - 198,000	100	-	<LOQ - 259,000	11.2
Skeet Shooting Range	Skeet Shooting Range	Algae	4	3060 (315)[c]	2,710 - 3,440	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 (6,830)[ab]	4,330 - 17,900	100	104 (110)[b]	<LOQ - 225	66.7	1,560 (2,160)[b]	180 - 4,050	100
	N. Kane'ohe Bay	Sediment	9	11,100 (1,460)	<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	Alga	3	37,70	10,2	100	-[b]	<LOQ	33.	91.7	63.2	100

	e	0 (23,800)[b]	00 - 52,700			- 74.6	3	(33.0)[a]	- 128		
He'eia	Sediment	4	18,800 (550)	<LOQ - 19,500	75	21,000 (23,900)[A]	<LOQ - 54,000	50	-	-	0

Table 3: Pb and As concentrations in blood (ng/g wm) and scute (ng/g dm) of recaptured turtles.

Turtle ID	Date		Days between sampling	SCL (cm)		Blood Pb		Blood As		Scute Pb		Scute As	
	1st Capture	2nd Capture		1st Capture	2nd Capture	1st Capture	2nd Capture	1st Capture	2nd Capture	1st Capture	2nd Capture	1st Capture	2nd Capture
460170554 79	7/10/12	7/9/13	364	55.2	56.7	193	236	224	686	99.3	135	397	890
460802162 7	7/11/12	7/9/13	363	52.6	54.6	355	32.8	41.2	151	253	288	114	185
41352260 4F	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
44525869 5E	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	7/12/1	7/8/13	361	44.1	46.6	16	317	44.2	1950	246	166	498	654

483A2C017D	23/30/11	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
4A397360 1B	7/10/12	7/8/13	363	44.8	46.9	344	682	759	178	59.3	184	852	594