TRACE ELEMENT CONCENTRATIONS IN GREEN TURTLES

TRACE ELEMENT CONCENTRATIONS IN BLOOD AND SCUTE TISSUES FROM WILD AND CAPTIVE HAWAIIAN GREEN SEA TURTLES (*CHELONIA MYDAS*)

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Abstract: Hawaiian green turtles (*Chelonia mydas*) are exposed to trace elements through water, sediment, and food. High concentrations of elements have been shown to decrease immune function, impair growth, and decrease reproduction in wildlife. This study compares trace element concentrations in green turtles in captivity at Sea Life Park Hawaii (SLPH, n = 6) to wild green turtles in Kapoho Bay, HI (n = 5 to 7). Blood and scute samples were collected and analyzed for eleven elements via inductively coupled plasma-mass spectrometry (ICP-MS). Seven elements were significantly different between captive and wild turtles. Selenium was significantly greater in the blood of captive turtles than wild turtles, while V, Ni, and Pb were significantly greater in the blood of wild turtles. In scute, V, Cu, Se, and Cr were significantly greater in captive turtles, while As was significantly greater in wild turtles. Pelleted food fed to the captive turtles and representative samples of the wild turtle diet were analyzed via ICP-MS to calculate trophic transfer factors (TTF) and daily intake values. TTFs > 1 indicated that V, Cr, and Sr potentially biomagnify in scutes of captive turtles; and Se in the scute and blood of wild turtles. Wild turtles had greater estimated daily intake than captive turtles of all elements except Cu and Se. Water samples were collected from the pond at SLPH and four elements were detected in the seawater: Sr > Cu > Se > Cr. However, the main source of exposure to sea turtles is through their food. Even though Se, Cu and V are essential elements, they are a possible husbandry concern because concentrations are significantly greater in the captive turtles. No toxic thresholds are known for sea turtles, but rehabilitation and managed care facilities should monitor sea turtle elemental concentrations to ensure the animals' health.

INTRODUCTION

Trace elements have been found in marine vertebrates around the world including cetaceans, seabirds, fish, and sea turtles.[3,21,23,25] Some elements are essential including K, Na, Mg, Ca, Mn, Fe, Co, Ni, Cu, Zn, Mo, and Se for enzymatic activity, cell structure, immune response, and other bodily functions. Essential elements can become toxic at high concentrations, while nonessential elements, e.g. As, Hg, Pb, can be toxic at very low concentrations.[11,20,32] These nonessential elements can gain access to cells by mimicking essential elements, potentially disrupting cellular processes.[32] This can adversely affect the health of organs, the central nervous system, and the immune system through acute or chronic exposure.[31]

Hawaiian green turtles (*Chelonia mydas*) face anthropogenic threats such as pollution, fisheries bycatch, and habitat loss. *C. mydas* are classified as threatened under the United States Endangered Species Act and listed in Appendix I of the Convention of International Trade in Endangered Species.[27,41] Age classes are defined based on straight carapace length (SCL). Juvenile sea turtles have a SCL < 75 cm, subadult turtles range between 75 cm to 80 cm SCL and adult turtles have a SCL > 80 cm. Hawaiian green turtles recruit to nearshore habitats at around 35 cm SCL. Once in nearshore habitats, the subadult and adult turtles exhibit high site fidelity to a particular location. Green turtles are bioindicators of the environment due to their long life, high site fidelity, and accumulation of contaminants from food, water, and sediment ingested while eating.[1,2,16,30]

Natural and anthropogenic contaminants, including metals, are one of the hazards potentially contributing to the decline of green turtles worldwide.[46] Wild sea turtles can live fifty years or longer, potentially bioaccumulating metals; though trace element concentrations are

generally one to two orders of magnitude lower in sea turtles than seabirds or marine mammals.[33,36]

Monitoring sea turtles can help elucidate the risk of trace elements to the species as well as to the ecosystem.[11] Blood samples can be used to estimate elemental contamination in liver, muscle, and kidney tissue of green turtles.[35] Whole blood allows measurement of elements in both intracellular and extracellular compartments and represents recent (weeks to months) exposure to contaminants.[45,47] Conversely, scutes are hard, keratinized plates that make up the shell of a sea turtle. Many trace elements bind to the keratin of the scutes, incorporating and storing contaminants over time, giving a history of exposure.[29] Once incorporated into scute, elements become metabolically inactive and are unavailable for remobilization.[14] These two tissues, collected non-lethally, along with food samples, can be used to monitor elemental contaminant exposure in live turtles. The trophic transfer factor (TTF) describes the ratio between the concentration of an element in the animals' tissue to the concentration of the element in its food.[15] A TTF value > 1 indicates elements may biomagnify, while a TTF <1 means biomagnification is unlikely to occur.[34]

The monitoring of captive turtles may help answer questions about wild turtles. Sea turtles have been raised and rehabilitated in captivity for decades. In addition to rehabilitation, sea turtles in captivity can provide valuable insight into disease pathways and physiology.[17] Captive turtles are fed a prescribed diet and offer a unique opportunity to study the trophic transfer of trace elements, and may potentially serve as a baseline of elemental concentrations in sea turtles.

This study quantified the mass fractions (hereinafter called concentrations) of the inorganic elements: V, Cr, Co, Ni, Cu, As, Se, Sr, Cd, Sb, and Pb in the blood and scutes of

captive and wild Hawaiian green turtles. Studies have previously been conducted on sea turtles in captivity, quantifying trace elements in headstarted (captive reared) Kemp's Ridley turtles (*Lepidochelys kempii*); and captive hawksbill (*Eretmochelys imbricata*), green, and loggerhead (*Caretta caretta*) turtles.[38,43,44] This is the second study comparing captive and wild green turtles and the first in the Central Pacific Ocean. To our knowledge this is the first study to measure elemental concentrations in captive turtle food and to calculate TTF and dietary intake values. This study determined elemental concentrations in adult green turtles to determine if captive turtles may serve as a baseline to better understand exposure in wild turtles.

MATERIALS AND METHODS

Sampling

Samples were taken according to the Biological and Environmental Monitoring and Archival of Sea Turtle Tissues (BEMAST) protocols. Blood, scute, algae, pelleted food and water samples were stored in liquid nitrogen vapor freezers (LN2, -150 °C) until shipped frozen to Texas Tech University for analysis.[28] Health status and morphometrics of all turtles can be found in SI Table 1. Wild green turtles were captured in November 2011 and April 2015 by hand or scoop net in Kapoho Bay on the eastern side of Hawaii Island (19.496291 N -154.820698 W, Figure 1), which is now covered by new lava from the 2018 eruption of Kilauea. After capture, turtles were brought ashore and blood samples taken. Briefly, double-ended stainless-steel needles were used to draw blood into glass sodium heparin Vacutainer blood collection tubes within 15 minutes of capture. Blood was kept on ice until aliquoted and frozen. Scute shavings were collected from the fifth central scute. The scute was cleaned of epiphytic/epibiotic organisms and sloughing keratin removed with a wet plastic scrubbing pad. The scute and knife

were cleaned with isopropanol and Millipore water, and dried with a cleanroom wiper. The top layer of scute that is penetrated with algae was shaved off and discarded. The knife and scute were cleaned again with isopropanol and Millipore water and dried. The knife was used to shave keratin layers off the entire surface of the 5th central scute, being careful to avoid scute seams or shaving too deep. Scute shavings were collected in a Teflon bag and sealed with a cable tie. Scutes were homogenized by mortar and pestle (pre-cleaned by soapy water sonication, Millipore water and acid rinsing) prior to analysis. Samples of known algae prey items (*Gracilaria salicornia* and *Amancia* spp.) were collected from Kapoho Bay in December 2013 while snorkeling to represent the wild green turtle diet.[5] Dive gloves were worn while collecting algae samples, samples were placed in centrifuge tubes, and stored in LN2 until analysis. Green turtles were brought into Sea Life Park Hawaii (SLPH) from the wild in the mid-to-late 1960s and are one of the few captive breeding colonies in the world. They are fed a commercially available pelleted floating compound (35% protein turtle finisher, Melick Aquafeed, Catawissa, PA 17820 USA) designed to meet the nutritional requirements of green turtles. The 14 adult green turtles (9 females, 5 males) living in the pond are fed 2.3 kg of pelleted food per day directly into the exhibit without turtle separation. Their diet is supplemented with fresh lettuce. Blood and scute were sampled from six adult captive turtles (three male and three female, without additional selection criteria) in August 2012 following the same protocol as the wild turtles. Seawater samples were collected October 2017 in 50 mL centrifuge tubes (Corning Inc., Corning, NY 14831 USA) from three points in the pond (the water inlet, the center of the pond and near the nesting beach). The Kapoho Bay turtles (three males, two females, and two of unknown sex) without externally visible fibropapillomatosis tumors were selected based on a similar SCL as the captive turtles. All sea turtles in this study

are adult or sub-adult sea turtles, with a SCL of at least 76 cm, reducing the variability in elemental concentrations due to life stage.

Sample Digestion and ICP-MS Analysis

A small mass digestion method was used.[19] Trace metal grade nitric acid (HNO_3; 0.2 mL, 5.53 mol/L, Fisher Scientific, Fair Lawn, New Jersey 07410, USA), trace metals grade hydrochloric acid (HCl; 0.1 mL, 0.99 mol/L, Fisher Scientific), and high-purity deionized water (resistivity = 18 MΩ cm^{-1}, 0.1 mL) were added to approximately 0.1 g scute in 15 mL centrifuge tubes. Tubes were heated in a reciprocal shaking hot water bath (Model 66800, Precision Scientific, Chennai, Tamil Nadu, India 600086) at 90 °C (± 5 °C) for 1 h. After cooling, 0.1 mL high purity 30% hydrogen peroxide (H_2O_2, Fisher Scientific) was added and samples were heated again for 30 min. Samples were subsequently diluted to 10 mL with high-purity deionized water and filtered through 0.45 µm polytetrafluoroethylene (PTFE) filters (Whatman 0.45 µm GMF-150, Maidstone, ME England). Captive turtle food (0.1 g) was digested in the same manner as the scute samples. For blood samples, 0.4 mL HNO_3, 0.1 mL HCl, and 0.1 mL high-purity deionized water were added to 0.2 mL blood in 15 mL centrifuge tubes; vortexed; sonicated for 10 min; and heated in a hot water bath at 95 °C ± 5 °C for 1 h. After cooling, 0.2 mL H_2O_2 was added to the blood samples, vortexed, sonicated, and heated for 30 min. This process was repeated two more times until the blood samples were completely digested. Blood samples were diluted to 10 mL with high-purity deionized water and filtered through 0.45 µm PTFE filters.

Algae samples were dried to complete dry mass overnight at 55 °C. A subsample of the algae (0.2 g) was combined with 0.2 mL HNO_3, 0.1 mL HCl, and 3 mL high-purity deionized water in a 15 mL centrifuge tube. The samples were placed in a hot water bath at 95 °C (± 5 °C)

for 1 h. After cooling, 0.2 mL H_2O_2 was added and the samples were heated for 30 min. Samples were filtered through 0.45 µm PTFE filters and diluted to 10 mL for analysis. Seawater samples collected from SLPH (0.5 mL) were acidified with 1.4 mL HNO_3 and subsequently diluted with high-purity deionized water in a 1:100 ratio. An in house matrix control material was created using a seawater and algae sample. The seawater and algae samples were spiked with a custom multi-element standard containing Al, Sb, As, Cd, Cr, Co, Cu, Pb, Hg, Ni, Se, Ag, Sr, Sn, V and Zn (TTUNIV-1, Inorganic Ventures, Christianburg, VA USA) at 100 ng/g. The trace element concentrations in the natural samples were subtracted from the spiked samples to account for the natural trace element mass fractions in the sample.

Elemental analyses were performed in helium collision mode using an Agilent Technologies 7900 inductively coupled plasma- mass spectrometer (ICP-MS) equipped with an Agilent Technologies ASX-500 Series autosampler (Agilent, Santa Clara, CA 95051 USA). A custom multi-element calibration standard (TTUNIV-1, Inorganic Ventures) was used to build a seven-point external calibration curve with concentrations ranging from 0.1 ng/g to 1000 ng/g. The calibration solutions were analyzed at the beginning and end of every run; and a check standard solution of 10 ng/g or 50 ng/g run every ten samples. Internal standards (Scandium (Sc), Germanium (Ge), Rhodium (Rh), Indium (In), Terbium (Tb), Bismuth (Bi), and Lutetium (Lu), (Agilent Technologies) were added online and samples were reanalyzed if the recovery was outside the acceptable recovery range of 80% to 120%. Quality assurance was conducted using method blanks, field blanks produced from the same lot numbers of blood collection supplies, and certified reference materials (CRMs; DOLT-5: Dogfish Liver from National Research Council Canada, Ottawa, Ontario, and Seronorm™ Trace Elements Whole Blood L-3 [REF 210313, LOT 1509408] from Sero AS, Billingstad, Norway). 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)[12]. Limit of quantification (LOQ) was calculated by multiplying the lowest concentration of the calibration curve by the dilution factor. Both field and method blanks were subtracted from the samples. All blood values are in ng/g wet mass (wm); scute and turtle chow in dry as received mass (dm), and algae in dry mass (dm).

Statistical Analysis

All statistical analyses were performed using the program R (version 3.2.3, The R foundation for Statistical Computing, Vienna, Australia) and the Nondetects and Data Analysis for Environmental Data (NADA) package, recommended for left censored data[26]. Mean, median, and standard deviations were calculated using Kaplan-Meier (K-M) or regression on order statistical (ROS) models. Shapiro-Wilk and Bartlett tests were used to test normality and homoscedasticity of data. Differences in elemental concentrations between captive and wild turtles were determined by parametric (using the NADA function cenmle) or nonparametric (using cendiff) tests. A Kendall's tau correlation was run (using cenken) to determine the relationships between SCL and elements in the blood or scute.

Trophic transfer factor was calculated as the ratio between the concentration of an element in the tissue (blood or scute) to the concentration in the diet (pelleted food or algae). For blood or scute concentrations below the LOQ, half the LOQ was used. Estimated daily intake (EDI) was calculated to estimate the daily exposure of captive and wild turtles to elemental contaminants. The fourteen captive turtles are fed approximately 2.3 kg of pelleted food each day, giving a consumption rate of 164 g pelleted food per turtle per day. The consumption rate was multiplied by the measured elemental concentration of the pelleted food, giving a daily

intake rate in µg per turtle per day. Captive turtles are fed additional, undocumented amounts of vegetables (lettuce) per day, but because of the amount uncertainties these were not included in the EDI. Wild turtle EDI was calculated using a daily food intake of 127 g (dm) per turtle per day as determined by Williams (1988).[50] *G. salicornia* made up approximately 40.9% of the green turtles' diet at Kapoho and *Amansia spp*. approximately 30%.[40] The remaining 29.1% was estimated using a 50:50 combination of the *Amansia* and *G. salicornia* concentrations.

RESULTS

The instrument detection limit ranged from 0.02 to 0.39 ng/g (SI Table 2). The LOQ were 10 ng/g, 0.29 ng/g, 2 ng/g and 0.83 ng/g for water, algae, scute/food pellets, and blood, respectively. Measured values of V, Cr, Co, Cu, As, Se, Cd, and Pb are in agreement with the certified values in the Seronorm CRM and Ni and Sb overlap with the certified value. Measured values of Cr, Co, Ni, Cu, and Pb are in agreement with the certified values in the Dolt 5 CRM. The remaining elements (V, As, Se, Sr, and Cd) were within 20% of the certified values. The in house matrix spike control material matched the spiked concentration by ± 21% (SI Table 2).

Individual measurements of element concentrations in scute and blood samples are provided in SI Tables 3 and 4. Eleven elements were above LOQ in at least one blood or scute sample of captive and wild turtles (Table 1). Essential element Sr was found at the greatest concentration in the blood and scute of all sea turtles in this study. Lead, a toxic heavy metal, was found in the blood of all wild and captive turtles.

Eleven elements were measured in the pelleted food at SLPH as well as representative samples of algae (*Amansia spp*. and *G. salicornia*) from Kapoho Bay (Table 2). Water samples from SLPH contained four elements > LOQ; Cr, Cu, Se and Sr (Table 3). Water samples from

Kapoho Bay were not analyzed in this study, however Cr, Cu and Se concentrations published previously for Kapoho Tidepool water[7] were less than those in SLPH (Table 3).

Significant differences ($P < 0.05$) were observed in blood and scute elemental concentrations between captive and wild turtles (Table 1). Captive turtles had significantly greater blood Se, an essential element, than wild turtles (Table 1). Three toxic elements, Cd, Ni, and Pb were significantly greater in the blood of wild turtles (Table 1). Captive turtles had significantly greater scute Cr, Cu, Se, and V, while As was greater in the scute of wild turtles (Table 1).

A Kendall's rank correlation showed three metals in the captive turtles scute that were significantly, positively correlated with SCL; V ($\tau = 0.87$, $P = 0.02$), Cd ($\tau = 0.8$, $P = 0.02$) and Pb ($\tau = 0.8$, $P = 0.04$) (SI Figure 1). Selenium was the only element in the blood of wild turtles to be significantly correlated with SCL ($\tau = 0.7$, $P = 0.03$) (SI Figure 1). No correlations were found between SCL and scute in wild turtles or between SCL and blood in captive turtles.

Three metals in captive turtle scutes, Cr, Sr, and V, had a TTF > 1 (Table 4). No TTF was > 1 in the blood of captive turtles. No elements in *Amansia spp.* had a TTF > 1 in scute or blood of wild turtles. Selenium in *G. salicornia* showed the potential for bioaccumulation in scutes and blood of wild turtles (Table 4).

Sex influenced differences in some element concentrations in captive turtles. Scute Pb was significantly greater in females than males. Blood Se was greater in males, and blood Sr was greater in females (SI Figure 2).

DISCUSSION

Elemental concentrations in sea turtles are affected by diet, species, sex, age, health status, type of tissue, and location, including captive vs wild. The differences in elemental

concentrations between captive and wild turtles are primarily due to their food source. Captive turtles are given a pelleted food that is a mixture of animal and plant protein products, with sodium selenite added as a dietary supplement. Consequently, the concentration of Se in the pelleted food was about 3 to 9 times greater than the samples of algae from Kapoho Bay. Captive turtles had significantly greater concentrations of Se in their blood and scute than wild turtles. This difference in Se concentrations was also seen in loggerhead turtles in captivity for rehabilitation[10]. The Se concentrations in loggerhead blood significantly increased through rehabilitation. Selenium concentrations in the present study are likely not of concern when compared to established avian toxicity reference values. Adequate Se concentrations in the whole blood of avian species ranges from 130 ng/g to 200 ng/g, sublethal effects such as weight loss and alopecia are seen at 900 ng/g and concentrations of 12,000 ng/g to 16,000 ng/g cause death.[37,42] Most sea turtle populations have adequate blood Se concentrations, with few populations including green turtles in San Diego Bay and Boyne River Estuary at or above sublethal concentrations in birds[22,29] (Figure 2).

Another essential element, Cu, was 11 times and 3 times greater in pelleted food then *G. salicornia* and *Amansia spp*., respectively. Due to the high concentrations of Cu in the pelleted food, scute Cu concentration was significantly greater in the captive turtles than the wild turtles. However, blood Cu concentrations were similar between the two groups, because Cu is known to be tightly regulated in the blood through excretion.[6] The similar blood Cu concentrations between the two groups, despite their major differences in exposure, indicates that sea turtles can regulate blood Cu concentrations. High concentrations of Cu in pelleted food led to increased sequestration of Cu in the scute as measured in the captive turtles. Though a decrease in the efficiency of copper excretion leading to copper toxicosis has not been shown in reptiles, zoo

managers should be aware of the possibility. However, aquarium and zoo managers likely do not need to worry about Cu in the turtle's food source due to their ability to manage blood Cu concentrations.

Vanadium, As, Cd and Pb were all at greater concentrations in both of the algae samples than the pelleted food and Ni was greatest in the *Amansia* spp. followed by the pelleted food. Some elements are naturally high in marine algae species. For example, V is an essential element for some brown and green algae, marine algae are known to accumulate As, and Cd is highly mobile between the environment and plants.[4,13,49] Similarly, these elements were all at greater concentrations in the blood of wild turtles with marine algae diets than captive turtles.

Turtle blood concentrations (ng/g wm) of V, Cd, Pb, As, Se and Ni in this study were compared to turtles around the world and toxicity reference values for related species (Figure 2). Toxicity reference values do not exist for green turtles. All sea turtle populations shown in Figure 2 have blood V concentrations greater than background levels in humans (0.05 ng/g), but less than the maximum measured in osprey blood from the Chesapeake Bay area (54 ng/g wm) with known pollution.[22,39] Cadmium concentrations in almost all the sea turtle populations are very similar, well below the value associated with toxic effects in birds (260 ng/g).[48] Blood Pb concentrations of ≤ 200 ng/g are considered background levels in three orders of birds (Anseriformes, Falconifomes, and Columbiformes), and 500 ng/g is considered clinical poisoning in Anseriformes and Falconiformes.[18,42] All sea turtle populations are well below these levels except turtles from San Diego Bay, CA. Arsenic blood concentrations were greatest in turtles from Boyne River Estuary in Australia, above ≈ 600 ng/g, which is potentially toxic in humans.[22] Nickel blood concentrations of turtles in this study were above background levels in humans (3 ng/g to 7 ng/g)[22] but toxicity reference values are unknown for all animal species.

Furthermore, reference values for one species may not accurately extrapolate to another. In the case of Pb, As and Se, sea turtles may be more tolerant to these elements than birds or humans because the populations with greater concentrations of these elements (Figure 2) are considered healthy.

Diet played the largest role in differences between captive and wild turtles, but sex is known to be a confounding factor. Female captive turtles had greater concentrations of scute lead than males. When females lay eggs, calcium (Ca) is mobilized from the bone for eggshell formation. Lead mimics Ca and is stored in and mobilized from bone following the same kinetics as Ca.[6] For example, blood Pb concentrations in leatherbacks have been shown to increase as the nesting season progresses.[24] In the wild, female turtles do not feed during the nesting season; their energy is supplied from fat stores.[8] One hypothesis to explain the greater scute lead concentrations in these captive female turtles is that as Ca or Pb was mobilized from bones, blood Pb concentrations increased. Unlike wild turtles, the captive turtles would continue to eat during the nesting season, and continue to be exposed to Pb and Ca. While some of those nutrients would be put into developing eggs, excess Pb could be sequestered in scute.

Three elements in captive turtle scutes, V, Cd and Pb, and one element in the wild turtle blood, Se, were positively correlated with SCL (SI Figure 1). Larger, potentially older, turtles have greater concentrations of these elements. This suggests accumulation through age of V, Cd, or Pb in their scutes. Accumulation through age is also suspected for Se in blood, based on these correlations. The biomagnifying potential, as seen by a TTF>1 for Se in blood of wild turtles, supports this suggestion.

One limitation to the current study was the lack of analysis of elements in the lettuce that captive turtles are fed at SLPH. This contribution could not be included in the daily intake or TTF calculations for the captive turtles, so the EDI and TTFs are conservatively underestimated. However, this study remains the first to analyze the food source of captive turtles and increases the knowledge of the impacts of pelleted food on captive turtles.

All metals found in the captive and wild Hawaiian green turtles in this study are similar to or less than concentrations found in sea turtles elsewhere (Figure 2). It is important to establish benchmark concentrations of elements in sea turtles to understand the toxicity threat of elements if and when they are found to be elevated and to follow contaminant exposure over time. Captive sea turtles are not the best population for establishing benchmark concentrations for elements. Sea Life Park Hawaii and other aquaria or rehabilitation centers feed captive sea turtles a diet very different than their natural prey. In the case of SLPH, the pelleted food they consume has greater concentrations of some elements than the wild turtles' food source, and thus their tissues have greater concentrations. Benchmark concentrations for some elements may be better established in healthy, wild populations living in relatively unpolluted areas.

NOTES

The authors declare no competing financial interest. Certain commercial equipment, instruments, or materials are identified in this paper 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.

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Table 1. Elemental concentrations in the scute (ng/g dm) and blood (ng/g wm) of captive turtles at Sea Life Park Hawaii and wild turtles at Kapoho Bay, HI.

	SCUTE					
	Captive			Wild		
Element	Median	Mean (SD)	% detected	Median	Mean (SD)	% detected
As*[a]	9.2	30.3 (55.8)	50	138.0	144 (22.8)	100
Cd[b]	9.72	15.5 (11.6)	66.7	14.7	14.4 (3.70)	80
Co[b]	-	-	0	-	-	0
Cr*[b]	119	855 (1825)	100	55.5	53.8 (37.2)	100
Cu*[a]	1030	1090 (372)	100	221.0	212 (29.0)	100
Ni[b]	180	270 (241)	100	111.0	134 (74.1)	100
Pb[a]	14.6	20.8 (16.8)	33.3	26.8	32.9 (12.0)	60
Sb	-	-	0	-	-	0
Se*[a]	350	306 (90.7)	100	126	110 (33.9)	100
Sr[a]	7370	9150 (4660)	100	5420	5920 (2160)	100
V*[b]	450	549 (310)	100	140	158 (80.9)	100

	BLOOD					
	Captive			Wild		
Element	Median	Mean (SD)	% detected	Median	Mean (SD)	% detected
As[a]	22.8	28.8 (17.3)	100	28.0	35.6 (24.2)	100
Cd	-	-	0	-	-	14.3
Co	-	-	0	4.23	4.61 (1.47)	42.9
Cr[b]	-	-	0	-	-	14.3
Cu[a]	611	609 (87.5)	100	28.0	35.6 (24.2)	100
Ni*[b]	-	-	16.7	38.0	40.4 (19.3)	100
Pb*[a]	25.1	24.6 (12.7)	100	55.3	69.3 (30.5)	100
Sb	-	-	0	-	-	0
Se*[a]	440	439 (103)	100	74.2	102 (61.4)	100
Sr[a]	717	789 (427)	100	631	694 (137)	100
V*[b]	-	-	0	11.6	12.7 (4.07)	71.4

[a] difference between captive and wildwas determined by a parametric function (R NADA cenmle)

[b] difference between captive and wild was determined by a nonparametric function (R NADA cendiff)

* significant difference ($p < 0.05$) between captive and wild turtles

Table 2. Mean elemental concentrations in pelleted food (n = 3) and algae (n = 1) in ng/g dm.

Element	Pelleted Food	*Amansia spp.*[*]	*Gracilaria salicornia*[*]
As	259 ± 5.8	1,520	4,270
Cd	53.5 ± 1.9	518	196
Co	95.5 ± 6.1	281	39.3
Cr	523 ± 105	1,630	663
Cu	10,400 ± 2,460	3,610	985
Ni	2,490 ± 118	8,970	532
Pb	97.5 ± 16.8	1,030	388
Sb	11.3 ± 0.5	14.9	14.1
Se	767 ± 54.3	237	77.6
Sr	8,730 ± 593	79,000	31,100
V	208 ± 24.7	2,020	3,370

[*] Moisture content of *Amansia spp*. was 46.2% and *G. salicornia* was 45.1%.

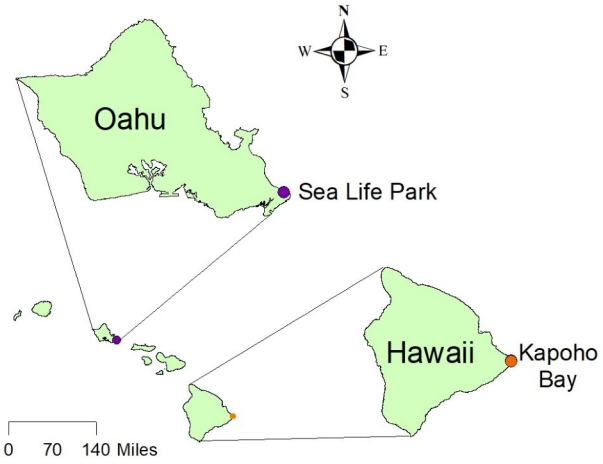
Table 3. Elemental concentrations (ng/mL) at the water inlet, middle of the pond, and near the nesting beach in the sea turtle habitat at SLPH compared to seawater from Kapoho Tidepools published previously.[7]

Element	Water Inlet	Middle	Nesting Beach	Kapoho Tide Pool
As	< LOQ	< LOQ	< LOQ	1.48
Cd	< LOQ	< LOQ	< LOQ	-
Co	< LOQ	< LOQ	< LOQ	0.01
Cr	15.8	14.1	15	0.14
Cu	280	< LOQ	< LOQ	0.19
Ni	< LOQ	< LOQ	< LOQ	0.24
Pb	< LOQ	< LOQ	< LOQ	0.01
Sb	< LOQ	< LOQ	< LOQ	-
Se	32.8	< LOQ	23.9	0.02
Sr	4,450	4,490	4,200	-
V	< LOQ	< LOQ	< LOQ	1.52

<LOQ = less than the limit of quantitation

Table 4. Trophic transfer values for blood and scute in captive and wild turtles. Estimated daily intake (µg/day) is calculated for captive turtles at SLPH using only pelleted food and wild turtles using a combination of *Amansia spp*. and *G. salicornia*.

	Captive			Wild				
	Pelleted Food			Amansia spp.		G. salicornia		
	Trophic Transfer Scute	Trophic Transfer Blood	Daily Intake	Trophic Transfer Scute	Trophic Transfer Blood	Trophic Transfer Scute	Trophic Transfer Blood	Daily Intake
As	0.12	0.11	42	0.09	0.02	0.03	0.01	387
Cd	0.29	< 0.01	9	0.03	< 0.01	0.07	< 0.01	43
Co	0.01	< 0.01	16	< 0.01	0.02	0.03	0.12	19
Cr	1.63	< 0.01	86	0.03	0.02	0.08	0.04	139
Cu	0.10	0.06	1700	0.06	0.01	0.22	0.04	273
Ni	0.11	< 0.01	408	0.01	< 0.01	0.25	0.08	544
Pb	0.21	0.25	16	0.03	0.07	0.11	0.24	85
Sb	0.01	< 0.01	2	0.07	0.03	0.07	0.03	2
Se	0.40	0.57	126	0.47	0.43	1.42	1.31	19
Sr	1.05	0.09	1430	0.07	0.09	0.19	0.02	6660
V	2.64	< 0.01	34	0.08	0.01	0.05	< 0.01	352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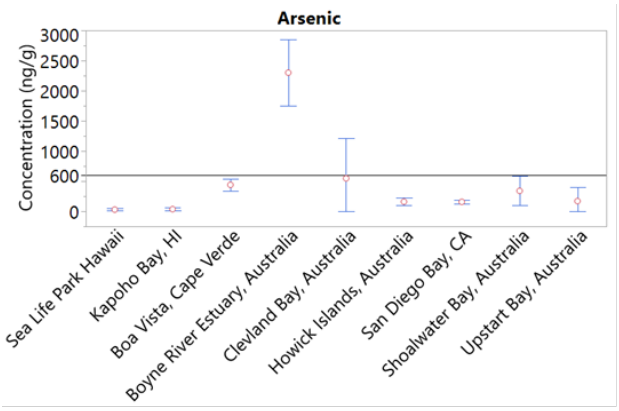
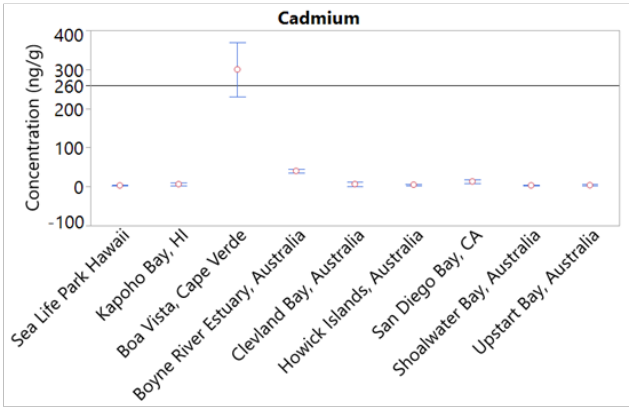
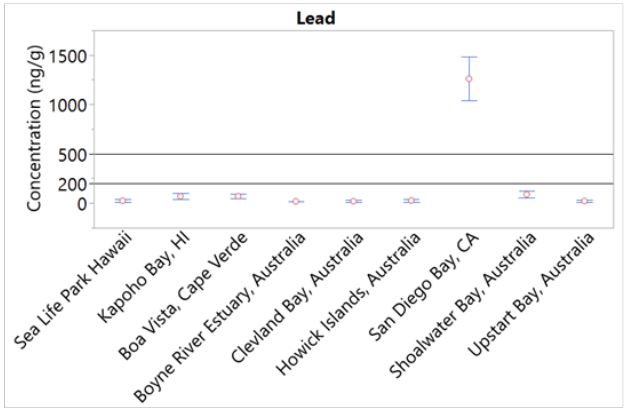
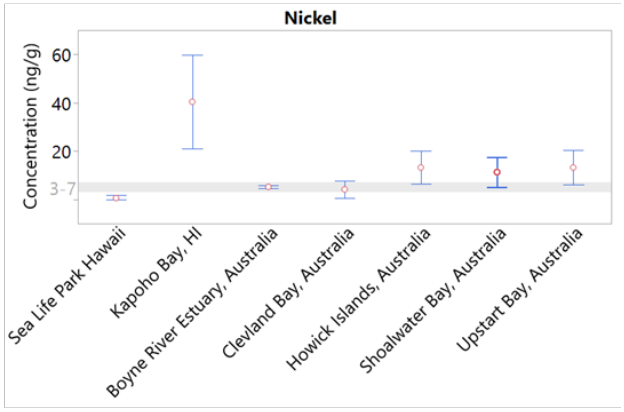
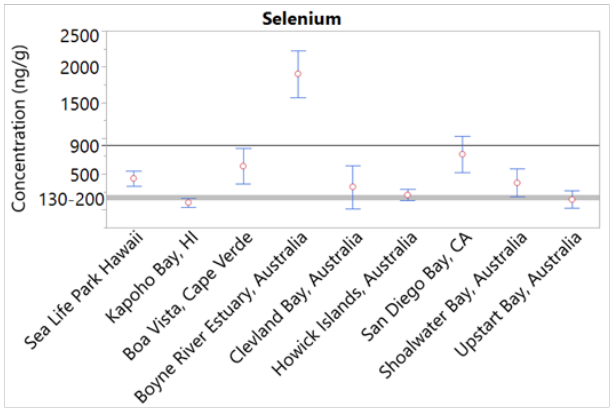
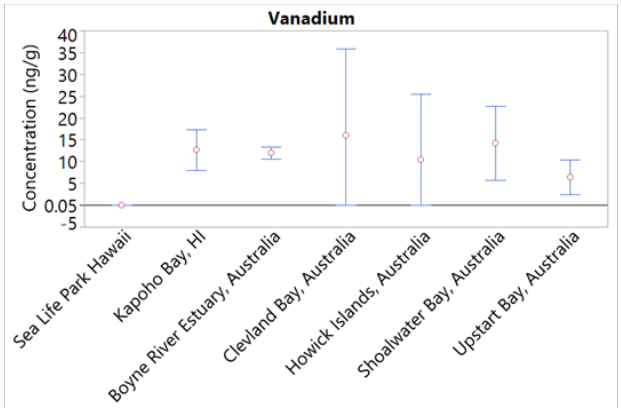












As: > 600 ng/g is toxic in humans

Cd: 260 ng/g causes toxic effects in avian species

Pb: < 200 ng/g is background levels in avian species, 500 ng/g is clinical poisoning in avian species

Ni: 3 ng/g to 7 ng/g: background concentration in humans

Se: 130 ng/g to 200 ng/g: adequate in avian species; 900 ng/g: sublethal effects (weight loss, alopecia) in avian species

V: 0.05 ng/g is the background concentration in humans

SI Table 1: Health and body information for individual sea turtles. None of the selected sea turtles had flippers amputated, fishhooks or fishing line on their bodies, signs of boat impact, were emaciated, or had external fibropapillomatosis tumors.

Turtle ID	Category	Year	Re-capture	SCL (cm)	Weight (kg)	Sex	PCV (%)	Carapace Damage	Shark Attack
4414642E78	Wild	2011	N	77.4	73.5	M	34.5	Y	Y
4414541222	Wild	2011	N	77.5	68.4	U	42	N	N
4414403833	Wild	2011	N	77.2	7	U	34	N	N
44130D7C31	Wild	2011	N	83.4	89.4	F	33	N	N
470C7B325B	Wild	2011	Y	80.1	81.7	M	36	N	N
443A113D41*	Wild	2015	Y	84.4	83.7	F	36	N	N
44545D612B*	Wild	2015	Y	76.2	66.7	M	29	N	N
A	Captive	2012	-	80.5	-	M	37	N	N
E	Captive	2012	-	94.5	-	F	43.5	N	N
J	Captive	2012	-	77.9	-	M	35	N	N
K	Captive	2012	-	82.8	-	F	34	N	N
L	Captive	2012	-	77.6	-	F	33	N	N
W	Captive	2012	-	78	-	M	39.5	N	N

Y= yes, N= no, M= male, F = female, U = unknown sex

* Turtles do not have scute samples.

- = Recapture does not apply to captive turtles and weights were not taken from captive turtles

SI Table 2: Instrument detection limit (ng/g) and % recovery of standard reference materials. Seronorm and DOLT 5 are represented as measured values ± detection limit and the certified/reference values in parentheses. Measured blood concentrations were corrected from mass-mass to mass-volume using a blood density of 1.05 g/mL. DOLT-5 was measured on a dry mass basis.

Element	Instrument Detection Limit (IDL)	Seronorm	DOLT 5	In House Water Control Material % recovery	In House Algae Control Material % recovery
As	0.17	27 ± 3.56 (21.8 - 32.7)	43.4 ± 1.56 (32.2 - 37)	101	95
Cd	0.04	11 ± 1.32 (7.9 - 11.9)	16.6 ± 0.69 (13.9 - 15.1)	96	112
Co	0.02	10.8 ± 0.71 (8.3 - 12.4)	0.29 ± 0.008 (0.241 - 0.293)	93	107
Cr^{+}	0.07	33.7 ± 6.62 (28.4 - 42.6)	2.50 ± 0.24 (1.77 - 2.93)	90	104
Cu	0.04	2440 ± 103 (1650 - 2500)	36.8 ± 1.43 (32.6 - 37.4)	90	116
Ni^{+}	0.07	7.09 ± 3.8 (8.8 - 13.3)	1.92 ± 0.40 (1.15 - 2.27)	102	100
Pb	0.29	376 ± 25.1 (310 - 467)	0.15 ± 0.009 (0.13 - 0.194)	113	96
$Sb^{\#}$	0.14	26.5 ± 0.3 (17.5 - 26.3)	0.025 ± 0.01 (0.013)	102	104
Se	0.39	215 ± 59.8 (158 - 238)	13.0 ± 0.73 (6.5 - 10.1)	107	110
Sr*	0.21	57.4 ± 15.7 (37)	4.22 ± 0.16 (3.47 - 3.98)	118	104
V	0.14	4.47 ± 1.09 (3.5 - 5.3)	0.64 ± 0.01 (0.45 - 0.57)	91	106

* Sr is a reference value in Seronorm

[+] Ni and Cr are reference values in DOLT 5

[#] Sb is an informational value in DOLT 5

15

SI Table 3. Elemental concentrations (ng/g dm) in scute for individual sea turtles.

Turtle ID	As	Cd	Co	Cr	Cu	Ni	Pb	Sb	Se	Sr	V
4414642E78	135	19.8	< LOQ	55.5	221	111	26.8	< LOQ	147	6840	190
4414541222	183	15.2	< LOQ	107	228	263	52.1	< LOQ	80.6	5420	281
4414403833	123	< LOQ	< LOQ	67.5	170	78.4	< LOQ	< LOQ	67.6	4780	78.4
44130D7C31	138	14.7	< LOQ	27.6	243	123	< LOQ	< LOQ	128	9120	140
470C7B325B	143	11.1	< LOQ	11.2	196	95.8	32.1	< LOQ	126	3450	98.8
443A113D41	-	-	-	-	-	-	-	-	-	-	-
44545D612B	-	-	-	-	-	-	-	-	-	-	-
A	16.2	15.5	< LOQ	117	1260	184	< LOQ	< LOQ	361	8000	509
E	143	37.5	91.1	4580	1690	758	49.4	< LOQ	371	18600	1170
J	< LOQ	< LOQ	< LOQ	62.0	778	138	< LOQ	< LOQ	366	7060	364
K	< LOQ	10.9	< LOQ	121	1230	176	32.1	< LOQ	339	7680	481
L	< LOQ	< LOQ	< LOQ	64.8	820	217	< LOQ	< LOQ	143	6620	352
W	19.5	9.72	< LOQ	187	756	149	< LOQ	< LOQ	256	6920	420

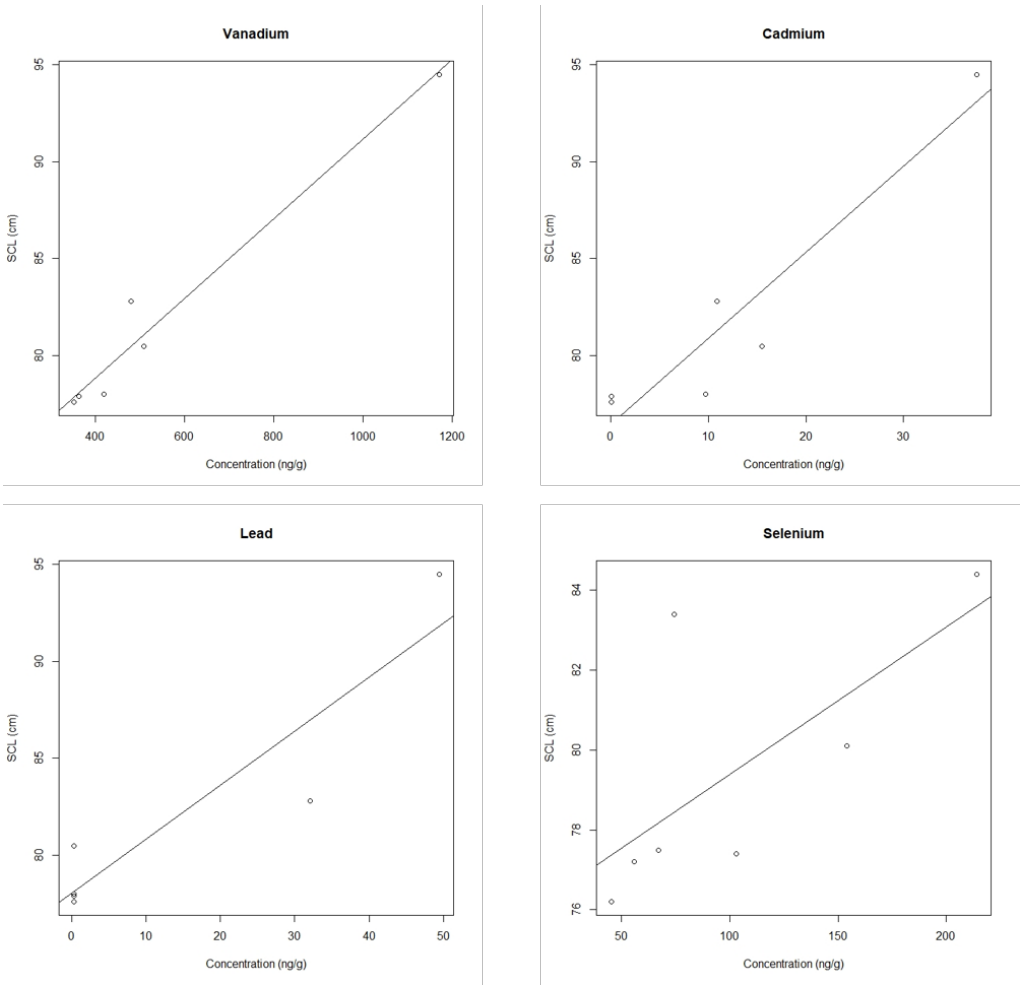
- Individual turtle without scute sample

<LOQ = less than the limit of quantitation

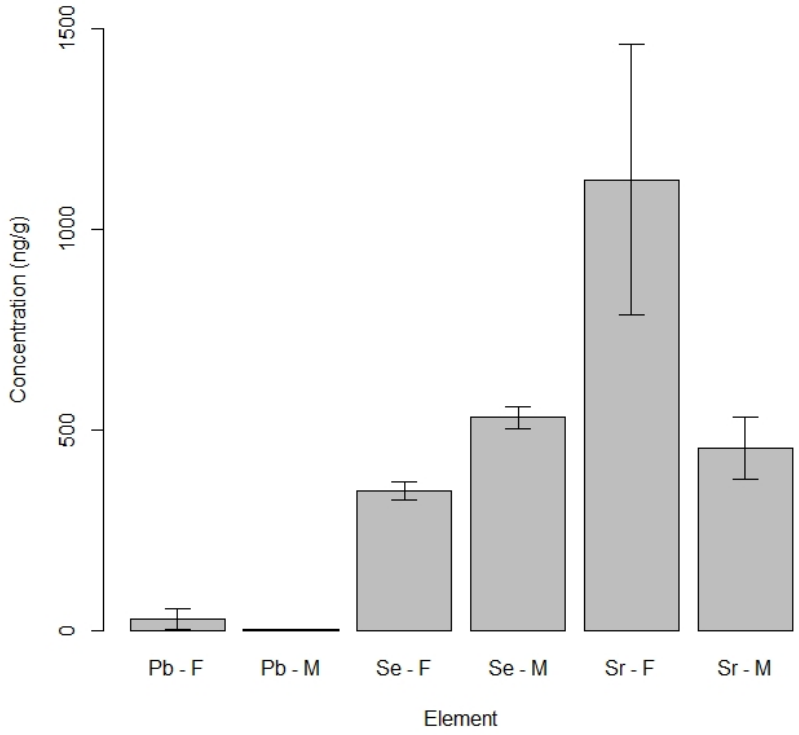
SI Table 4. Elemental concentrations (ng/g wm) in blood for individual sea turtles.

Turtle ID	As	Cd	Co	Cr	Cu	Ni	Pb	Sb	Se	Sr	V
4414642E78	57.2	< LOQ	< LOQ	< LOQ	640	49.9	90.2	< LOQ	103	854	15.7
4414541222	28.0	< LOQ	< LOQ	< LOQ	606	27.3	55.3	< LOQ	67.2	568	9.22
4414403833	20.9	< LOQ	< LOQ	< LOQ	697	20.9	39.6	< LOQ	56.0	822	< LOQ
44130D7C31	21.1	< LOQ	6.89	< LOQ	471	38.0	121	0.58	74.2	533	14.3
470C7B325B	71.2	< LOQ	5.97	1.80	680	78.7	46.1	0.63	154	619	19.3
443A113D41	48.8	15.0	< LOQ	< LOQ	645	38.2	85.8	< LOQ	214	833	< LOQ
44545D612B	1.94	< LOQ	< LOQ	< LOQ	659	29.6	46.5	< LOQ	45.2	631	11.6
A	19.1	< LOQ	< LOQ	< LOQ	647	< LOQ	23.6	< LOQ	524	417	< LOQ
E	63.7	< LOQ	< LOQ	< LOQ	576	< LOQ	8.29	< LOQ	336	1510	< LOQ
J	23.9	< LOQ	< LOQ	< LOQ	738	< LOQ	13.0	< LOQ	506	544	< LOQ
K	21.7	< LOQ	< LOQ	< LOQ	561	< LOQ	34.6	< LOQ	374	889	< LOQ
L	19.1	< LOQ	< LOQ	< LOQ	648	2.76	41.8	< LOQ	332	972	< LOQ
W	25.3	< LOQ	< LOQ	< LOQ	486	< LOQ	26.6	< LOQ	561	401	< LOQ

<LOQ = less than the limit of quantitation



SI Figure 1: A Kendall's rank correlation showed three metals in the captive turtles scute that were significantly, positively correlated with SCL; V ($\tau = 0.87$, $P = 0.02$),Cd ($\tau = 0.8$,$P = 0.02$) and Pb ($\tau = 0.8$, $P = 0.04$) and one metal in the wild turtles blood; Se ($\tau = 0.7$, $P = 0.03$) .



SI Figure 2: Sex influenced differences in Pb concentrations in the scute captive turtles. In the blood of captive turtles, Se was greater in males and Sr was greater in females.

M = male

F = female