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Trace element concentrations in green sea turtles

Trace Element Concentrations In Blood And Scute Tissues From Wild And Captive Hawaiian Green Sea Turtles (*Chelonia Mydas*)

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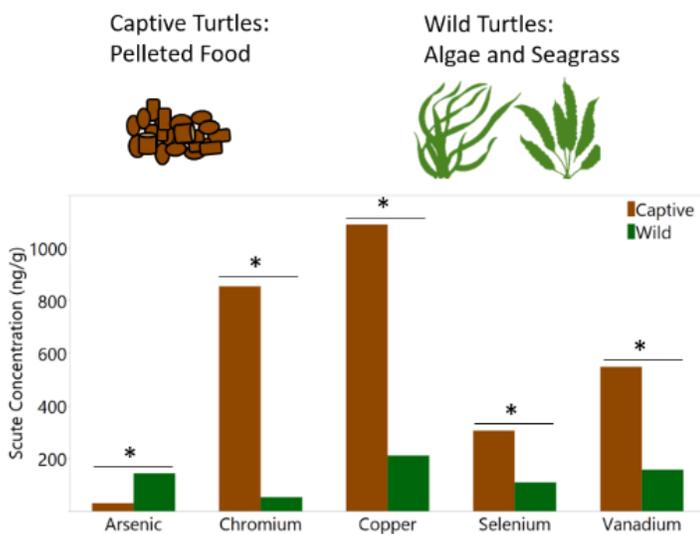
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Abstract: Sea turtles are exposed to trace elements through water, sediment, and food. Exposure to these elements has been shown to decrease immune function, impair growth, and decrease reproductive output in wildlife. This study compares trace element concentrations in green turtles in captivity at Sea Life Park Hawaii (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). Selenium was significantly greater ($p < 0.05$) in the blood of captive turtles compared to 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 and daily intake values. Wild turtles had greater estimated daily intake than captive turtles for all elements except Cu and Se. Because captive turtles are fed a diet very different from their wild counterparts, captive turtles do not represent control or reference samples for chemical exposure studies in wild 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.

Graphical Abstract

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 additives, while wild turtles have a primarily herbivorous diet.



Keywords: Marine turtle, reptile, aquarium, Hawaii, captive, heavy metals

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INTRODUCTION

Trace elements have been found in marine vertebrates around the world including cetaceans, seabirds, fishes, and sea turtles (Gochfeld et al. 1999; Gardner et al. 2006; Araújo and Cedeño-Macias 2016; Hansen et al. 2016). Some elements, including K, Na, Mg, Ca, Mn, Fe, Co, Ni, Cu, Zn, Mo, and Se, are essential 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, and Pb, can be toxic at very low concentrations (Gadd 1992; Liu et al. 2008; Cortes-Gomez et al. 2017). These nonessential elements can gain access to cells by mimicking essential elements, potentially disrupting cellular processes (Liu et al. 2008; Perrault et al. 2017). This can adversely affect the health of organs, the central nervous system, and the immune system through acute or chronic exposure (Liang et al. 2004).

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 (IUCN 2019; Sinaei and Bolouki 2017). Age classes are defined based on straight carapace length (SCL) measured from nuchal notch to the tip of the posterior-most marginal scute. 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, subadult and adult turtles exhibit high site fidelity to a particular location. Green turtles are bioindicators of the environment due to their long lives, high site fidelity, and accumulation of contaminants from food, water, and sediment

inadvertently ingested while eating (Aguirre and Lutz 2004; Lam et al. 2007; Andreani et al. 2008; Duncan et al. 2019).

Natural and anthropogenic contaminants, including metals, are one of the hazards potentially contributing to the decline of green turtles worldwide (Villa et al. 2015). Wild green sea turtles have a lifespan of 75 years, potentially bioaccumulating metals; though trace element concentrations in tissues are generally one to two orders of magnitude lower in sea turtles than seabirds or marine mammals (Lutcavage et al. 1997; Milton and Lutz 2003; Mayne et al. 2020).

Contaminant monitoring in sea turtles can help elucidate the risk of trace elements to the species as well as to the ecosystems in which they inhabit (Cortes-Gomez et al. 2017). Blood samples can be used to estimate elemental contamination in liver, muscle, and kidney tissue of green turtles (Van de Merwe et al. 2010). Whole blood allows measurement of elements in both intracellular and extracellular compartments and represents recent (weeks to months) exposure to contaminants (Takeuchi et al. 2016; Villa et al. 2017). 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 (Sakai et al. 2000; Day et al. 2005; Innis et al. 2008; Van de Merwe 2008; Komoroske et al. 2011; Bezerra et al. 2013; Bryan 2013; Perrault et al. 2017). Once incorporated into scute, elements become metabolically inactive and are unavailable for remobilization (Day et al. 2010). All species of sea turtle are threatened or endangered and protected by the Endangered Species Act, making non-lethal samples a requirement. Blood and scute, along with food samples, can be used to monitor elemental contaminant exposure in live turtles. Trophic

transfer factor (TTF) is defined as the ratio between the concentration of an element in the animals' tissue to the concentration of the element in its food (DeForest et al. 2007). A TTF value > 1 indicates elements may biomagnify, while a TTF < 1 means biomagnification is unlikely to occur (Mathews and Fisher 2008).

Contaminant monitoring in captive turtles may help answer questions about wild turtles. Sea turtles have been raised and rehabilitated in captivity for decades (Owens and Blainvillain 2013). In addition to rehabilitation, sea turtles in captivity can provide valuable insight into disease pathways and physiology (Falk et al. 2007). 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.

The present study quantified the mass fractions (hereinafter called concentrations) of the inorganic elements: As, Cd, Co, Cr, Cu, Ni, Pb, Sb, Se, Sr, and V in the blood and scutes of adult 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*); a green turtle in rehabilitation, and captive hawksbill (*Eretmochelys imbricata*), green, and loggerhead (*Caretta caretta*) turtles (Bezerra et al. 2014; Orvik 1997; Suzuki et al. 2012a,2012b). To our knowledge this is the first study to measure elemental concentrations in captive turtle food and to calculate TTF and dietary intake values. Past studies have incorrectly suggested that captive turtles may serve as control or reference samples for health assessments, contaminant exposure, or toxicology studies because they might be exposed to lesser contaminants than wild turtles (Arthur et al. 2008; Casal et al. 2009; Basile et al. 2012;

Suzuki et al. 2012a, Suzuki et al. 2012b). Since sea turtles receive most of their contaminant load from diet, captive turtles are still exposed to chemicals through provided food, whether wild caught or formulated, but likely at different concentrations than wild turtles (Keller 2013).

MATERIALS AND METHODS

Sampling

Samples were collected according to the Biological and Environmental Monitoring and Archival of Sea Turtle Tissues protocols (Keller et al. 2014). 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. Health status and morphometrics of all turtles can be found in Table S1. 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 min of capture. An average of 16.4 mL of blood was taken from each turtle (range: 13 mL – 19 mL). 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 70% isopropanol and Millipore high purity 18 M Ω cm⁻¹ water (hereinafter referred to as high purity deionized 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 70% isopropanol and high purity deionized 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. An average of 0.86 g of scute was collected from each individual (range: 0.69 g – 1.04 g). Scutes were homogenized by mortar and pestle (pre-cleaned by soapy water sonication, high purity deionized 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 (Arthur and Balazs 2008). Dive gloves were worn while collecting algae samples, which 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 in 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 (nuchal notch to tip) as the captive turtles. All sea turtles in this study were adult or sub-adult sea turtles, with a SCL of at least 76 cm. A Welch's two sample t-test ($p = 0.43$, $t = 0.83$) showed no difference in mean SCL between the two groups, reducing the variability in elemental concentrations due to life stage.

Sample digestion and ICP-MS analysis

A small mass digestion method was used (French et al. 2017). 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 (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 $90\text{ }^\circ\text{C} (\pm 5\text{ (SD)}\text{ }^\circ\text{C})$ 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 $0.45\text{ }\mu\text{m}$ polytetrafluoroethylene (PTFE) filters (Whatman $0.45\text{ }\mu\text{m}$ 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 $95\text{ }^\circ\text{C} \pm 5\text{ (SD)}\text{ }^\circ\text{C}$ 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. Moisture content was determined by subtracting the dry weight from the wet weight and dividing that value by the wet weight. Moisture content of both samples was 46%. 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 (SD) °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 Ag, Al, As, Cd, Co, Cr, Cu, Hg, Ni, Pb, Sb, Se, Sn, Sr, V, and Z (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. The in house matrix spike control material matched the spiked concentration by $\pm 20\%$ (Eastern-Research-Group 2012, Table S2).

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 Bismuth (Bi), Germanium (Ge), Indium (In), Lutetium (Lu), Rhodium (Rh), Scandium (Sc), and Terbium (Tb; Agilent Technologies) were added online and samples were reanalyzed if the recovery was outside the acceptable 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). Measured values of As, Cd, Co, Cr, Cu, Pb, Se, and V are in agreement with the certified values in the Seronorm CRM and Ni and Sb overlap with the certified value (Table S2). Measured values of Co, Cr, Cu, Ni, and Pb are in agreement with the certified values in the Dolt 5 CRM (Table S2). The remaining elements (As, Cd, Se, Sr, and V) were within 20% of the certified values. 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's *t*-test value (3.143), giving an IDL ranging from 0.02 ng/g to 0.39 ng/g (Table S2; Creed et al. 1994). Limit of quantification (LOQ) was calculated by multiplying the lowest concentration of the calibration curve by the dilution factor. The LOQ (ng/g) were 10, 0.29, 2, and 0.83 for seawater, algae, scute/food pellets, and blood, respectively. Both field and method blanks were subtracted from the samples. All blood values are in ng/g wet mass (wm); scute and

captive turtle food in ng/g dry mass as received (not dried in an oven; dm) and algae in ng/g dry mass (oven dried).

Statistical analysis and data handling

All statistical analyses were performed using the program R (version 3.2.3, The R foundation for Statistical Computing, Vienna, Austria) and the Nondetects and Data Analysis for Environmental Data (NADA) package, recommended for left censored data (Helsel 2005), with a $p \leq 0.05$ considered significant. 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 as well as to determine the relationship between blood and scute elemental concentrations.

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; DeForest et al. 2007). 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 (Perrault 2014).

Concentrations of elements in algae was measured using the dry mass of the algae. Algae concentrations were converted from ng/g dm to ng/g wm using the moisture content of the algae (46%). 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 earlier (Williams 1988). *G. salicornia* made up approximately 40.9% of the green turtles' diet at Kapoho and *Amansia spp.* approximately 30% (Russell and Balazs 2009). The remaining 29.1% was estimated using a 50:50 combination of the *Amansia* and *G. salicornia* concentrations.

RESULTS

Individual measurements of element concentrations in scute and blood samples are provided in Tables S3 and S4. 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. Turtle E, the largest of the captive turtles, had the greatest scute concentrations of all elements amongst the captive turtles.

Ten 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). Seawater samples from SLPH contained four elements $>$ LOQ; Cr, Cu, Se, and Sr (Table 3). Seawater samples from Kapoho Bay were not analyzed in this study, however Cr, Cu, and Se concentrations published previously for Kapoho Tidepool water (Bienfang et al. 2009) 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 ($\tau = 0.80, p = 0.02$) and Pb ($\tau = 0.80, p = 0.04$, Figure S1). Selenium was the only element in the blood of wild turtles to be significantly correlated with SCL ($\tau = 0.70, p = 0.03$, Figure S1). 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). Only one element, Cu, was negatively correlated between blood and scute tissue in wild turtles ($\tau = -1.0, p = 0.027$; Figure S2). No elements were correlated between blood and scute in captive turtles.

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 (Figure S3).

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 in this study 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 rehabilitation whereby blood selenium concentration increased through rehabilitation (Camacho et al. 2014b). 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 are seen at 900 ng/g, and concentrations of 12,000 ng/g to 16,000 ng/g cause death (O'Toole and Raisbeck 1997; Stout et al. 2010). Furthermore, yellow-bellied sliders (*Trachemys scripta scripta*) were exposed to Se at doses of 15,000 ng/g and 30,000 ng/g for 5 weeks. Sliders in the 30,000 ng/g treatment group had a blood Se concentration of $14,000 \pm 900$ ng/g and exhibited anemia. Mortality was only seen in the 30,000 ng/g treatment group and not in the control or 15,000 ng/g groups (Haskins et al. 2017). However, leatherback sea turtles have been documented with blood Se concentrations up to 10,300 ng/g, indicating leatherback turtles may tolerate high Se concentrations (Innis et al. 2010; Perrault et al. 2013). Selenium may represent a greater hazard to eggs and

hatchlings than adult sea turtles. A study of metals in green sea turtle eggs determined that the Se concentrations (464 ± 26 ng/g wm) were likely to affect hatching success (van de Merwe et al. 2009). 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 (Figure 2; Komoroske et al. 2011; Gaus et al. 2012). Chelonian species are likely more resilient than avian species from Se toxicity, as seen from high blood Se concentrations in green sea turtles from Boyne River Estuary, Australia, leatherbacks from the US Virgin Islands, and effects observed in laboratory-exposed yellow-bellied sliders (Gaus et al. 2012; Perrault et al. 2013; Haskins et al. 2017).

Another essential element, Cu, was 11 times and 3 times greater in pelleted food than *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 (Liu et al. 2008). The similar blood Cu concentrations between the two groups, despite their major differences in exposure, indicate 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. A negative relationship was seen in Cu between scute and blood tissues of wild turtles (Figure S2). This relationship is difficult to explain, but may have to do with (1) blood representing recent exposure to Cu that has not yet been incorporated in the scute tissue, (2) Cu is tightly regulated in the blood through rapid elimination pathways that do not involve scute growth, and/or (3)

turtles with higher Cu exposure have induced metallothioneins or other elimination pathways that eliminate the Cu before it is sequestered into scute (Storelli et al. 2008). A decrease in the efficiency of copper excretion leading to copper toxicosis has not been shown in reptiles.

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 including V, As, and Cd. Marine algae may be high in V because V is an essential element for some species while As is high because some marine algae can accumulate As. Cadmium is highly mobile between the environment and algae and will bioaccumulate in seaweed (Wever and Kustin 1990; Dawczynski et al. 2007; Ardiyansyah et al. 2019). Similarly, these elements were all at greater concentrations in the blood of wild turtles than captive turtles.

The $TTF > 1$ seen in V, Cr, and Sr in captive turtle scutes indicates these elements may biomagnify in captive turtles. Turtle E had much greater concentrations of all elements in its scute tissue, and may have influenced the TTF calculations. When Turtle E was removed, only V had a $TTF > 1$. A larger sample size of captive turtles is needed to conclusively determine which elements may biomagnify in green turtles.

Turtle blood concentrations (ng/g wm) of V, Cd, Pb, As, Se, and Ni in this study were compared to green turtles around the world, with associated toxicity reference values for related species, as toxicity reference values do not exist for green turtles (Figure 2). All sea turtle populations shown in Figure 2 have blood V concentrations greater than background levels in humans (0.05 ng/g), but show concentrations less than

the maximum concentration measured in osprey blood from the Chesapeake Bay area (54 ng/g ww) with known pollution from metal-working and petroleum refinery activities (Rattner et al. 2008; Gaus et al. 2012). A population of sea turtles at an uncontaminated site in the Howick Islands, Australia had blood V concentrations ranging from 1.23 ng/g – 94.3 ng/g (Villa et al. 2017). All green sea turtle populations considered in Figure 2 are within this range.

Cadmium concentrations in almost all green sea turtle populations are very similar, well below the value associated with toxic effects in birds (260 ng/g ww) and similar to concentrations seen in Howick Island sea turtles (Wayland and Scheuhammer 2011; Villa et al. 2017). Leatherback turtles have been shown to have higher Cd concentrations than green turtles, from 14 ng/g – 182 ng/g (Harris et al. 2011; Innis et al. 2010).

Blood Pb concentrations of ≤ 200 ng/g are considered background levels in three orders of birds (Anseriformes, Falconiformes, and Columbiformes), and 500 ng/g is considered clinical poisoning in Anseriformes and Falconiformes (Stout et al. 2010; Franson and Pain 2011). However, crocodiles (*Crocodylus porosus*) with sustained blood Pb concentrations of 3,420 ng/g for several months showed no adverse effects (Hammerton et al. 2003). All green sea turtle populations are well below these levels.

Arsenic blood concentrations were greatest in turtles from Boyne River Estuary in Australia, above ≈ 500 ng/g, which is potentially toxic in humans (Saha et al. 1999; Gaus et al. 2012). Sea turtles in the Howick Islands had blood As concentrations of 160 ± 66 ng/g. Kapoho Bay and SLPH turtles are below these values, indicating As is not a concern for these two populations.

Nickel blood concentrations of turtles in this study were above background levels in humans (3 ng/g to 7 ng/g; Gaus et al. 2012). The worst case hazard quotient for Ni in green sea turtle eggs from Hong Kong was established to be 26.4 ng/g (Lam et al. 2007). Kapoho Bay turtles in this study had mean blood Ni concentrations greater than 26.4 ng/g (40.4 ± 19.3 ng/g), but toxicity reference values are unknown for adult sea turtle blood. However, adult sea turtles are likely less sensitive to Ni than embryos and hatchlings.

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 often 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 (Assi et al. 2016). For example, blood Pb concentrations in leatherbacks have been shown to increase as the nesting season progresses (Guirlet et al. 2008). In the wild, female turtles are capital breeders and in general do not feed during the nesting season, though opportunistic feeding can occur. Instead their energy is supplied from fat stores (Bjorndal 1985; Hays et al. 2002; Tucker and Read 2017; Page-Karjian et al. 2020). One hypothesis to explain the greater scute lead concentrations in these captive female turtles is that as Ca, and hence Pb as a Ca mimic, was mobilized from bones during egg vitellogenesis/egg production, 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 offloaded 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 (Figure S1). Larger, potentially older, turtles have greater concentrations of these elements, as seen with largest captive Turtle E. 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. In addition, the bioaccumulation of Se has been documented in leatherbacks from the northwestern Atlantic Ocean (Perrault 2012).

This study only found one element, Cu in wild turtles, to be significantly correlated between blood and scute tissues. In previous studies, total mercury concentrations were correlated between scutes and blood in loggerhead sea turtles, scute manganese, zinc, and Hg concentrations were correlated with whole body burdens of these elements in loggerhead and green turtles, and Se concentrations in the blood of green turtles were correlated with concentrations in the liver, kidney, and muscle (Day et al. 2005; Sakai et al. 2000; van de Merwe 2008). The sample size in this study was small and may be the reason why more elements were not correlated between blood and scute. Additional studies should be done for green turtles to determine relationships between blood and scute element concentrations to prevent unnecessary sampling and stress to these threatened and endangered species.

One limitation of 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.

CONCLUSION

All metals found in the captive and wild Hawaiian green turtles in this study are similar to or less than concentrations found in green sea turtles elsewhere (Figure 2). It is important to establish baseline 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 do not represent wild turtles' exposure to trace 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. Reference concentrations for some elements may be better established in healthy, wild populations living in relatively unpolluted areas.

Supplemental Data—The Supplemental Data are available on the Wiley Online Library at DOI: 10.1002/etc.xxxx.

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Data availability statement—Data, associated metadata, and calculation tools are available from the corresponding author (katherine.shaw@nist.gov).

Author Contribution Statement—J. Lynch, G. Balazs, and J. Pawloski collected samples. K. Shaw, A. French, J. Liu, and G. Cobb performed sample analysis. J. Lynch and D. Klein provided technical and editorial assistance. K. Shaw wrote manuscript. J. Lynch, G. Balazs, M. Rice and T. Jones conceived and designed experiment.

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Figure 1: Location of Sea Life Park Hawaii on the island of Oahu and Kapoho Bay on the island of Hawaii.

Figure 2: Elemental concentrations (mean \pm SD, ng/g wm) in the whole blood of green turtles worldwide. Data taken from Komoroske et al. (2011), Gaus et al. (2012); Camacho et al. (2014a), and Villa et al. 2017). Horizontal lines indicate thresholds as defined as: As toxicity in humans (500 ng/g, Saha et al. 1999); Cd toxicity in avian species (260 ng/g, Wayland and Scheuhammer 2011); Pb 200 ng/g is background levels in avian species and 500 ng/g is clinical poisoning in avian species (Stout et al. 2010; Franson and Pain 2011); Ni 3 ng/g to 7 ng/g is background concentration in humans (Gaus et al. 2012); Se weight loss in avian species (900 ng/g, O’Toole and Raisbeck

1997; Stout et al. 2010); and V 0.05 ng/g is the background concentration in humans (Gaus et al. 2012). Grey shaded areas are 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).

Figures

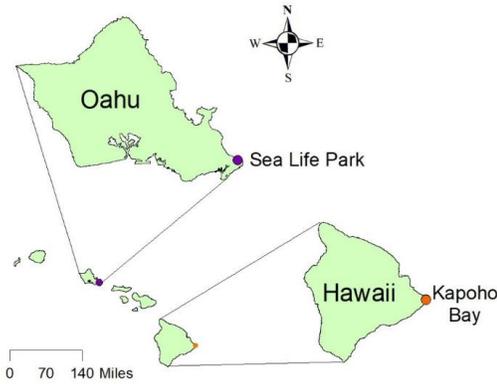


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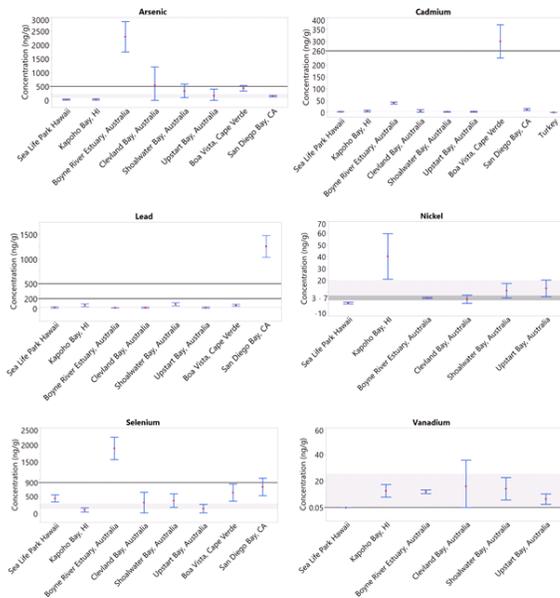


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Table 1. Elemental concentrations in the scute (ng/g dm) and blood (ng/g wm) of captive turtles at Sea Life Park Hawaii and wild turtles at Kapoho Bay, HI.

Element	SCUTE					
	Captive			Wild		
	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	-	-	16.7	-	-	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

Element	Captive			Wild		
	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	645	628 (75.3)	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 wild was 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	<i>Amansia spp.</i> [*]	<i>Gracilaria salicornia</i> [*]
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 (Bienfang et al. 2009).

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 ($\mu\text{g}/\text{day}$) is calculated for captive turtles at SLPH using only pelleted food and wild turtles using a combination of *Amansia spp.* and *G. salicornia*. Bold values highlight a TTF > 1, indicating potential toxicological risk for that element.

	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	0.04	0.03	0.02	387
Cd	0.29	< 0.01	9	0.03	< 0.01	0.07	< 0.01	43
Co	0.01	< 0.01	16	< 0.01	0.03	0.03	0.21	19
Cr	1.63	< 0.01	86	0.03	0.03	0.08	0.07	139
Cu	0.10	0.06	1700	0.06	0.02	0.22	0.07	273
Ni	0.11	< 0.01	408	0.01	0.01	0.25	0.14	544
Pb	0.21	0.25	16	0.03	0.13	0.11	0.33	85
Sb	0.01	< 0.01	2	0.07	0.05	0.07	0.05	2
Se	0.40	0.57	126	0.47	0.80	1.42	2.39	19
Sr	1.05	0.09	1430	0.07	0.02	0.19	0.04	6660
V	2.64	< 0.01	34	0.08	0.01	0.05	0.01	352