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TRACE METALS IN GREEN SEA TURTLES (*CHELONIA MYDAS*) INHABITING TWO SOUTHERN CALIFORNIA COASTAL ESTUARIES

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Abstract

Foraging aggregations of east Pacific green sea turtles (*Chelonia mydas*) inhabit the Seal Beach National Wildlife Refuge (SBNWR) and San Diego Bay (SDB), two habitats in southern California, USA, located near urbanized areas. Both juvenile and adult green turtles forage in these areas and exhibit high site fidelity, which potentially exposes green turtles to anthropogenic contaminants. We assessed 21 trace metals (TM) bioaccumulated in green turtle scute and red blood cell (RBC) samples collected from SBNWR (n = 16 turtles) and SDB (n = 20 turtles) using acid digestion and inductively coupled plasma mass spectrometry. Principal component analyses of TM composition indicate that SBNWR and SDB turtles have location-specific contaminant signatures, characterized by differences in cadmium and selenium concentrations: SBNWR turtles had significantly more cadmium and selenium in RBC and more selenium in scute samples, than SDB turtles. Cadmium and selenium concentrations in RBC had a strong positive relationship, regardless of location. SBNWR turtles had higher selenium in RBCs than previously measured in other green turtle populations globally. Due to different retention times in blood vs. scute, these results suggest that SBNWR turtles have high long- and short-term selenium exposure. Turtles

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from SBNWR and SDB had higher trace metal concentrations than documented in green turtle populations that inhabit non-urbanized areas, supporting the hypothesis that coastal cities can increase trace metal exposure to local green turtles. Our study finds evidence that green turtle TM concentrations can differ between urbanized habitats and that long-term monitoring of these green turtles may be necessary.

Keywords

selenium; mercury; marine; cadmium; metal; turtle

Introduction

Understanding environmental contamination and its effects on threatened species is a critical first step for habitat restoration and conservation of species inhabiting these areas. Sea turtles are one taxon that, because of their coastal existence and proximity to human-altered landscapes, is potentially vulnerable to pollutants (Hamann et al., 2010). By pinpointing which populations are most affected by such anthropogenic stressors, wildlife managers are able to develop well-informed management strategies to protect coastal sea turtle populations and ideally reduce contaminant inputs into these areas.

East Pacific green sea turtles (*Chelonia mydas*) are a regional population of green sea turtles (hereby, green turtles) that inhabit the eastern Pacific Ocean from California, U.S. to Chile (Wallace et al., 2010). In recent years, green turtles from the eastern Pacific were reclassified from an endangered to a threatened species, citing improvements in at-sea conservation and recovery of nesting populations (Seminoff et al., 2015). However, egg harvesting, bycatch, pollution, and other human activities still threaten green turtle population recovery in the eastern Pacific (Seminoff et al., 2015). This species exhibits an ontogenetic shift from pelagic habitats as small juveniles to neritic habitats as larger juveniles where they reside and forage for long periods of time until they reach sexual maturity. As adults, the turtles leave the foraging ground periodically for mating and egg-laying at their mating/nesting location before returning back to their foraging ground (Lutz and Musick, 1997). These coastal foraging habitats are critical for the recovery and support of green turtle populations, yet many of these areas have been heavily impacted by urbanization thus presenting health risks with anthropogenic stressors such as pollution from trace metals (Gardner and Oberdorster, 2005; Finlayson et al., 2016).

Trace metals, often separated into essential and non-essential metals, occur in the environment at low concentrations, but can be artificially elevated via human activities (e.g. dredging, runoff, shipyard activity, etc.) (Pugh and Becker, 2001; Deheyn and Latz, 2006; Dodder et al., 2012; Dodder et al., 2016). Trace metal exposure in many organisms is mainly through diet (Rozman and Klaassen, 2007). Essential trace metals (i.e., selenium and iron), are needed for normal biological functions and can bioaccumulate in the tissues of organisms (Gardner and Oberdorster, 2005; Rozman and Klaassen, 2007). However, when accumulated to high enough levels, essential metals can overwhelm molecular signaling pathways, and alter an organism's ability to compensate for other stressors (Gardner and

Oberdorster, 2005; Rozman and Klaassen, 2007). Non-essential trace metals, those not needed for biological functions, can bioaccumulate over time in tissues (e.g., bone or kidney) and cause negative health effects, such as neurological damage or sudden death (i.e., mercury and cadmium) (Rozman and Klaassen, 2007). Long-lived species, such as green turtles, are vulnerable to bioaccumulation of non-essential trace metals due to their long lifespan and extended residence in specific habitats (Finlayson et al., 2016). As a result, studying both essential and non-essential metals could help elucidate and characterize how urban areas affect green turtle contaminant loads.

Previous research has linked various bioaccumulated trace metals to negative effects on physiology and reproduction in multiple sea turtle species (van de Merwe et al., 2010a; Komoroske et al., 2011; Perrault et al., 2011; Keller et al., 2014a; Finlayson et al., 2016). For example, mercury in adult leatherback turtle (*Dermochelys coriacea*) blood samples and hatchling liver samples correlated with reduced hatching success (Perrault et al., 2011). However, co-exposure of mercury with selenium was shown to improve hatching success in leatherback turtles relative to mercury alone, suggesting that selenium may help detoxify mercury (Perrault et al., 2011). Another trace metal study using green turtle blood samples found copper and lead to be positively correlated with indicators of oxidative stress (i.e., lower 3-hydroxy-3-methylglutaryl-CoA reductase, and increased lipid peroxidation), and green turtles with high oxidative stress were more likely to have fibropapillomatosis, a tumor-bearing disease affecting many green turtle populations worldwide (da Silva et al., 2016). While research on trace metal health effects has been expanding, their impact on sea turtle health remain poorly understood (Finlayson et al., 2016). The above studies indicate that trace metal monitoring in sea turtles is an important component for addressing sea turtle health concerns (Gardner and Oberdorster, 2005; Finlayson et al., 2016). Considering the possible negative effects of high trace metal exposure on sea turtle physiology, assessing the degree of trace metal exposure in green turtles occupying habitats influenced by heavy urbanization, where exposure to trace metals is elevated, is essential for evaluating possible health risks due to anthropogenic activities.

Along the western coast of the U. S., the northernmost resident foraging aggregations of green turtles inhabit San Diego Bay (SDB) and the Seal Beach National Wildlife Refuge (SBNWR) (Eguchi et al., 2010; MacDonald et al., 2012; Crear et al., 2016). SDB is home to a major U.S. Navy base and subject to other anthropogenic activities such as ship maintenance, dredging, fishing, and boating, which have resulted in the bay being listed (303D) as an impacted body of water (Fairey et al., 1998). SBNWR is located within a naval weapons station and green turtles have been shown to forage in the surrounding area (Crear et al., 2016; Crear et al., 2017). The SBNWR is within 15 km of the Port of Los Angeles, has a naval weapons station, and has input from many impacted waterways and water bodies influenced by industrial and residential runoff (Schiff et al., 2011; Dodder et al., 2016). The different industries and anthropogenic influences in these locations have led to distinct trace metal sediment compositions in SDB versus SBNWR (Dodder et al., 2016). Urbanization, such as ship yard use, dredging and residential and commercial development can continue to change trace metal profiles in these two urban habitats (Schiff et al., 2011; Dodder et al., 2016). Therefore, green turtles inhabiting these habitats may exhibit changes in their trace metal concentrations over time.

Previous studies on green turtles have shown that SDB green turtles bioaccumulate anthropogenic contaminants such as trace metals (Komoroske et al., 2011). However, the green turtle aggregation in the SBNWR was only recently discovered as a permanent foraging aggregation and has not been previously studied for trace metals accumulation levels (Crear et al., 2016). Considering the different sediment trace metal profiles (Dodder et al., 2016), the high site fidelity green turtles exhibit (Eguchi et al., 2010; Crear et al., 2016), and previous research demonstrating green turtle trace metal bioaccumulation from urbanized habitats (Komoroske et al., 2011), we hypothesized that green turtles from the SBNWR and SDB would have location-based differences in trace metal bioaccumulation. By assessing trace metal concentrations in both aggregations we can: (1) determine whether there are differences in trace metal concentrations between sampling locations, (2) assess if trace metal concentrations have changed since previous studies in SDB green turtles, and (3) compare SBNWR and SDB turtle trace metal concentrations with those found in previous research around the world.

Previous studies have shown that whole blood and red blood cell trace metals were positively correlated with trace metals in kidney and liver tissues (Keller et al., 2004; van de Merwe et al., 2010a), albeit at significantly lower levels. Measuring trace metals in blood and scute samples has been used to provide both short- and long-term exposure signatures that have been correlative to organ burden (Day et al., 2005; Komoroske et al., 2011). Using these non-lethal measurements, we assessed and compared trace metal bioaccumulation differences of green turtle foraging aggregations influenced by heavy urbanization to determine if trace metal composition differed in both tissue types across locations. These data will enhance our understanding of how green turtles accumulate trace metals in two different impacted environments, as well as assess green turtles as environmental sentinels of trace metals.

Methods

Green Turtle Capture and Sampling

Green turtles were captured and tagged in two locations (Figure 1): SBNWR (33° 44' 06.8" N, 118° 03' 51.9" W) and SDB (32° 36' 54" N, 117° 6' 4" W), using previously established techniques (Eguchi et al., 2010; Crear et al., 2016), and in collaboration with National Marine Fisheries Service (NMFS) under Permit #16803. One to three 100-m long entanglement nets were set and checked every 30 min in areas where green turtles are known to inhabit. Once captured, green turtles were brought ashore or onboard a support vessel for processing. Sex of captured green turtles were determined using morphology for adults (as mature males have longer tails) and hormone concentration (data not shown) for immature turtles (as they do not have external morphology to distinguish sex until maturity) (see Caldwell, 1962; Allen et al., 2015), weighed (to the nearest 0.1 kg), and several morphometric measurements (e.g., curved carapace length; to the nearest 0.1 cm) were obtained.

National Institute of Standards and Technology (NIST) protocols (Keller et al., 2014b) were followed with moderate modifications to reduce sample contamination and increase sensitivity to lower detection limits. Briefly, blood was collected via the dorsal cervical sinus

using 21-gauge and 3.8 cm needles (Owens and Ruiz, 1980). Blood was drawn into sodium-heparinized 10-mL glass vacutainers (Becton Dickson, San Jose, California). To account for varying keratin concentrations throughout the carapace (Schneider et al., 2015), scutes were sampled via numbering each scute and using a random number selector to randomly choose scutes to sample. Scutes were only sampled if there were no injuries on the scutes chosen or if the scute exhibited a thin keratin layer. Scute samples were collected with sterile stainless steel blades by scraping a thin layer of carapace to remove algae and microorganisms, followed by scraping scute shavings into a whirl-pak sampling bag, modified from Day et al. (2005). All scute and blood samples were placed in a cooler with cold packs and a cloth barrier to prevent hemolysis and point contact freezing until transported back to laboratory for processing. Scute samples were only collected from green turtles with no carapace injuries. To account for possible equipment and container contamination, Millipore water (Q-Pod®, Burlington, MA) was used as a field blank and collected into the same materials as would be used for collecting blood. After morphological measurements and sample collection, turtles were tagged and released near the location of capture. Typically, the entire procedure took approximately one hour. Whole blood in vacutainers was spun at 3000 rpm for 10 min to separate plasma, buffy layer, and red blood cells (RBCs). After removal of plasma and white blood cells for companion studies, RBCs were pipetted into 2 mL cryovials using sterile low-density polyethylene pipette tips. Samples were placed at -20°C overnight, then transferred to -80°C freezers until trace metal analysis. Whole blood and scute samples were collected from 20 green turtles in SDB and 16 in SBNWR.

Trace Metal Analyses

To assess the degree of trace metal accumulation in green turtles, RBC and scute samples were digested using concentrated Nitric/Hydrochloric acid and sonicated for 3 hours (EPA Method 200.15). RBC samples were used instead of whole blood to avoid possible errors caused by hematocrit as seen in Komoroke et al. (2011) and Day et al. (2005). Twenty-five trace metals (B, Al, Ti, V, Cr, Mn, Fe, Co, Ni, Cu, Zn, As, Se, Sr, Ag, Cd, Sn, Sb, Ba, Pb, and Hg) were analyzed using an Agilent (7500) Inductively Coupled Plasma Mass Spectrometer (ICPMS) equipped with a reaction/collision cell (Agilent, Santa Clara, CA). Internal standard curves were based on a 6-point linear regression calibration curve with an R^2 value of 0.99. Calibration standards were purchased from a NIST traceable commercial supplier (AccuStandard, New Haven, CT). The limit of detection for all elements was determined via EPA method 200.15 (see Kazi et al., 2009). Blanks with Millipore water and blank spikes were analyzed along with all samples for quality assurance. The limit of detection (LOD) for most metals was below $0.0005\text{ }\mu\text{g/g}$, with the exception of total mercury (LOD = $0.025\text{ }\mu\text{g/g}$; Supplementary Table 1). Each sample type was digested with a replicate sample from one individual, a corresponding matrix spike sample from the same individual, a blank, and a blank spike. Percent recovery of blank spikes was calculated as $(\text{measure}/\text{expected}) \times 100\%$ and matrix spike recovery was calculated as $([\text{measured} - \text{measured non-spiked replicate}]/\text{expected}) \times 100\%$.

Statistical Analyses

All statistical analyses were done in R (version 3.3.3; R Core Team, 2018) with significance based on an alpha of 0.05. Due to low detection limits, all non-detections were treated as

zeros for statistical purposes. Zeros reported throughout the study are non-detections. Turtles captured more than once had their values averaged. Principal component analyses (PCA) were used to determine location-based differences in green turtle trace metal concentrations and to identify specific metals for further comparison by location (R package *vegan*; Oksanen et al., 2017). Separate PCAs were conducted for scute and RBC trace metals concentrations to examine long and short-term trace metal exposure, respectively. PCA included only trace metals found at detectable levels, and the analyses focused on prevalent elements that were detected in more than six out of 36 turtles. K-means clustering analyses were done on the first two principal components to assess location-based differences (R package *cluster*; Maechler et al. 2016). T-tests were used to compare size and element concentrations between the two locations. Relationships between metal concentrations and turtle body size and between metal concentrations with other metal concentrations were assessed using a linear regression.

Results

Principal Component Analyses

SBNWR green turtles had significantly ($p < 0.001$) smaller carapace length (median $\pm$ SE = 70.7 ± 1.39 cm, range = 50.8 – 82.6 cm) than SDB green turtles (mean $\pm$ SE = 89.8 ± 3.86 cm, range = 61.6 – 114.1 cm). PCA for turtle body size and metals detected in scute tissue had two principal components (PC) that accounted for 60% of the variance (Figure 2A). Variables with the strongest loading factors for PC1 were cadmium (−0.414) and selenium (−0.388), and the strongest loading factors for PC2 were zinc (−0.470) and iron (−0.410). For scute samples, eleven SDB green turtles and eleven SBNWR green turtles fell within one 95% confidence ellipse, whereas eleven SBNWR green turtles and twelve SDB turtle fell within the second ellipse (Figure 2A). Cadmium, selenium, mercury, and silver accounted for the majority of the differences between PCA groups for scute samples (Figure 2A). PCA for turtle body size and metals detected in RBCs had two PCs that accounted for 44% of the variance (Figure 2B). Variables with the strongest loading factors for PC1 were vanadium (−0.316) and cobalt (−0.305), and the strongest loading factors for PC2 were silver (0.318) and mercury (−0.333). For RBC samples, all SDB and seven SBNWR green turtles fell within one 95% confidence ellipse, whereas one SDB individual and all SBNWR turtles were within a second 95% confidence ellipse (Figure 2B). Based on PC loadings, metals that accounted for the majority of the difference between the PCA groups for RBCs included aluminum, lead, cadmium, and selenium (Figure 2B).

Carapace Scute

Of 23 elements measured, 20 were detected in scute samples in SDB turtles and 19 elements were detected in SBNWR scute samples (Table 1). There were no significant differences between green turtle's essential metal concentrations, except selenium, in scute samples. Only SBNWR green turtles had detectable levels of mercury in scutes, whereas no mercury was detected above the LOD (LOD = $0.025 \mu\text{g/g}$) in SDB green turtles. SBNWR mercury scute concentrations displayed a significant negative relationship ($R^2 = 0.62$; $p < 0.001$) with turtle size. SDB green turtles had significantly higher ($p = 0.002$) concentrations of cadmium in their scute samples than SBNWR turtles (Table 1). SBNWR green turtles ($n = 16$) had

significantly higher ($p < 0.001$) selenium in scute samples than scute samples of SDB green turtles ($n = 20$). No statistically significant relationship was found between selenium and cadmium in scute samples ($p = 0.22$).

Red Blood Cells

Of 23 elements measured, 16 elements were detectable in RBCs (Table 2) in SDB and only 15 elements were detected in SBNWR. There were no significant differences between green turtle's essential metal concentrations, except selenium, in RBC. SDB green turtles had significantly higher ($p = 0.01$) aluminum in their RBCs than SBNWR green turtles (Table 2). SBNWR green turtles ($n = 16$) had significantly higher ($p < 0.001$) selenium in RBC samples than RBC samples from SDB green turtles (Figure 3A; $n = 20$). SBNWR green turtles had significantly higher ($p < 0.001$) cadmium in RBCs than SDB green turtles (Figure 3B). Green turtle selenium concentrations have a positive and significant relationship ($p < 0.001$, $R^2 = 0.71$) with cadmium concentrations in RBCs, regardless of location (Figure 3C).

Carapace Scute and Red Blood Cell Differences

With the exception of selenium, essential metal (B, Mn, Fe, Co, Cu, and Zn) concentrations measured in turtles did not vary between locations, regardless of sample type. Chromium, antimony, tin, barium and mercury were all below detection limits in green turtle RBCs but were detected in scute (LOD ~ 0.005 $\mu\text{g/g}$ for all but mercury, see Supplementary Table 1). Only SDB green turtles had detectable levels (LOD < 0.0005 $\mu\text{g/g}$) of tin in scute and silver in both RBCs and scute samples (Table 1 & 2).

Discussion

Site-specific element differences

Overall, we found that green turtles residing within the SBNWR and SDB tended to display site-specific trace metal contaminant signatures. Whereas most individual elements were not significantly different between locations, there were some elements that differed (e.g., aluminum, cadmium, and selenium) between green turtles from SBNWR and SDB. Most essential elements, except selenium, were not significantly different across locations or individuals, indicating that essential element concentrations are maintained within similar ranges. Whereas size was not related with any elements measured (except nickel and mercury in scute), there were size differences in turtles sampled from each location, which may introduce indirect influences (such as behavior/age) that could not be measured by our study. Green turtle trace metal differences were expected to be related to sediment trace metal profiles of San Diego and Seal Beach. However, the concentrations of some metals in this study did not reflect location-based sediment concentrations, which could indicate that trace metal profiles in sediment are not necessarily reflected in green turtle tissue. In scute samples, only SDB green turtles had detectable amounts of silver despite both regions having similar sediment silver concentrations (~ 6.15 $\mu\text{g/g}$; Dodder et al., 2016). Since silver concentrations were higher in SDB prior to more stringent regulations (Schiff et al., 2011; Dodder et al., 2016), larger/older SDB turtles may have silver concentrations in scutes that reflect these patterns. Silver has been shown to bioaccumulate in green turtle food sources in higher concentrations than sediment (Komoroske et al., 2012) indicating that silver in the

SDB region may be more readily available through diet than SBNWR, or that large individuals from SDB have accumulated enough silver over time to be detectable in scute samples. Aluminum concentration differences in green turtles were reflective of sediment patterns found in their respective locations (~ 34838 µg/g dw Al in SBNWR and ~54348 µg/g dw Al in SDB), indicating that aluminum input into San Diego Bay, possibly from shipyard activity and urban runoff, is bioaccumulating into green turtle food sources (Dodder et al., 2016). However, previous research has shown that aluminum does not readily bioaccumulate in eelgrass (*Zostera marina*), sponge, red algae, or green algae, implying that aluminum could be coming from other food sources, such as mobile invertebrates which inhabit plant material that sea turtles are known to consume (Lemons et al., 2011; Komoroske et al., 2012). Only SBNWR turtles had detectable levels of mercury in scute samples, despite both locations having similar mercury concentrations in sediment (Dodder et al., 2016). Mercury concentrations were negatively related to SBNWR turtle size, indicating that once turtles recruit to SBNWR their mercury concentrations decrease over time. Previous research (Komoroske et al. 2011) had found mercury concentrations above 0.025 µg/g in scute samples, while the current study did not find any SDB scute samples above the LOD. However, the current study and Komoroske et al. (2011) had two different methods used with two different LODs. These results possibly indicate that mercury concentrations in green turtle scute has decreased as they recruit to these coastal habitats and that SDB green turtle mercury exposure may have decreased in recent years. Additional monitoring with similar methods will be needed to better assess these patterns in scute mercury. PC analyses identified two elements, cadmium and selenium, as likely major drivers for the differences between metal signatures in southern California green turtles.

Cadmium

Consistent with the differences in cadmium levels in RBC, sediments from SBNWR (0.52 – 1.13 µg/g dry weight) have higher levels of cadmium compared to sediments from SDB (0.09 – 0.24 µg/g dry weight; Dodder et al., 2016). However, compared to SBNWR green turtles, SDB green turtles had higher scute cadmium concentrations, suggesting that SDB turtles have had more long-term cadmium exposure while SBNWR turtle have had higher short-term exposure. Considering the Seal Beach region has higher concentrations of cadmium in sediment than SDB (Dodder et al., 2016), and SBNWR turtles are generally smaller than SDB turtles, SDB green turtles could be bioaccumulating cadmium with age while residing in SDB (Dodder et al., 2016). These contaminant patterns suggest that sediment cadmium is accumulating in green turtle food sources, most likely eelgrass, which readily bioaccumulates cadmium and deposit cadmium in stem and leaf tissue (Lyngby and Brix, 1984). However, green turtles from SBNWR have been observed within the San Gabriel River, where no eelgrass habitat is present, which implies that green turtles that forage within the river may have a more algae biased diet (Crear et al., 2016; Crear et al., 2017). SBNWR green turtles move between SBNWR and the San Gabriel River, which may indicate that trace metal accumulation could be occurring from foraging items in both locations, possibly explaining differences from SDB. Previous research has shown evidence that green turtles have a high capacity to metabolize and detoxify cadmium at a high rate (Sinaei, 2016). However, SBNWR green turtles have one of the highest observed cadmium concentrations in green turtle red blood cell samples reported to date, 0.305 µg/mL in RBCs,

suggesting that cadmium levels in SBNWR green turtles are abnormally high compared when compared to other green turtle studies that use whole blood (Villa et al., 2016).

Selenium

Selenium concentrations in sediment at our two study sites were similar (approximately 0.48 µg/g); however, green turtle selenium concentrations in blood and scute samples were greater than sediment patterns (Dodder et al., 2016). Previous research has shown various green turtle food sources, such as many algae and plant species, accumulate higher selenium concentrations than surrounding sediment within SDB (Komoroske et al., 2012). Eelgrass beds are important foraging habitat for green turtles in SDB, and presumably in SBNWR, suggesting that eelgrass habitats may be one of the main sources of trace metal exposure for green turtles from southern California (Lemons et al., 2011; Seminoff et al., unpubl. data; Komoroske et al., 2012). Because the SBNWR area has extensive eelgrass habitat, it is expected that SBNWR green turtles feed on eelgrass habitat similar to SDB green turtles. However, SBNWR turtles may also feed within the San Gabriel River, which may result in a more algae-based diet providing an additional avenue for high selenium exposure (Seminoff et al., unpubl. data). These results suggest that either eelgrass habitat in the SBNWR or algae in the San Gabriel River has significantly more selenium than SDB eelgrass habitat or that other factors, such as non-essential metal exposure, explain increased selenium concentrations in SBNWR green turtles.

The presence of other, non-essential metals alters the uptake of selenium. For example, in many other organisms, selenium tissue burdens display a positive relationship with cadmium tissue concentrations, and co-exposure of cadmium with selenium has been found to reduce cadmium toxicity (e.g. Pugh and Becker, 2001; Gardner and Oberdorster, 2005; Gardner et al., 2006; Rozman and Klaassen, 2007; Perrault et al., 2011). While selenium and cadmium absorption and distribution mechanisms are not well studied, it is thought that selenium forms insoluble complexes with cadmium that aid in cadmium detoxification and excretion (Ohlendorf, 2003; Rozman and Klaassen, 2007). Therefore, the observed relationship between cadmium and selenium concentrations in green turtle RBCs in the current study suggests that green turtle selenium uptake may be influenced by co-occurring cadmium levels.

Green turtle selenium contamination represented the starkest difference in trace metals between SDB and SBNWR green turtles, suggesting that SBNWR green turtles are possibly at higher risk of selenium toxicity than SDB green turtles. Most other studies have found adult and juvenile green turtles had selenium concentrations roughly between 0.3 to 7 µg/mL in whole blood and RBC (van de Merwe et al., 2010a; Komoroske et al., 2011; Labrada-Martagon et al., 2011; Ley-Quinonez et al., 2013; Camacho et al., 2014; Villa et al., 2016), while SBNWR green turtles RBCs selenium ranged from 1.02 to 33.06 µg/mL. In many organisms, including banded water snakes (*Nerodia fasciata*), mallard ducks (*Anus platythynchos*), and African clawed frogs (*Xenopus laevis*), excess selenium, without other trace element exposure, has been shown to reduce hatchling success, cause dermal damage and loss of neurological function (e.g. Heinz, 1996; Ohlendorf, 2003; Rozman and Klaassen, 2007). Avian research into egg contamination has been used as a proxy to model and predict

risks in green turtles (Heinz, 1996; Lam et al., 2006). These studies have suggested that green turtle eggs with selenium loads higher than 600 µg/g could experience reduced hatchling success (Heinz, 1996; Lam et al., 2006). Considering green turtles can maternally offload contaminants (van de Merwe et al., 2010b), including cadmium (non-essential) and selenium (essential), to their eggs, future studies could examine what affect cadmium and selenium have on hatchling development and health in green turtles. All but one SBNWR green turtles sampled were female (unpublished results), which indicated that selenium offloading could be a potential issue as these immature turtles become adults.

Comparisons to other studies

A previous study has determined reference intervals of trace metal concentrations using whole blood from a “reference” green turtle population that inhabits pristine habitat in the West Barrier Reef in Australia (Villa et al., 2016). These reference intervals are ranges of trace metal concentrations found in a green turtle population not exposed to anthropogenic contaminants. Therefore, concentrations below or above these ranges indicate altered trace metals concentrations in blood (Villa et al., 2016). Most green turtles in our study from both locations had RBC trace metal concentrations above established reference intervals (Villa et al., 2016). This supports the hypothesis that the burden of trace metals found in SBNWR and SDB green turtles are above what is considered background levels. Green turtles in this study had vanadium, chromium, iron, and copper levels (all essential elements) that were within background levels (Villa et al., 2016). However, SDB and SBNWR green turtles had higher than background levels of cobalt, manganese, zinc, strontium, arsenic, cadmium, nickel, and selenium (Villa et al., 2016). Comparison of trace metal concentrations in green turtles from the current study and green turtle trace metal studies around the world (Table 3) suggest that green turtles inhabiting waterways impacted by urbanization have different trace metal accumulation patterns than non-exposed green turtle populations (van de Merwe et al., 2010a; Komoroske et al., 2011; Labrada-Martagon et al., 2011; Ley-Quinonez et al., 2013; Camacho et al., 2014; Villa et al., 2016). The current study’s turtles may have high cadmium and selenium concentrations, associated with kidney failure and reduced hatchling success. This information supports increased pollution-based health risks in green turtles living in urbanized coastal zones.

Conclusions

Our study found evidence that a region’s unique anthropogenic activity (e.g. ship-yard, power plant) and pollution can affect how green turtles accumulate trace metals. Particularly, SBNWR green turtles had significantly higher selenium and cadmium in their RBCs than green turtles from other recent studies. There was a difference in size observed between the two populations, which was positively related to nickel concentrations in scute and negatively related to mercury concentrations in SBNWR scute samples. Most elements were similar to previous measurements in SDB turtles, with the exception of mercury. Mercury concentrations are lower in current SDB turtle scute samples, indicating a possible reduction of exposure or intake, however previous studies used different methods. Future studies should investigate larger SBNWR individuals to assess whether adults in the SBNWR have more selenium than immature green turtles. SBNWR turtles had higher than average

selenium concentrations and comparisons to other studies show evidence that urban habitats continue to expose green turtles to trace elements. With each green turtle foraging aggregation accumulating different trace metal concentrations, there is potential for differential health risks across these two foraging aggregations that future studies could elucidate. Because of their long life spans and extended residency patterns to specific foraging sites, continued monitoring of SBNWR and SDB green turtles will be necessary as trace metal concentrations can potentially change as green turtles continue to inhabit areas impacted by urbanization.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1. Green sea turtle capture locations (red star). Top left circle is the Seal Beach (SB) National Wildlife Refuge; bottom middle circle is San Diego Bay (SDB). CA: California.

Figure 2.

Principal component analysis of detectable metals in scute (A) and red blood cells (B) of green sea turtles from San Diego Bay (n = 20 turtles; orange points) and the Seal Beach National Wildlife Refuge (n = 16 turtles; blue triangles). Dotted ellipses indicate 95% confidence ellipses determined by K-means clustering. Elements are displayed in scientific abbreviations; CCL = curved carapace length.

Figure 3.

A) Selenium in red blood cells of green sea turtles in Seal Beach (n = 16 turtles) and San Diego Bay (n = 19 turtles). B) Cadmium in red blood cells of green sea turtles in Seal Beach (n = 16 turtles) and San Diego Bay (n = 19 turtles). C) Linear regression (dotted line; equation) depicting the relationship between cadmium and selenium in green turtle red blood cells. Boxes are 50% quartiles with line being median and whiskers are range. Asterisks indicate significant differences via one-way ANOVA (***) $p < 0.001$.

Table 1:

Trace metal concentrations detected in scute samples of green sea turtles from Seal Beach National Wildlife Refuge (n = 16 green turtles) and San Diego Bay (n = 20 green turtles). Note: LOD = limit of detection and values are µg/g. Samples below LOD are treated as zero.

Elements	Seal Beach National Wildlife Refuge			San Diego Bay		
	n>LOD	Scute Mean ± SE	Scute Median (Range)	n>LOD	Scute Mean ± SE	Scute Median (Range)
Boron	16	33.39 ± 3.82	31.01 (13.73 – 63.65)	20	42.93 ± 4.40	35.93 (15.69 – 107.30)
Aluminum	16	318.62 ± 63.71	206.87 (14.62 – 829.64)	20	209.99 ± 40.18	134.47 (12.44 – 627.79)
Titanium	16	24.15 ± 4.60	14.92 (2.20 – 63.77)	20	13.98 ± 2.94	8.15 (2.27 – 61.38)
Vanadium	16	1.03 ± 0.14	0.94 (0.14 – 2.14)	20	1.08 ± 0.17	0.77 (0.13 – 2.69)
Chromium	16	0.43 ± 0.07	0.41 (0.04 – 1.11)	20	0.61 ± 0.10	0.49 (0.04 – 1.75)
Manganese	16	13.81 ± 1.90	13.21 (1.00 – 26.58)	20	15.81 ± 4.30	9.06 (3.10 – 91.46)
Iron	16	430.89 ± 77.20	327.52 (24.30 – 1070.64)	20	340.53 ± 56.92	233.44 (28.15 – 840.31)
Cobalt	16	0.25 ± 0.03	0.23 (0.08 – 0.51)	20	0.4 ± 0.09	0.28 (0.03 – 1.80)
Nickel*	16	0.95 ± 0.12	1.05 (0.16 – 1.83)	20	2.37 ± 0.44	1.81 (0.35 – 8.68)
Copper	16	1.79 ± 0.21	1.72 (0.35 – 3.26)	20	5.62 ± 0.72	4.34 (1.01 – 11.72)
Zinc	16	201.85 ± 22.03	187.53 (59.18 – 362.25)	20	225.39 ± 19.15	231.20 (65.63 – 398.58)
Arsenic	16	0.56 ± 0.05	0.55 (0.16 – 0.94)	20	0.58 ± 0.07	0.47 (0.20 – 1.31)
Selenium***	16	2.59 ± 0.56	2.05 (0.29 – 9.10)	20	0.48 ± 0.06	0.38 (0.15 – 1.06)
Strontium	16	19.04 ± 5.18	13.47 (6.74 – 100.27)	20	16.55 ± 3.74	12.39 (7.61 – 87.43)
Silver	0	0	0 (0)	8	0.06 ± 0.02	0 (0 – 0.28)
Cadmium**	14	0.07 ± 0.01	0.09 (0 – 0.15)	20	0.30 ± 0.06	0.23 (0.05 – 1.09)
Tin	0	0	0 (0)	2	0.01 ± 0.004	0 (0 – 0.06)
Antimony	2	0.01 ± 0.01	0 (0 – 0.12)	5	0.03 ± 0.02	0 (0 – 0.43)
Barium	16	5.62 ± 1.16	4.47 (0.34 – 16.19)	20	5.64 ± 1.02	4.59 (0.80 – 16.18)
Lead	16	4.49 ± 0.91	3.26 (0.34 – 11.44)	20	3.10 ± 0.96	1.88 (0.39 – 20.67)
Mercury*	8	0.07 ± 0.0003	0.05 (0.02 – 0.19)	0	0	0 (0)

Asterisks indicate significant differences between locations via one-way ANOVA

*
< 0.05

< 0.001.

Table 2:

Trace metal concentrations detected in red blood cell (RBC) samples of green sea turtles from the Seal Beach National Wildlife Refuge (n = 16 turtles) and San Diego Bay (n = 20 turtles). Note: LOD = limit of detection, and all values are µg/mL. Samples below LOD are treated as zero.

Elements	Seal Beach National Wildlife Refuge			San Diego Bay		
	n>LOD	RBC Mean ± SE	RBC Median (Range)	n>LOD	RBC Mean ± SE	RBC Median (Range)
Boron	16	2.017 ± 0.159	2.003 (1.012 – 3.415)	20	1.525 ± 0.102	1.469 (0.632 – 2.742)
Aluminum *	16	0.184 ± 0.010	0.181 (0.101 – 0.249)	20	0.252 ± 0.021	0.232 (0.136 – 0.495)
Titanium	16	0.085 ± 0.003	0.084 (0.064 – 0.102)	20	0.081 ± 0.003	0.086 (0.058 – 0.100)
Vanadium	15	0.010 ± 0.005	0 (0 – 0.058)	8	0.006 ± 0.002	0 (0 – 0.021)
Manganese	16	0.494 ± 0.124	0.248 (0.027 – 1.439)	20	0.465 ± 0.127	0.181 (0.058 – 1.811)
Iron	16	581.25 ± 25.54	567.41 (459.46 – 834.51)	20	601.38 ± 29.49	571.42 (392.61 – 864.67)
Cobalt	16	0.045 ± 0.008	0.039 (0.007 – 0.122)	20	0.026 ± 0.003	0.021 (0.013 – 0.058)
Nickel	15	0.107 ± 0.008	0.114 (0 – 0.156)	20	0.087 ± 0.008	0.068 (0.057 – 0.156)
Copper	16	0.789 ± 0.051	0.733 (0.501 – 1.368)	20	0.681 ± 0.040	0.705 (0.394 – 1.014)
Zinc	16	25.540 ± 1.354	24.77 (18.47 – 37.94)	20	28.121 ± 1.071	28.29 (18.50 – 36.14)
Arsenic	16	2.442 ± 0.922	0.689 (0.139 – 13.603)	20	0.397 ± 0.052	0.394 (0.055 – 0.833)
Selenium ***	16	13.154 ± 2.836	11.478 (1.017 – 33.060)	20	1.639 ± 0.465	1.05 (0.241 – 9.399)
Strontium	16	0.064 ± 0.006	0.064 (0.031 – 0.111)	20	0.060 ± 0.005	0.053 (0.034 – 0.135)
Silver	0	0	0 (0)	2	0.002 ± 0.001	0 (0 – 0.023)
Cadmium ***	14	0.131 ± 0.020	0.113 (0.028 – 0.305)	10	0.025 ± 0.007	0.006 (0 – 0.095)
Lead	16	0.449 ± 0.062	0.369 (0.154 – 0.859)	20	0.532 ± 0.083	0.433 (0.171 – 1.643)

Asterisks indicate significant differences between locations via one-way ANOVA

*
< 0.05

< 0.001.

Table 3:

Trace metal concentrations found in other green turtle studies using whole blood and red blood cell samples as well as reference intervals (RI) established in Villa et al. (2016). All values are ($\mu\text{g/mL}$) blood. RIs are (min – max), and other studies' values are mean $\pm$ SE.

Elements	Reference Intervals	California, San Diego ¹	West Africa ²	Mexico, Baja ³	Queensland, Australia ⁴	Mexico, Sonora ⁵
Boron	NA	NA	NA	NA	NA	NA
Aluminum	NA	0.140 $\pm$ 0.026	1.95 $\pm$ 2.35	NA	NA	NA
Titanium	0.018 – 0.040	NA	NA	NA	NA	NA
Vanadium	0.001 – 0.037	NA	NA	NA	NA	NA
Manganese	0.0077 – 0.035	1.010 $\pm$ 0.169	0.03 $\pm$ 0.02	NA	NA	1.22 $\pm$ 0.99
Iron	210 – 410	NA	NA	343.57 $\pm$ 12.02	NA	NA
Cobalt	0.0071 – 0.033	NA	NA	NA	0.036 $\pm$ 0.006	NA
Nickel	NA	NA	2.76 $\pm$ 3.54	76.47 $\pm$ 11.02	NA	1.03 $\pm$ 1.01
Copper	0.290 – 0.690	0.934 $\pm$ 0.040	0.25 $\pm$ 0.12	NA	1.019 $\pm$ 0.099	1.71 $\pm$ 0.73
Zinc	7.3 – 14	NA	1.04 $\pm$ 0.45	13.92 $\pm$ 0.49	7.924 $\pm$ 0.668	63.58 $\pm$ 17.06
Arsenic	0.072 – 0.350	0.286 $\pm$ 0.061	0.44 $\pm$ 0.10	NA	4.361 $\pm$ 1.414	NA
Selenium	0.04 – 0.380	1.520 $\pm$ 0.673	0.61 $\pm$ 0.25	1.59 $\pm$ 0.19	2.447 $\pm$ 0.631	7.66 $\pm$ 3.19
Strontium	NA	0.316 $\pm$ 42.6	NA	0.28 $\pm$ 0.03	NA	NA
Silver	NA	0.002 $\pm$ 0.001	NA	NA	NA	NA
Cadmium	0.0024 – 0.0083	0.029 $\pm$ 10.6	0.30 $\pm$ 0.07	0.06 $\pm$ 0.00	0.035 $\pm$ 0.009	NA
Lead	0.0057 – 0.075	3.260 $\pm$ 0.626	0.07 $\pm$ 0.02	NA	0.022 $\pm$ 0.005	NA

¹Komoroske et al., 2011 (RBC)

²Camacho et al., 2014 (Whole Blood)

³Labrada-Martagon et al., 2011 (Whole Blood)

⁴van de Merwe et al. 2010a (Whole Blood)

⁵Ley-Quinonez et al., 2013 (Whole Blood)