Environmental Pollution xxx (2016) 1–12

Contents lists available at ScienceDirect

Environmental Pollution

journal homepage: www.elsevier.com/locate/envpol

Trace element reference intervals in the blood of healthy green sea turtles to evaluate exposure of coastal populations☆

C.A. Villa [a, *], M. Flint [b, c], I. Bell [d], C. Hof [e], C.J. Limpus [f], C. Gaus [a, **]

[a] The University of Queensland, National Research Centre for Environmental Toxicology (Entox), 39 Kessels Road, Coopers Plains, QLD 4108, Australia
[b] School of Forest Resources and Conservation, University of Florida, The Florida Aquarium's Center for Conservation, Apollo Beach, FL 33572, USA
[c] Vet-MARTI, School of Veterinary Science, The University of Queensland, Gatton Campus, QLD 4343, Australia
[d] Queensland Department of Environment and Heritage Protection, Townsville, QLD 4810, Australia
[e] WWF-Australia, Level 1, 17 Burnett Lane, Brisbane, QLD 4000, Australia
[f] Queensland Department of Environment and Heritage Protection, Brisbane, QLD 4102, Australia

ARTICLE INFO

Article history:
Received 21 July 2016
Received in revised form
28 October 2016
Accepted 28 October 2016
Available online xxx

Keywords:
Metals
Trace elements
Marine turtles
Reference intervals
Great Barrier Reef

ABSTRACT

Exposure to essential and non-essential elements may be elevated for green sea turtles (*Chelonia mydas*) that forage close to shore. Biomonitoring of trace elements in turtle blood can identify temporal trends over repeated sampling events, but any interpretation of potential health risks due to an elevated exposure first requires a comparison against a baseline. This study aims to use clinical reference interval (RI) methods to produce exposure baseline limits for essential and non-essential elements (Na, Mg, K, Ca, Ti, V, Cr, Mn, Fe, Co, Ni, Cu, Zn, As, Se, Mo, Cd, Sb, Ba, and Pb) using blood from healthy subadult turtles foraging in a remote and offshore part of the Great Barrier Reef. Subsequent blood biomonitoring of three additional coastal populations, which forage in areas dominated by agricultural, urban and military activities, showed clear habitat-specific differences in blood metal profiles relative to the those observed in the offshore population. Coastal turtles were most often found to have elevated concentrations of Co, Mo, Mn, Mg, Na, As, Sb, and Pb relative to the corresponding RIs. In particular, blood from turtles from the agricultural site had Co concentrations ranging from 160 to 840 μg/L (4–25 times above RI), which are within the order expected to elicit acute effects in many vertebrates. Additional clinical blood biochemistry and haematology results indicate signs of a systemic disease and the prevalence of an active inflammatory response in a high proportion (44%) of turtles from the agricultural site. Elevated Co, Sb, and Mn in the blood of these turtles significantly correlated with elevated markers of acute inflammation (total white cell counts) and liver dysfunction (alkaline phosphatase and total bilirubin). The results of this study support the notion that elevated trace element exposures may be adversely affecting the health of nearshore green sea turtles.

1. Introduction

Tidal and subtidal seagrass meadows and algal turfs are important foraging grounds for maturing and adult green sea turtles (Limpus, 2008). Some of these habitats include shallow coastal embayments close to shore, which are subject to natural and diverse anthropogenic impacts (e.g. floods, agricultural and industrial runoff, urbanisation, coastal dredging) that can elevate concentrations of a wide range of pollutants, including metals, metalloids and their compounds. In polluted areas, trace elements are typically enriched in surrounding water and sediments, as well as in the seagrass and algae (Talavera-Saenz et al., 2007) that make up the bulk of the diet of neritic green turtles (*Chelonia mydas*). Having high fidelity to relatively small (few km^2) foraging areas (Hazel et al., 2013; Shimada et al., 2016) over several decades (Chaloupka et al., 2008), resident coastal sea turtles can experience long-term exposure to contaminant impacted areas. Both chronic and acute exposures may pose significant risks to their health and survival (Camacho et al., 2013; da Silva et al., 2016; Day et al., 2010; Faust et al., 2014; Flint et al., 2014; Labrada-Martagon et al., 2011). Their life-history traits are particularly relevant given frequent

☆ This paper has been recommended for acceptance by Maria Cristina Fossi.

* Corresponding author.
** Corresponding author.
E-mail addresses: c.villa@uq.edu.au (C.A. Villa), c.gaus@uq.edu.au (C. Gaus).

http://dx.doi.org/10.1016/j.envpol.2016.10.085

observations that chronic diseases (e.g. fibropapilloma) are more prevalent in green turtles from inshore, shallow, and polluted waters (Foley et al., 2005; Van Houtan et al., 2010). There is also evidence suggesting metal exposure is involved in the aetiology and development of fibropapillomatosis, impaired immune functions (da Silva et al., 2016; Grillitsch and Schiesari, 2010), and as a contributing factor in mass strandings (Flint et al., 2014).

The composition, concentrations, and bioaccessibility of trace elements in coastal sediments and aquatic plants can vary considerably on a regional scale depending on local sources and environmental conditions. Moreover, species-specific toxicokinetic processes (absorption, distribution, metabolism, and elimination), which are poorly understood for reptiles in general, govern the trace element differences among various tissues. Only a few descriptions of these processes exist for freshwater turtle species, and even then only for Cd, Se, and Hg (Blanvillain et al., 2007; Dyc et al., 2016; Guirlet and Das, 2012; Schwenter, 2007). Since internal tissue concentrations reflect the integration of all trace element exposure pathways, they are more informative as biomonitoring matrices than external ones (i.e. diet, air, sediment). Given the ethical considerations of collecting the ideal biomonitoring tissues (i.e. liver, kidney, and muscle) from free-ranging protected species, blood, which acts as a transport medium and reservoir for all endogenous and exogenous chemicals in the body, can be collected using minimally invasive methods (Faust et al., 2014). At constant exposure, trace element concentrations in blood are correlated to those in organs (Grillitsch and Schiesari, 2010). With blood elimination rates of many trace elements generally on the order of weeks to months (depending on the element and its speciation), blood is considered a suitable matrix for evaluating relatively recent exposure (Angerer et al., 2007; Burger et al., 2005), since abrupt changes in exposure are rapidly reflected in blood first and can be observed depending on the time of sampling relative to the time of exposure (Blanvillain et al., 2007; Day et al., 2010; Guirlet and Das, 2012).

Several studies have investigated trace element concentrations in blood from free-ranging green sea turtles around the world (Camacho et al., 2014a; Komoroske et al., 2011; Ley-Quiñónez et al., 2013; McFadden et al., 2014; Suzuki et al., 2012; van de Merwe et al., 2010). These data, however, are generally from regions suspected to be or known to be, environmentally impacted with ongoing or legacy mining and industrial activities, making them unsuitable as control populations. In addition, much of the data originates from stranded, moribund or recovering individuals. Since poor health and nutrition can alter toxicokinetic processes, trace element concentrations in the blood can be considerably altered in compromised individuals (Camacho et al., 2014b). In the absence of a physiological healthy or baseline range in trace elements for sea turtles, a major gap exists in our ability to interpret reported element concentrations in context to adverse health risks. Some blood and other tissue-based effect concentrations have been derived for immunotoxicity associated with Hg exposure (Day et al., 2010) in an exposed group of loggerhead turtles (*Caretta caretta*), and fibropapillomatosis associated with Fe, Cu and Pb exposure (da Silva et al., 2016) for juvenile green turtles. However, the paucity of any tissue-based effect data and the ever-changing composition of elements to which these species are potentially exposed to further adds to the difficulty in extrapolating baselines from exposed populations. Alternatively, blood from healthy turtles that forage in areas distant to point sources allows us to determine the likely concentration limits of non-essential elements associated with low exposure and functionally healthy concentration ranges for essential elements (Aggett et al., 2015). The heterogeneous global distribution of elements in the environment and the ability of anthropogenically enriched elements to disperse via long-range transport mechanisms (water, land, air) makes it improbable that a truly pristine site might exist; nonetheless, offshore and distant foraging grounds within marine zones protected from anthropogenic activity offer us the closest alternative to sample healthy and minimally exposed turtles.

Blood-based reference intervals (RIs) are standard tools for interpreting health diagnostics in humans and animals. They commonly include biomarkers of metabolic stress and disease as well as free-ion trace element biochemistry and haematology and have recently been described for Queensland green turtles by Flint et al. (2014). While green turtle RIs for health now exist, the RI approach has yet to be employed in determining population level trace element exposure. In general, RIs define an analyte upper and lower 95% prediction intervals in an asymptomatic population. Methodologies to derive RIs are well established (CLSI-IFCC, 2010; Friedrichs et al., 2012), with validated statistical approaches for handling complex data features such as excessive skew (common for many blood analytes), outliers, and small sample sizes (Horn and Pesce, 2003). The ability to develop robust RIs using between 20 and 100 carefully selected individuals, makes these approaches ideal for describing trace element baselines in difficult to catch, free-ranging, and protected species such as green turtles.

Thus, our objective for this study was to calculate blood-based biomonitoring RIs for 20 trace elements (11 essential and 9 non-essential), using a reference group (baseline cohort) of clinically healthy green turtles foraging in a northern Great Barrier Reef habitat that is located off-shore and presumably distant to point or diffuse anthropogenic sources. Those 20 exposure baselines were then used to identify and evaluate elevated trace element exposure in the blood of three different turtle populations foraging in three different coastal habitats, which were categorised based on their distinct anthropogenic influences; agricultural, urban, and military activities. Finally, clinical health data (biochemistry and haematology) were assessed for each coastal turtle to identify any associations between elevated exposure and adverse health.

2. Materials and methods

2.1. Turtle populations

2.1.1. Baseline cohort

For the development of the trace element exposure RIs, we analysed whole blood from n = 49 subadult green turtles from a population foraging at the Howick Group of Islands (HWK), a collection of sheltered mid-shelf, uninhabited reefs which lie within the remote northern Great Barrier Reef Marine Park (−14.416695 °S, 144.880484 °E; Fig. 1). HWK is located approximately 30 km offshore from the Cape York region catchment, a remote coastal wetland with low pressures from nutrient, sediment, and pesticide loads, or water regime changes and habitat alterations (Senior et al., 2015). Therefore, this remote and offshore sea turtle foraging ground was predicted to be a suitable surrogate for a site free from anthropogenic contaminant point sources.

2.1.2. Three coastal populations

To evaluate trace element exposure of green turtles foraging near shore, whole blood was collected from subadults foraging at three different coastal sites (Fig. 1): Cleveland Bay (CLV; n = 28; −19.235,428 °S, 146.938,284 °E) adjacent to the city of Townsville (population >175,000) with metal processing (Zn, Cu, Ni, and Co) and other heavy industries, and home to a major port (Esslemont, 2000), Upstart Bay (UPB; n = 35; −19.767,554 °S, 147.702,955 °E), a rural coastal area approximately 100 km south of CLV and 50 km north of an international coal terminal, with a system of mostly ephemeral creeks receiving wet-season discharges from one of Queensland's largest catchments