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Levels of trace elements, methylmercury and polybrominated diphenyl ethers in foraging green turtles in the South China region and their conservation implications☆

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A B S T R A C T

Sea turtles are globally endangered and face daily anthropogenic threats, including pollution. However, there is a lack of ecotoxicological information on sea turtles, especially in the Asia-Pacific region. This study aims to determine pollutant levels of foraging green turtles (*Chelonia mydas*) in South China, including Hong Kong, Guangdong and Taiwan, as a basis for their conservation. Scute, liver and muscle tissues of stranded green turtles were analysed for levels of 17 trace elements and methylmercury (MeHg) ($n = 86$ for scute and $n = 14$ for liver) and polybrominated diphenyl ethers (PBDEs) ($n = 11$ for muscle and $n = 13$ for liver). Ten-fold higher levels of Pb, Ba, V and Tl and 40-fold greater Cd levels were measured in green turtle livers in South China relative to other studies conducted over 10 years ago. Measured PBDE levels were also 27-fold and 50-fold greater than those reported in Australia and Japan. These results warrant further investigation of potential toxicological risks to green turtles in South China and their source rookeries in Malaysia, Micronesia, Indonesia, Marshall Islands, Japan and Taiwan. Research should target monitoring pollutant levels in sea turtles within the West Pacific/Southeast Asia regional management unit spanning East Asia to Southeast Asia to fill in knowledge gaps, in particular in areas such as Thailand, Vietnam, Indonesia, Malaysia and the Philippines where less or no data is available and where foraging grounds of sea turtles have been identified.

1. Introduction

Of the seven sea turtles species in the world, five species are found in the South China Sea: the green turtle (*Chelonia mydas*), leatherback (*Dermochelys coriacea*), olive ridley (*Lepidochelys olivacea*), loggerhead (*Caretta caretta*), and hawksbill (*Eretmochelys imbricata*) turtles (Wang, 1993; Chan et al., 2007). These sea turtle

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species are migratory with circumtropical distribution, with adults traveling hundreds to thousands of kilometres between nesting beaches and foraging grounds. Among the five sea turtle species recorded in the South China region, the green turtle is the most common and is the only species that nests in the area (Wang, 1993; Chan et al., 2007; Wang and Li, 2008; Ng et al., 2014). Foraging grounds of green turtles are mainly distributed along the coasts of mainland China (Hainan Island and Guangdong Province), as well as of Taiwan, and on outlying islands in the South China Sea and East China Sea. Green turtles migrating for breeding and during the inter-nesting period usually stop foraging (Ng, 2015). Green turtles in the South China Region mainly forage on red algae and occasionally marine biota such as fishes and crabs (Ng et al., 2016). Their primary source rookeries are in Malaysia, Micronesia, Indonesia, the Marshall Islands, Japan and Taiwan (Ng et al., 2017). The globally endangered green turtle (IUCN, 2016) faces various anthropogenic threats, such as direct take and by-catch (Cheng and Chen, 1997; Wang and Li, 2008), trade pressure (Pilcher et al., 2009; Lam et al., 2011), habitat degradation (Wang and Li, 2008) and pollution and marine debris (Lam et al., 2006; Wabnitz and Nichols, 2010). Nesting populations of green turtles in South China have been dwindling for decades (Chan et al., 2007; Wang and Li, 2008).

Sea turtles are known to be exposed to trace elements and persistent organic pollutants (POPs) (Lam et al., 2004; Storelli and Marcotrigiano, 2003; Day et al., 2005; Van de Merwe et al., 2010). Some trace elements (e.g., Se, Zn), are essential to the health and biological function of organisms but can be detrimental above optimal levels; others such as As and Pb have no nutritional value and can be toxic at low doses (Hoffman et al., 2003). POPs such as the additive flame retardants polybrominated diphenyl ethers (PBDEs) remain intact for long time periods in the environment, are highly soluble in lipids in living organisms and can cause adverse impacts to humans and wildlife (Hites, 2004). Although the production and use of PBDEs (mainly the penta- and octa-BDEs) have been banned/restricted under the Stockholm Convention since 2004, PBDEs remain in the environment. PBDEs have been detected in marine microplastics and reported in abdominal adipose tissue of oceanic seabirds in the North Pacific Ocean (Hirai et al., 2011).

Coastal waters and habitats in South China are contaminated by organic pollutants and trace elements due to the recent rapid increase in industrialized activities in the region (Blackmore, 1998; Mai et al., 2005; Wong et al., 2006; Vane et al., 2009). Levels of PBDEs in two small cetaceans from the South China Sea, the Indo-Pacific humpback dolphin (*Sousa chinensis*) and finless porpoise (*Neophocaena phocaenoides*), increased from 1990 to 2000 (Isobe et al., 2007) as well as 1997–2007 (Lam et al., 2009). Studies on trace elements in biota in South China have mostly focused on benthic fauna (Che and Cheung, 1998; Wong et al., 2005) and birds (Burger and Gochfeld, 1993; Connell et al., 2002). Toxicology studies in sea turtles in China are limited to measurement of levels of selected trace elements in muscle and organs of stranded green turtles (Lam et al., 2004) and in green turtle eggs (Lam et al., 2006) in Hong Kong, and trace elements (including As, Cd, Cr, Cu, Hg, Ni, Pb and Se) in blood of stranded, by-catch or captive sea turtles and nesting green turtles in Taiwan (Kuo, 2015). Bioaccumulation of trace elements and POPs in sea turtles pose potential risks to their health, in particular physiological and immune function, development and growth, and reproductive success (Grillitsch and Schiesari, 2010; Jakimska et al., 2011). Despite the threats posed by these pollutants, most previous studies generally have quantified pollutant loading in sea turtles (Cortés-Gómez et al., 2017), with less consideration given to assessing potential impacts (Hopkins et al., 2001; Weir et al., 2010).

The objectives of this study are to determine the recent baseline levels of trace elements, methylmercury (MeHg) and PBDEs in green turtles in the South China region and to compare these concentrations to data reported 10 years ago as part of the information needed for management and conservation of this species. Risk assessment of selected trace elements and MeHg measured in green turtle livers using a hazard quotient approach was also conducted.

2. Materials and methods

2.1. Sample collection

Samples were collected from foraging green turtles collected as by-catch or stranded individuals in the South China Region, including Hong Kong, Guangdong and Taiwan, from 2005 to 2013. Necessary CITES import and export licences were granted by the Endangered Species Import and Export Management Office of the People's Republic of China, the Bureau of Foreign Trade Ministry of Economic Affairs and the Council of Agriculture of Taiwan, the U.S. Fish and Wildlife Service Division of Management Authority and the Agriculture, Fisheries and Conservation Department of the Government of the Hong Kong Special Administrative Region of the People's Republic of China.

Scute scrapings, liver and pectoral muscle tissues of green turtles were sampled. Approximately 1–2 g of scute scrapings were obtained by scratching ~1 mm depth of the scutes at the posterior end of the carapace (Day et al., 2005; Sakai et al., 2000a) using a ceramic knife, and the scrapings were stored at −20 °C in polyethylene bags for analysis of trace elements and MeHg. 10 g of liver and muscle tissue were dissected from freshly dead turtles using a hexane-rinsed scalpel (Keller et al., 2004; Lazar et al., 2011) during post-mortem examinations and placed in hexane-rinsed aluminum foil and then in polyethylene bags and stored at −20 °C for PBDE analysis. An additional 10 g of liver was dissected using a ceramic knife and stored in only a polyethylene bag at −20 °C for trace element analysis. Curved carapace length (CCL) of each green turtle sampled was recorded and is presented as mean ± standard deviation and range. Life stage was defined using carapace length intervals described for the best-available and geographically closest green turtle population, that in Hawaii described by Balazs (1980). The following age classes were used: a juvenile is a post-hatchling to an individual of 65 cm straight carapace length (SCL); a sub-adult is an individual of SCL from 65 cm to 81 cm; and an adult is an individual of SCL > 81 cm and reproductively mature. According to the conversion between SCL and CCL where $CCL = -0.414 + 1.039$ SCL (Bjorndal and Bolten, 1989), the life stage of green turtles based on CCL was defined as: juvenile, a post-hatchling to an individual of 67 cm CCL; sub-adult, CCL from 67 cm to 84 cm; and adult, CCL > 84 cm.

2.2. Laboratory analysis

Laboratory analysis of trace elements generally followed the methods reported in Connell et al. (2002) and Lam et al. (2004) with modifications. Detailed methods can be found in the Supplementary Material. Briefly, scute scrapings and liver samples were freeze-dried and homogenized before microwave digestion in duplicate in 5 ml of concentrated nitric acid (Aldrich Sigma, trace metal grade, ≧65%). Levels of As, Ag, Ba, Cd, Cu, Cr, Co, Cs, Fe, Mn, Pb, Ni, Se, Sr, Tl, V and Zn were measured with inductively coupled plasma-atomic emission spectroscopy (ICP-AES) (PerkinElmer 2100DV, PerkinElmer, Waltham, MA, USA).

Reagent blanks and sample matrix spikes were included in every digestion batch. Matrix recoveries ranged from 75% to 125%. A certified reference material (DOLT-2 dogfish liver tissue; National Research Council, Ottawa, Canada) was analysed for method