



# Mercury and selenium concentrations in leatherback sea turtles (*Dermochelys coriacea*): Population comparisons, implications for reproductive success, hazard quotients and directions for future research



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## HIGHLIGHTS

- We examined Hg and Se concentrations in leatherback sea turtles.
- Reproductive success did not correlate with Hg and Se concentrations.
- Blood Se concentrations were higher in remigrants than neophytes.
- Liver Hg and Se concentrations of dead-in-nest hatchlings were correlated.
- Hazard quotients indicate that Se may cause physiologic harm to hatchlings.

## ARTICLE INFO

### Article history:

Received 1 March 2013

Received in revised form 16 May 2013

Accepted 17 May 2013

Available online xxx

Editor: F. Riget

### Keywords:

*Dermochelys coriacea*

Mercury

Selenium

Hatching success

Emergence success

Hazard quotients

## ABSTRACT

Leatherback sea turtles (*Dermochelys coriacea*) are long-distance migrants that travel thousands of km from foraging grounds to breeding and nesting grounds. These extensive journeys are fueled by ingestion of an estimated 300–400 kg of prey/d and likely result in exposure to high concentrations of environmental toxicants (e.g., mercury compounds). Increased bodily concentrations of mercury and its compounds in nesting female turtles may have detrimental effects on reproductive success. Leatherbacks have relatively low reproductive success compared with other sea turtles (global average hatching success ~50–60%). To assess toxicants and necessary nutrients as factors affecting leatherback turtle reproductive success at Sandy Point National Wildlife Refuge (SPNWR), St. Croix, U.S. Virgin Islands, we collected blood from nesting female leatherbacks and tissues from their hatchlings (blood from live turtles, liver and yolk sac from dead turtles). We compared the concentrations in those tissues to hatching and emergence success. We found that on SPNWR, hatching and emergence success were more closely related to seasonal factors than to total mercury and selenium concentrations in both nesting females and hatchlings. Selenium concentrations of nesting females were positively correlated with those of their hatchlings. Mercury and selenium in the liver of hatchlings were positively correlated with one another. Turtles with greater remigration intervals tended to have higher blood selenium concentrations, suggesting that selenium accumulates in leatherbacks through time. Through hazard quotients, we found evidence that selenium may be at or above concentrations that may cause physiologic harm to hatchlings. We also found evidence that population level differences exist for these trace elements. The concentrations of mercury and selenium established in this manuscript form a baseline for future toxicant studies.

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## 1. Introduction

Toxic effects of contaminants on reptiles are a relatively underrepresented field of study and there is a paucity of data regarding this topic (Wolfe et al., 1998). Impacts of heavy metals and organic contaminants on the nervous, immune and reproductive systems are well documented for other taxa, including fishes, birds and mammals (Heinz and Wiemeyer, 1991; Hoffman et al., 2002; Milton and Lutz,

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2003). Those studies on reptilian toxicology are few and focus mostly on organic contaminants in relatively few taxa; studies of the consequences of heavy metals are more limited (Wolfe et al., 1998; Gardner, 2006; Willingham, 2006). Even fewer are those investigations into the effects of toxicants on reproductive success, although these studies do exist (Bishop et al., 1991; Guillette et al., 1994). If reproductive output is compromised due to environmental contaminants, populations may suffer, especially those of already vulnerable species.

Heavy metals (e.g., arsenic, cadmium, chromium, mercury, lead, etc.) enter the environment from a variety of sources, both natural (Nriagu, 1989) and anthropogenic (Pacyna and Pacyna, 2002). Of these metals, mercury (Hg) and its compounds are the most toxic (Bryan, 1971) and have no known biological function (Chowdhury and Chandra, 1987). Atmospheric and ocean surface concentrations of this element and its compounds have tripled in the past century (Mason et al., 1994). Of particular concern is the capacity of Hg to travel long distances from its point source to deposition thousands of miles away into the oceans (Ebinghaus et al., 2001), where it can impact marine organismal health.

Mercury can affect a variety of functional processes in wildlife, including effects on the nervous, excretory and reproductive systems (Wolfe et al., 1998); yet, most studies of reptiles to-date focus on establishing concentrations of contaminants in the tissues. Rarely do those studies attempt to identify links between physiological and/or reproductive effects and contaminants. Over 700 species in the class Reptilia are listed in the CITES (Convention on International Trade in Endangered Species of Wild Fauna and Flora) Appendices. Appendix I (those threatened with extinction) includes all seven species of marine turtles (CITES, 2012). One threat to health and recovery of many of the marine turtle populations is pollution, including that from heavy metals (Lutcavage et al., 1997; Hamann et al., 2010). Keller et al. (2004a) and Day et al. (2007) suggest that certain toxicants may affect sea turtle health. Additionally, Lam et al. (2006) and van de Merwe et al. (2009) established hazard quotients (HQs) for selenium (Se) (a necessary detoxifying nutrient that is toxic at high doses, Naganuma et al., 1983) in green turtle (*Chelonia mydas*) eggs. These HQs were at levels that may negatively impact green turtle hatching success.

Among sea turtles, the leatherback (*Dermochelys coriacea*) has the lowest hatching and emergence success of the seven species (Eckert et al., 2012, for review). This reduced reproductive success has been shown to correlate with maternal health (Bell et al., 2004; Rafferty et al., 2011; Perrault et al., 2012), chemical contaminants (e.g., Hg), and the essential mineral Se (Perrault et al., 2011). Additionally, hatchling leatherbacks from Florida exhibited muscular pathologies (Miller et al., 2009) that were similar to bovine neonates with Se deficiency (Enjalbert et al., 1999), which can be caused by high bodily Hg concentrations. These findings led us to hypothesize that Hg and/or Se could impact leatherback reproductive success in multiple populations.

Northern Caribbean nesting leatherback females mostly forage in similar waters as nesting females from Florida. Food and water intake are the main source of Hg and Se uptake (Caurant et al., 1999; Guirlet et al., 2008). The post-nesting migrations of Caribbean females diverge somewhat from those of Floridian turtles (Thompson, 2006), which could expose them to differing concentrations of these elements and their compounds. In this study, our objectives were to (i) identify correlations with Hg and/or Se and leatherback turtle hatching and emergence success, (ii) determine if maternal blood Hg and/or Se concentrations were related to hatchling blood, liver and yolk sac Hg and/or Se concentrations and concentrations in the eggshell and shelled albumen globs (SAGs), (iii) establish trends in Hg and Se concentrations across the nesting season, (iv) compare Hg and Se concentrations in leatherbacks from Sandy Point National Wildlife Refuge, St. Croix, U.S. Virgin Islands to Florida, and (v)

establish HQs for Hg and Se in tissues (i.e., blood, liver and yolk sac) of hatchling leatherbacks.

## 2. Materials & methods

### 2.1. Study period & site description

Nesting leatherback sea turtles and their young were sampled along the beach (2.4 km) at Sandy Point National Wildlife Refuge (SPNWR), St. Croix, U.S. Virgin Islands (17°40'40" N, 64°54'0" W, Fig. 1). The beach is marked with numbered stakes, each 20 m apart. This rookery has been monitored nightly for leatherback nesting activity since 1981 (Thompson, 2006). Sampling and nest monitoring began 1 April and ended 1 September, 2009. Over 1000 individual leatherbacks have been tagged at SPNWR since tagging programs began (Garner and Garner, 2010). The population of nesting females on SPNWR exhibit high nest site philopatry, which allowed for repeated sampling of individuals within a season. Leatherbacks typically nest four to six times (or more) in a season.

A portion of the nesting beach at SPNWR experiences annual sand erosion and accretion patterns that result in a substantial loss of beach (Eckert, 1987). The sand that moves from the western erosion zone is deposited on the northern sandy side of the beach, known as the accretion zone (Fig. 1). The sand from the accretion zone returns to the erosion zone in the winter months. Nests deposited in the erosion zone were relocated to the accretion zone (Conrad et al., 2011); however, relocated nests were not included in statistical analyses as non-natural factors may have influenced the development of the embryos (e.g., handling, reburial time, loss of cloacal fluid due to handling, non-natural nest chamber, etc.).

### 2.2. Nesting females

The beach was patrolled nightly from 2000 h to 0500 h (or until the last female finished nesting); the entire beach was checked for nesting activity every 60 min. Nesting females were approached during the nesting fixed action pattern phase, which begins after oviposition commences (Dutton and Dutton, 1994). Turtles were identified by the presence of flipper tags and/or passive integrative transponder (PIT) tags. Flipper and/or PIT tags were applied if turtles lacked either of these types of tags. During oviposition, approximately 5 mL of blood was collected aseptically into 7 mL K<sub>3</sub>EDTA (purple-top) Vacutainer® tubes, using an 18G venous collection needle fitted into a Vacutainer® tube holder. The femoral rete system was used as the blood collection site (Dutton, 1996). The area was then swabbed with betadine and pressure was applied to promote hemostasis. Whole blood samples were chilled immediately and subsequently frozen before being shipped to the University of Georgia's Veterinary Diagnostic and Investigational Laboratory (UGA-VDIL, Tifton, GA, USA). When possible, subsequent blood samples were collected from nesting females that were sampled earlier in the season, allowing us to track Hg and Se concentrations across the season. Following blood collection, minimum curved carapace length (CCL<sub>min</sub>) and curved carapace width (CCW) were recorded (after Wyneken, 2001). After collection of samples and maternal morphometric data were taken, the location of the egg chamber was recorded using triangulation (Boulon et al., 1996).

### 2.3. Hatchlings

Nests were monitored for signs of hatchling emergence starting at days 50–55 of incubation. As hatchlings emerged, up to 10 turtles were selected, weighed, and measured (straight carapace length [SCL]; straight carapace width [SCW]; body depth [BD]) to the nearest 0.05 mm using Vernier calipers. Body condition was calculated by dividing the mass by SCL (van de Merwe et al., 2010a). The venipuncture site (dorsal cervical sinus) was swabbed with isopropanol, and

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