

Abstract: Leatherback sea turtles (*Dermochelys coriacea* V.) have low hatch success compared to other sea turtle species. We documented heart muscle cell degeneration in hatchlings that was similar to that observed in bovine neonates born to mothers with low selenium (Se). We hypothesized these and other anomalies found in dead-in-nest leatherbacks are correlated with mercury compounds (hereafter Hg) and Se levels. Hg is detoxified by the liver through the formation of Hg-Se complexes and elevated Hg may exhaust *in vivo* Se. We conducted a dead hatchling survey for evidence of Hg exposure and other indicators of compromised vigor to better understand low leatherback nest success. Blood was collected from nesting leatherbacks and analyzed for Hg, Se, and routine blood parameters. We then correlated those results with nest success. Next, blood was collected from a subset of live hatchlings as they emerged from the nests, and livers were collected from a subset of dead-in-nest hatchlings. The nests were excavated and inventoried for mass emergence. We found a mean emergence success of 41% (range = 0 – 33%). Maternal blood Hg and Se levels ranged from 6.3 – 87.6 ppb and 0.4 – 20.0 ppm, respectively. Neither Hg nor Se had a significant effect on emergence success. Hg levels in turtles that nested more than once decreased between sampling events, suggesting maternal transfer to the offspring. Se tended to increase as the season progressed, which may indicate that these turtles are feeding on Se-rich marine food sources. For hatchlings, Hg was only measured in the liver (8.8 – 29.34 ppb) because an insufficient blood quantity was available. Hatchling liver Se ranged from 1.0 – 2.1 ppm. Hatchling blood Se ranged from 1.4 – 7.0 ppm. Two metabolic measures from blood surveys significantly correlated with emergence success (anion gap, blood urea nitrogen); it is unclear at this time how these parameters relate. Continued sampling will allow us to determine if blood values are accurate predictors of leatherback turtle nest success.

INTRODUCTION

Leatherback sea turtles (*Dermochelys coriacea* V.) are critically endangered internationally and show a worldwide population decline, largely due to anthropogenic practices (Spotila et al. 1996, IUCN 2007). Current assessments suggest as few as 34,000 leatherbacks remain in the Atlantic (TEWG 2007). Sea turtle populations rebound poorly because these turtles are late-maturing (Magnuson et al. 1990). They also incur very high mortality in their earliest life stages (Davenport 1997, Bell et al. 2003). Nest success is low for reasons that remain conjectural (e.g., maternal reproductive health, chemical contaminants, bacterial infection; Bell et al. 2003). Here we explore uninvestigated sources of hatchling mortality and reduced vigor associated with a toxicant (mercury, hereafter given as Hg) and compromised maternal health.

When Hg concentrations are abnormally high, a selenium (Se) deficiency can occur (Fig 1, after Chauvaud et al. 1996). But if Hg levels increase, Se is depleted and muscle damage can occur (Orr & Blakey 1997). Studies of calves deficient in Se and leatherback hatchlings show similar patterns of myocyte degeneration (Fig 2, Miller et al. 2006). Here we identify correlations between nest success and the concentrations of Hg and Se found in the blood of hatchlings and their mothers, nesting in Florida.

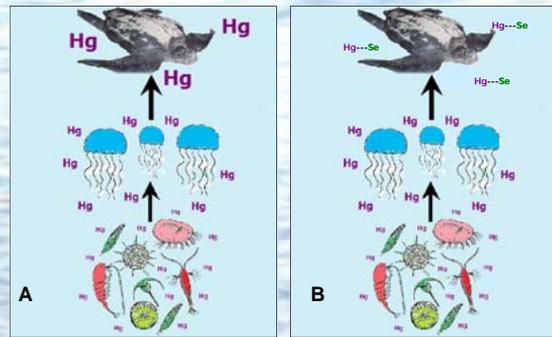


Figure 1. (A) Methylmercury bioaccumulation is magnified when Se is absent. Se acts as an antioxidant and detoxifies Hg in the liver. (B) Hg bioaccumulates less drastically with Se present (from Ralston et al. 2005).

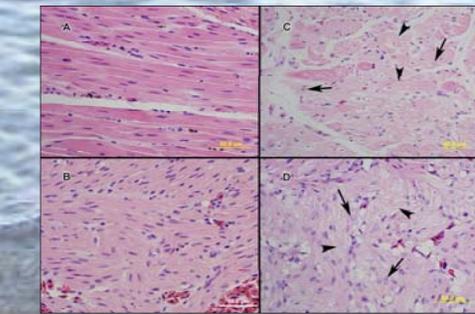


Figure 2. (A) Normal skeletal and (B) normal heart muscle from a hatchling leatherback. (C) Degenerative skeletal and (D) degenerative heart muscle from a hatchling leatherback. Arrows show swollen nuclei and arrowheads show swollen muscle fibers that are fragmented and missing cross striations.

Why is nest success low? Mercury, selenium, and baseline blood parameters in nesting leatherback sea turtles (*Dermochelys coriacea*) and their young.

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MATERIALS and METHODS

Study Site & Sampling: Blood was collected from nesting females between March and June, 2007 and 2008, at Juno and Jupiter Beach, Florida, U.S.A. (Fig 3). Approximately 10 mL of blood, were taken from the hind leg rete system for analyses. Because females nest 4-8 times/season, we resampled females whenever possible to track across season changes.



Figure 3. Juno Beach - Jupiter Beach are located on the east coast of Florida.

Five – 10 hatchlings from nests deposited by the study females were sampled. Blood (< 0.2 µl) was collected from the external jugular vein of the hatchlings as they emerged and was used for Se analyses. We could not collect sufficient blood from hatchlings to conduct blood Hg surveys. Instead, we also collected up to 5 dead-in-nest hatchlings in good condition and their livers were used for measurement of Hg and Se concentrations.

We conducted standard blood counts, biochemical assays, analyses of Hg and Se levels in mothers and their offspring and opportunistically analyzed Hg and Se levels in captive-reared hatchling (from a different study) that died after their started feeding.

Data Analysis Simple linear regressions were run to determine if individual blood parameters had an effect on nest success. Multiple regressions were run to determine if both Hg and Se affected nest success. Data were transformed when they did not meet parametric assumptions.

RESULTS

Table 1. Blood concentrations of Hg and Se from nesting females. Hg tended to decrease on average in subsequent samples while Se increased, suggesting that Hg was eliminated early in the nesting season. Se may increase if the turtles resumed feeding.

	Hg (ppb)	Se (ppm)
Mean ± SD (1 st sampling)	30.46 ± 16.81	8.40 ± 5.25
N (1 st sampling)	31	59
Mean ± SD (repeated samples)	27.67 ± 5.83	9.48 ± 5.07
N (repeated samples)	6	12

Table 2. Averages, standard deviations, and sample sizes for hatchlings tested for Hg and Se.

	Liver Hg (ppb)	Liver Se (ppm)	Blood Se (ppm)
Mean ± SD	16.23 ± 7.03	1.77 ± 1.27	3.99 ± 1.39
N	14 from 14 nests	13 from 10 nests	22 nests

Two blood parameters correlated with hatch success. Anion gap, an indicator of kidney function, positively correlated with hatch success (Fig 5A). Blood urea nitrogen, also an indicator of kidney function, negatively correlated with hatch success. It is unclear if these parameters are biologically meaningful or are spurious findings.

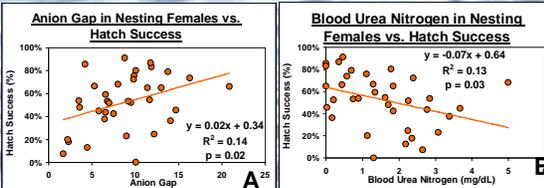


Figure 4. (A) Anion gap significantly increased with hatch success (N = 35). (B) Blood urea nitrogen significantly decreased with hatch success (N = 35).

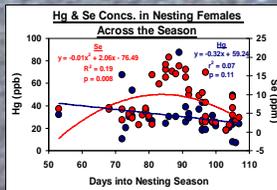


Figure 5. Hg concentrations significantly decreased as the season progressed (N = 36). Se first increased and then decreased, so that the relationship was a significant polynomial trend (N = 46). Early season leatherbacks may pass on Se to their offspring, lowering their bodily concentrations. Mid-season nesters may dump Hg into their eggs, reducing the need to detoxify. Late season nesters may be unable to replenish their bodily Se concentrations.

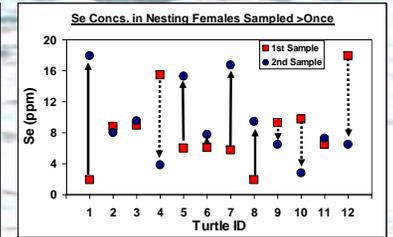
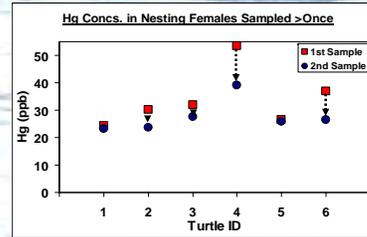


Figure 6. Changes in Hg and Se blood concentrations in nesting females repeatedly sampled during the nesting season. (A) As the nesting season progressed, Hg concentrations decreased in 6 of 6 females. (B) Selenium levels increased in 7 of 12 females.

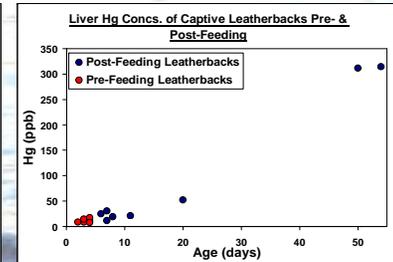
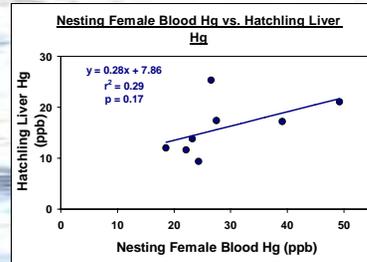


Figure 7. Liver Hg concentrations in hatchlings tended to increase as maternal blood Hg concentrations increased, supporting our hypothesis that leatherback females dump Hg into their eggs and thus, their hatchlings (N = 8).

Figure 8. Mercury concentrations in captive hatchlings significantly increased with age. Low levels were found before feeding commenced (<5 days old, red). Higher levels were found in the livers of captive hatchlings (>5 days) that fed on fish and shellfish based foods (blue).

CONCLUSIONS

- We found that Hg levels tended to decrease as the season progressed, suggesting that nesting females may “dump” some of their Hg loads into their eggs.
- Early season leatherbacks exhibit low Se, presumably associated with Hg detoxification before the nesting season, which decreases the bodily concentration of both.
- Late season leatherbacks exhibit lower Se concentrations, perhaps due to limited ability to replenish their stores.
- Mid-season nesters may dump their Hg loads into their eggs, reducing bodily Hg concentrations to presumably tolerable ranges (with little or no need to detoxify).
- Se increased in 7 of the 12 samples, suggesting that nesting female turtles feed/drink during the nesting season.
- Hg concentrations of hatchling leatherbacks tended to increase as maternal Hg increased, but not significantly so. Because of our small sample size (n = 8), we are exploring if the relationship persists with added samples from a second year of data collection.
- Hg concentrations tended to increase with age in hatchlings that died before feeding, indicating Hg is mobilized from the yolk sac and the detoxification process does not begin immediately.
- Neonate Hg concentrations increased once feeding commenced, suggesting that Hg accumulation is greater from food than from yolk mobilization.
- Hg concentrations in Juno Beach leatherbacks were lower than Hg concentrations reported from Gabon, Africa (Deem et al. 2006), but higher than those reported from French Guiana (Guiret et al. 2008). Differences may reflect different feeding locations and or trophic level.
- Current and future lines of inquiry:
 - Hg and Se analyses of natural food sources of these animals.
 - Hg and Se analyses of yolk sacs of dead-in-nest hatchlings.
 - Increase sample sizes of blood parameters to characterize inter-annual variation in this population.
 - Experimental dosing of eggs with Hg and Se to determine the tolerance limits of embryos and hatchlings.

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Literature Cited
 Bell et al. 2003. Low reproductive success of leatherback turtles, *Dermochelys coriacea*, is due to high embryonic mortality. *Biol Con* 115:131–138.
 Chauvaud et al. 1996. Mercury in pilot whales: possible limits to the detoxification process. *Sci Tot Emv* 156: 95–104.
 Davenport, J. 1997. Temperature and the life-history strategies of sea turtles. *J Theor Biol* 22:679–688.
 Guiret et al. 2008. Maternal transfer of trace elements of leatherback turtles of French Guiana. *Aquatic Tox* 88: 267–276.
 IUCN 2007. *IUCN Red List of Threatened Species*. www.iucnredlist.org.
 Magnuson, J. J., et al. 1990. Decline of the Sea Turtles: Causes and Prevention. National Academy Press, Washington, DC.
 Miller et al. 2006. Loss in a group of leatherback sea turtle (*Dermochelys coriacea*) posthatchlings: Pathological findings and speculations on pathogenesis. *Orn. J P & Blakely, B. R.* 1997. Investigation of the selenium status of aborted calves with cardiac failure and myocardial necrosis. *J Vet Diag Invest* 9: 172–179.
 Perrault et al. 2008. Do maternal blood characteristics predict nest success and hatchling mortality in the leatherback sea turtle (*Dermochelys coriacea*)? Proceedings of the 26th Sea Turtle Symposium, International Sea Turtle Society, Baja California Sur, Mexico, January 2008, abstract 2625.
 Potts, R. 1994. *Mineral Levels in Animal Health: Diagnostic Data*, 2nd Edition. Sherna International, Caslerbrook, British Columbia, Canada.
 Ralston et al. 2005. Mercury-Selenium Interactions in Aquatic Ecosystems (Grant proposal to the Energy & Environmental Research Center’s Center for Air Toxic Metals).
 Spotila et al. 1996. Worldwide population decline of *Dermochelys coriacea*: are leatherback turtles going extinct? *Choi Con Bio* 2: 209–222.
 Turtle Expert Working Group. 2007. An assessment of the leatherback turtle population in the Atlantic Ocean. NOAA Technical Memorandum NMFS-SEFSC-555. 11pp.