

Brevetoxin exposure in nesting loggerhead sea turtles (*Caretta caretta*) and effects on immune system health

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Introduction

- Florida's Gulf Coast experiences frequent red-tide events caused by the toxic dinoflagellate *Karenia brevis*
- K. brevis* releases neurotoxins (brevetoxins) causing fish kills, marine mammal mortalities and adverse effects on human health¹
- Marine turtles are exposed to brevetoxins through inhalation of aerosolized toxins and ingestion of red-tide exposed prey
- As predators on filter-feeding organisms, loggerhead sea turtles (*Caretta caretta*) are especially susceptible to the effects of bioaccumulation²; little is known about potential sublethal effects this toxin has on marine turtles
- Marine turtles fast during the nest season, thus stored toxin can be metabolized from fat stores³
- Brevetoxin persists in the environment and in prey items for over a year, which could result in prolonged exposure in marine turtles⁴
- The last major red tide event on Florida's Gulf Coast occurred May 2013

Objectives

- (1) Determine brevetoxin concentrations (ng PbTx-3/ml) in the plasma and scute of nesting loggerhead sea turtles
- (2) Determine if brevetoxin exposure was related to immune system health

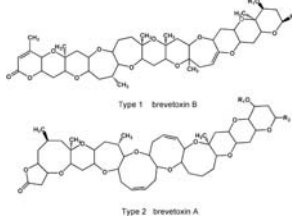


Figure 4. The two different brevetoxin chemical structures, type I and II.

Materials and methods

- Blood and scute samples were collected from nesting loggerhead turtles on Nokomis Beach, Florida USA
- Scute samples were prepared for ELISA through solid phase extraction
- Plasma and scute samples were analyzed for brevetoxins using a competitive ELISA
- Plasma was also analyzed for:
 - Superoxide dismutase (SOD) activity using an SOD Assay kit
 - Total protein using a handheld refractometer
 - Plasma protein fractions (albumin, α_1 , α_2 , β , γ) using agarose gel electrophoresis
 - Lysozyme activity using a lysozyme assay kit
 - Leukocyte differentials using thin blood smear slides
 - Extracted plasma was analyzed for specific brevetoxin congeners through LC-MS/MS
- Plasma from captive turtles was tested for brevetoxin concentrations, SOD activity and lysozyme activity to serve as a control

Results

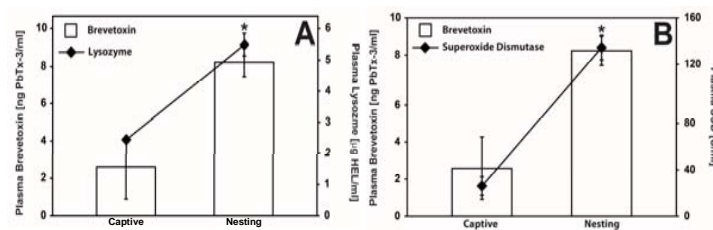


Figure 1. Mean (\pm SE) plasma brevetoxin concentrations and (A) lysozyme activity (B) and superoxide dismutase activity in captive and nesting loggerhead sea turtles. Brevetoxin concentrations, lysozyme activity and superoxide dismutase activity were significantly higher in nesting turtles in comparison to captive, unexposed animals. *Brevetoxin concentrations, lysozyme activity and superoxide dismutase activity were significantly different between the captive and nesting turtles ($P < 0.05$).

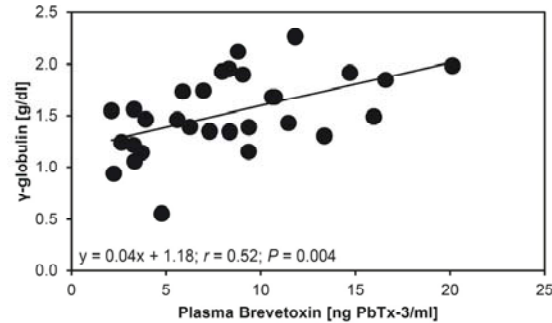


Figure 2. Plasma brevetoxin concentrations (ng PbTx-3/ml) in wild-caught nesting loggerhead sea turtles showed a significant positive correlation with γ -globulin proteins ($P < 0.05$).

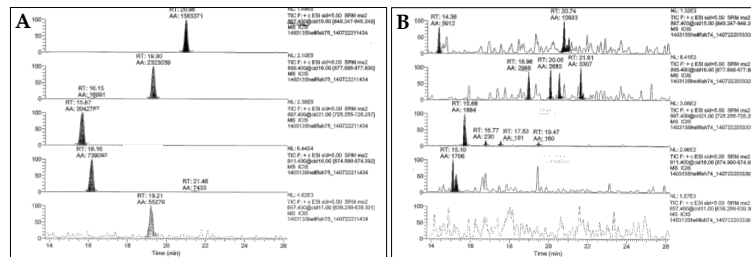


Figure 3. (A) LC-MS/MS standard chromatogram for brevetoxin congeners and (B) LC-MS/MS chromatogram for extracted plasma that shows the presence of PbTx-3.

Discussion

- All nesting females ($n = 29$) tested positive for brevetoxin exposure, indicating that this toxin is extremely persistent in tissues and the environment
- There were significant differences between plasma brevetoxin concentrations, lysozyme activity and SOD activity between captive and nesting turtles (Fig. 1) indicating that brevetoxin may cause upregulation of certain enzymes that are symptomatic of immune and oxidative stress
- Plasma brevetoxin concentrations showed a significant positive correlation with γ -globulins (Fig. 2). The γ -globulins consist of immune proteins and so an increase in these proteins may be indicative of immune system stress
- PbTx-3 was found through LC-MS/MS in low concentrations in the plasma of nesting turtles (Fig. 3) potentially suggesting recent exposure to this toxin and/or toxin metabolism
- Brevetoxin was detected in the scute of nesting loggerheads through the ELISA procedure. Therefore, the scute may serve as a site of elimination for toxin metabolites
- These findings show that red-tide events can affect the health of organisms for a long time after the event. This data is important for conservation efforts, as well as for turtles that enter rehabilitation facilities due to brevetoxicosis
- In the future, maternal transfer of this toxin should be studied to understand how the lingering affects of this toxin on offspring



Literature cited

- (1)Fauguer, D.A., Flewelling, L.J., Maucher, J., Manire, C.A., Socha, V., Kinsel, M.J., Stacy, B.A., Henry, M., Gannon, J., Ramsdell, J.S., Landsberg, J.H., 2013. Brevetoxin in blood, biological fluids, and tissues of sea turtles naturally exposed to *Karenia brevis* blooms in central west Florida. *Journal of Zoo and Wildlife Medicine* 44: 364-375.
- (2)Borndal, K.A., 1996. Foraging Ecology and Nutrition of Sea Turtles. In: *The Biology of Sea Turtles Vol. 1*. (Lutz, P., Musick, J.A., eds) CRC Press, Boca Raton, Florida, pp 199-232.
- (3)Keller, J.M., Kucklick, J.R., Harms, C.A., McClellan-Green, P.D. 2004. Organochlorine contaminants in sea turtles: correlations between whole blood and fat. *Environmental Toxicology and Chemistry* 23: 726-738.
- (4)Nair, J.P., Flewelling, L.J., Lenz, A., Abbott, J.P., Ganholm, A., Jacocks, W.M., Gannon, D., Henry, M., Pierce, R., Baden DG, Wolny, J., Landsberg, J.H. 2007. Brevetoxins, like ciguaterins, are potent ichthyotoxic neurotoxins that accumulate in fish. *Toxicol* 50: 707-723.

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