# Toxicity in the Tissues: Examining Brevetoxin Bioaccumulation in Blacktip Sharks

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## INTRODUCTION & HYPOTHESES

Brevetoxins are a family of lipid-soluble neurotoxins produced by the dinoflagellates, most notably by the algae *Karenia brevis,* commonly known as red tide (Fleming et al. 2011). Harmful Algae Blooms (HABs) occur when the population of an algae or dinoflagellate increases dramatically. In addition to producing toxins, HABs can also reduce oxygen by blocking light to photosynthetic organisms during a bloom (Tester and Steidinger 1997). Brevetoxins, which are neurotoxins produced by *K. brevis* during harmful algal blooms, cause respiratory distress (Watkins 2008) and bioaccumulate in marine organisms (Landsberg et al. 2009). *Karenia brevis* blooms can cause severe health issues for coastal residents, including neurotoxic shellfish poisoning (Poli et al. 2000; Flewelling et al. 2010). Fish kills are common during a *K. brevis* bloom, though this statistic seldom includes sharks. Sharks have been shown to accumulate brevetoxins in their tissues and serve as good subjects for studying accumulation over time (Naar et al. 2007; Flewelling et al. 2010). However, they sink when they die, limiting the data that can be obtained during red tide blooms.

**Hypotheses**

We investigated brevetoxin accumulation in blacktip sharks (*Carcharhinus limbatus*) from Sarasota Bay. The liver likely accumulates the most brevetoxins due to its role in filtering, detoxification, and metabolizing contaminants, which leads to prolonged toxin retention. Additionally, brevetoxins are lipid-soluble, allowing them to easily integrate into the liver's fatty tissue. We predict that livers will contain the highest concentration of PbTx-3 from a Brevetoxin enzyme-linked immunosorbent assay, which is the primary detectable toxin produced by *K. brevis* algae.

## METHODS AND MATERIALS

**Collection & Tissue Preparation (Fig. 1)**

- Sharks were collected between 2020 and 2021 by Dr. Jayne Gardiner and frozen at 0℃.
- We collected samples from a total of 26 blacktip sharks comprised of 23 muscle, 20 gill, 19 stomach, 8 brain, 6 liver and 2 stomach content samples
- Samples collected (liver, stomach, muscle, brain, and gill arch) were dissected and frozen at -20℃

**ELISA - enzyme-linked immunosorbent assay**

- Wells in the competitive Brevetoxin ELISA by Attogene contains brevetoxin binding sites that brevetoxin in samples could bind to
  - Higher absorbance = lower brevetoxin concentration, as brevetoxins were not present to bind to the binding sites
- ELISA analysis was run following Attogene protocol including:
  - Homogenizing and diluting (1:50 sample:90% methanol) the sample supernatant
  - Placing samples into 96 well plate and mixing with antibodies
  - Analyzing samples through 450 nm spectrophotometer

**Statistical Analysis**

- We conducted a comparison of means test to determine whether there were significant differences in each tissue type.

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**Figure 1.** Pictures from the experiment, including (top to bottom): hook-and-line fishing, tissue dissection, tissue homogenizing, ELISA plate preparation

## RESULTS

- We detected brevetoxins in 14/20 gill, 12/19 stomach, 9/23 muscle 6/6 liver, and 3/8 brain samples (**Fig. 2**)
- An ANOVA test indicated there were significant differences between tissue types ($F = 3.66$, $p=0.0053$)
  - Based on a Tukey HSD test, livers have significantly higher brevetoxin concentrations than muscle ($Q=5.18$, $p=0.0061$) and brains ($Q=4.53$, $p=0.024$) (**Fig. 2**)
- The three highest concentrations came from three separate tissues
  - Liver had highest sample at 2.19 ppb for a 2020 shark
  - Second highest from stomach sample with around 1.94 ppb from a 2021 shark
  - Third highest from gill sample with around 1.46 ppb from 2021 shark
- Blooms were reported in March-August during 2019, only in December during 2020, and January-November during 2021 (**Table 2**)
  - Blooms consisted of 100,00 cells per liter
- Of the three sharks from 2021 noted in **Table 2**, the shark caught three days prior to the other two had the lowest concentration of the three, supporting the hypothesis that accumulation builds up over time.

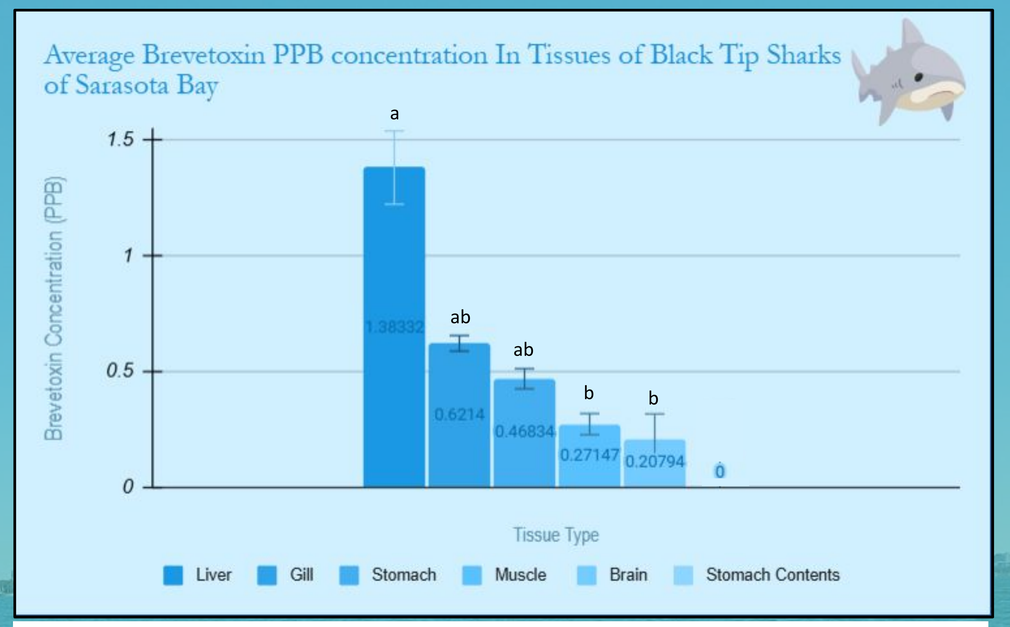


**Figure 2.** Bar graph showing mean Brevetoxin PPB concentration in tissues of *Carcharhinus limbatus* sharks. The error bars represent standard error. Letters indicate significant differences as determined by the Tukey HSD test.

**Table 2**

Brevetoxins Concentrations in Blacktip with Corresponding Red Tide Status

| Sharks | Year | Muscle | Liver | Gill | Brain | Stomach | Red Tide Status |
|---|---|---|---|---|---|---|---|
| 2019-1014-05-06 | 10/14/2019 | 0.00 | | 0.87 | 0.00 | 0.00 | |
| 2020-0522-06-02 | 5/22/2020 | 0.10 | | | | | |
| 2020-0528-06-02 | 5/28/2020 | | 1.44 | 0.00 | | 0.00 | |
| 2020-0528-06-03 | 5/28/2020 | 0.00 | | 0.00 | 0.00 | 0.00 | |
| 2020-0622-03-01 | 6/22/2020 | 0.00 | 2.19 | 0.35 | 0.26 | 0.00 | |
| 2021-0210-06-28 | 2/10/2021 | | | 1.83 | | 1.94 | |
| 2021-0810-06-25 | 8/10/2021 | | | | 1.15 | | |
| 2021-0810-06-28 | 8/10/2021 | 0.00 | | 1.46 | | 0.97 | |

Red tide data were obtained from the the Florida Fish and Wildlife Conservation Commision document, "Over 100 Years of Red Tides off Florida's West Coast". Blue fill indicates a reported bloom (>100,000 cells/L), blank cells indicate no reported bloom.

## CONCLUSIONS

- Our data support our hypothesis that *Carcharhinus limbatus* sharks bioaccumulate brevetoxins produced by *K. brevis* in all of the tissues tested.
- Of the 26 sharks used in this experiment, 20 had brevetoxin in at least one tissue sample signifying that *Carcharhinus limbatus* do accumulate brevetoxins in their liver, gills, and stomach tissues when there are blooms in the water, even when they are not significant blooms.
- Flewelling 2010 also found that the liver is where the highest concentration of brevetoxins, likely due to its lipid solubility.
- It is reasonable that the highest concentrations were found in the liver tissues, as shark livers represent a significant portion of their internal volume and the liver serves as a filter for all nutrients (and lipids) that pass through the shark.
- It is also reasonable that the gills would be the second highest as this area is in direct contact with the water column and would pick up high concentrations of brevetoxin and accumulate.
- As for stomach contents, it makes sense that this would be the lowest concentration found as the stomach acids could deteriorate the brevetoxins, thereby reducing accumulation over time.
  - Our data indicate brevetoxins do accumulate in the stomach lining, possibly from brevetoxin-contaminated prey ingestion.

**Future research**

- There are more brain samples to be run as well as blood. If brevetoxins are found in shark blood, it could result in a novel, non-invasive way of testing for brevetoxin exposure in sharks.