# Physiological Impacts of Heavy Metals in the Blood of Blacktip Sharks in Biscayne Bay, Florida

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## Introduction

In populated coastal areas, such as Miami, Florida, abundant anthropogenic activity contributes to heavy metal contamination in marine habitats. Some metals may accumulate in the tissues of marine life with exposure. Blacktip sharks (*Carcharhinus limbatus*), which seasonally migrate along the East Coast of the United States and seek warm waters in the winter, may be vulnerable to impacts from South Florida's pollution.

Historically, heavy metal measures in sharks have been analyzed from a food safety point of view, and the physiological consequences to sharks have not been well documented. Heavy metals are known to cause neurodegenerative effects, deregulated enzymatic and tissue function, compromised immune function, disrupted cell signaling, and increased oxidative stress. These effects may threaten the foraging efficiency, migratory behavior, and overall health condition of sharks.

Figure 1: A blacktip shark *(Albert kok, CC BY-SA 4.0 <https://creativecommons.org/licenses/by-sa/4.0>, via Wikimedia Commons)*

Figure 2: Shark blood sampling in Biscayne Bay.

## Methods

Over four months, sharks were caught-and-released in Biscayne Bay using a system of drumlines. For 16 blacktips, blood was drawn to collect plasma and create peripheral blood smears. Biometric measurements were taken to evaluate body size and body condition.

For each blood smear, a 100-cell differential leukocyte count (1000x) and 5 high-powered-field total leukocyte counts (400x) were performed.

Counts were used to calculate the granulocyte-to-lymphocyte ratio and leukocyte total for each shark. Plasma samples were submitted for serum protein electrophoresis (EPH), an elasmobranch blood chemistry panel, and metal concentration evaluation using Inductively Coupled Plasma-Mass-Spectrometry (ICP-MS).

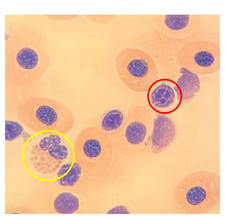

Figure 3: Peripheral blood smear under light microscopy (1000x). A lymphocyte is circled in red, and a granulocyte is circled in yellow.

## Results

### Regional Overview

Miami-Dade County has distinct stratification in land use with urbanization in the north and agriculture in the south. This presents a unique distribution of pollutants and elevated contaminant concentrations in the neighboring bay due to high anthropogenic activity on the coast. Spatial analysis of historic metal levels in sediment reveals consistent hotspots concentrated in areas of drainage, wastewater effluent, and industry (see Figure 4). Sharks residing in these regions are most vulnerable to metal exposure and the resulting physiological effects.

### Physiological Analyses

Table 1: Biometric data for each sampled shark. GLR is the granulocyte to lymphocyte ratio, and body condition (C) was evaluated via C = ∑(LS + FS+ PS + CKC)/(PCL) where LS is the lateral span, FS is the frontal span, PS is the proximal span, CKC is the caudal-keel circumference, and PCL is the precaudal length.

| Tag | Sex | Total Length | Body Condition | GLR |
|---|---|---|---|---|
| N409185 | F | 184 | 1.191 | 0.635 |
| N409183 | F | 161 | 1.235 | 0.318 |
| N409200 | F | 170 | 1.138 | 0.379 |
| N398511 | F | 162 | 1.402 | 0.378 |
| N398480 | M | 156 | 1.037 | 0.526 |
| N398476 | M | 129 | 1.268 | 0.425 |
| N398485 | M | 148 | 1.361 | 0.192 |
| N398479 | M | 155 | 1.377 | 0.424 |
| N409158 | F | 156 | 1.328 | 0.449 |
| N409157 | F | 137 | 1.163 | 0.341 |
| N409187 | F | 145.5 | 1.286 | 0.444 |
| N409156 | M | 173 | 1.211 | 0.594 |
| N409173 | M | 140 | 1.431 | 0.256 |
| N409208 | M | 154 | 1.351 | 0.319 |
| N409189 | M | 156 | 1.289 | 0.254 |
| N395393 | F | 163 | 1.447 | 0.301 |

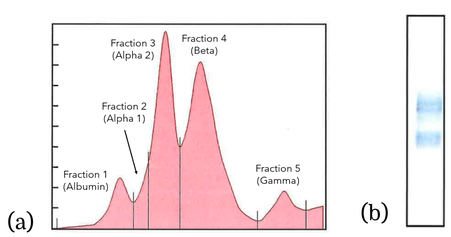


Figure 5: a. EPH diagram with protein fractions labelled and peaks showing concentrations in plasma in g/dL. The ordered fractions with corresponding means are: 1) albumin (0.36 ± 0.01), 2) alpha 1 (0.17 ± 0.01), 3) alpha 2 (1.20 ± 0.04), 4) beta (1.78 ± 0.05), 5) gamma (0.32 ± 0.02). b. Gel electrophoresis results with Fraction 3 and 4 bands most visible.

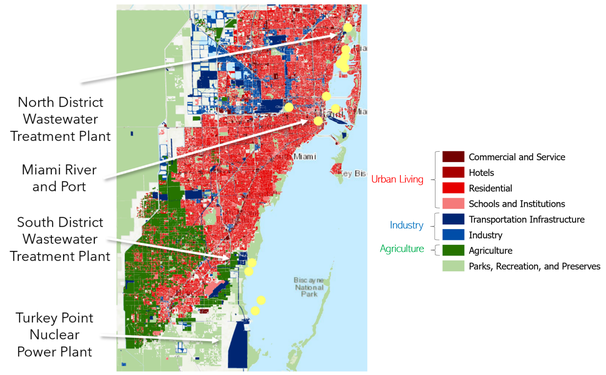


Figure 4: Land use in Miami-Dade county with yellow points indicating areas with arsenic levels in the top 50% of the total value range measured in Biscayne Bay, aiming to highlight the hotspots of metal pollution in the Bay. *(Data sources: Miami-Dade Open Data Hub, NCCOS National Status and Trends)*

Table 2: Metal levels in µg/L. Cadmium and Nickel were also measured, but values had little to no detection or were not reproducible.

| Metal | Mean | Std Dev | Std Error | Minimum | Maximum |
|---|---|---|---|---|---|
| Fe | 846.05 | 351.81 | 87.95 | 464.71 | 1849.81 |
| Cu | 496.89 | 47.60 | 11.90 | 432.14 | 593.35 |
| Zn | 979.11 | 155.47 | 38.87 | 796.65 | 1298.17 |
| As | 2024.36 | 850.05 | 212.51 | 915.43 | 3881.69 |
| Se | 1276.93 | 485.59 | 121.40 | 243.95 | 2169.43 |
| Pb | 0.91 | 0.58 | 0.15 | 0.00 | 2.14 |

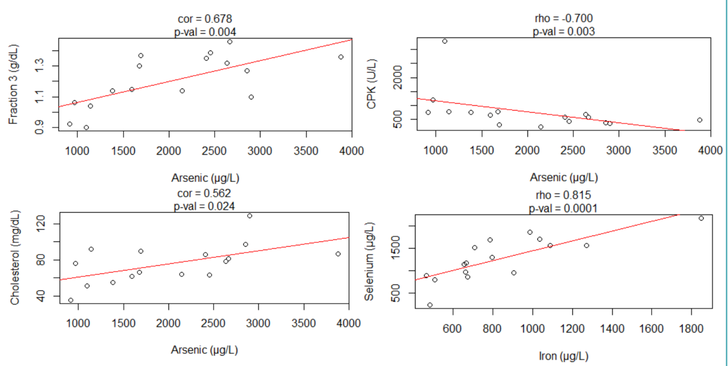


Figure 6: Significant correlations found between metal levels and other parameters measured. Values below the quantitation limit were replaced with the LOQ/√2. A Pearson correlation test was used for normally distributed parameters, and a Spearman test was used for all others.

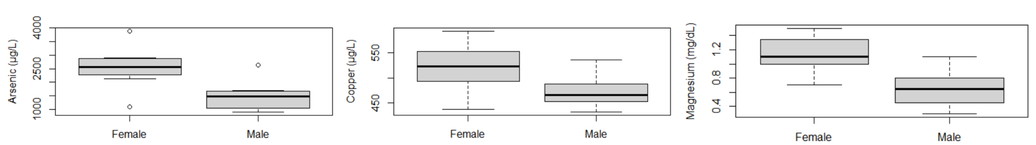


Figure 7: Significant differences found between females and males for levels of arsenic, copper, and magnesium. Evaluations were made via a simple t-test after checking for normality.

## Discussion

- No anomalies were observed in biometric data for sampled sharks
- The GLR had a narrow range of 0.192 – 0.635
- Relationships between arsenic and both cholesterol and fraction 3 (includes proteins for lipid transport) concentration, suggest impacts to lipid metabolism
  - Arsenic-induced dyslipidemia has been observed in other animals
- High arsenic levels were associated with reduced creatine phosphokinase (CPK) in the blood – may be indicative of disruption to muscle metabolism
- Mean arsenic levels were over 140% higher than values reported in great white shark plasma in similar study
- Mean copper levels were about 25% higher than reported in great white shark plasma
- Out of all metals, only iron and selenium were correlated with each other, suggesting similar sourcing
- Higher heavy metal concentrations in females may influence reproductive status, and therefore, population sustainability
- Higher magnesium levels in females may provide some protection from heavy metal toxicity through chelation
- There was no significant correlation between metal levels and body condition or GLR
- Copper was the only metal correlated with size

## Significance

Here we used noninvasive techniques to assess the physiological effects of environmental toxicants. This study presents previously unknown blood metal levels for this species and reveals relationships between these levels and biometric/physiological parameters which typically have only been evaluated in other tissues. Similar techniques may be applied to future projects evaluating the impacts of other pollutants. The analyses utilized are useful for assessing the health of local sharks, determining the magnitude of impact of coastal anthropogenic activity, and informing conservation decisions.

## Acknowledgements

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