

A metabolomic approach to health assessment in the whale shark, *Rhincodon typus*

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Background. No studies have evaluated the biochemistry of whale sharks. The illness of two animals in the collection at Georgia Aquarium provided an opportunity to evaluate the blood chemistry of healthy and unhealthy animals in a search for biomarkers of health in this species.

Methods. In the absence of existing data on whale shark biochemistry, we used a discovery based approach called metabolomics, wherein the compounds (and sometimes their concentrations) in a fluid sample can be determined using proton NMR spectroscopy and mass spectrometry. Samples from both healthy and unhealthy whale sharks were compared by both methods. NMR data were analysed by principal components analysis whereas the MS data were evaluated for presence/absence frequency for each analyte

Results. PCA of proton NMR data could clearly discriminate between samples from healthy and unhealthy whale sharks. Analysis of PC loadings identified a shift region that was driving this discrimination and subsequent experiments determined that this region consisted of homarine, or n-methyl picolinic acid. Mass spectrometric data revealed 26 compounds that varied meaningfully, implying that they may be biomarkers of health in this species. A set of keto-acids was more frequently encountered in unhealthy animals, which was consistent with their primary clinical presentation (inappetence)

Conclusion. Metabolomic approaches proved a powerful way to explore biochemistry of whale sharks, discriminating healthy from unhealthy and offering a selection of potential biomarker compounds for use in future studies of this species. The obvious next step is to attempt to obtain samples from field populations so that this approach can be used for conservation benefit, but this is a significant logistic challenge

Key words: metabolomics, biomarkers, homarine, *Rhincodon*, whale sharks, aquarium, biochemistry