BIOMARKER DEVELOPMENT FOR MANATEE HEALTH ASSESSMENTS

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FLORIDA'S MANATEE

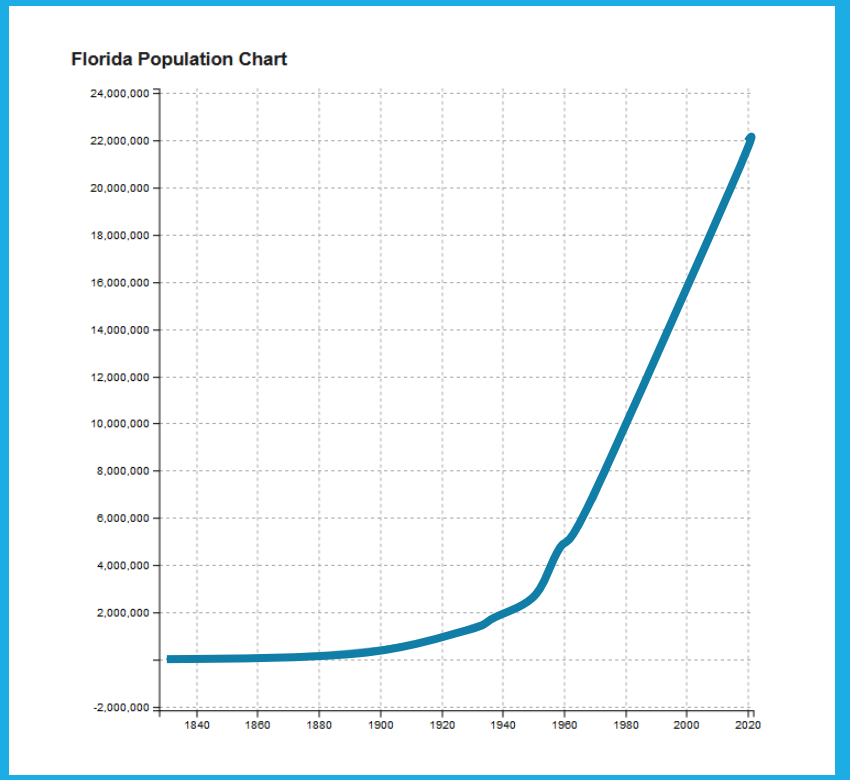
Population has recovered due to conservation efforts

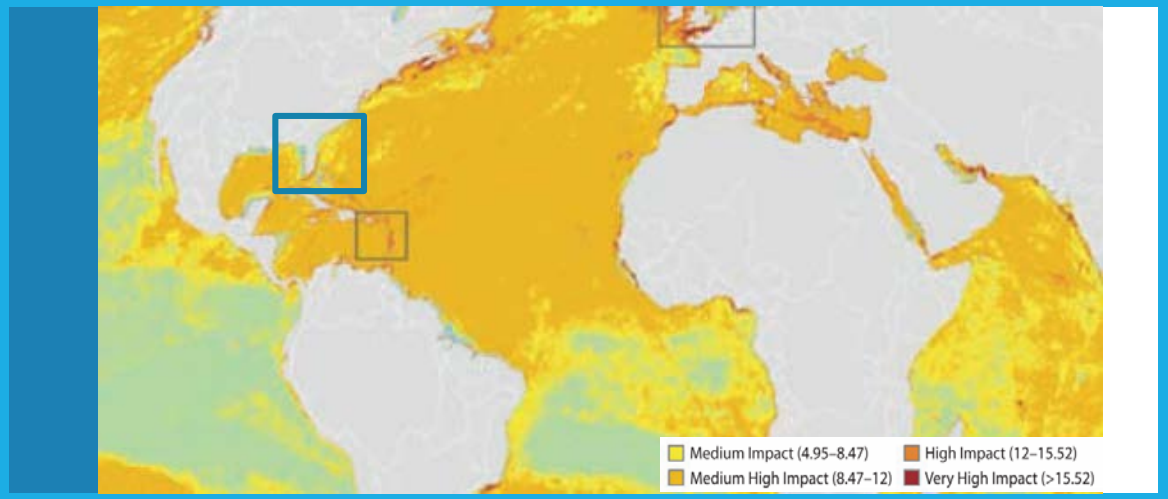
Human population in Florida

→ increasing threats to the environment and to the population

→ aquatic environment considered the ultimate sink for anthropogenic chemicals

→ **New threats to the population**





http://worldpopulationreview.com/states/florida-population/

Halpern et al. 2007

MANATEES VULNERABILITY

O'Shea and Tanabe (2002) recorded a 1250-fold increase in the number of organic pollutants and 50 elements detected in marine mammals from 1960 until 2000.

Manatees life-traits: vulnerable

Chemicals can affect :

- hormone balance
- oxidative damage
- genotoxicity
-alter specific enzyme or even can produce epigenetic modifications

→ chronic disease and new biomarkers in the context of manatees' modern health assessments.

POSSIBLE BIOMARKERS

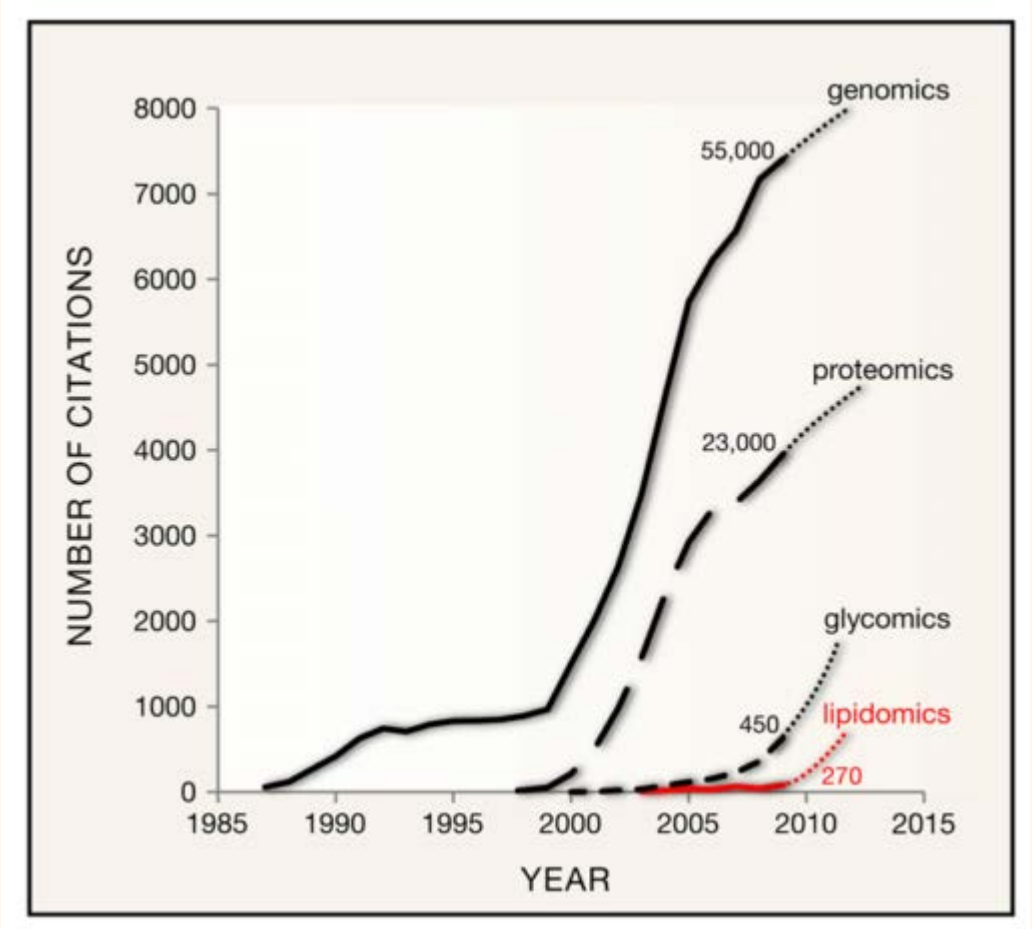
Objective, quantifiable characteristics of biological processes

the biological effect of chemical compounds and indicates a normal or diseased state

Genomics: study of genes and their functions

Proteomics: structure, function, and interactions

Lipidomic: different profile, pathways and networks of cellular lipids



Wenk et al. 2010

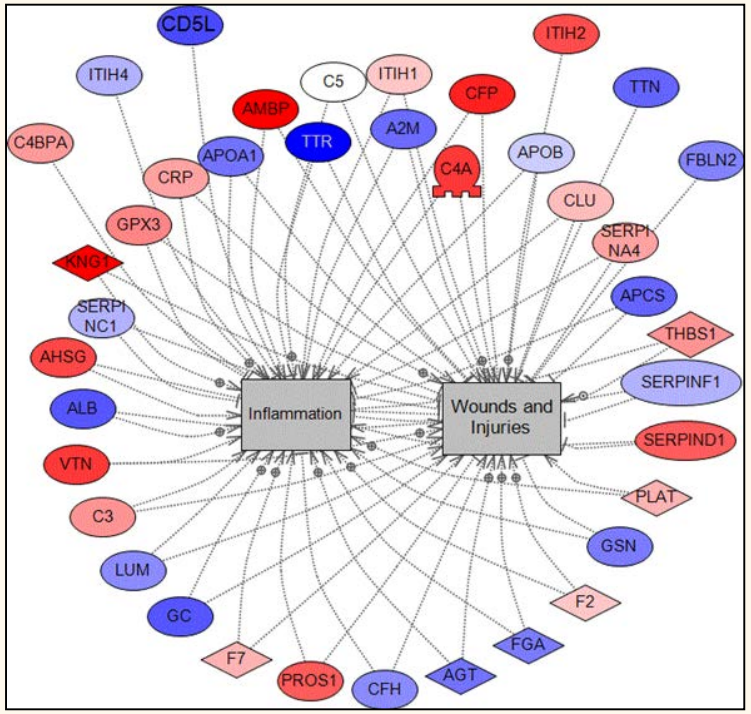
PROTEOMICS IN MANATEES

Lazensky et al. in preparation

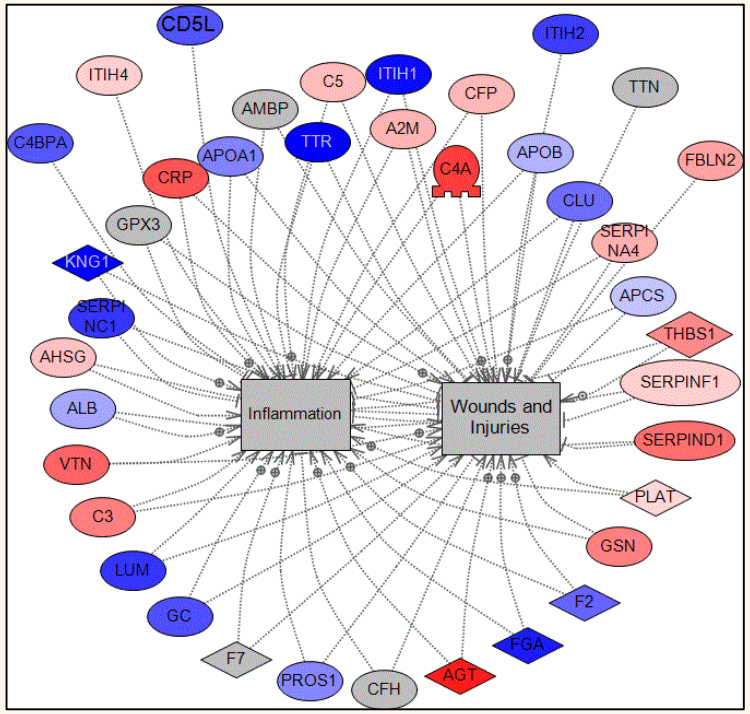
Plasma samples were collected from 12 manatees from three separate groups

- Lowry – Red tide group
- Brevard – Unknown mortality group
- Crystal River – Controls

→biomarkers useful to diagnose, distinguish from other illnesses



Unknown Mortality



Red tide

HERBICIDE: GLYPHOSATE

Main ingredient: Round up/Rodeo®

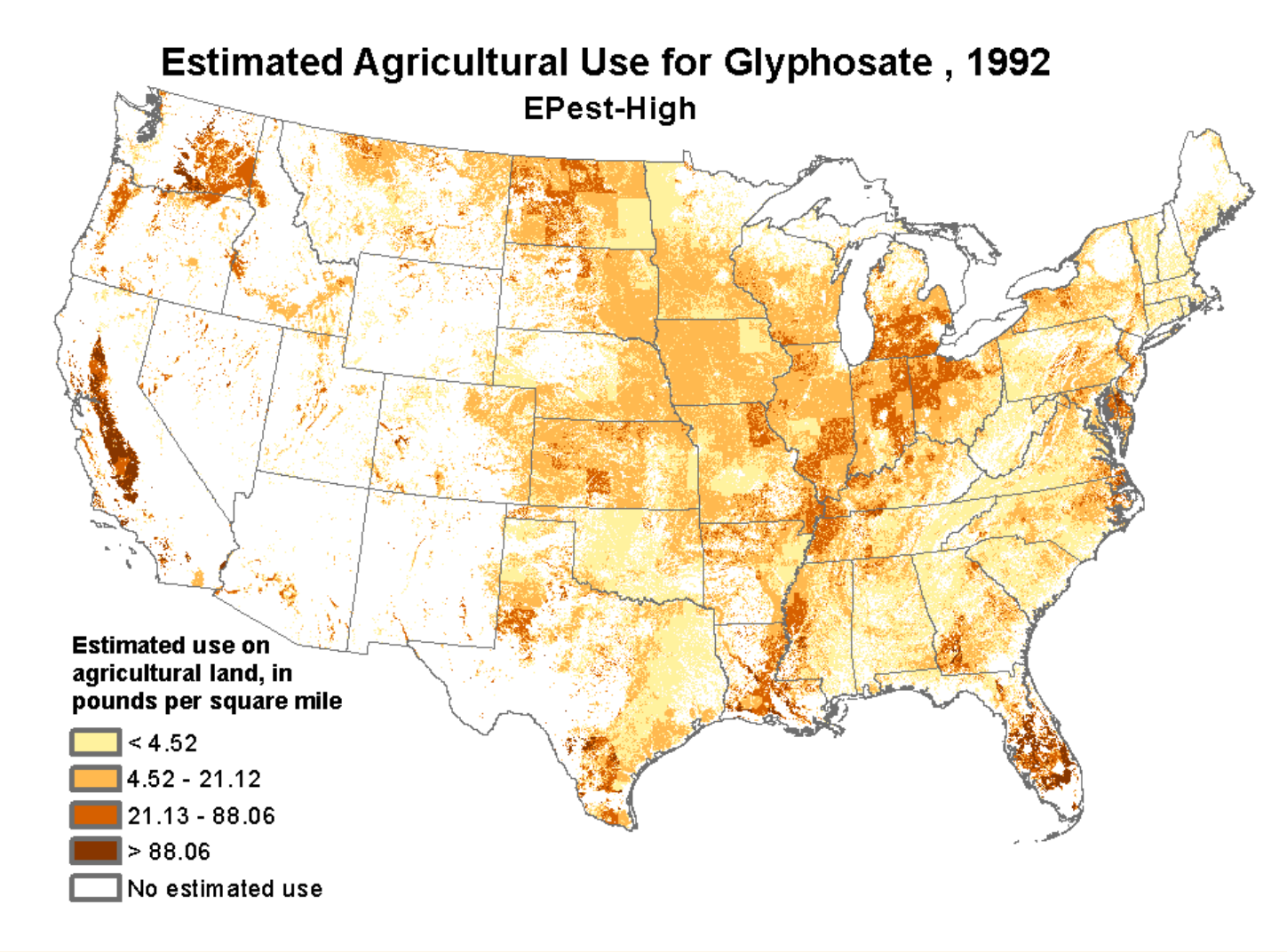
Florida: sugar cane ripener

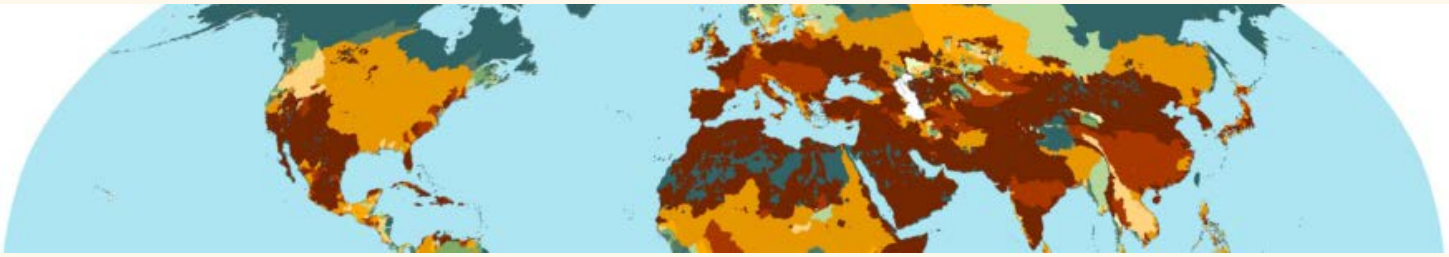
Extended use + run off + direct application = exposure of aquatic animals

Half-life fresh water: 60-70 days

Phosphorus in water: less degradation

Manatees: summer bouts for fresh water and warm water refugees





USGS 2018
Mekkonen & Hoekstra 2018

CONSEQUENCES OF THE EXPOSURE

Epidemiological studies: idiopathic chronic kidney disease

Rats: ultra-low concentration bellow allowed limits produce kidney and liver disease. Even endocrine disruption (decrease in testosterone).

Fish: liver and kidney toxicity, tissue damage. Reproductive impairment

Toxicity mechanism: reactive oxygen species

→ **oxidative damage**

Chaufan et al. 2014

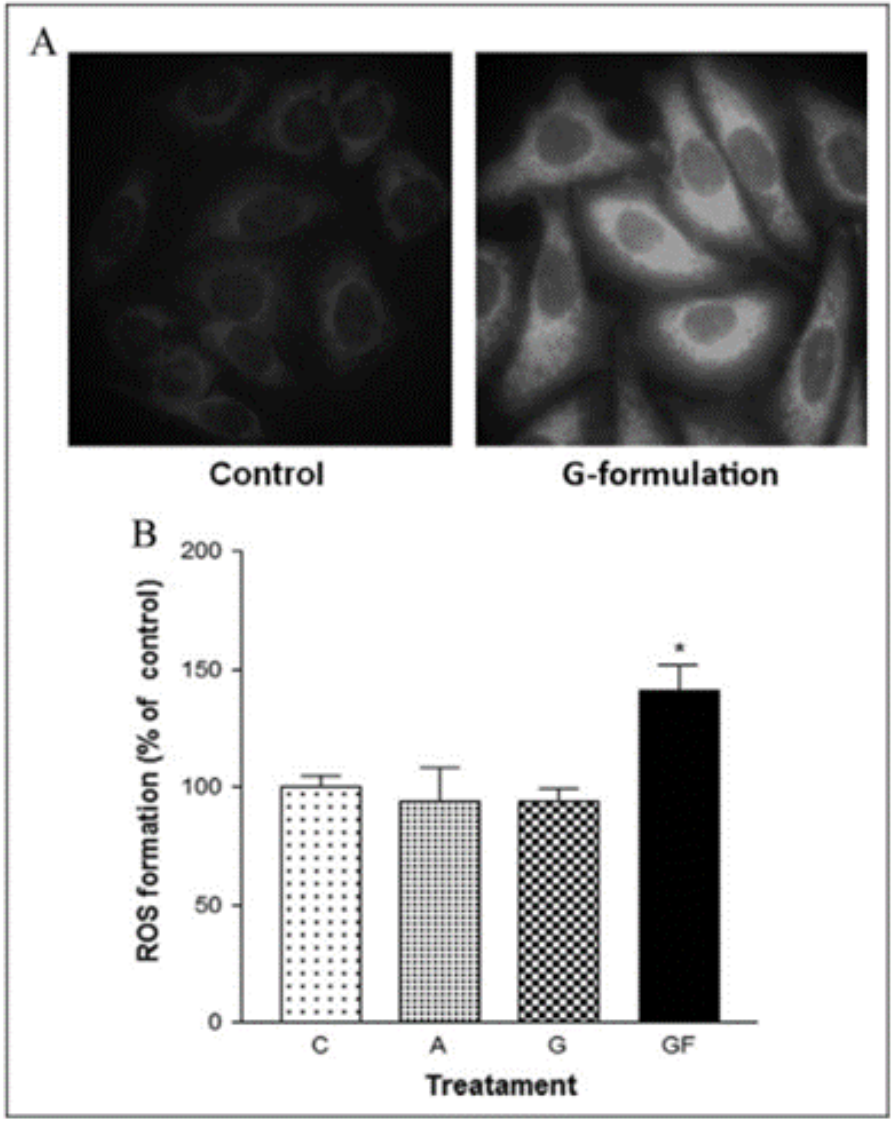
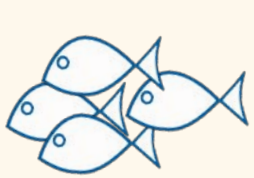
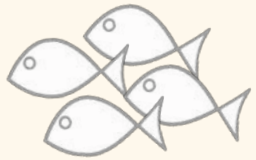


Figure 2. Reactive oxygen species formation by glyphosate (G), AMPA (A), or G formulation (GF) in HepG2 cells. Cultures were exposed to 900 mg/L of glyphosate, 900 mg/L of AMPA, or 40 mg/L of G formulation for 24 hours. A, Cytoplasmic ROS analysis. The ROS production was visualized by fluorescence microscopy using filters B-2A (λexc: 450-490 nm; λem: 515 nm). Control and G formulation treatment: 100×. B, ROS production is presented as percentage

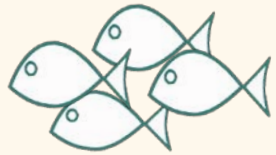
ASSESS GLYPHOSATE/RODEO® KIDNEY INJURY TO LARGEMOUTH BASS: DETECTION OF POSSIBLE BIOMARKERS



CONTROL

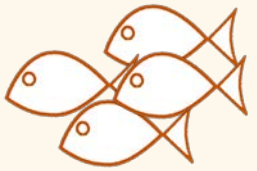


GLYPHOSATE 0.5 mg/L

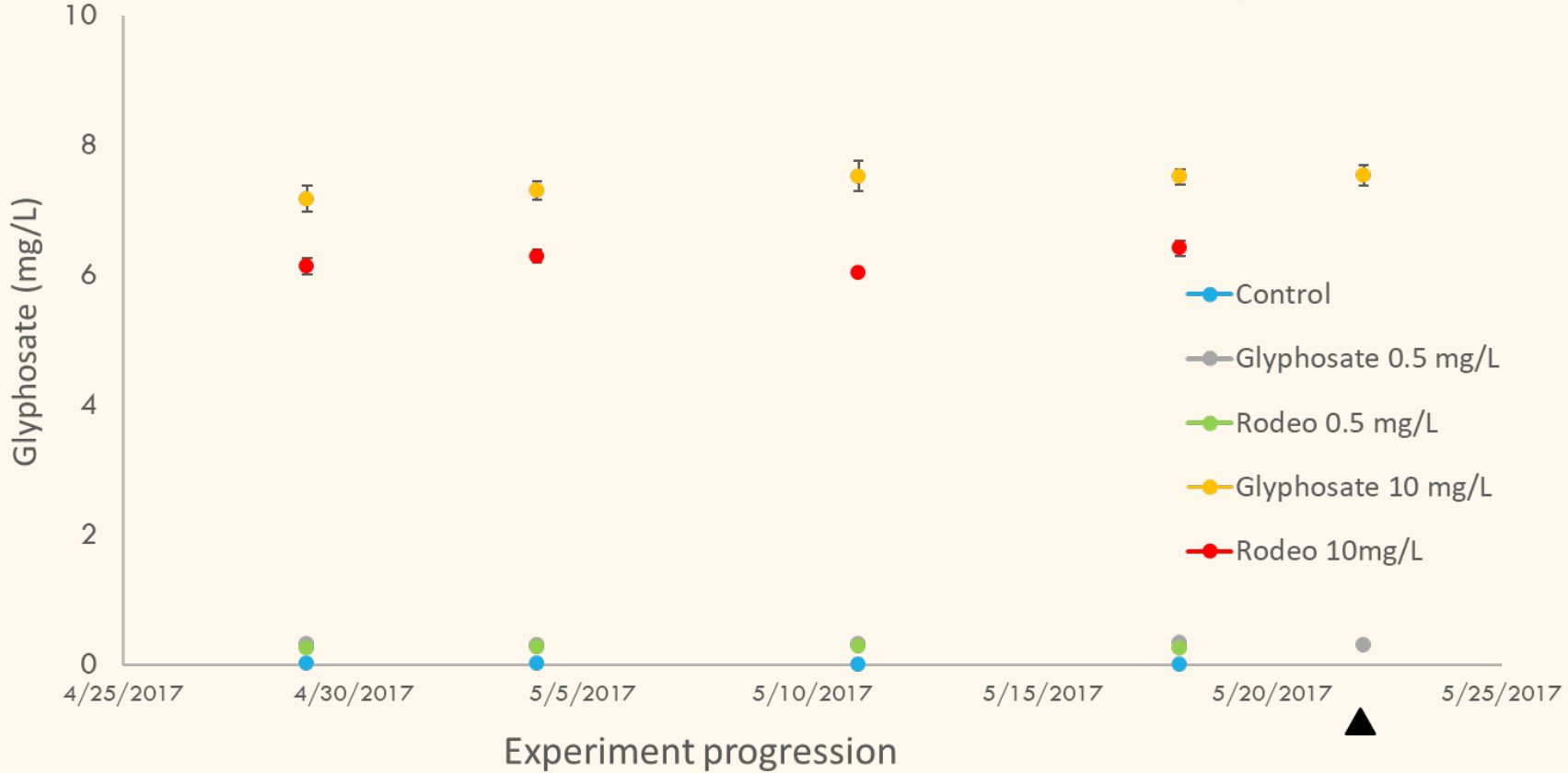


RODEO 0.5 mg/L

GLYPHOSATE 10 mg/L

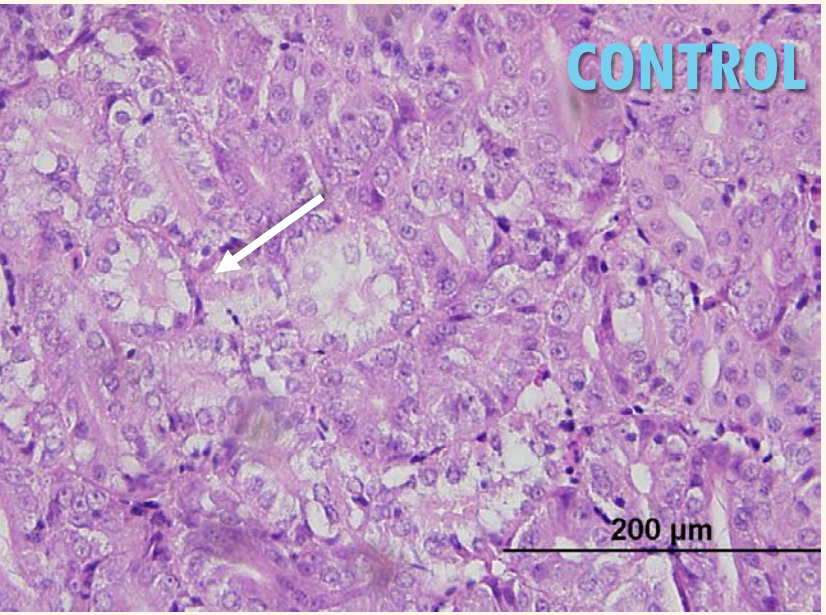


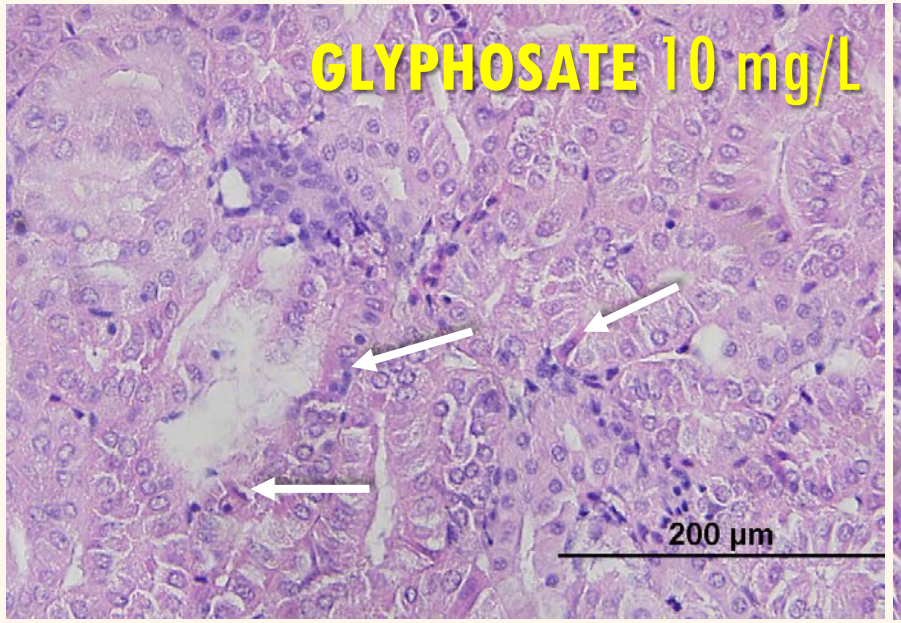
RODEO 10 mg/L

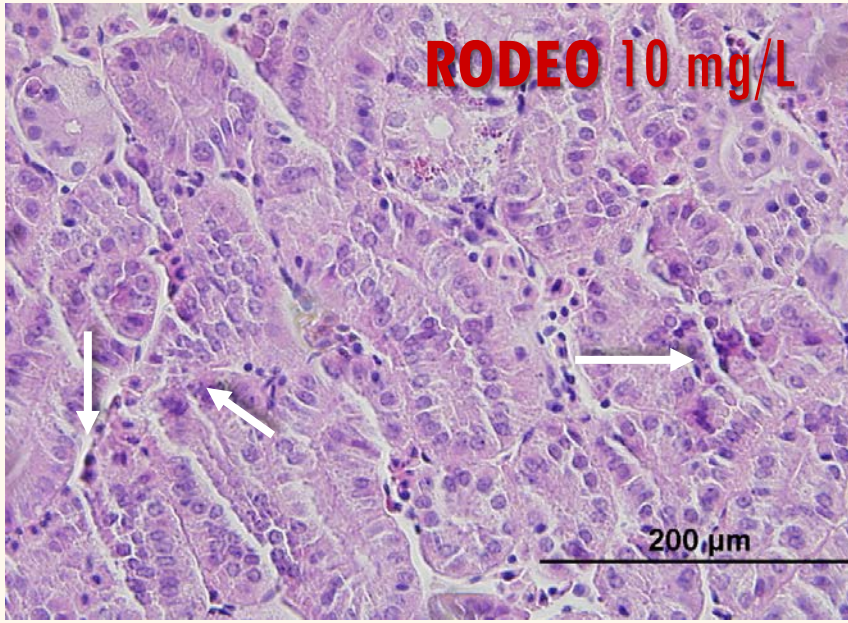


PRELIMINARY RESULTS

Tissue damage is minor: some necrotic/degenerative tissue in the highest doses



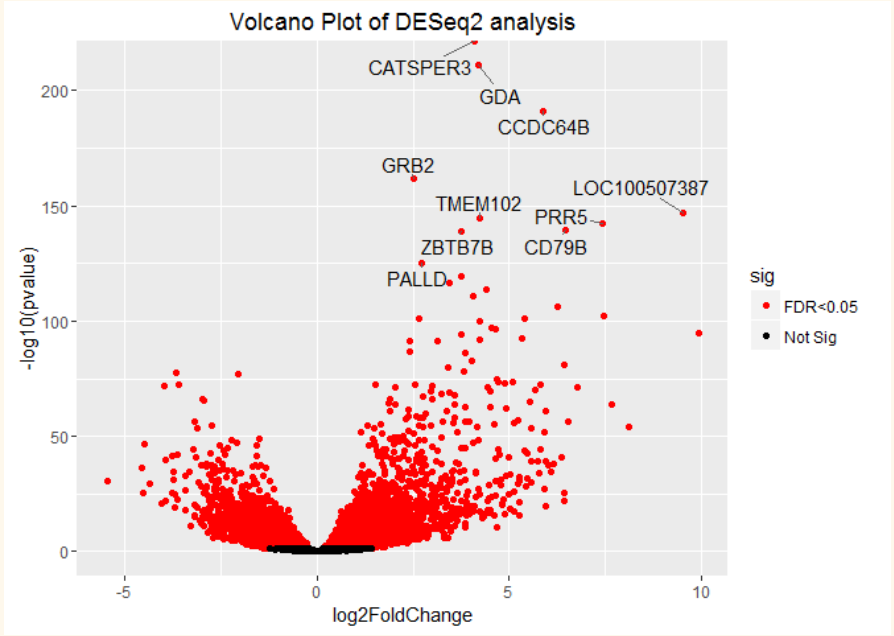


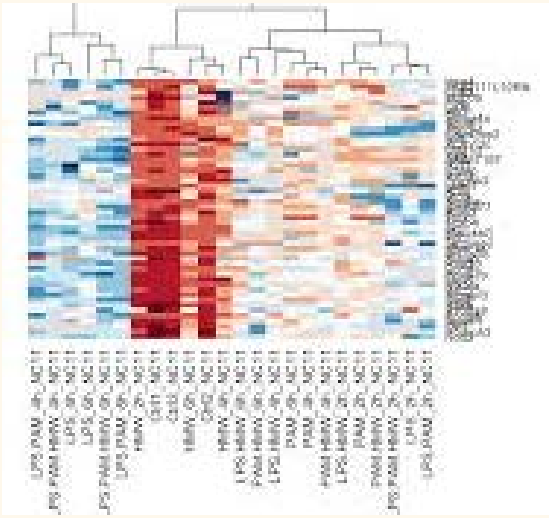


DIFFERENTIAL GEN EXPRESSION

RNA-seq:

→ Differential gene expression





KIM-1, NGAL, beta-2 microglobulin

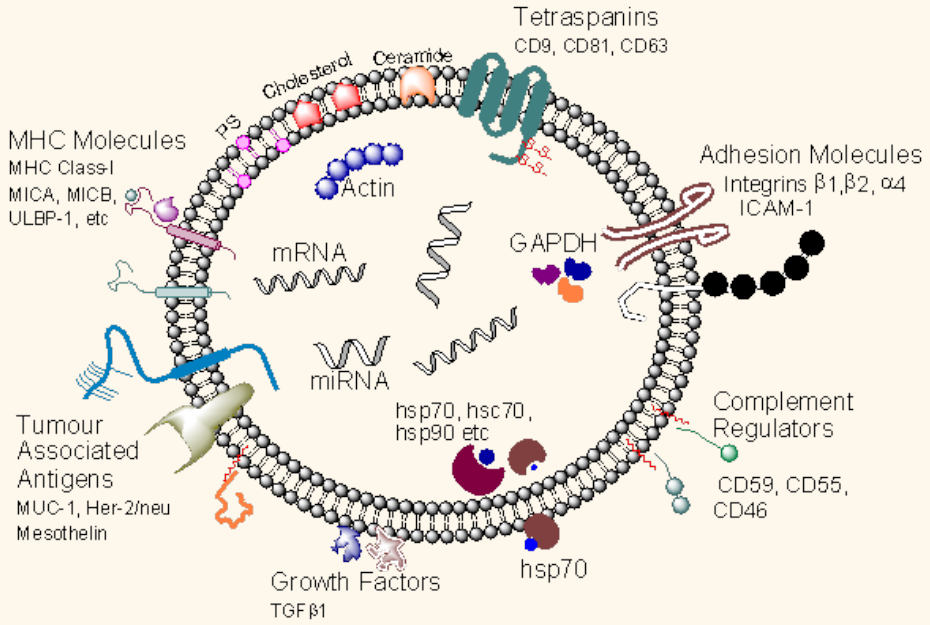
→ Detect possible biomarkers of kidney dysfunction related to glyphosate and rodeo exposure

EXOSOMES

40-100 nm in diameter

Newly identified method of communication between cells

Membrane vesicles, bilayer spherical structures which contain RNA such as mRNA, microRNA they are enriched in lipids and proteins.

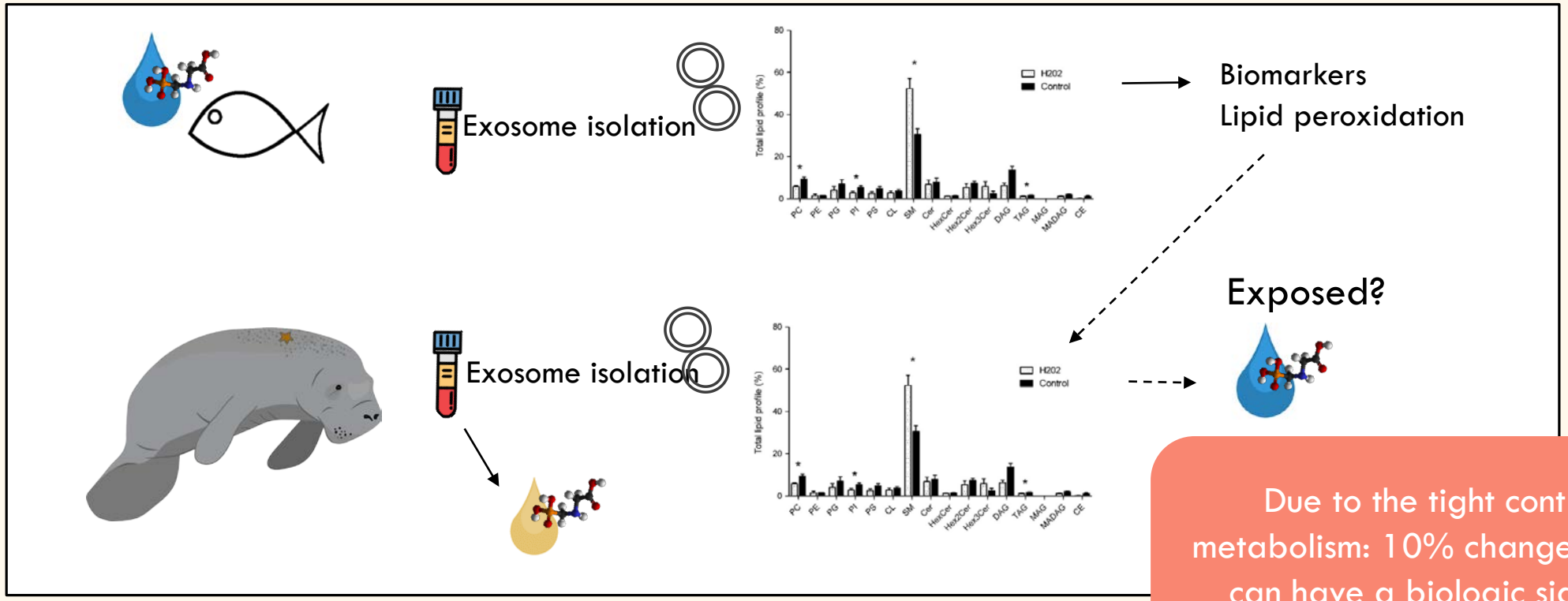


LIPIDS AS BIOMARKERS

Non-lethal sample: isolation from plasma!

Lipids can be a new system-level of analysis: influenced by the environment

Profiling exosome lipids is now possible: sensible analytical technics that detect lipids



Due to the tight control of lipid metabolism: 10% change in lipid profile can have a biologic significance.

→ **Early diagnostic tool?**

EXPECTED RESULTS

Identify genes and lipids as biomarkers for chronic kidney damage and lipid peroxidation as a result of glyphosate exposure

- Apoptosis
- Tissue damage
- Lipid metabolism
- Immune response

→ Measure in other species through non-lethal samples
→ Exosomes

Isolate exosomes: Crystal River – Health assessments
Possibly exposed manatees South of Florida

→ Higher concentration Glyphosate/AMPA in blood
More lipid peroxidation in exposed manatees

Developing:
Non-lethal samples to assess manatee health in relation to exposure to glyphosate, measure kidney damage lipid peroxidation or even alternations in the immune system

Florida manatee, the possible consequences in the immune system should be addressed due to other environmental challenges this population has to cope with like cold stress and red-tide

The development of these techniques can help understand the manatees' vulnerability.

Analyze the metabolome of the animal under different conditions, and define **healthy** through from a wider range of possibilities

THANK YOU

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