

# Organotypic cultures open new avenues of research in reptilian toxicology





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#### INTRODUCTION

- ♦ Sea turtles are listed as endangered by IUCN and ESA.
- ◆ Sea turtles play an important role in marine ecosystems; maintenance of coastal habitat, food webs, and nutrient cycling².
- ♦ Environmental contaminants have been implicated as a contributor to population declines<sup>1</sup>.
- ◆ Federal protection prohibits *in vivo* studies; critical need to develop non-lethal methods to expand current knowledge on chemical stressors in sea turtles.
- ◆ Fetal bovine serum (FBS) is commonly used in culture medium, however specific properties could interfere with toxicological applications<sup>3,4</sup>.
- ♦ Tissue viability tends to decline with increasing time in culture<sup>5</sup>.

### **OBJECTIVES**

- 1) Determine whether sea turtle skin organotypic cultures remain viable up to 96h in the laboratory.
- 2) Assess the effect of fetal bovine serum (FBS) and culture time on viability.
- 3) Utilize three end points to assess viability and determine which method is the best indicator of viability.



Left: A captive loggerhead sea turtle at the NOAA sea turtle facility.Below: Biopsy collection at the NOAA sea turtle facility.

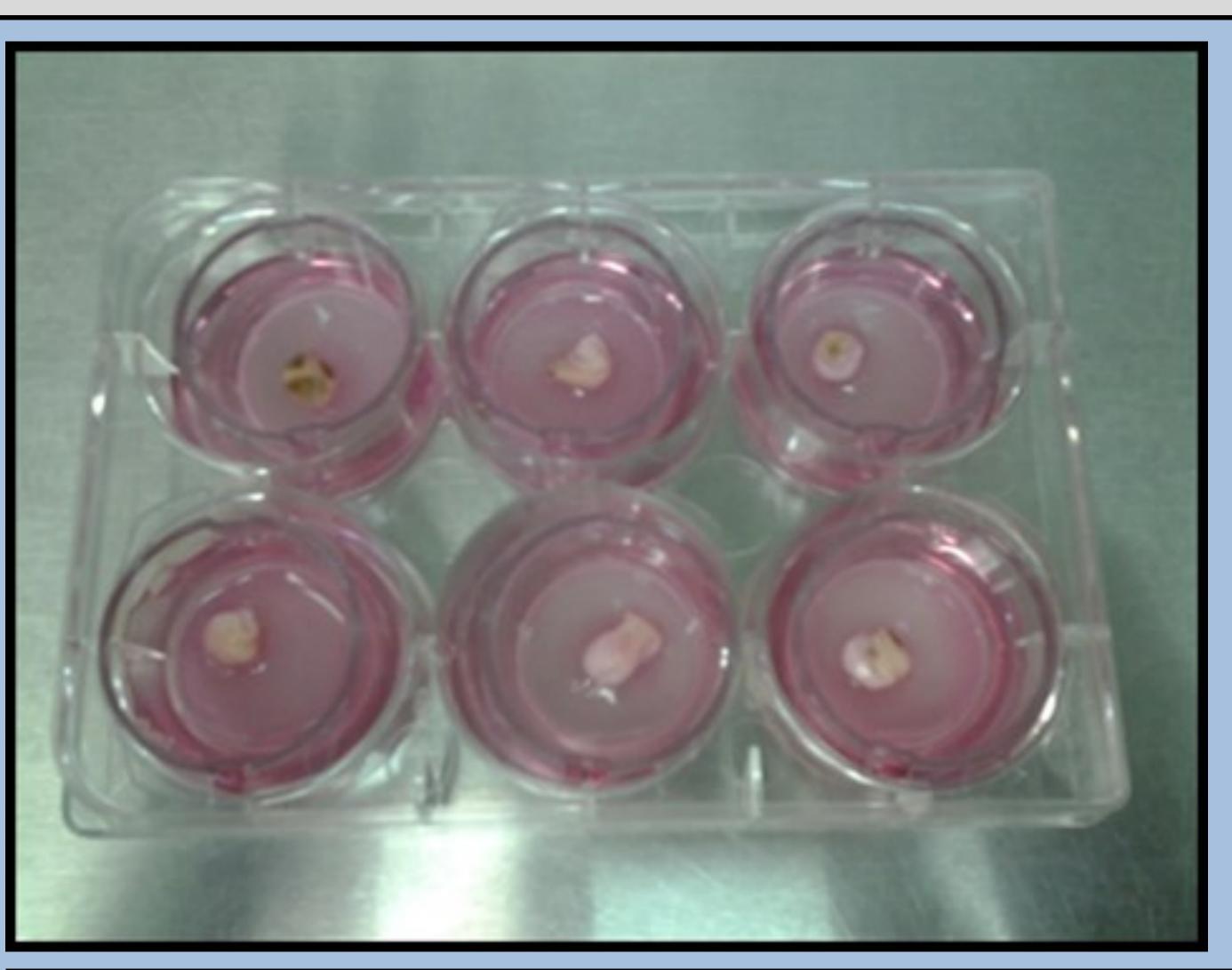


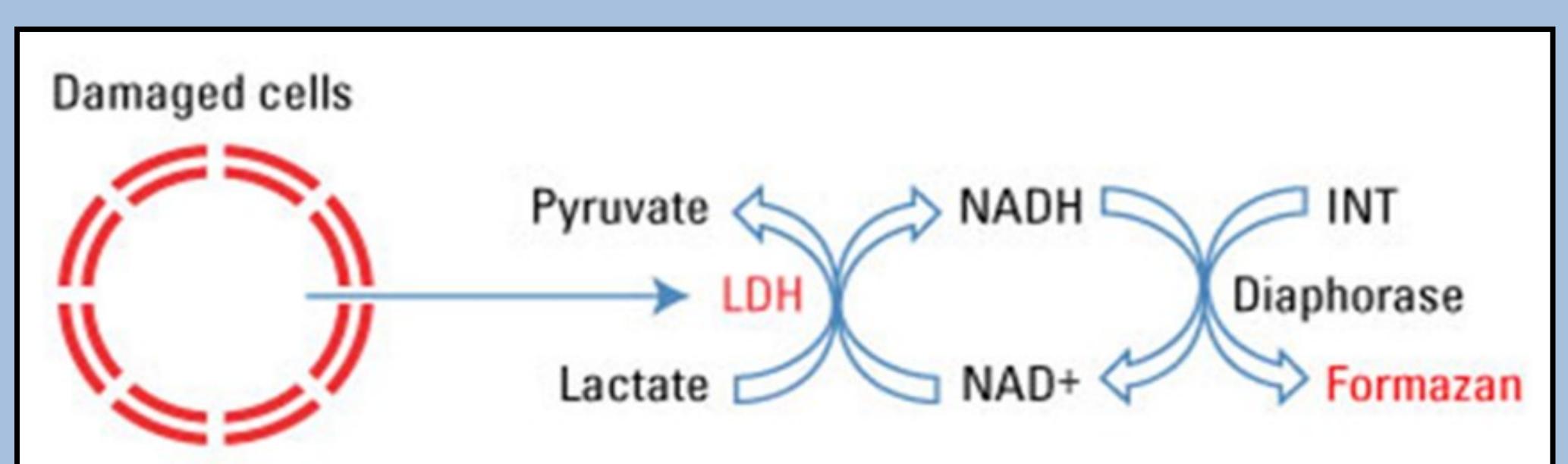
# **PERMITS**

- ♦ U.S. Fish and Wildlife Service Endangered Species Act Section 10a(1)a Scientific Research Permit# TE-676379-5
- ♦ Florida Fish and Wildlife Conservation Commission Permit# FWC MTP-01. MTP #015

#### **METHODS**

- I) Sample collection: Double punch biopsies (8mm) from ten captive raised loggerhead sea turtles were collected USDOC/NOAA/NMFS in Galveston TX.
- 2) **Organotypic culture:** Skin explants were cultured for 24, 48, 72, or 96h in serum free (SF) media or media containing 10% fetal bovine serum (FBS).
- 3) **Cell culture:** Fibroblast cell cultures were created via explantation from all of the organotypic tissues upon completion of the culture time points.
- 4) Internal K+ analysis: Tissue subsamples were digested in 70% nitric acid and 30% hydrogen peroxide; K+ in the solution was quantified by flame atomic absorption spectroscopy. Results were analyzed with a three-stage nested ANOVA.
- 5) Lactate Dehydrogenase (LDH) assays: Media was collected from shipping tubes and twice daily from culture plates until completion of the culture time points. LDH activity in media was quantified by measuring absorbance of formazan with a plate reader.





Left: Photo of sea turtle skin explants on tissue culture membranes in a 6 well culture plate.

**Above:** Diagram of the production of formazan catalyzed by LDH released from damaged cells. From http://www.piercenet.com/media/88953-001-LDH-Cytotox.jpg

#### RESULTS

- 1) **Cell culture:** All skin explants exhibited fibroblast growth, indicating viable tissue after all organotypic culture time points and culture in both media types.
- 2) Internal K+: Tissue subsamples had a range of internal K+ from 90-893 ug/g wet weight. The ANOVA indicated no significant effect of individual animal, culture time, or media content on internal K+.
- 3) **LDH in media:** LDH in SF media was below background levels for all time points indicating no loss of membrane integrity. LDH in media with FBS was highly variable, demonstrating incompatibility of this assay with media containing FBS.

Results	Serum free	10% FBS
LDH	No activity detected	Activity highly variable
% CV	5.49 ± 2.10	13.18 ± 9.27

Top right: Fibroblast cell cultures grown from skin explants

Left: LDH activity in organotypic culture media with and without FBS.

# CC-13-1787 FBS, 24h CC-13-1787 SF, 24h CC-13-1788 FBS, 24h CC-13-1788 SF, 24h CC-13-1789 FBS, 48h CC-13-1789 SF, 48h CC-13-1790 FBS, 48h CC-13-1790 SF, 48h CC-13-1791 FBS, 72h CC-13-1792 FBS, 72h CC-13-1792 SF, 72h CC-13-1791 SF, 72h CC-13-1793 FBS, 96h CC-13-1793 SF, 96h CC-13-1794 FBS, 96h CC-13-1794 SF, 96h

#### CONCLUSIONS

- 1) Organotypic culture methodology is applicable to sea turtle skin tissue.
- 2) Sea turtle skin explants remain viable in culture for at least 96h in serum free media or media with 10% FBS.
- 3) Cell culture is an excellent indicator of viability, however this method is lengthy and requires many supplies.
- 4) LDH assays are a non-destructive indicator of viability when using serum free media but are incompatible with media with FBS, as expected from the literature.
- 5) Further investigation of internal K+ in sea turtle skin tissue is needed to establish a threshold of viability.
- 6) Investigation of the suitability of skin organotypic cultures to investigate the effects of marine contaminants in sea turtles is currently underway.

## ACKNOWLEDGEMENTS

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- Dr. Chris Colise
- ♦ Dr. Chris Salice

Caitlin Ryan

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