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QIBC Analysis on Killifish Cells Under Anoxic Conditions

Jaina Canlas
Portland State University

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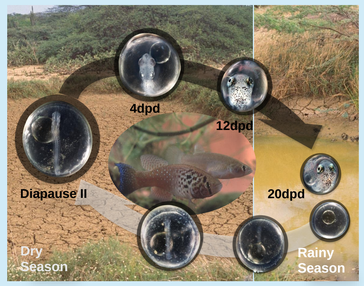
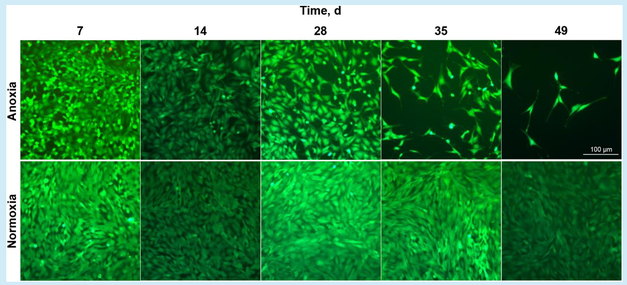
Jaina Canlas, Riley Roth-Carter, and Jason Podrabsky | PSU Biology Department | CLEE

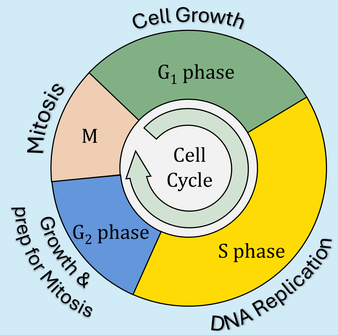
BACKGROUND

- The annual killifish, *Austrofundulus limnaeus*, is native to ephemeral ponds in Venezuela. These embryos have the capability to enter a form of embryonic dormancy called diapause.
- Embryos of *A. limnaeus* are resistant to genotoxic stressors such as anoxia (the absence of oxygen) and irradiation.
- When other vertebrate cells are exposed to anoxic conditions, cell death occurs due to cellular damage and cell cycle disruption.
- All work done in this experiment was conducted on the PSU-AL-WS40NE cell line that shows similar anoxia tolerance to embryos.





Live/dead stain indicates cells are still alive after 7 weeks in anoxia.



RESEARCH QUESTION

Can we determine how cell cycle stages affect the annual killifish's ability to repair DNA damage under anoxic conditions?

HYPOTHESIS

Due to previously proven anoxia tolerance, the annual killifish have enhanced DNA repair capabilities under anoxic conditions.

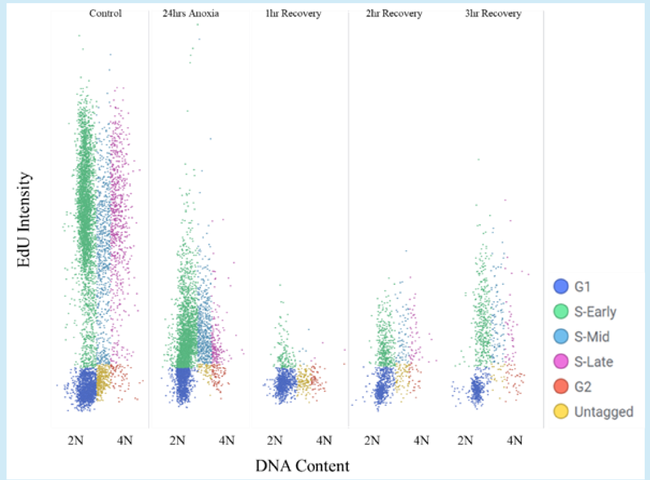
QIBC

- Quantitative image-based cytometry.
- Quantifies a large number of individual cells on culture plates using immunofluorescent images.
- By using an EdU pulse and DAPI fluorescence we can also differentiate cell cycle stages efficiently.

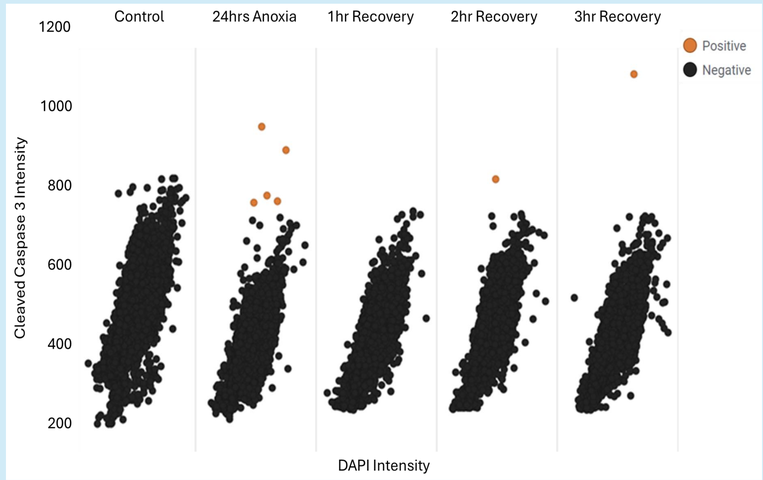
METHODS

- Anoxic conditions conducted in the Bactron EZ Anaerobic Chamber.
- Cells pulsed with EdU and fixed at 24hrs anoxia along with 1, 2, and 3 hours of recovery.
- QIBC immunofluorescent staining for Edu (DNA replication) and cleaved caspase-3 (apoptosis) and DAPI used to stain nuclei of all cells to determine DNA content.

RESULTS



QIBC analysis of the cell cycle in anoxia exposed cells. EdU intensity is compared to DNA content to visualize stages of the cell cycle for each cell.



QIBC analysis of cleaved caspase-3 intensity as a marker for apoptotic cells.

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REFERENCES

Wagner, J. T., Knapp, M. J. & Podrabsky, J. E. Antioxidant capacity and anoxia tolerance in Austrofundulus limnaeus embryos. *Journal of Experimental Biology* **222**, (2019).

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Claire, L., Riggs *et al.* Establishment and characterization of an anoxia-tolerant cell line, PSU-AL-WS40NE, derived from an embryo of the annual killifish *Austrofundulus limnaeus*. *Science Direct.* **232**, (2019). DOI: 10.1016/j.cbpb.2019.02.008

CONCLUSIONS & MOVING FORWARD

- Cells exposed to anoxia continue to replicate their DNA and there are minimal cells in the G_2 phase.
- Previous work has shown that DNA damage occurs during anoxic exposures, yet here we show that this damage does not stop DNA replication or cause an increase in apoptosis.
- There is no relation between the cell cycle stage and apoptosis when cells are exposed to anoxia.
- Still unknown how cells can continue faithful replication through damage. Further investigation is needed including determining a potential relationship between cell cycle and DNA damage and the role of the core proteins ATM and ATR.