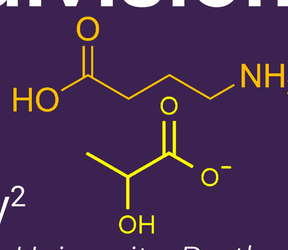
GABA and lactate preconditioning increases cell division in annual killifish cell line during anoxia



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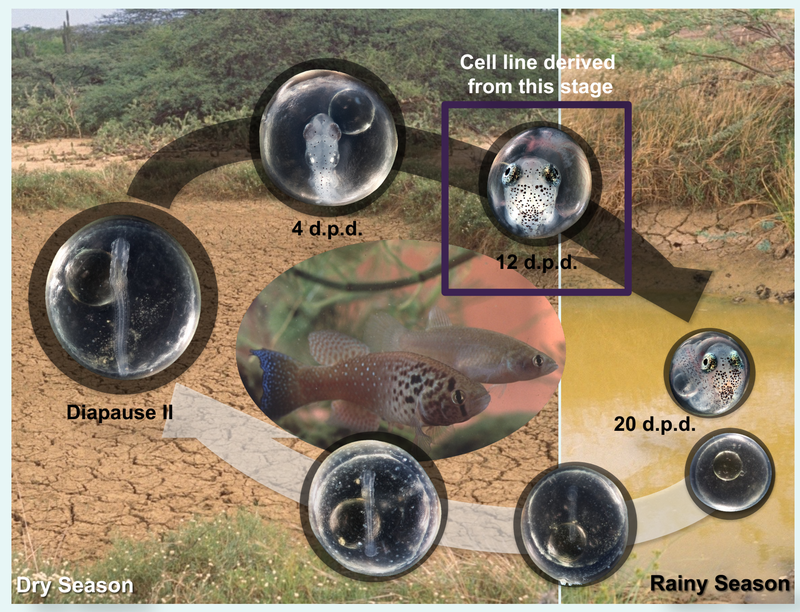
Background:

- Annual killifish (*Austrofundulus limnaeus*) live in arid environments with temporary ponds (Maracaibo Basin, Venezuela)[1]
- Species survives by laying drought- and anoxia-tolerant embryos in substrate
- Embryos can survive many months without oxygen (anoxia)
- Embryos survive by arresting development (via diapause or quiescence)[2]
- When exposed to anoxia, embryos produce lactate and GABA[3]
- GABA is an inhibitory neurotransmitter and has been shown to be critical in surviving long-term anoxia in *A. limnaeus* embryos
- Cultured *A. limnaeus* cells survive 40+ days in anoxia despite not producing GABA[4]
- Brief exposures to anoxia (preconditioning) has been shown to increase anoxia tolerance in certain stages of embryos[1]
- **No research has explored if preconditioning affects anoxia tolerance of these cells**



The life cycle of the annual killifish, *Austrofundulus limnaeus*.

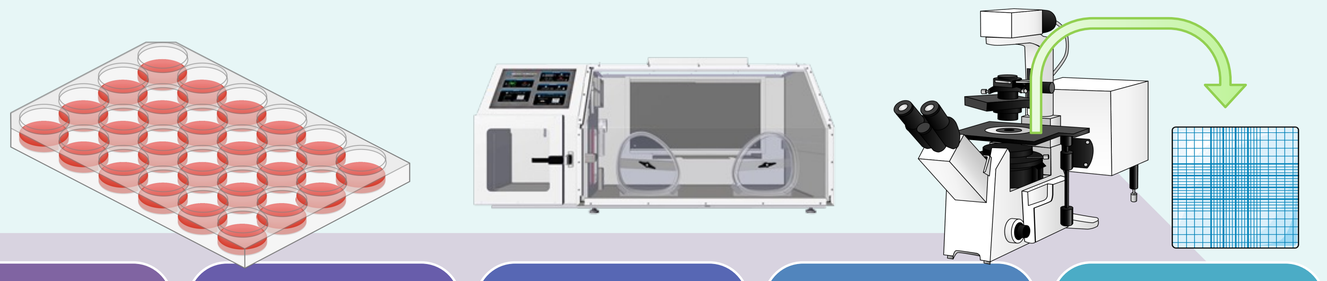
Research Question:

How does preconditioning with anoxia or lactate, or GABA supplementation affect cell survival in anoxia?

Hypothesis: *A. limnaeus* WS40NE cells preconditioned with **anoxia** or **lactate**, or in **GABA** supplemented cell media will result in an **increase** in **anoxia tolerance** compared to non-preconditioned or supplemented cells.

Methods (1): Preconditioning Experiment

1. Precondition/Treat, Recover, Expose to Anoxia, and Monitor Survival

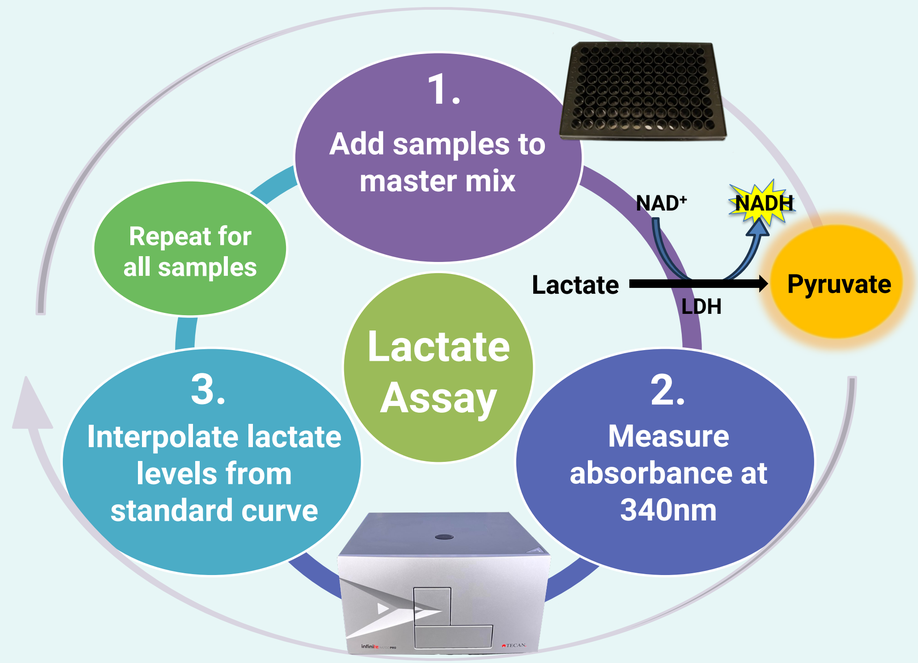


Precondition cells for 24 hours in: - Anoxia - 3 mM Lactate - 3 mM GABA*	Recover cells for 24 hours* in normoxia	Place cells in anoxic chamber (95% N_2, 5% H_2)**	Sample plates every four days. Count cells and verify survival via live/dead stain***	Collect cell media from wells for lactate measurement (Methods 2)

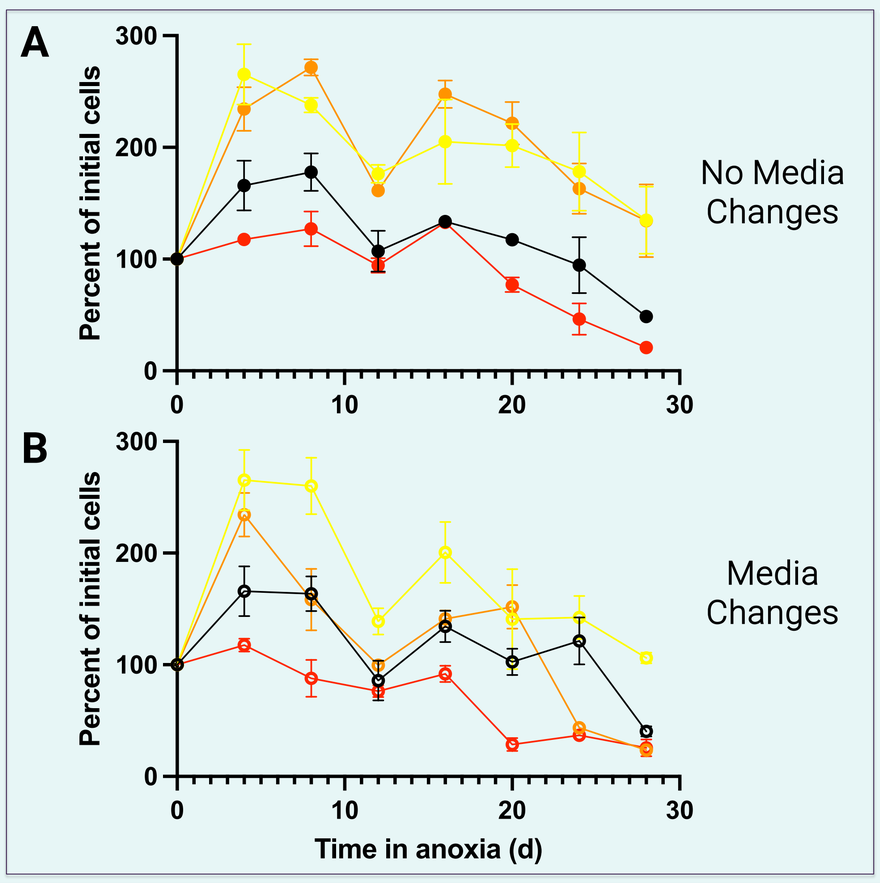
*GABA remained in the media for the duration of the experiment for GABA treatment
**Media was changed every four days or remained static for entire duration (n=4 for both treatments)
***Cells were treated with LIVE/DEAD™ viability/cytotoxicity kit (ThermoFisher) using 1.0 µM calcein AN (stains live cells green) and 2.5 µM ethidium homodimer-1 (stains dead cells red)

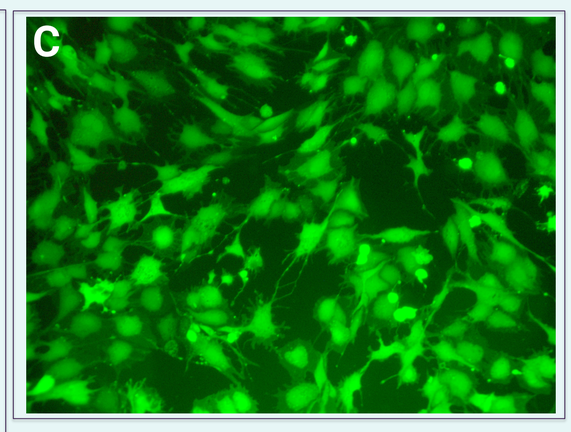
Methods (2): Lactate Assay

2. Measuring lactate production in cell media samples



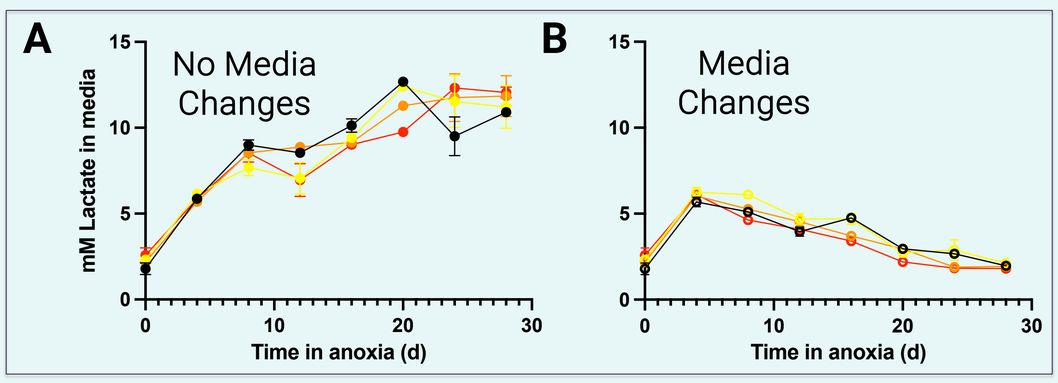
Survival Results (1): ● Control ● GABA (3 mM) ● Lactate (3 mM) ● Anoxia



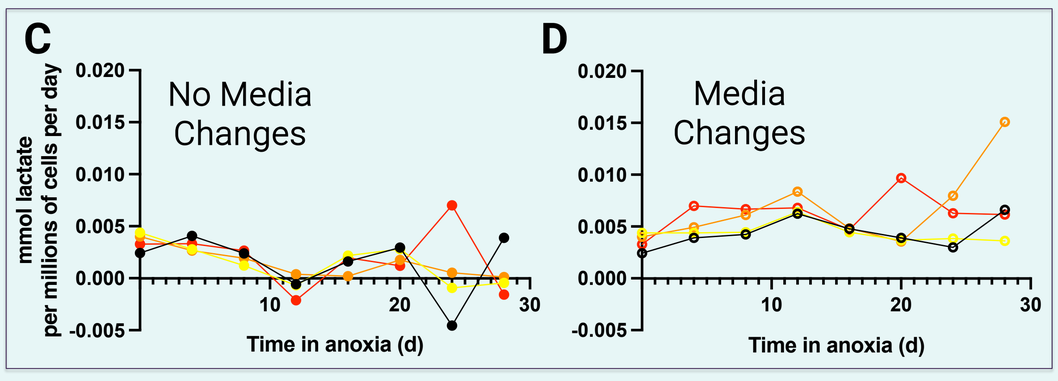


A–B. Survival of *Austrofundulus limnaeus* WS40NE (Wourms' stage 40 neuroepithelial) cells during anoxia at 30°C when exposed to various treatments (anoxia or lactate preconditioning, or GABA supplementation). Control cells were not preconditioned and went directly into anoxia. Values are mean cell counts ± s.e.m. (n = 3 wells). Media remained static (**A** - filled circles) or was changed every four days (**B -** open circles).
C. Sample image of live/dead staining of WS40NE cells exposed to long-term anoxia. This image is of cells preconditioned with 3mM lactate and taken at t = 12 days in anoxia. Note: dead cells are infrequent in image as they are rinsed away in the staining process.

Lactate Results (2):



A–B. Lactate levels in media from *A. limnaeus* cells exposed to anoxia at 30°C. Panel **A** shows levels in static media. Panel **B** shows when media was changed every four days. Values are means ± s.e.m. (n = 3).



C–D. Rate of lactate accumulation in media of *A. limnaeus* cells exposed to anoxia. Rates are adjusted to account for number of cells. **C** = static media; **D** = media changes. Values are means ± s.e.m. (n = 3).

Conclusions & Future Directions:

- **Lactate preconditioning** and **GABA supplementation** **increased** **cell division** in *A. limnaeus* cells during **initial** exposure to anoxia
- **Anoxia preconditioning** saw the **lowest rate** of cell proliferation
- **Lactate** and **GABA** treated cells may lead to cells surviving longer in anoxia – there appears to be a trend– *further experiments needed*
- Lactate accumulation **slows** when media is not changed
- Lactate accumulation is **mostly constant** when media is replenished
- **Next:** Repeat experiment until all cells are dead
- **Next:** Increase GABA and lactate concentrations – does it help?

Scan for a digital poster, references, and more!

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