Sirtuins as a Regulator of Metabolic Activity in *Mytilus californianus*

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Introduction / Background / Goals

- Sirtuin (SIRT protein family) are key regulators of metabolism under caloric restriction and affect the cellular stress response in intertidal mussels of the genus *Mytilus* during heat and salinity stress (Vasquez and Tomanek, 2019).
- Sirtuins are NAD+-dependent deacetylases, which remove acyl groups that attach to proteins through increasing levels of metabolites such as acetyl- and succinyl–CoA that are produced by core metabolic pathways.
- Acylation generally reduces while de-acylation increases enzyme activity within the cell
- Three mitochondrial sirtuins affect dozens of Krebs cycle and electron transport chain enzymes and therefore respiration, although this has apparently not been shown.
- We therefore **hypothesize** that sirtuin inhibitors affect respiration (O_2 consumption) rates in gill preparations of California Mussels (*Mytilus californianus*).

Methods

IN THE LAB:

- A Loligo Microplate Respiration System was used to measure (mmol O_2/L*s) consumption.
- Suramin/nicotinamide sirtuin inhibitors were used to inhibit mitochondrial sirtuins 3 and 5 and nuclear/cytosolic sirtuin 1.
- *Mytilus californianus* were collected along the Avila Beach coastline right before dissection.
- Gill tissue was dissected and immediately placed into wells

ANALYSIS

- Gill tissue remained in wells for 1 hour
- Wet weight was measured post oxygen readings
- A one-way ANCOVA test was used to measure significance in Rstudio

Fig 1. The Loligo Microplate System used for analysis of sirtuin inhibition in Mytilus californianus. Two of these well plates were used for respiration data collection

Results

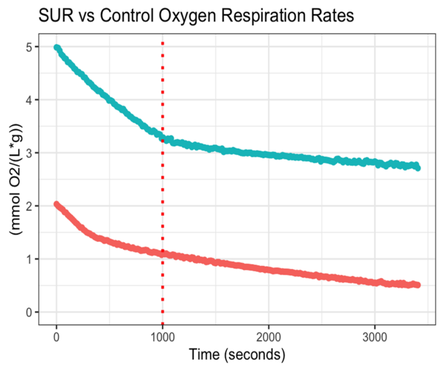

Figure 2a. Comparative oxygen respiration rates of *Mytilus californianus* gill tissue measured in mmol of O_2 per L per gram that was exposed to **Suramin or SUR** (orange) and control group (blue) over 0~3500 seconds.

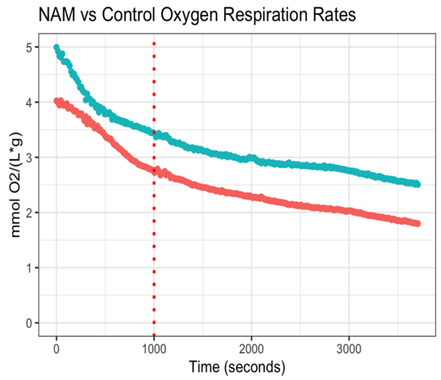

Figure 2b. Comparative oxygen respiration rates of *Mytilus californianus* gill tissue measured in mmol of O_2 per L per gram that was exposed to **Nicotinamide or NAM** (orange) and control group (blue) over 0~3500 seconds.

- Across all gill tissue samples in the experiment, oxygen uptake was highest within the first 0–1000 seconds. Tissue exposed to suramin initially consumed O_2 at a rate of 7.708×10^{-6} O_2/s*g*L, which then declined to 8.889×10^{-7} mmol O_2/s*g*L over time. Tissue exposed to nicotinamide initially consumed O_2 at a rate of 1.325×10^{-4} mmol O_2/s*g*L, which then declined to 2.850×10^{-4} mmol O_2/s*g*L over time
- After initial steep decline, gill tissue exposed to suramin exhibited a significantly reduced oxygen uptake rate of 2.671×10^{-7} mmol O_2/s*g*L lower compared to tissue maintained in seawater alone ($p < 0.001$).
- After initial steep decline, gill tissue exposed to nicotinamide also showed a reduction in oxygen uptake, with a decrease of 3.613×10^{-5} mmol O_2/s*g*L compared to seawater controls. This reduction was statistically significant, though less pronounced than with suramin exposure ($p = 1.228 \times 10^{-9}$).
- Post steep decline, nicotinamide had a significantly greater consumption of O_2 (2.850×10^{-4} mmol O_2/s*g*L) compared to suramin (8.889×10^{-7} mmol O_2/s*g*L); which shows greater metabolic inhibition from suramin ($p < 0.001$).

Conclusions / Discussions

- Suramin reduced oxygen consumption in *Mytilus californianus* gill tissue compared to the control, indicating effective inhibition of sirtuin function and its role in regulating oxidative metabolism.
- Nicotinamide also reduced oxygen consumption, but to a lesser extent than suramin, suggesting it is a less potent inhibitor or may target different sirtuin isoforms.
- Given that suramin primarily inhibits SIRT1, SIRT2, and SIRT5 - localized in the nucleus, cytosol, and mitochondria, respectively - whereas nicotinamide predominantly targets SIRT1 (nuclear) and SIRT3 (mitochondrial), the observed differences suggest that cytosolic sirtuins (particularly SIRT2) may play a significant role in mitigating oxidative stress. California Mussels (*Mytilus californianus*)
- Our results support the hypothesis that sirtuins play a role in mitochondrial regulation and oxidative stress response in marine invertebrates.
- Understanding sirtuin involvement in oxidative metabolism may help predict how *Mytilus californianus* responds to environmental changes such as increased temperature or ocean acidification.

Future Directions / Next Steps

Follow-up experiments:
- will use additional sirtuin inhibitors to target specific sirtuins.
-evaluate the effect of temperature stress on respiration during sirtuin inhibition.
-use the tide simulator to investigate the effect of temperature acclimation.

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