HONORS COLLEGE & COLLEGE OF SCIENCE RESEARCH SHOWCASE

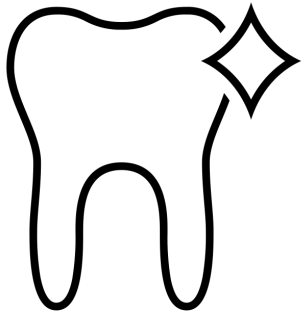
Oregon State University

“Hard” Protein Chemistry

Phil McFadden lab
Department of Biochemistry and Biophysics

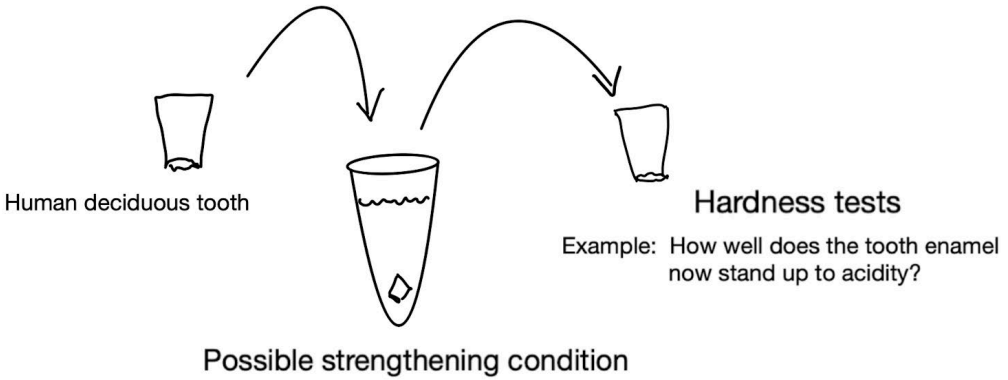
1. QUESTION

While it is known that tooth enamel is strengthened by fluoride, what other factors affect tooth strength?



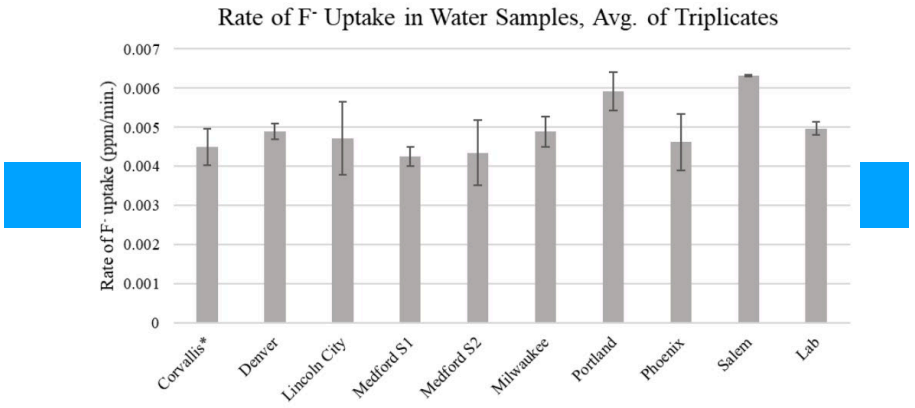
2. EXPERIMENTAL SYSTEM

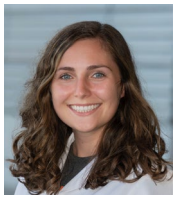
Tooth treatment followed by tests of strength



3. RESULTS

In her Honors College thesis, Karissa Renyer studied fluoride uptake. She found that pH 6 is optimal for fluoride uptake into tooth mineral. Ten different municipal water sources showed remarkably similar fluoridation potentials (graph to the right). We are now examining other possible tooth strengthening factors including protein factors in the water and in the teeth, with the goal of understanding biomineral versatility, improving health, and brightening smiles.





Karissa Renyer
D.M.D. Candidate, Class of 2024
OHSU School of Dentistry

Current OSU undergraduate students on the project include: Bereket Berhanu, Alyssa Abonitalla, Nazrawit Berhe, and Abigiya Bekele

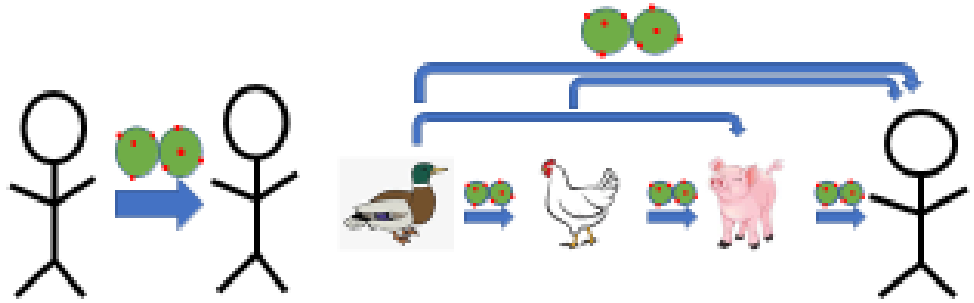
The Rowe lab in the department of Microbiology studies direct and indirect interactions between Influenza viruses and the microbiome of the host.

Hannah Rowe
Nash 434
hannah.rowe@oregonstate.edu

Role of Bacterial-Viral Interactions in Transmission

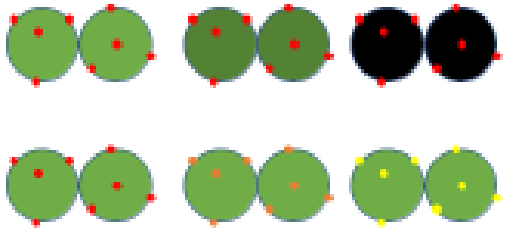
Respiratory Transmission

Zoonotic Transmission

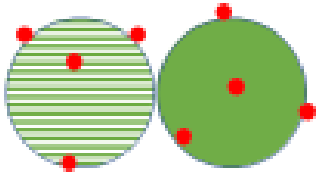


Role of Bacterial-Viral Interactions in Pathogen Evolution

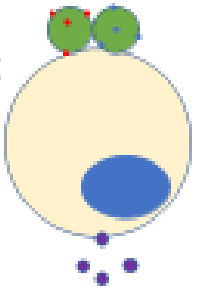
Genetic Drift During Co-Infection



Bacterial Horizontal Gene Transfer

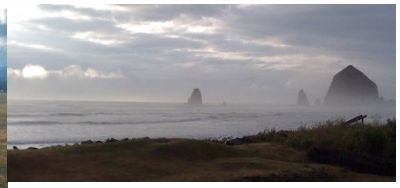


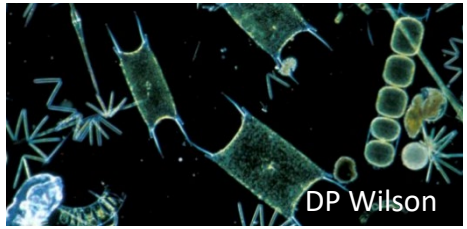
Viral Reassortment

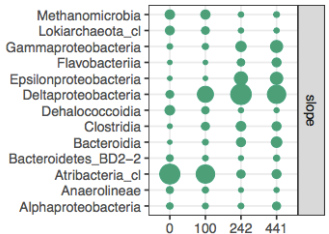


Research

Our **Geomicrobiology Lab** investigates microbes in earth and ocean systems (diversity, abundance, and function).
Opportunity: Thiamine deficiency complex (TDC) leads to high mortality in early life stages of salmonids. Causes of TDC are hard to pin down. What is the relationship between thiamine deficiency in fish and microbial communities?







Thiamine?
Which microbes?
What season?
Habitat conditions?

Rick Colwell
Weniger 519
541-737-5220
rick.colwell@oregonstate.edu

OSU Oregon State University

Research skills you'll learn

Lab and field experience
- ***Sampling***
- ***DNA extraction and sequencing***
- ***Characterization of microbial communities***
- ***Bioinformatic (computational) analysis***
- ***Data synthesis***

General scientific practice
- ***Oral/poster presentations***
- ***Technical writing (thesis, technical papers)***
- ***Teamwork***
- ***Outreach***

Dr. Jim Rivers - Animal ecology and conservation

jim.rivers@oregonstate.edu http://people.forestry.oregonstate.edu/jim-rivers/

Does snow control streamflow in the Willamette Basin?

Roy Haggerty

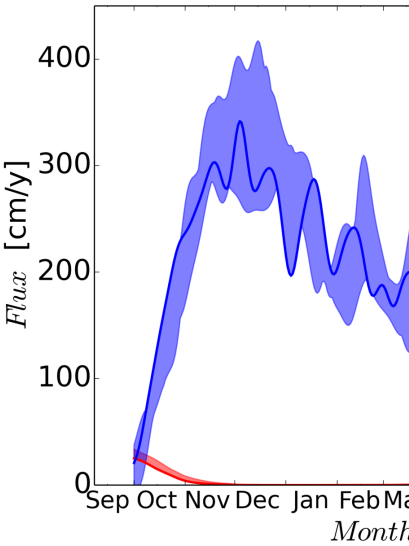
roy.haggerty@oregonstate.edu

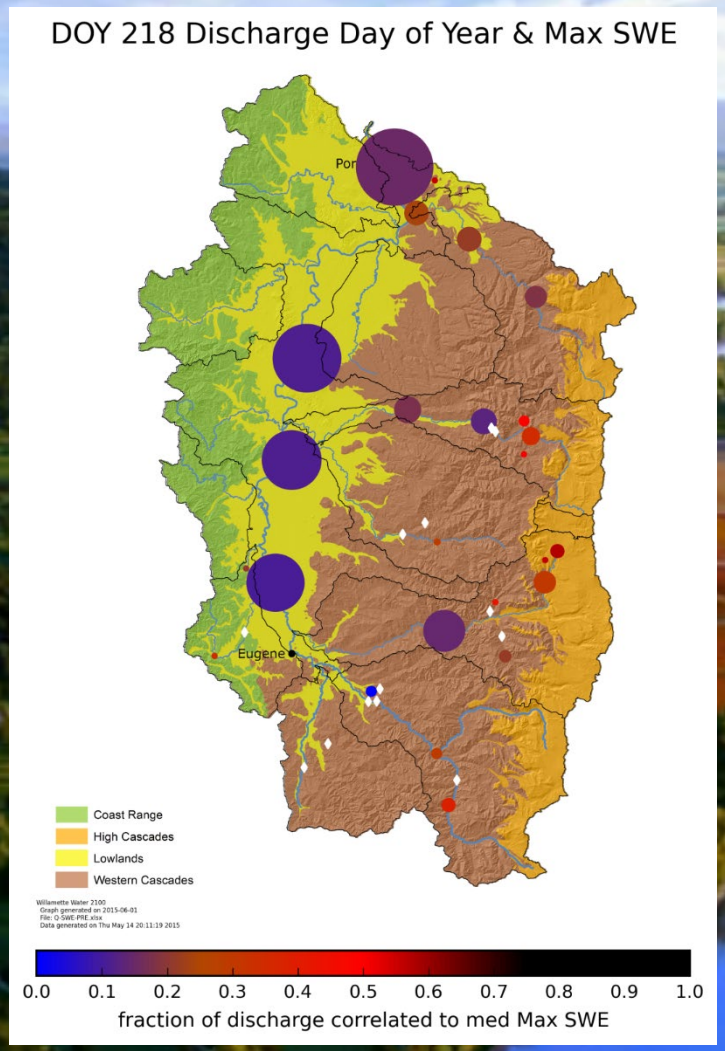
Dean of Science

COLLEGE OF SCIENCE

Building leaders in science.

Oregon State University





Most people, including most hydrologists, think that snow is the most important source of water for summer streamflow in the Willamette Basin.

I hypothesize this is not true – that the most important source of water for summer streamflow is spring rain.

Diagram at left shows results from all 85 stream gauges in the basin. If snow is correlated to summer streamflow, a dot is shown (only about 20). Size of dot = flow. Blue/brown is low correlation. Red/black is high correlation. Dot not shown = no correlation.

Help me test this hypothesis.

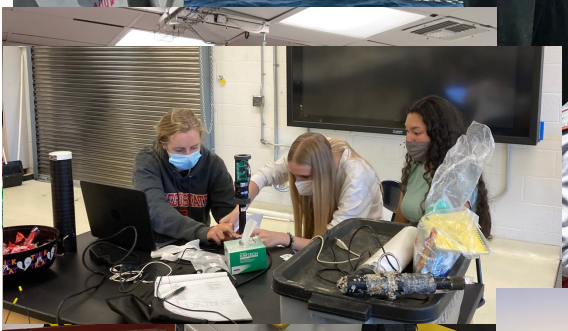
Background needed:
- Any major in Science; Experience coding in Python; Lots of curiosity

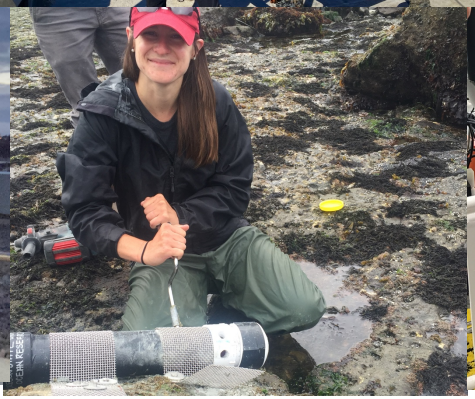
Ecosystem Ecology in a Changing Ocean

Francis Chan
Integrative Biology

Are we facing a “hot, sour, and breathless ocean?” What are the consequences and what can we do about it?

Let’s find answers together

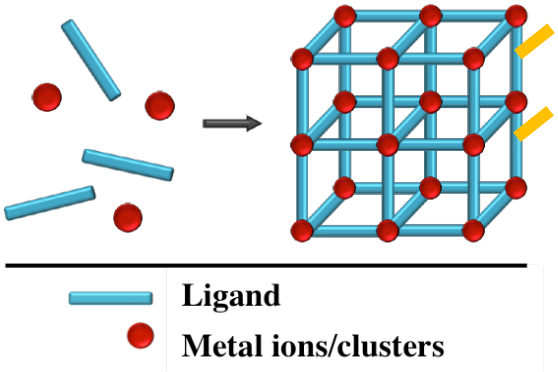


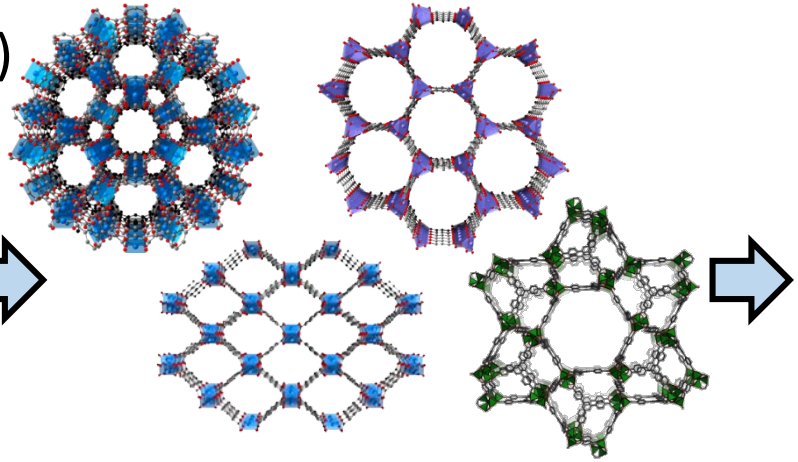


Materials **D**iscovery **Lab**oratory (MaD Lab)

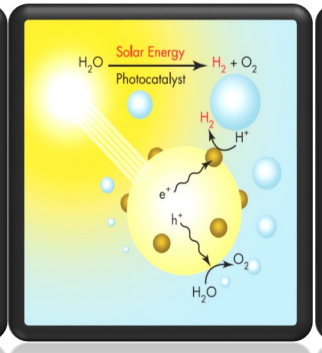
Oregon State University

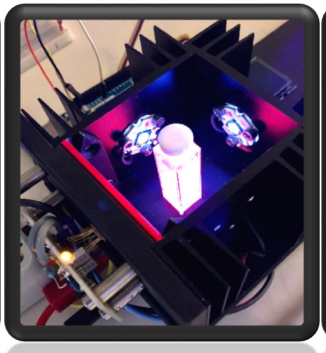
Metal-organic Frameworks (MOFs)

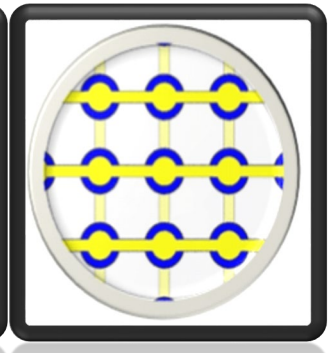




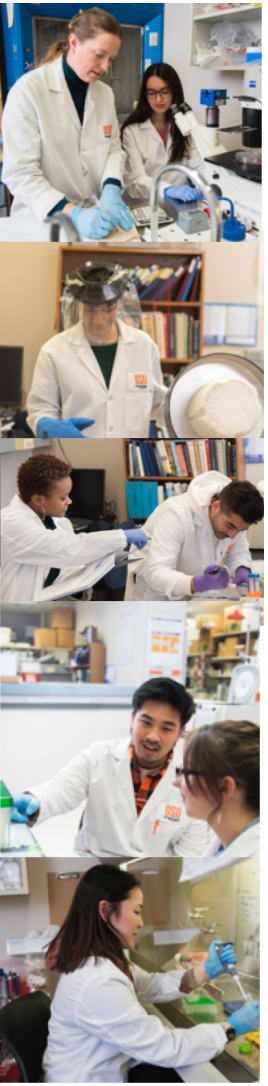
Applications







Prof. Kyriakos C. Stylianou, Department of Chemistry, Oregon State University

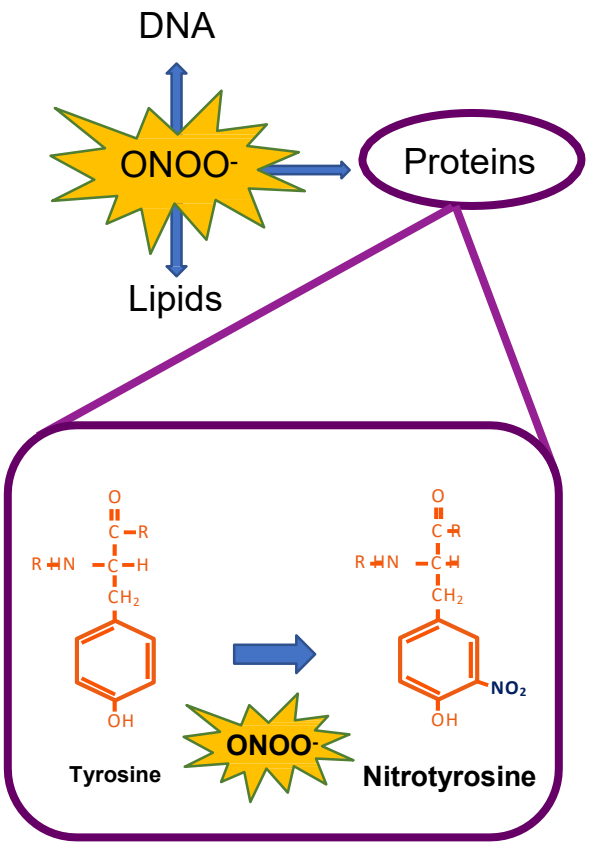


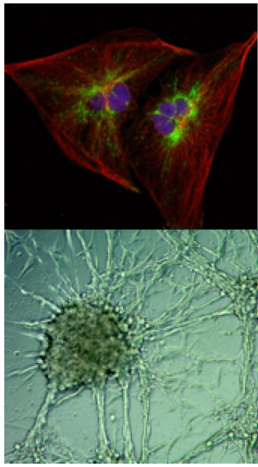
The Franco Lab

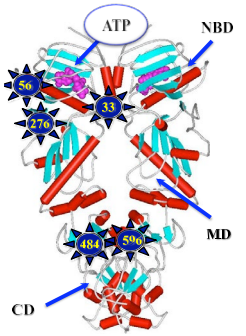
Role of Oxidants in Tumor Development and Growth

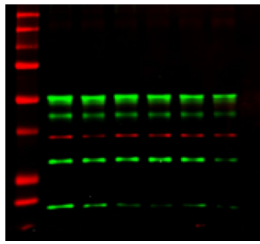
Peroxynitrite & Tyrosine nitration

Tumors of the nervous system

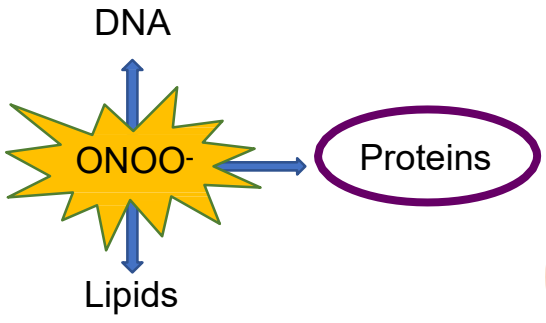








The Franco Lab



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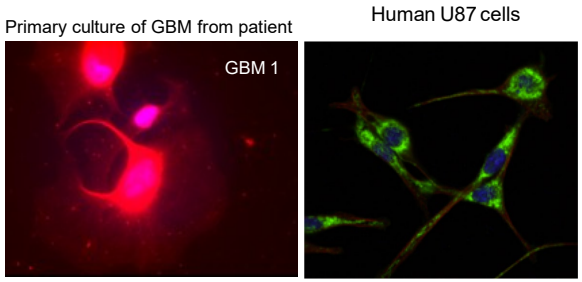
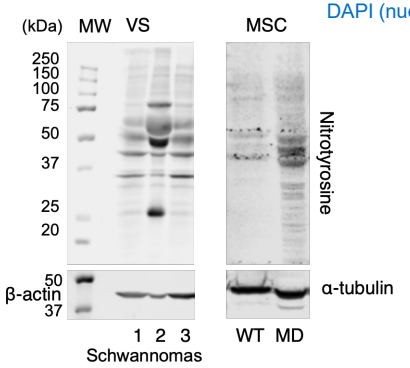
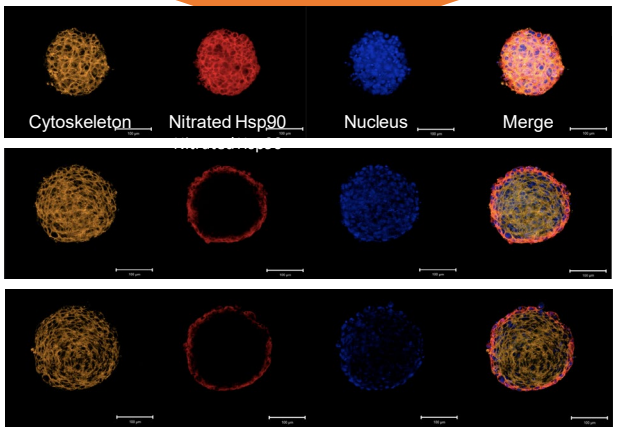
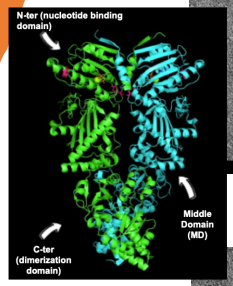




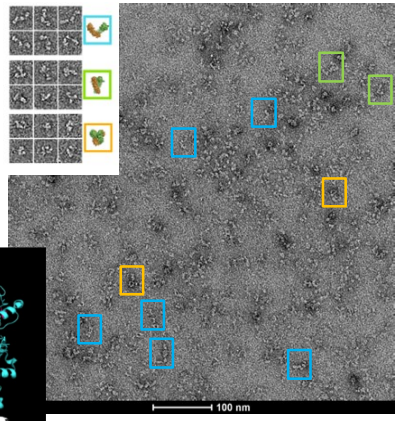
3D cell culture model



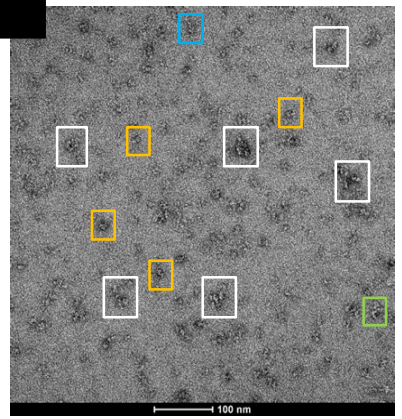
Protein structure



Hsp90



Nitrated Hsp90



The Franco Lab

Maca Franco, Ph.D.

(She, her, hers)

Biochemistry and Biophysics

maria.franco@oregonstate.edu

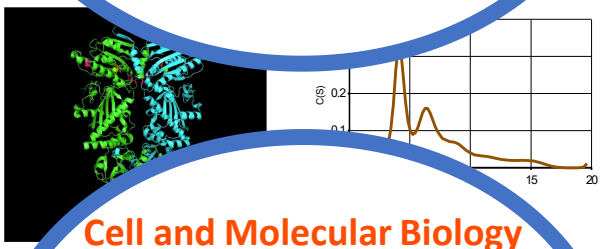
ALS 2103 / (541) 737-4997

Structural Biology

Protein gain-of-function

How does nitration affects protein structure to induce a gain-of-function?

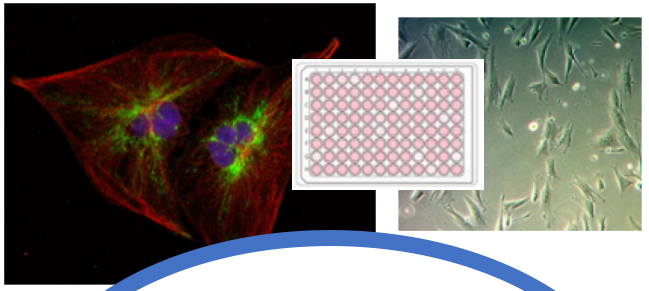
- Analytical ultracentrifugation
- Negative stain
- Cryo-EM



1. Identification of oxidized proteins with activities relevant to disease process
   - 2A. Mechanism of action
   - 2B. Structural changes induced by oxidation
3. Drug development

Role of Oxidants in Tumor Development and Growth

Peroxynitrite & Tyrosine nitration

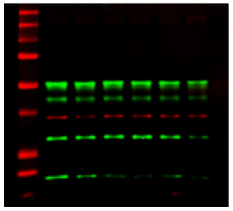


Cell and Molecular Biology

Role of Nitration in tumor growth

- Relevant nitrated proteins
- Cell metabolism
- Signaling pathways
- Mechanism of action
- OMICS

In vitro and *in vivo* models



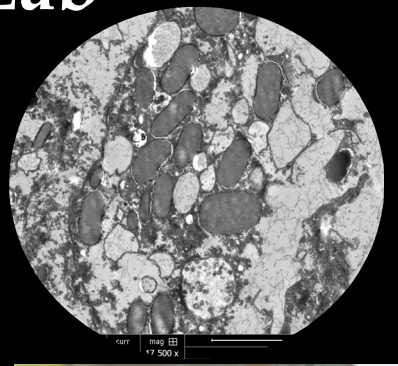
Translational Research

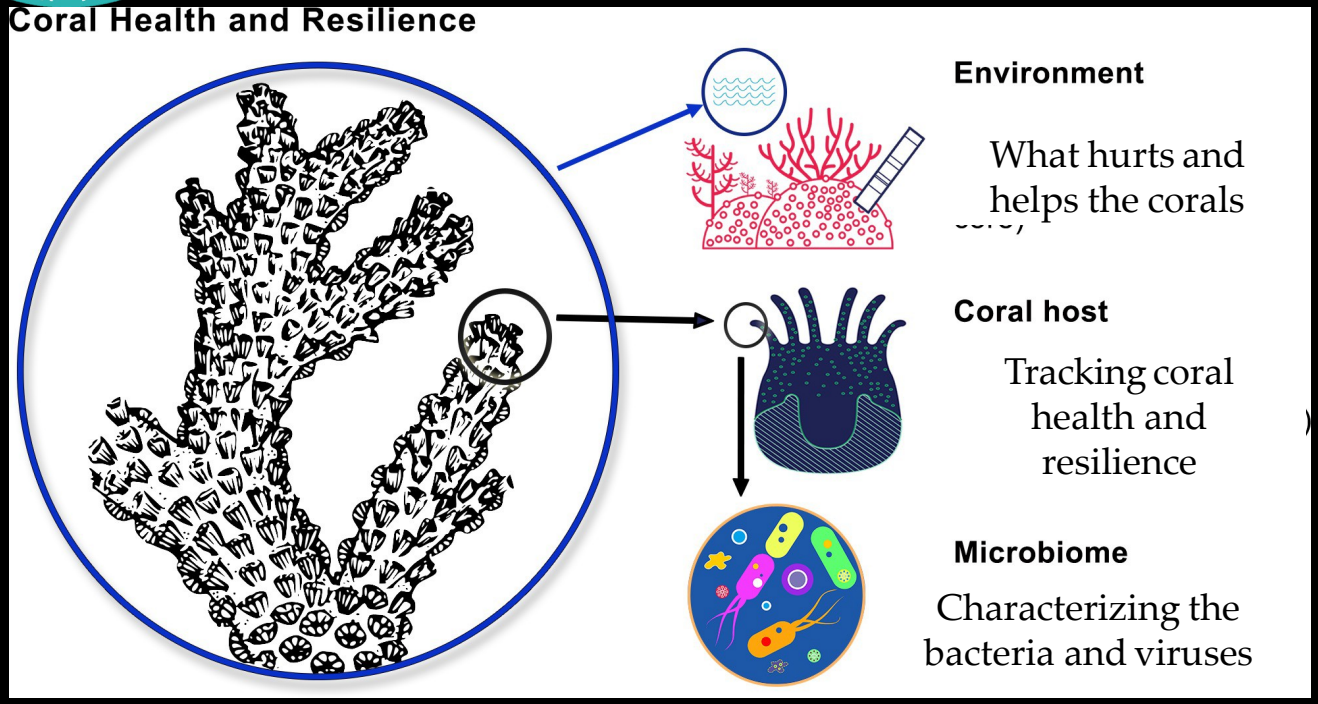
Drugs that specifically target the nitrated form of the protein

- Surface Plasmon resonance
- *In vitro* functional assays
- Drug screenings

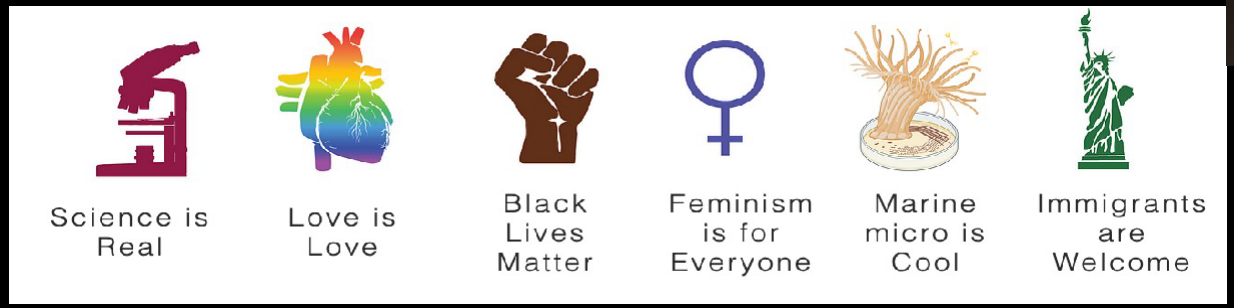
A microbiological and viral view of the causes and consequences of coral reef decline

Rebecca Vega Thurber Lab





In our lab



Microbial Evolution in Ocean Deserts: How do microorganisms adapt to nutrient loss in warming seas?

Stephen Giovannoni, Department
of Microbiology

Dedicated to Lynn Margulis, 1938-2011

Global warming is causing ocean gyres to expand, increasing habitat for some of the simplest and most successful microorganisms known. These organisms are different – they have undergone extreme transitions in their genomes and cell architecture that enable them to efficiently use the diminished nutrients in ocean gyres, which are sometimes called ocean deserts because of their high water clariity and low productivity

We're trying to understand how many different cell types underwent similar changes when they expanded their ranges to ocean gyres. It was once thought this process, called genome streamlining, was caused by the loss of genes for repairing mutations. Now we know that is not true.

We want undergraduate students interested in computational science and microbiology to help us study these cells and to understand their unique evolution

How does metallicity influence the types and distribution of extrasolar planets that form?

We have now discovered 4390 planets in 3313 planetary systems:

Preliminary research (diagrams to the right) shows that these planets fall into three "groups" based on their mass and distance from their parent star.

Questions:

1. What is the statistical likelihood of these groupings? Cluster analysis.

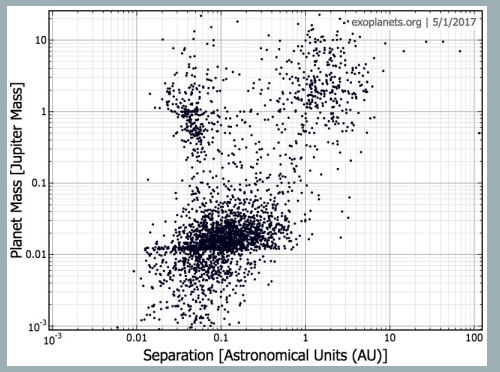
2. Do the planets in these groups have different metallicities? Do their parent stars? Do newer generations of stars produce more metal rich planets, and will this affect the types of planets that form in the future?

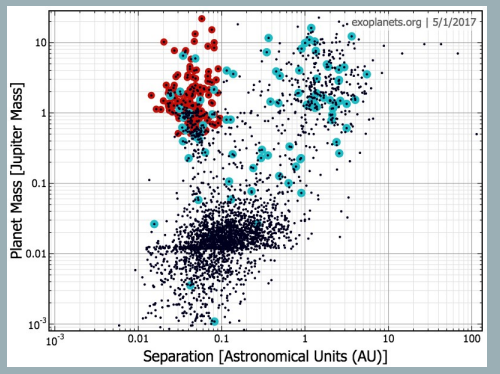
3. Is there evidence for two different types of gas giants, one that form more metal rich and one metal poor? Do planets in binary stars tend to belong to one group versus the other?

Dr. Rebecka Tumblin - Physics

Help me explore these questions using data analysis and data mining!

Background needed: Any science major with experience coding in python; the ability to fail at something and keep trying; an infectious enthusiasm for scientific inquiry; unbridled curiosity.





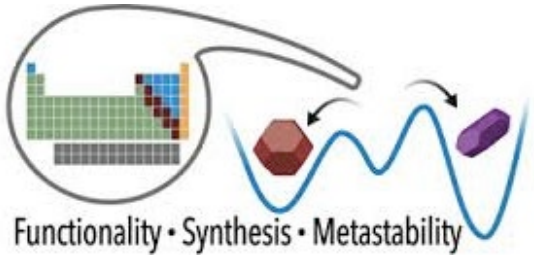
Planets in Binary stars are highlighted in blue and hot Jupiter's in red.

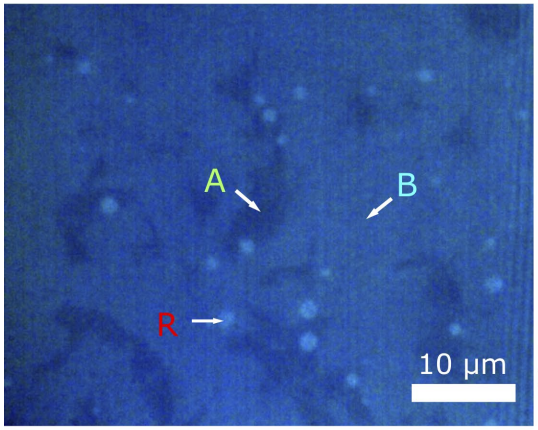
Metastable semiconductors: TiO_2

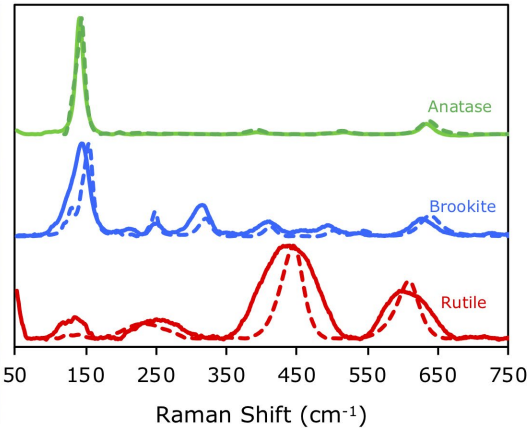
Important photocatalyst, pigment, and electrode

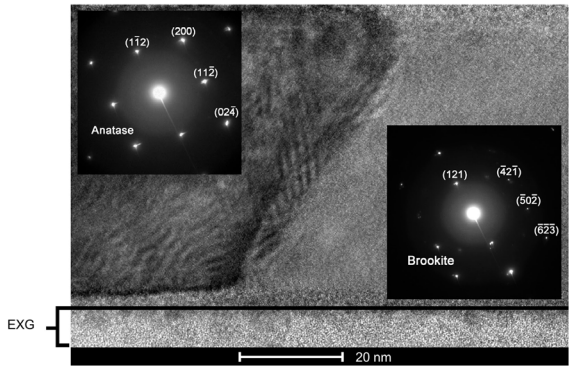
Some versions aren't "stable" but have better properties!

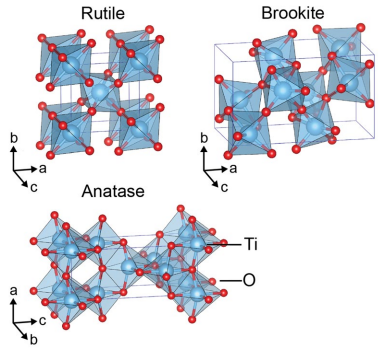
How do metastable versions form?











Janet Tate, Department of Physics, College of Science, OSU (Janet.Tate@oregonstate.edu)

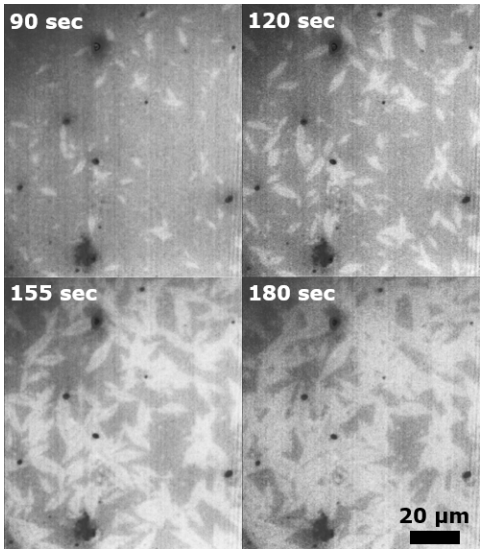
Project:
How fast do crystals grow?

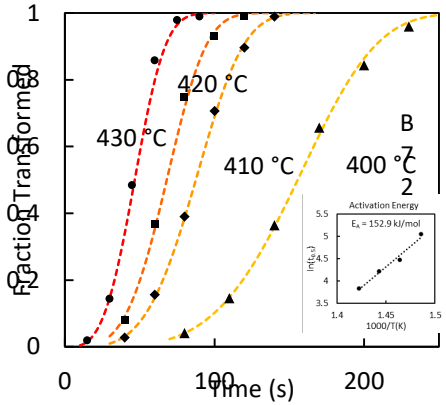
When amorphous films crystallize …

- what determines how fast they grow?
- can we model the growth and make sense of the model parameters?

You need

- some curiosity about materials
- to know a bit about image-processing software or be willing to learn quickly
- to be detail-oriented and organized





Janet Tate, Department of Physics, College of Science, OSU (Janet.Tate@oregonstate.edu)

Department of Chemistry

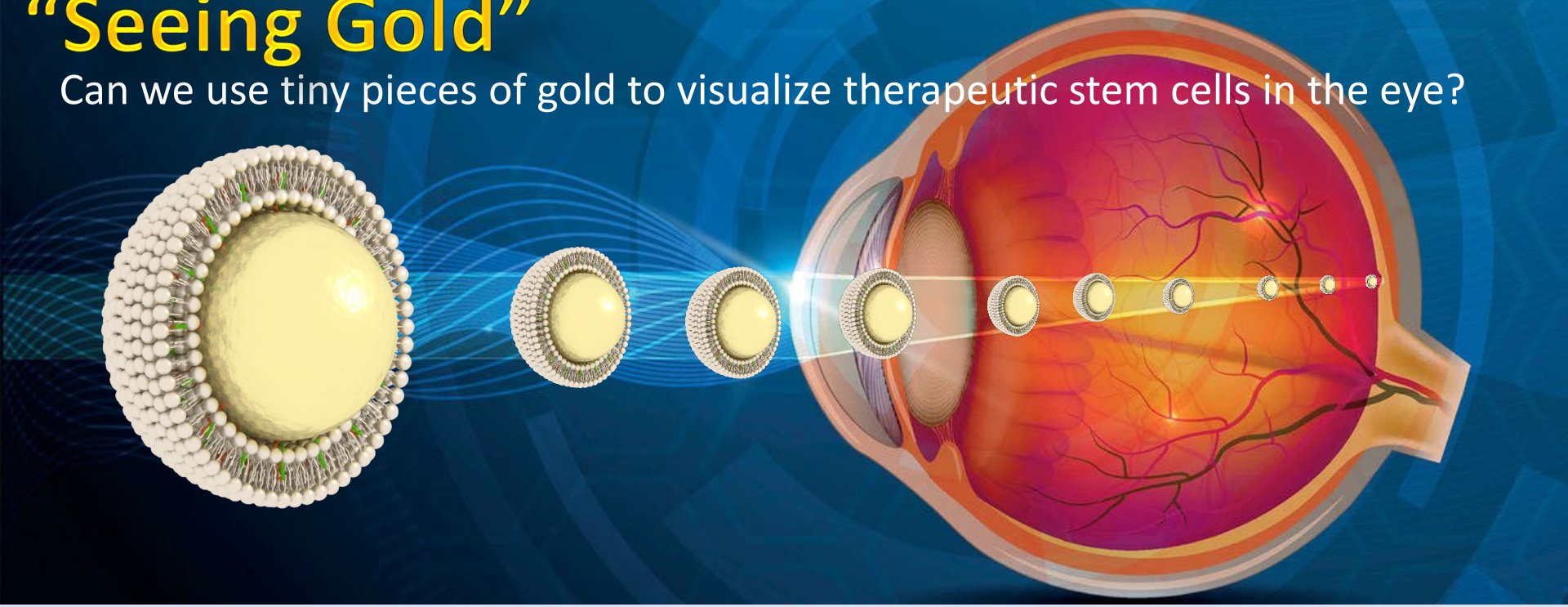
MARILYN RAMPERSAD MACKIEWICZ

MARILYN.MACKIEWICZ@OREGONSTATE.EDU

Oregon State University

“Seeing Gold”

Can we use tiny pieces of gold to visualize therapeutic stem cells in the eye?



Cell transplantation is a promising therapy for degenerative retinal diseases and is currently being investigated in multiple clinical trials to treat Age-related Macular Degeneration (AMD), which affects over 196 million people worldwide. In rodent model's cell transplantation has been shown to rescue rod and cone photoreceptors and preserve eyesight. Characterization of cell-based therapies relies on specific information regarding cell survival, migration, and integration in the host that is primarily derived from post-mortem histological assessments. However, the serial nature of this method requires large numbers of animals for these studies at multiple time points since there is currently no method for evaluating efficacious cell-based therapies longitudinally *in vivo.* Consequently, there is a critical need for the development of technology that would enable us to understand the consequence of transplanting cells into the eye to visually track transplanted cells survival and migration *in vivo*.

We hypothesize we can use tiny pieces of gold to visually track therapeutic cells *in vivo*?

Help us test this hypothesis by joining our research group and publishing our results. To learn more, check out the MackLab

Background needed: any science major who want to learn how to do materials synthesis, work with a diverse and awesome team, and who want to engage their curiosity.

2021 Thesis Poster Fair Winner in Science

College of Public Health and Human Sciences

Simone Burton, Dr. Diana Rohlman

REDUCING PLASTIC POLLUTION: A COMMUNICATION STRATEGY

Studying the most effective modes of marine science communication on plastic pollution in the ocean.

INTRODUCTION

Ocean health and human health are inextricably linked. Consumer choices, and the prevalence of plastic, have led to significant marine plastic pollution. This thesis project evaluated communication strategies to reduce marine plastic pollution. We hypothesize that the visual modes of science communication will be more effective at increasing knowledge of plastic pollution in the oceans and inspiring changes in plastic use.

METHODS

1. An online survey was sent out to various organizations. The survey included 4 different science communication products (Figures 1-4). Participants were asked to:
   - Self assess marine plastic pollution knowledge
   - Rank the products from best to worst and explain why
   - Select likelihood of reducing plastic use
2. Data were analyzed in Excel. ANOVA tests were conducted to identify differences in participants based on location and self-assessed knowledge.
3. A thematic analysis was conducted to better understand which products were most effective, in addition to a weighted rank analysis using numeric ranking scores.

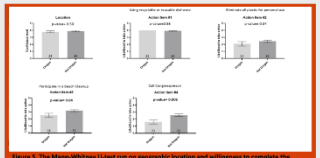


Figure 5. The Mann-Whitney U-test run on geographic location and willingness to complete the action items, along with the corresponding bar graph.

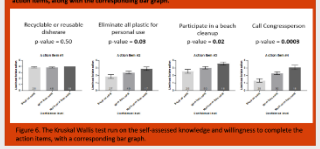


Figure 6. The Kruskal Wallis test run on the self-assessed knowledge and willingness to complete the action items, with a corresponding bar graph.

Figure 1. The image product shown to the survey participants. Credit to the NOAA Marine Debris Program.

Figure 2. A text-only product from the NOAA Marine Debris Program. It was 195 words and identified what plastic pollution is, why it threatens marine life, and how plastic can get into the ocean. [Link to URL]

Figure 3. The Problem with Plastics. A video from the Shedd Aquarium [3:24]. The video identified what plastic pollution is, why it threatens marine life, and how plastic can get into the ocean. [Link to URL]

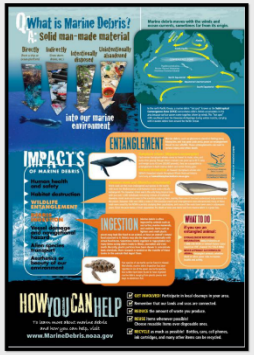


Figure 4. The infographic by the NOAA Marine Debris Program.

RESULTS

- Participants were mostly college-educated with a high level of marine plastic pollution knowledge.
- Ranking: video #1, infographic #2, image #3, and text #4.
- The higher the self-assessed knowledge, the more willing people are to complete action items (Figure 6).
- Most participants were very likely to use recyclable dishware (86%) and participate in a beach cleanup (47%).
- People residing outside of Oregon were more likely to contact their congress people and participate in beach cleanups than using recyclable dishware or eliminating all plastic for personal use (Figure 5).
- Common themes: 1) The video was most engaging. 2) The infographic was cluttered. 3) The text needed visuals. 4) The image invoked emotion but needed context.

DISCUSSION

Overall, study participants exhibited an existing knowledge of marine plastic pollution and a willingness to reduce individual plastic use.

- Location may be associated with the likelihood of completing plastic pollution action items
- Visual modes of communication were preferred over text and the image would have been rated #1 had solutions to the problem and more context been included. We conclude that graphical products are best when communicating the plastic pollution problem.
- Best practices: use visuals, include context and solutions, don't clutter the graphics, don't overstimulate the viewers visually, and keep the message succinct.
- Rather than using harsh images to make the point, pair the harsh image with a solution-oriented image.[1,2]

NEXT STEPS

- These practices were used to create an original science communication product (Figure 7) that was aimed at the plastic pollution inflated by the COVID-19 pandemic. The graphic targets the action items of wearing a disposable mask, using sanitizer instead of disposable gloves, and shopping locally or opting to ship all packages together when ordering online.
- This product will be published shortly in a second online survey and its effectiveness evaluated by the same groups the first survey was sent to.

ACKNOWLEDGEMENTS

Special thanks to:

- Kerry Carlin-Morgan, Oregon Coast Aquarium
- Katharine Bear Nalven, Defenders of Wildlife
- Briana Goodwin, Surfrider Foundation
- All the groups who sent out the survey
- Our participants!

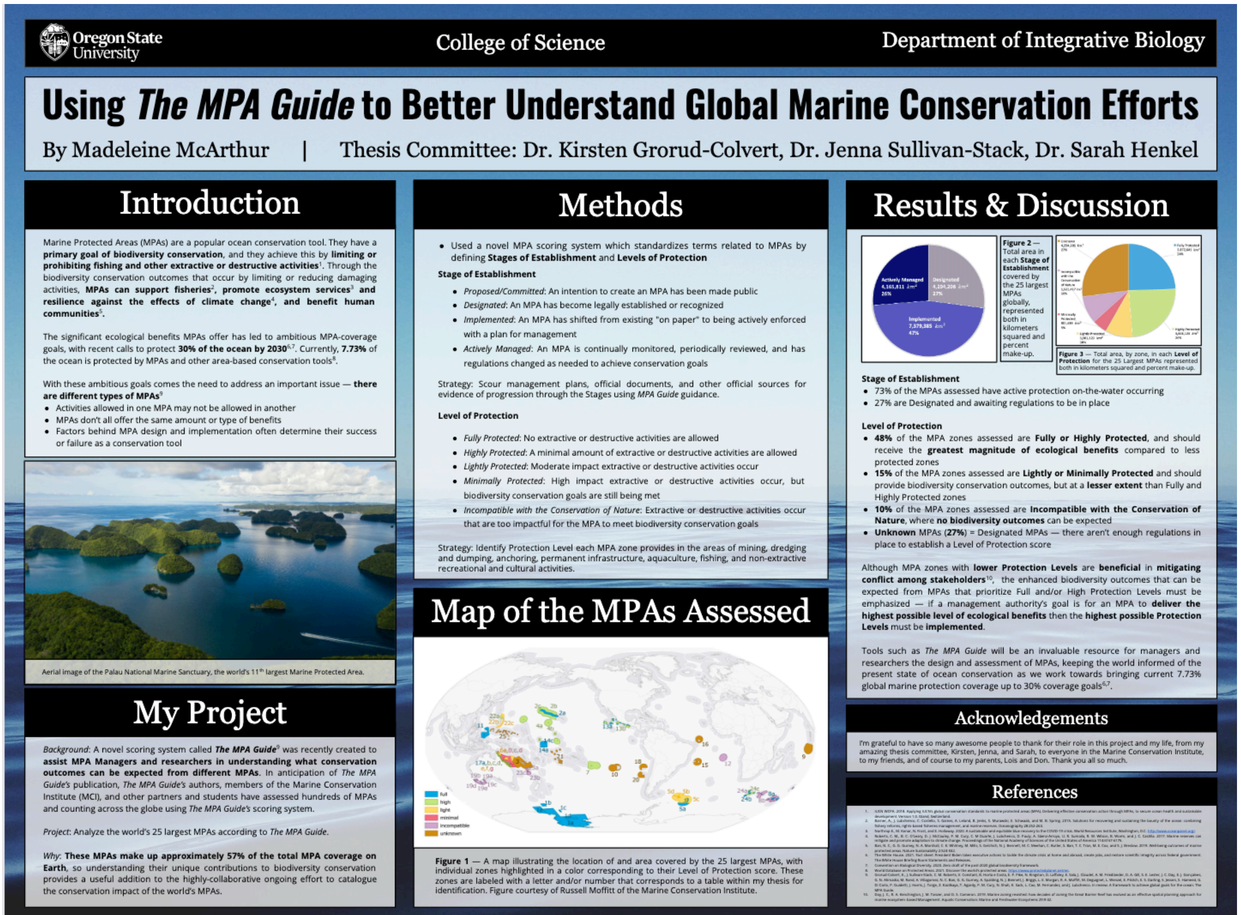
1. Corner A, Webster R, Teriete C. 2016. Climate Visuals. Glob Environ Chang. 9(2):1–41.
2. Nabi RL. 2015. Emotional Flow in Persuasive Health Messages. Health Commun. 30(1):114–124.

Figure 7. The new graphic created, aimed at plastic consumed in the COVID-19 pandemic.

Oregon State University

Oregon State University
Honors College

2021 Thesis Poster Fair
Honorable Mention in Science



Oregon State University
Honors College

College of Science Department of Biochemistry and Biophysics

THE ROLE OF NITRATED HSP90 IN SCHWANNOMA CELL SURVIVAL

Oregon State University

Sharon R. Kim, Maria Clara Franco

CDMRP
Neurofibromatosis Research Program

ABSTRACT

Neurofibromatosis Type 2 (NF2) is a genetic tumor disorder caused by mutations that inactivate the gene coding for the merlin tumor suppressor. Patients with NF2 develop multiple tumors throughout the nervous system, mainly schwannomas, for which there is no cure or effective treatment. In conditions like NF2, redox signaling and oxidative stress have been found to contribute to tumorigenesis. Reactive oxygen and nitrogen species play an essential role in regulating various pathways favoring proliferation, survival, and the malignant progression of tumor cells. In particular, peroxynitrite is a powerful oxidant produced by cells that causes the nitration of tyrosine residues in proteins. Tyrosine nitration has been detected in several tumor types and we have confirmed that there is increased tyrosine nitration in the schwannomas of NF2 patients. Furthermore, the Franco lab showed that peroxynitrite and tyrosine nitration regulate schwannoma cell metabolism and support survival and that nitrated forms of the molecular chaperone Heat shock protein 90 (Hsp90) regulate key metabolic processes in pathological conditions. Our long-term goal is to develop pharmaceutical approaches targeting nitrated Hsp90 to prevent schwannoma growth in NF2.

The goal of this project was to determine the role of nitrated Hsp90 in schwannoma growth by employing 2D and 3D mouse cell culture models of NF2 in the form of merlin-deficient (MD)-Schwann cells. To this end, we performed survival and metabolic assays and intracellularly delivered nitrated Hsp90 into MD-Schwann cell in conditions of prevention of tyrosine nitration using compounds such as urate and edaravone. We also utilized immunocytochemistry and confocal microscopy to determine the localization of the different forms of nitrated Hsp90 (Hsp90 nitrated at tyrosine 33 and 56) and other markers for cell proliferation and death in the 3D schwannoma cell culture model. We found that the treatment of mouse MD-Schwann cells with urate and edaravone induced the activation of apoptotic pathways and cell death. However, this effect was counteracted in schwannoma cells with the intracellular delivery of nitrated Hsp90. We also uncovered that upon merlin loss-of-function, there is a peroxynitrite-mediated decrease in mitochondrial activity. Furthermore, we determined that the spatial distribution of nitrated Hsp90 in a tumoroid was dependent on the site of tyrosine nitration. These observations suggest that peroxynitrite plays an essential role in regulating cell survival and reprogramming the metabolic phenotype in mouse MD-Schwann cells.

INTRODUCTION

Neurofibromatosis Type 2

Single-gene disorder of the nervous system
1:25,000 people

Mutations in the *NF2* gene that codes for merlin tumor suppressor

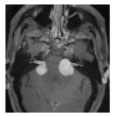
⇩

merlin loss of function

⇩

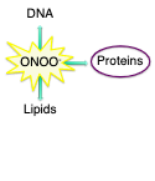
Development of benign, slow growing tumors: schwannomas, meningiomas, and ependymomas

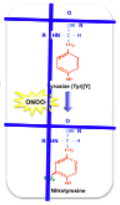
BILATERAL VESTIBULAR SCHWANNOMAS are diagnosis of NF2

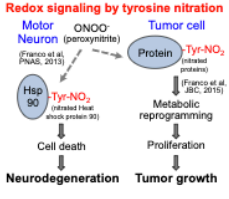


Bilateral vestibular schwannomas in NF2 patient (MRI) (http://www.nanagement.org)

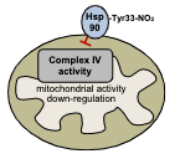
Peroxynitrite (ONOO-) and Tyrosine Nitration







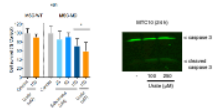
Nitrated Heat shock protein 90 (Hsp90) decreases mitochondrial activity by inhibiting cytochrome *c* oxidase



Franco et al., JBC, 2015

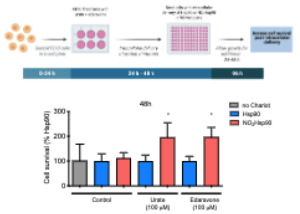
RESULTS

Tyrosine nitration supports cell survival in mouse MD-Schwann cells

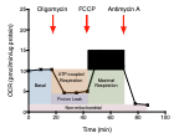


* $p < 0.05$ vs. untreated control by One-Way ANOVA followed by Bonferroni post-test.

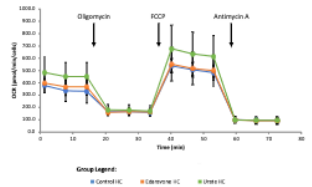
Intracellular delivery of nitrated Hsp90 increases schwannoma cell survival

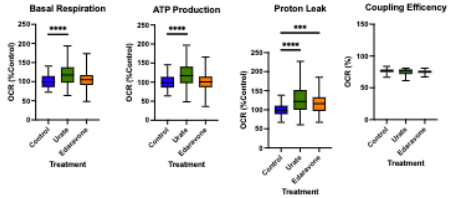


Peroxynitrite down-regulates multiple parameters of mitochondrial activity in mouse MD-Schwann cells



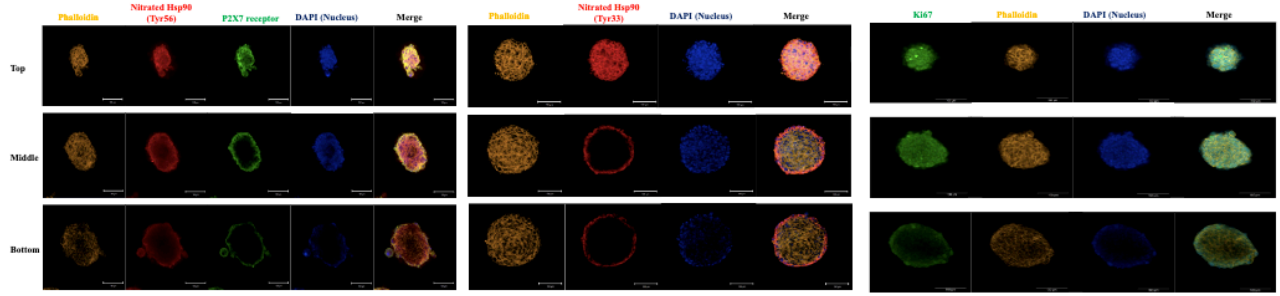
Compound	Target in Electron Transport Chain
Oligomycin	ATP synthase
FCCP	Inner mitochondrial membrane
Antimycin A	Complex Ⅰ and Ⅲ





*** $p < 0.0001$ vs. untreated control; *** $p < 0.001$ vs. untreated control by Kruskal-Wallis test followed by Dunn's post test or One-Way ANOVA followed by Bonferroni post test.

Spatial distribution of nitrated Hsp90 in a 3D tumoroid is dependent on the tyrosine that is nitrated



CONCLUSIONS

- Peroxynitrite and nitrated proteins are essential for schwannoma cell survival, as prevention of tyrosine nitration induces cell death by apoptosis.
- Upon merlin-loss-of function, there is a peroxynitrite-mediated metabolic reprogramming to decrease activity of mitochondrial oxidative phosphorylation.
- Nitration of Hsp90 at specific residues alters the spatial distribution of the protein; nitrated Hsp90 could potentially play a fundamental role in regulating schwannoma cell proliferation and survival.

Future Directions:

- Determine the effects of nitrated Hsp90 on other metabolic pathways relevant to tumor energy metabolism such as glycolysis and glutaminolysis.
- Identify other endogenously nitrated proteins and perform additional survival/proliferation assays to determine the effects on tumor cell survival.

Acknowledgements:

This work was supported by the Office of the Assistant Secretary of Defense for Health Affairs, through the Neurofibromatosis Research Program (NRP), New Investigator Award (NIA), under Award No. W81XWH-17-1-0409, and R01NS102479 from NINDS/NIH to MCF.

I would also like to thank Dr. Maria Clara Franco, Dr. Alvaro Estevez, Carrie Marean-Reardon, Jeanine C. Pestoni, Tilottama Chatterjee, Kyle Nguyen, and all of the undergraduate and graduate students of the Franco and Estevez lab for their assistance and support in the completion of this project.

College of Science | Department of Chemistry

METHOD VALIDATION OF QUANTITATIVE ANALYSIS OF OXYLIPINS IN HUMAN PLASMA VIA MASS SPECTROMETRY

Author: Madeline B. Bloom

Committee Members: Dr. Claudia Maier, Dr. Manuel Garcia-Jaramillo, Dr. Gerd Bobe

BACKGROUND

Oxylipins are a class of bioactive lipid metabolites derived from polyunsaturated fatty acids (PUFAs) via enzymatic and non-enzymatic pathways. Overall, oxylipins mediate a variety of physiological functions that regulate apoptosis, tissue repair, blood clotting, blood pressure regulation, and inflammation[1]. Their function in the body shows their potential for chemical biomarkers in various human diseases that are associated with inflammatory events. It is essential to assess the entire oxylipin profile because there are competing effects from each compound that will alter the positive or negative outcomes in the human body[1]. Quantitative analysis of oxylipins is still very difficult with traditional analytical methods because of their low endogenous concentrations and structural similarity between compounds. A reproducible, robust, and sensitive analytical method is needed in order to validate the quantification results.

Human plasma sample collection → Protein precipitation → Solid phase extraction → Liquid chromatography → Mass spectrometry → Quantification

METHODS

- **Participants:** Oxylipins were analyzed in 200 human plasma samples collected from OHSU collaborators. Their sex, age, and health status were unknown in this study.
- **Sample Preparation:** A mixture of 19 deuterated internal standard (IS) was added in order to quantify the oxylipin species. Samples were extracted using Waters OSTRO flow-through 96-well plate to remove phospholipids and proteins. CUDA was added as an IS to correct for injection volume differences and platform stability.
- **Analysis:** Samples were separated using liquid chromatography (LC) with a Shimadzu system and mass analyzed using an AB SCIEX QTRAP 4000 mass spectrometer (MS/MS) in multiple reaction monitoring (MRM) mode. Data was processed using an in-house compound library in MultiQuant.
- **Validation:** The analytical method was validated using the following parameters: intra- and interday accuracy and precision, IS recovery, and matrix effect from solvents and plasma matrix.

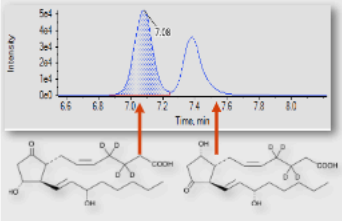


Figure 2: Extracted ion chromatogram of critical pair compounds, PGE2-d_4 (left, RT = 7.08 min) and PGD2-d_4 (right, RT = 7.40 min). These deuterated internal standards share a *m/z* transition of 355>375 and were separated in the LC column.

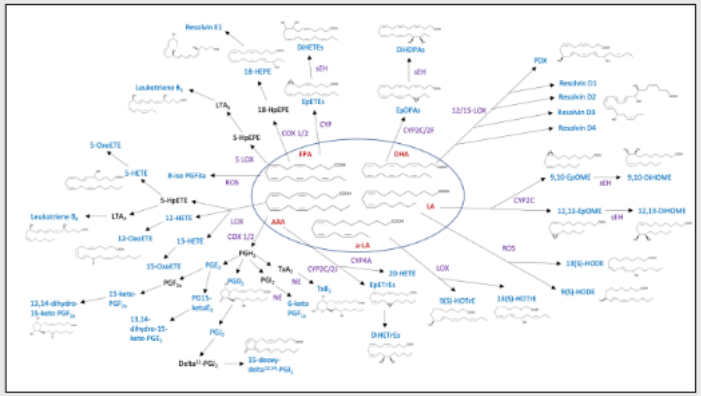


Figure 1: Formation of oxylipins via enzymatic and non-enzymatic pathways from precursor fatty acids: arachidonic acid (ARA), linoleic acid (LA), α-linolenic acid (ALA), eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA) (shown in red). The pathways are modified from Gabbs et al[2]. Oxylipins shown in blue were analyzed and quantified by LC-MS/MS. Enzymes involved in PUFA and oxylipin metabolism are shown in purple. CYP: cytochrome 450; sEH: soluble epoxide hydrolase; LOX: lipoxygenase; COX: cyclooxygenase; ROS: reactive oxygen species; NE: non-enzymatic

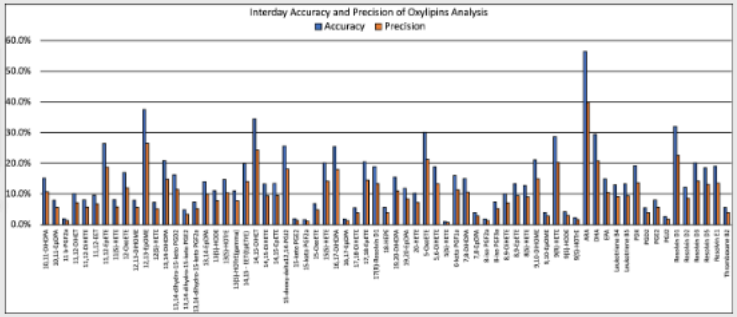


Figure 3: Interday accuracy and precision of oxylipin compounds and precursor PUFAs in calibration curve standard mixtures injected on two different days and analytical runs.

RESULTS

- **Quantification:** This analytical method was able to quantify 68 oxylipin species and 3 PUFAs from a wide range of metabolomic pathways (Figure 1). The limit of quantifications (LOQ) for the oxylipins ranged from 6 pM to 17 nM by evaluating the signal-to-noise ratio from the blank samples to the plasma samples.
- **Method validation:**

Intraday accuracy and precision between IS in quality control samples had an average of 9.7% (± 2.4%, n = 22) accuracy and 13.5% (± 2.9%, n = 22) precision.

Figure 3 shows over 80% **interday accuracy and precision** for 86% of the oxylipin and PUFA analytes (n = 74).

Recovery of IS was over 100% for 75% of compounds (n = 20).

Between the EtOH and ACN:MeOH calibration curves, a **matrix effect** was observed as the peak area for the ACN:MeOH mixtures was on average 20% (n = 48) greater at the same concentrations. Additionally, the analysis of the extraction blanks with IS showed an average of a 38% (n = 22) difference in peak area from the plasma samples after extraction through the OSTRO plate.

DISCUSSION

Concentration ranges of oxylipins are comparable to those in previous LC-MS/MS quantitative studies, which shows correlation with other published methods.

Further studies should attempt to decrease the LOQ as endogenous concentrations may be lower in other tissues such as human brain. More sensitive mass spectrometers such as the Waters XEVO TQ-XS have illustrated positive preliminary data for this improvement.

Conclusions

This **dynamic LC-MS/MS quantitative analysis** method of oxylipins that showed a high amount of sensitivity and reproducibility at pM concentrations. Many compounds showed **improvement in LOQ and specificity** from previous studies, which shows that this method can be applied to clinical chemistry analyses with success.

Method validation parameters revealed a robust analysis that can evaluate the comprehensive oxylipin profile as **both pro- and anti-inflammatory lipid mediators were quantified** successfully.

Quantifying oxylipins at this level of specificity continues to demonstrate their **potential for biomarkers** of human disease that can lead to new **diagnosis protocols or drug treatment designs.**

ACKNOWLEDGEMENTS

The use of the OSU Mass Spectrometry Center and their instrumentation was funded by the NIH grant #1S10RR022589-01 and my project was funded by SURE through the College of Science.

LITERATURE CITED:

1. Gladine, C.; Ostermann, A. I.; Newman, J. W.; Schebb, N. H. MS-Based Targeted Metabolomics of Eicosanoids and Other Oxylipins: Analytical and Inter-Individual Variabilities. *Free Radic. Biol. Med.* **2019**, *144* (April), 72–89.
2. Gabbs, M.; Leng, S.; Devassy, J. G.; Aukema, H. M. Advances in Our Understanding of Oxylipins Derived from Dietary PUFAs. *Am. Soc. Nutr.* **2015**, *6*, 513–540.
3. Wolfer, A. M.; Gaudin, M.; Taylor-Robinson, S. D.; Holmes, E.; Nicholson, J. K. Development and Validation of a High-Throughput Ultrahigh-Performance Liquid Chromatography-Mass Spectrometry Approach for Screening of Oxylipins and Their Precursors. *Anal. Chem.* **2015**, *87* (23), 11721–11731.

Oregon State University

Chemistry and Grit: The Impact of Prerequisites vs Perseverance on Academic Success in Biology

Author: Kiersten Sparks **Mentor: Lori Kayes**

Oregon State University College of Science, Honors College

INTRODUCTION

- Biology is interdisciplinary and relies on chemistry background, especially when covering biochemistry in a general biology course (AAAS, 2011).
- Research has shown that general biology students who take chemistry as a prerequisite earn higher course grades than those who take chemistry as a corequisite (Kulesza, 2019).
  - OSU's Principles of Biology for Life Science Majors has a mandatory chemistry corequisite.
- When students do not have the background in chemistry that would help them better understand biochemical concepts in the course, they must rely on other skills and motivations to succeed.
  - "Academic success" is multifaceted an accounts for multiple parts of a student's success, but in this study academic success will be measured by academic achievement (course and exam grades).

Figure 1: Adapted version of the Revised Conceptual Model of Academic Success by York, Gibson, and Rankin (York et al., 2015).

- We propose that in some students, grit may be able to increase student's performance in the class and make up for the pre-requisite knowledge students may lack when they take biology.
  - Grit is a personality trait defined as "passion and perseverance for long term goals" (Duckworth et al., 2007).
- This study examined the relationship between a student's academic success (measured by exam grades and final course grades) and their level of 1) chemistry exposure, and 2) grit.

RESEARCH QUESTIONS

The research was guided by the following questions:

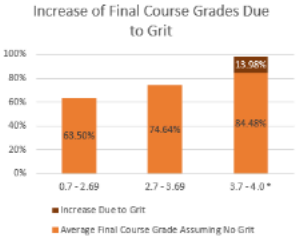
1. How much influence does a student's grit have on their academic success and performance on assessments covering biochemistry?
2. How much influence does a student's prior chemistry exposure have on their academic success and performance on assessments covering biochemistry?
3. Does grit or chemistry exposure provide a greater increase in student academic success on assessments of biochemical content?

METHODS

- Data were collected via a survey from two classes of the BI 212 quarter of the Principles of Biology series.
- Survey included demographic questions, questions about prior chemistry courses taken, and a 12-item Grit Scale questionnaire.
- Data was processed in bins according to GPA using a multiple linear regression, with course grades or exam grades as the dependent variables, and chemistry exposure, grit, and gender as independent variables.
- Models displayed low correlation (R^2) but were found to be significant, and the best fitting models were selected for analysis based on AIC score.

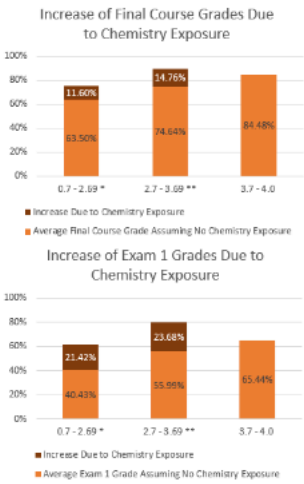
RESULTS

How much influence does a student's grit have on their academic success and performance on assessments covering biochemistry?



- NOTE: Grit had no impact on Exam 1 grades regardless of the student's cumulative GPA.
- * = $p < 0.05$. ** = $p < 0.01$

How much influence does a student's prior chemistry exposure have on their academic success and performance on assessments covering biochemistry?



Does grit or chemistry exposure provide a greater increase in student academic success on assessments of biochemical content?

- Grit is only significant in the highest achieving students (GPAs between 3.7 to 4.0), and only for final course grades.
- Chemistry exposure is the most significant predictor of success in all students with a GPA of 3.69 or below, for both exam grades and final course grades.
- Chemistry exposure provided the greatest additive value on student grades (exam or course) and impacted students in both the GPA2 and GPA3 bins (those with GPAs of 3.69 and below).

Model	Model Estimate (β)	Grit estimate (β)	Grit Std. Error	Chem estimate (β)	Chem Std. Error	Gender (M) estimate (β)	Gender Std. Error	Residual Std. Error	Model P value	F value [degrees of freedom]	Adjusted R^2	AIC Value
GPA4												
Course grade ~ grit + chem + gender	0.8448	0.1398*	0.0665	-0.0179	0.0519	0.0330 .	0.0175	0.0700	0.0689 .	2.448 (3 and 88)	0.0455	**-222.1279**
Exam 1 ~ grit + chem + gender	0.6544	0.1032	0.1122	0.0073	0.0876	0.0625	0.0295	0.1182	0.1776	1.678 (3 and 88)	0.0219	**-125.9294**
GPA3												
Course grade ~ grit + chem + gender	0.7464	0.0526	0.0613	0.1476 **	0.0500	0.0305 *	0.0142	0.0928	0.0040 **	4.581 (3 and 195)	0.0514	**-375.1060**
Exam 1 ~ grit + chem + gender	0.5599	-0.0524	0.0659	0.2368***	0.0538	0.0436**	0.0152	0.0998	2.058e-05 ***	8.642 (3 and 195)	0.1038	**-346.2646**
GPA2												
Course grade ~ grit + chem	0.6350	0.16717	0.1320	0.1160 *	0.0459	N/A	N/A	0.0588	0.0306 *	3.893 (2 and 32)	0.1454	**-94.13982**
Exam 1 ~ grit + chem	0.4043	0.1340	0.1851	0.2142**	0.0644	N/A	N/A	0.0825	0.0075 **	5.717 (2 and 32)	0.2172	**-70.49386**

*Asterisks denote significance (p) *** = [0, 0.001] ** = (0.001, 0.01] * = (0.01, 0.05] . = (0.05, 0.1] = (0.1, 1]* (β) = standardized value

DISCUSSION

Grit

- Grit was only found to be significant in the highest achieving groups, aligning with the pattern seen in previous literature ((Duckworth et al., 2007; Bazelais et al., 2016; Credé et al., 2017).
- The model in which the variable grit was significant was not significant itself, so conclusions about whether grit impacts biology students in a meaningful way is still unclear.
- However, student's level of grit can be increased by:
  - "Deliberate practice" which increases both content knowledge and perserverance.
  - Goal setting, which better prepares students to persevere to attain their long-term goals.
  - Encouraging a growth mindset.

Chemistry

- In all cases except the highest achieving group (GPAs of 3.7-4.0) chemistry exposure provided the greatest additive value to both exam 1 grades and final course grades.
  - Note: Chemistry exposure score is measuring the amount of chemistry courses taken, and only accounts for the understanding of chemistry by proxy, it may not fully represent the student's grasp of chemical concepts.
- The magnitude of the impact of chemistry exposure is called into question based on the low significance of the overall model, but it is reasonable to assume based on this data that chemistry has a small but significant role in student success in biology.
- This may suggest that increasing students' prerequisite knowledge of chemistry may help improve their performance in biology, especially regarding biochemistry topics.
- Students' prerequisite knowledge of chemistry can be improved by:
  - Scaffolding more fundamental chemistry skills into the biology course.
  - Providing supplemental chemistry materials for biology students.
  - Consideration of the implementation of a chemistry prerequisite rather than corequisite.

Other Considerations and Limitations

- Data were a sample of convenience, subject to response bias.
- GPA was the largest explanatory variable examined in the preliminary analysis, and its removal in order to reveal the nuanced factors at play impacted the significance of the overall models, leaving R^2 values low, explaining between 2 and 21% of the variance in the data.
- GPA and chemistry were highly correlated, so impacts due to chemistry may be inflated due to the association with GPA.
- Grit may also be correlated with another variable not present in this analysis, like a student's self concept or other factors.
- Both knowledge of biology and knowledge of chemistry were measured by proxy, and therefore are not perfect in their estimation of a student's subject knowledge in these areas.

CONCLUSIONS

- All variables considered provide only a small percentage of influence over students' grades.
- Chemistry provides more additive value on student grades than grit.
- It may prove beneficial to promote increased chemistry knowledge before taking Principles of Biology.
- More studies should be conducted to elucidate what traits or knowledge best prepare students for Principles of Biology.

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Oregon State University

Aquarickettsia rohweri Dynamics in Disease Susceptible and Resistant *Acropora cervicornis* Corals

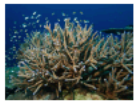
Shalvi Patel[1], Grace Klinges[1], Rebecca Vega Thurber[1] PhD

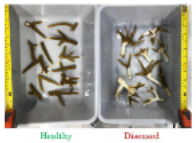
[1]Department of Microbiology, Oregon State University, Corvallis, OR, USA

Oregon State University Honors College

INTRODUCTION

- Coral reefs are one of the most diverse ecosystems
- Their structural complexity prevent erosion of shorelines and loss of life
- Over half a billion people depend on coral reefs for food, income, and culture
- Direct threats such as fertilizer pollution severely impacts the ability of the corals to survive
- Having a diverse microbiome with beneficial bacteria allows the coral to optimally adjust to changing environments



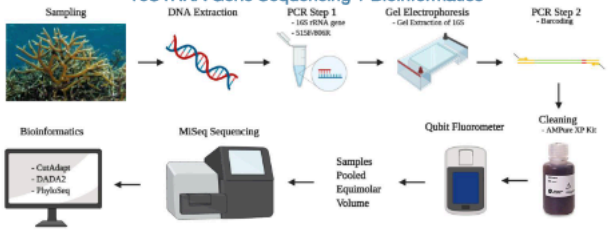


- *Acropora cervicornis* corals are among the most dominant reef building corals found in the Caribbean
- In the 1980s, they declined 80-98% from anthropogenic stressors and diseases placing them on the Endangered Species list
- One of the primary diseases responsible is White Band Disease (WBD) seen on the left, but little is known about the etiology.
- Previous work has discovered a parasite, *Aquarickettsia rohweri*, that is associated with WBD and is strongly stimulated by nutrient pollution.
- *A. cervicornis* genotypes that are susceptible to WBD have a high abundance of *A. rohweri* compared to resistant genotypes.
- This study hopes to further our understanding of the impact of nutrient exposure on the abundance of parasite, *A. rohweri*

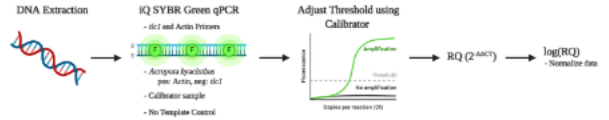
We hypothesize that nutrient exposure variably stimulates proliferation of *A. rohweri* in different *A. cervicornis* genotypes with combined nutrient exposure inducing the largest change.

METHODS

16S rRNA Gene Sequencing + Bioinformatics



qPCR + Normalization



RESULTS

16S Sequencing Richness between Weeks of Nutrient Exposure

Richness Between Weeks of Nutrient Exposure

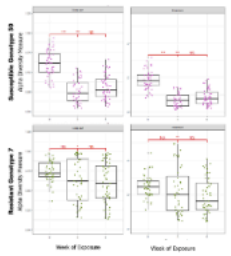


FIGURE 1. Boxplot comparing richness (alpha diversity) of genotype 50 and 7 *A. cervicornis* coral samples between the weeks of nutrient exposure using Simpson's and Shannon Index. Statistical analysis done using a Paired Wise Wilcoxon Rank Sum test. "****"=0.001, "***"=0.01, "**"=0.05

The significant decrease in richness in Genotype 50 samples at Week 3 from Week 0 suggests that three weeks is enough to induce a response in microbial community compared to genotype 7 samples which did not have significant decrease in richness until Week 6.

RESULTS

16S Sequencing Richness between Genotypes

Richness Between Genotypes

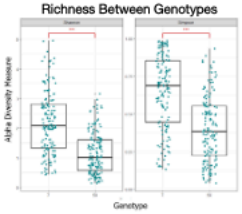
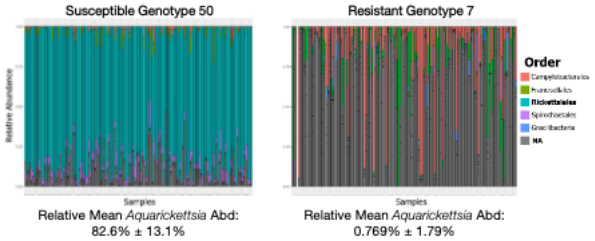


FIGURE 2. Boxplot comparing richness (alpha diversity) of genotype 50 and 7 *A. cervicornis* coral samples using Simpson's and Shannon Index. "****"=0.001

Genotype 7 samples had a significantly greater average richness than genotype 50 sample likely due to genotype 50 samples being dominated by *A. rohweri*.

16S Sequencing Relative Abundance



Relative Mean *Aquarickettsia* Abd: 82.6% ± 13.1%

Relative Mean *Aquarickettsia* Abd: 0.769% ± 1.79%

FIGURE 3. Relative abundance of top 10 most abundance taxa in genotype 50 and 7 *A. cervicornis* samples. Genotype 50 is dominated by *Aquarickettsia*, order Rickettsiales (teal). Genotype 7 is dominated by unclassified taxa (gray).

16S Sequencing Beta Diversity

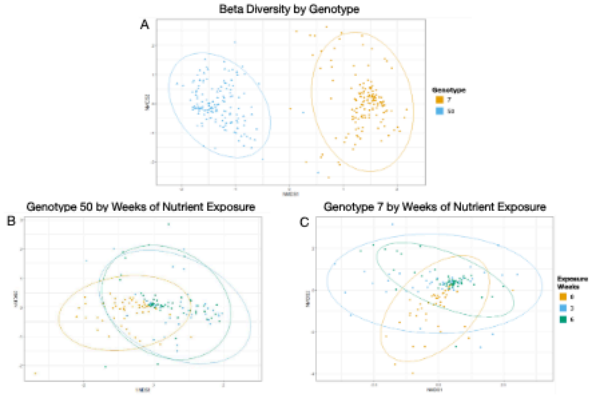


FIGURE 4. Beta diversity calculated using Bray-Curtis dissimilarity colored by **A.** *A. cervicornis* genotypes, **B.** Genotype 50 by weeks of nutrient exposure, and **C.** Genotype 7 by weeks of nutrient exposure. PERMANOVA test was done to determine significance.

A shows significant ($p<0.001$) clustering by *A. cervicornis* genotype (50 vs. 7)
B and C shows significant ($p<0.001$) clustering between Week 0 and 3/6 but not between Week 3 and 6 in *A. cervicornis* genotype 50 and 7 samples.

RESULTS

Dynamics of Parasite *A. rohweri* in Genotype 50 – Relative and Absolute Abundance

A. rohweri Abundance of High and Low Treatments Combined

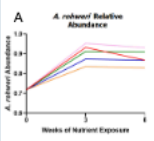
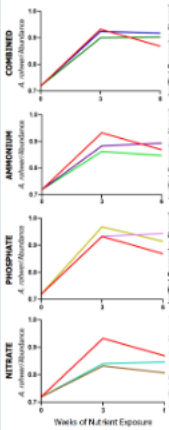


FIGURE 5. Line graph of **A.** relative *A. rohweri* abundance and **B.** log transformed absolute *A. rohweri* abundance from 3x and 2x ambient treatments combined for each nutrient.

Overall, the absolute abundance results corroborate relative abundance trends except in Control samples.

A. rohweri Abundance



Nutrient	2010s Looe Keys Water (µM)	2017 Canal Water (µM)
SRP	0.11 ± 0.01	0.01 ± 0.01
Total N	0.55 ± 0.05	3.10 ± 0.31
Ammonium	0.57 ± 0.04	1.33 ± 0.72

FIGURE 6. Line graph of relative *A. rohweri* abundance and log transformed absolute abundance of *A. rohweri* of 3x and 2x ambient nutrient exposure compared to control untreated corals for each nutrient.

Corals amended with additional nitrate had the lowest abundance of *A. rohweri* in absolute and relative abundance (not significant).

Corals amended with additional phosphate (phosphate treated and combined nutrient treated) had the biggest response in *A. rohweri* abundance (not significant).

CONCLUSIONS & FUTURE DIRECTIONS

Conclusions:

- qPCR results don't always reflect findings in amplicon data: highlights importance of performing qPCR along with 16S amplicon sequencing when evaluating a dominant microbial community member
- Time and off-reef incubation have a larger impacts on *A. rohweri* abundance in genotype 50 *A. cervicornis* samples than nutrient amendments in this experiment: most likely due to high nutrient concentration in canal water used in the aquaria
- Alarming that *A. rohweri* proliferates under control conditions: we may be inadvertently increasing disease susceptibility in reefs during reef restoration efforts
- *A. rohweri* abundance was lower than expected in genotype 7 *A. cervicornis* samples

Future Directions:

- Better assess which nutrient most stimulates proliferation of *A. rohweri* by conducting additional off-reef aquarium experiments using baseline water that is closer in nutrient concentration to Looe Keys water

ACKNOWLEDGMENTS

I would like to thank my mentor, **Dr. Rebecca Vega Thurber**, and **Grace Klinges, Ph.D. candidate** for their constant guidance throughout the project; my committee members, **Dr. Maude David** and **Dr. Nathan Kirk**; and all the members of the **Vega Thurber Lab.**

Department of Biochemistry and Biophysics, Oregon State University

Cloning the zebrafish otoferlin B wildtype and a transmembrane mutant to characterize changes in cellular localization

Rebecca France, Aayushi Manchanda, Josephine Bonventre, Colin P. Johnson

INTRODUCTION

- Ferlins are a family of large, eukaryotic calcium binding proteins that play an important role in membrane trafficking events.[1]
- Otoferlin is a protein essential in the process of hearing and mutations in otoferlin are associated with profound recessive deafness.[1]
- Otoferlin is known to bind calcium and is believed to play a role in calcium dependent exocytosis that leads to neurotransmitter release.[2] However, the mechanism by which this happens remains poorly understood. Additionally, the large size of the *OTOF* gene makes it a challenging model for gene therapy.[3]
- This project sought to characterize the difference between wildtype (WT) and a truncated mutant (MUT) otoferlin *in vitro* to go alongside *in vivo* studies on mutant zebrafish presenting with deafness and vestibular effects.
- The long-term goal of the Johnson lab is to understand how otoferlin contributes to hearing and why certain mutations result in deafness to be able to inform potential treatments.

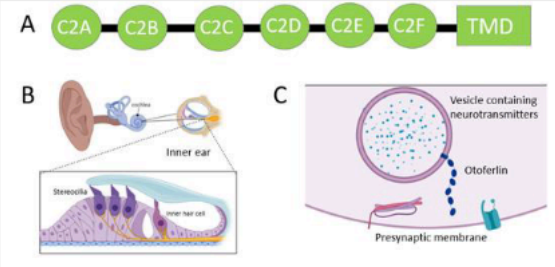


Figure 1. Otoferlin as a multi C2 domain protein. A) Schematic of the six C2 domains of otoferlin and the transmembrane domain. B) Diagram of the cochlea, inner ear, and inner hair cells, where otoferlin is expressed. C) Otoferlin binding to a vesicle with neurotransmitters right before it releases at the presynaptic membrane, which results in hearing.

METHODS

- The WT C2F-TM region of the zebrafish otoferlin B gene was amplified through a polymerase chain reaction. This was then inserted into a pcDNA plasmid containing Green Fluorescent Protein through Ligation Independent Cloning. The plasmid was transformed into bacterial cultures and miniprepped. Sequencing confirmed that the proper region was amplified and inserted into the pcDNA plasmid.
- Site Directed Mutagenesis created a mutant otoferlin with an early stop codon in the transmembrane domain that results in a truncated protein.
- The WT and MUT otoferlin plasmids were transfected into HEK 293 cells. A Hoechst blue nuclear stain and a red wheat germ agglutinin membrane stain were used when imaging the cells.
- Three representative images were selected for both the WT C2F-TM plasmid transfected cells and the MUT plasmid transfected cells. The percentage of cells containing otoferlin was calculated by dividing the cells showing colocalization by the total number of cells. This process was repeated twice more.

RESULTS

Wildtype Cell Images

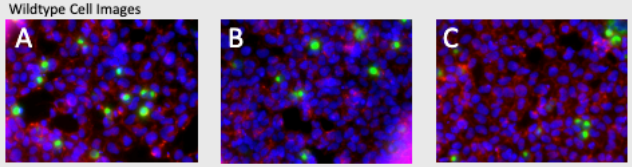


Figure 2. Overlay images of HEK293 cells transfected with WT zfotoB C2F - TM. A red membrane stain and a blue nuclear stain were used to visualize the cells. The overlay image indicates that at least some of the cells successfully took up the plasmid containing GFP and otoferlin.

Mutant Cell Images

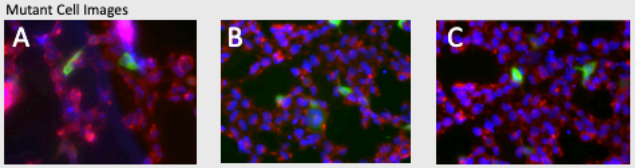


Figure 3. Overlay images of HEK293 cells transfected with MUT zfotoB C2F plasmid that is missing most of its transmembrane domain. Similar to the WT, the overlay image indicates that at least some of the cells successfully took up the plasmid containing GFP and otoferlin.

DISCUSSION

- HEK293 cells appeared to express more WT otoferlin (7.90%) relative to MUT otoferlin (6.37%) despite transfecting the same amount of plasmid.
- The MUT otoferlin may be trafficked differently and potentially degraded by the cell. However, selected cell images may not be representative of the entire plate.
- Future work includes transfecting a control with the C2F domain alone, repeating the transfections of WT C2F-TM and and MUT C2F-TM, and attempting to clone the full length zebrafish otoferlin B and transfect in HEK293 cells.

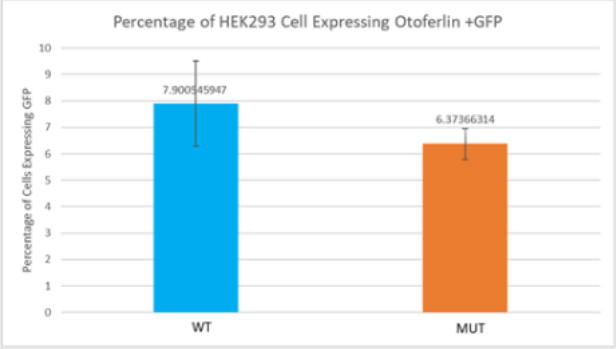


Figure 4. The percentage of imaged cells that showed green fluorescence localization with the red and blue fluorescence. The WT (M = 7.90, SD = 2.80) demonstrated a higher percentage of cells uptake the pcDNA plasmid containing otoferlin than the MUT (M = 6.37, SD = 1.00), but the results are not statistically significant, $t(4) = 0.889$, $p = 0.212$. Error bars represent the differences in counting the total number of cells in the three replicates.

CONCLUSIONS

- The MUT zebrafish otoferlin B C2F-TM appears to be trafficked differently than the WT and is possibly degraded by the cell.
- The transmembrane domain seems to be a critical component for any future gene therapy that is designed to treat deafness and should thus be included in any designed treatment.
- Studying the cellular localization of otoferlin *in vitro* allows us to learn more about this particular mutation as it relates to hearing loss.
- The long-term goal is to help recover hearing loss in affected individuals by better characterizing otoferlin for any future designed therapies.

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Oregon State University

Role of Nitrated Proteins in Glioblastoma Multiforme Cell Survival and Migration

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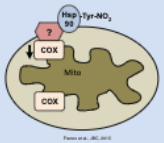
Honors Undergraduate Research Thesis Project

ABSTRACT

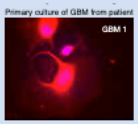
Glioblastoma multiforme (GBM) is the most aggressive brain tumor. GBM tumor samples show high levels of tyrosine nitration, an oxidative post-translational modification to proteins that occurs in pathological conditions. The molecular chaperone Heat-shock protein 90 (Hsp90) is one of the nitrated proteins that we detected in GBM. Hsp90 is involved in proteostasis, aiding in natural client protein folding and conservation as well as preventing unfolding in stressful environments. When normal Hsp90 becomes nitrated, it promotes disease progression by regulating glioblastoma cell metabolism. Intracellularly delivered nitrated Hsp90 activates the purinergic P2X7 receptor in neurons, inducing motor neuron death. The activation of the P2X7 receptor plays multiple roles: causing cell death in motor neurons, and supporting proliferation in cancer cells[2]. *We hypothesize that in GBM cells, nitrated Hsp90 activates the purinergic P2X7 receptor, supporting cell proliferation and migration.* In this projects, we employed two different cell culture models of GBM: human-derived U87 cells grown at low and high density. When U87 cells grow overconfluent in culture (high density), they form tumor-like cell aggregates (tumoroids) which we use as a three-dimensional cell culture model. In contrast, U87 cells maintained below 90% confluence (low density) grow as a two-dimensional monolayer. We found that prevention of tyrosine nitration using urate and edaravone significantly decreased survival of U87 cells at both low and high density, with a more pronounced effect in cells grown at low density. Addition of an antagonist of the P2X7 receptor, brilliant blue G (BBG), had similar effects in significantly decreasing cell viability in U87 cells. We also cultured GBM cells at low and high density in the presence and absence of urate and edaravone to study cell migration by performing wound healing assays. We found that preventing tyrosine nitration in U87 cells cultured at low density had a marked effect on wound healing with respect to control. In cells cultured at high density, preventing tyrosine nitration played two roles: decreasing wound healing and inhibiting the migration of U87 cells to form tumoroids when compared to control. The same result occurred when the P2X7 receptor was inhibited using BBG. Together, these results suggest that tyrosine nitration and the P2X7 receptor support GBM cell survival and migration.

INTRODUCTION

Nitrated Hsp90 downregulates Mitochondrial Metabolism

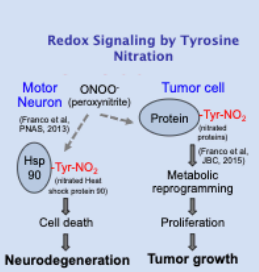


Human glioblastoma multiforme (GBM) Nitrotyrosine Staining

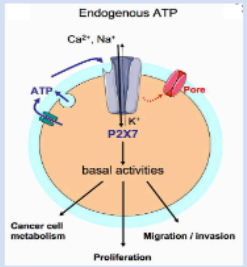


Nitrotyrosine – DAPI (nuclear staining)

Redox Signaling by Tyrosine Nitration

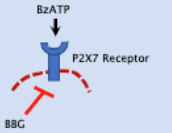


Role of P2X7 Receptor in cell growth

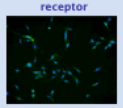


Urate and Edaravone Prevent Tyrosine Nitration

BBG Inhibits and BzATP activates P2X7 Receptor

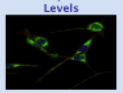


GBM cells express P2X7 receptor



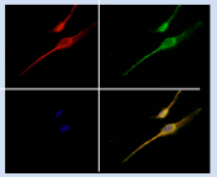
P2X7 receptor
DAPI

Nitrotyrosine Levels



Nitrotyrosine
Tubulin
DAPI (nuclear staining)

Nitrated Hsp90 is associated with mitochondria in GBM Cells (Confocal Images)

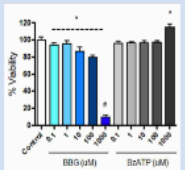


Mitochondrial complex IV
Nitrated Hsp90
DAPI

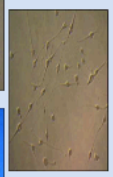
RESULTS

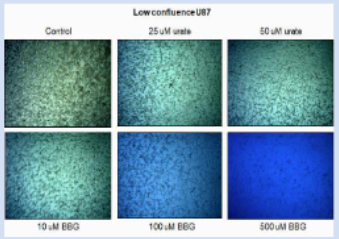
The P2X7 Receptor regulates Survival/Proliferation of GBM Cells

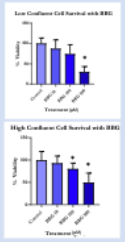
Low confluence U87 cells

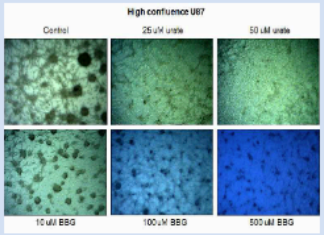


Pictures provided by Dr. Franco





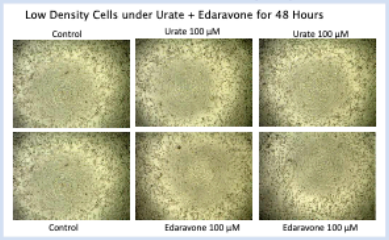


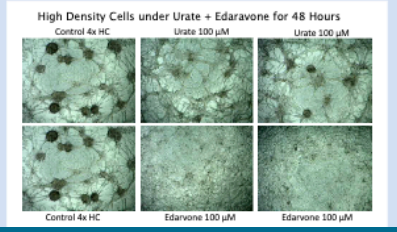


* p < 0.05 by one-way ANOVA and Bonferroni post-test

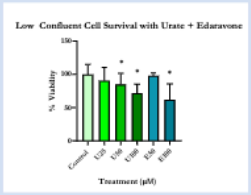
Nitrated proteins regulate Survival/Proliferation and Migration of GBM cells

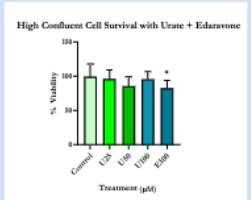
Tumoroid formation (48 h)





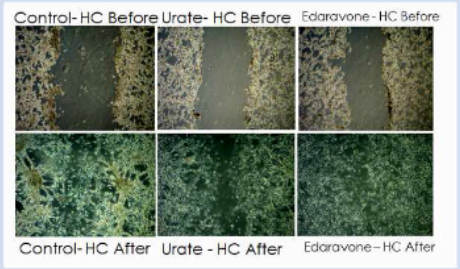
Cell viability (48 h)





* p < 0.05 by Krushkall –Walis and a Dunns post-test

Wound healing assay (48 h)



CONCLUSIONS

- **Nitrated proteins and P2X7R regulate cell survival, proliferation and migration in low-density and high-density human GBM U87 cells.**
- Our findings indicate that treatment of U87 cells with the P2X7 receptor inhibitor Brilliant Blue G, as well as urate and edaravone, to compounds used to prevent tyrosine nitration significantly reduced cell survival after 48 hours in both low and high density cell cultures.
- When cells were incubated for 48 hours, urate and edaravone also had an effect on the ability of cell to migrate to fill the gap in wound healing assays.

FUTURE DIRECTIONS

- Future directions include intracellularly delivering nitrated Hsp90 into normal human astrocytes to dissect the mechanisms by which nitrated Hsp90 regulates the metabolic phenotype of tumor cells.
- Additionally, cell migration will be quantitively measured to ensure that there are quantitative findings of the affected migration due to scavengers.

ACKNOWLEDGEMENTS

- We thank Jeanine Pestoni, Oliver Valdivia Camacho, Carrie Marean-Reardon, and Kyle Nguyen for their supervision and help in performing these experiments. We would also like to thank Sarah Gitto for the confocal images of U87 cell (Introduction).
- Mihir Palan was an URSA and CURE Fellow
- This work was supported by the Office of the Assistant Secretary of Defense for Health Affairs, through the Neurofibromatosis Research Program (NRP), New Investigator Award (NIA), under Award No. W81XWH-17-1-0409, and R01NS102479 from NINDS/NIH to MCF.

Social Context Affects Response to an Acute Stressor in Captive Red Crossbills (*Loxia curvirostra*)

Natsumi Tsuchihashi and Jamie Cornelius
Integrative Biology, Oregon State University

Introduction

- Social animals can use available information to inform behavioral and physiological responses to situations, but the way that information is processed can be influenced by the social context in which an event occurs.
- Consequently, the presence or absence of conspecifics can alter stress perception.
- In a recent experiment, red crossbills – a social songbird – that were housed in pairs (i.e., doubly-housed) lost mass when moved to a new room and presented with a neighbor cage, whereas individually-housed birds did not lose mass in the same situation.

- We tested the following non-mutually exclusive hypotheses in relation to how social grouping affects responses to a change in environment and the presentation of a neighbor.

Dominance Hypothesis: The weight loss observed in doubly-housed birds is due to the introduction of a neighbor, which may have temporarily disrupted the pre-existing dominance hierarchy.

Social Buffering Hypothesis: The maintenance of weight observed in previously isolated, singly-housed birds is due to social interactions mitigating the stress of moving rooms.

Methods

- 96 adult red crossbills were placed into cages based on their treatment group:

Alone Control – Individually-housed birds on a natural photoperiod, not paired on move day
Singly-housed – Individually-housed birds on a natural photoperiod, paired with a neighbor cage on move day
Doubly-housed – Doubly-housed birds on a natural photoperiod, paired with a neighbor cage on move day

- Visual barriers were put up during the pre-pairing period (8 weeks) such that individually-housed birds in each cage could hear, but not see, the birds in other cages. Doubly-housed birds could see and interact with their cage-mate.
- Birds experienced a change in environment (i.e., a new room) on move day, which is known to be a stressor, and were allowed visual access to a second cage of the opposite housing type (i.e., singles were paired next to doubles). Alone controls remained visually isolated in their new room.
- Mass and food intake was measured pre and post pairing to the nearest 0.01 gram using an electronic scale. Activity was recorded continuously pre and post pair using an infrared activity monitoring system by Starr Life Sciences. Activity was summed per hour and averaged across 24-hour periods for daily activity estimates.
- A least squares method was used to test for the impact of food intake, activity, treatment group, and interactions on the change in mass.

Results and Discussion

- The mean change in mass was significantly different by treatment (Figure 1, ANOVA – $F_{2,93} = 70.3$; $P < 0.0001$; $R^2 = 0.61$), with doubly-housed birds losing significantly more mass than singly-housed birds and alone controls (Tukey Comparison of Means, $P < 0.05$).
- These data suggest that treatment group (i.e., social context) affects whether mass was retained or lost after exposure to a stressor.

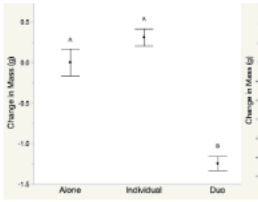


Figure 1. The mean change in mass (g) by treatment group, where the mean change in mass of doubly-housed birds (B) differs significantly from singly-housed birds (A). Error bars denote S.E.M. Groups with different letters are significantly different.

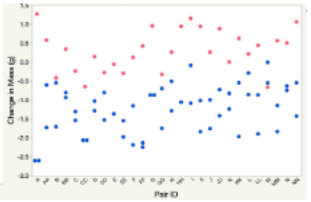


Figure 2: Changes in mass (g) by social pairing, where red represents individually-housed birds (individuals) and blue represents doubly-housed birds (duos). The change in mass is often similar within doubly-housed pairs (e.g., pairs A, BB, C, CC, D, etc).

Predictions and Results

1. If pairing induces stress in doubly-housed birds by upsetting dominance hierarchies, then we predicted that we would see increased activity, reduced food intake, and asymmetric weight loss in doubly-housed partners given that one bird of the duo would be excluded from food.

- For social pairings, there was almost no overlap between the change in mass for individually-housed birds and doubly-housed birds (Figure 2).
- Individually-housed birds consistently maintained or gained weight, while doubly-housed birds consistently lost weight.
- Pair ID was a nearly significant random effect ($P = 0.06$) in explaining mass loss in doubly-housed birds.
- These data suggest that doubly-housed birds may have responded similarly to their partner in response to the stressor.
- Because changes in mass were not asymmetric between duos, we can conclude that one bird was not being excluded from food. Therefore, we can reject the dominance hypothesis.

2. If social buffering mitigates stress in previously isolated, singly-housed birds, then we predicted that we would see a reduction in activity levels and/or an increase in food intake following pairing in singly-housed birds relative to those that remained in social isolation.

- The difference in the change in mass between singly-housed birds and alone controls was not statistically significant (Figure 1).
- There was no significant interaction between food intake and treatment group on the change in mass (LMM, $P = 0.88$; Figure 3b).
- There was a weak trend suggesting the interaction between activity and treatment group impacted the change in mass (Figure 3a, $P = 0.11$). In doubles, there was an inconsistent relationship between change in activity and change in mass (Regression, $P = 0.34$), but singles that had an increase in activity tended to lose more mass (Regression, $P = 0.08$).
- Treatment group significantly predicted change in food intake (Figure 4a; LMM, $R^2 = 0.2$; $t_{1,55} = 3.6$; $P = 0.0006$), but not change in activity (LMM, Figure 4b).
- Across all birds, treatment group was the only significant factor driving change in mass (LMM; $p < .0001$).

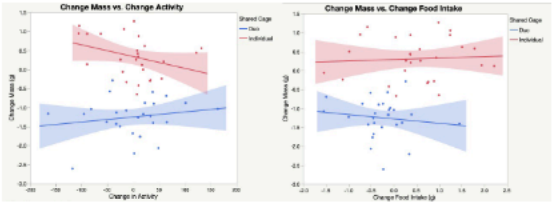


Figure 3a (left): Change in mass (g) versus change in food intake (g) by treatment group / Figure 3b (right) Bottom – Change in mass (g) versus change in activity by treatment group, with "duo" (blue) representing doubly-housed birds and "individual" (red) representing singly-housed birds. Best fit line and 95% confidence intervals shown.

- These data do not provide any direct support for the social buffering hypothesis, and it appears that social buffering did not occur in any context.
- However, stress physiology may still be playing an important role in our observations. Singly-housed birds and alone controls may have had an altered physiological state from being in long-term isolation, which affected their ability to activate the hypothalamic-pituitary adrenal axis in response to an acute stressor (i.e., moving to a new room).

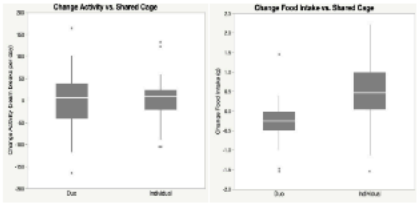


Figure 4a (left): Change in mass (g) versus change in activity (left) and Figure 4b (right): Change in mass (g) versus change in food intake by treatment group. Box plots denote first quartiles from median and whiskers denote 95% CI. Outliers shown as points.

Conclusions

- The changes in mass in doubly-housed birds did not seem to be due to dominance by any one individual and the social buffering hypothesis was not directly supported by these data.
- These data suggest that the mechanism driving this phenomenon may be a more complex intersection between social context and physiological decision-making in response to stress.

Lanthanide-Based Metal Organic Frameworks for CO_2 Capture and Conversion

David Le[1] and Kyriakos Stylianou[2]

[1]College of Engineering, School of Chemical, Biological, and Environmental Engineering
[2]College of Science, Department of Chemistry

Oregon State University

Goals

- Evaluate the performance of the metal organic framework, CeHTCPB, for the capture of CO_2 and catalysis of propylene oxide into propylene carbonate.
- Study the implications of using different lanthanides for CO_2 catalysis.
- Investigate the stability of CeHTCPB under repeated reaction cycles.

Background

- Metal–organic Frameworks (MOFs) are crystalline materials formed by a network of metal ions coordinated to organic ligands which are extended into one, two or three dimensions[1].

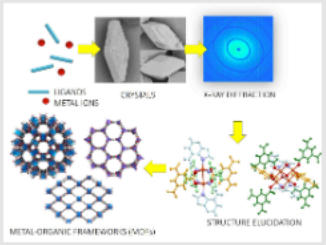


Figure 1. Typical steps for synthesis and structural elucidation of MOFs.

- The unlimited combination of metal ions (active sites) and ligands (structural functionalization) can generate infinite structures with applications in:
  - Carbon Capture and Utilization[2]
  - Photocatalysis[3]
  - Analyte sensing and capture[4]
  - Capture and separation of hazardous molecules[5]
- MOFs have key advantages over other materials being highly modular, structurally versatile, and possessing high porosity (100 – 4500 $m^2 g^{-1}$)[1].
- Lanthanides are excellent hard Lewis Acids due to their high charge valency. This means that are exceptional for binding to lone electron pairs of oxygen containing molecules[5].
- In particular, the MOFs studied in this project, CeHTCPB and other lanthanide derivatives, are water stable, synthesized under green conditions, and thermally robust[6].

Why Propylene Carbonate?

- Propylene carbonate is widely used as a film-forming and high boiling point solvent in paints, industrial cleaners, polymer plasticizers, pesticides, dyes, and cosmetic products[7].
- It can also be used as an amine alternative for CO_2 and H_2S removal from acid gases and natural gases[7].
- The high dielectric constant of propylene carbonate makes it an important solvent in electrolytes for lithium-ion batteries[7].

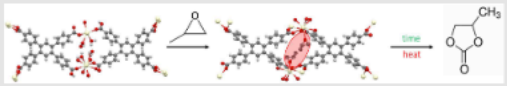


Figure 2. Reaction pathway for incorporating CO_2 into propylene oxide using CeHTCPB. Binding of CO_2 was confirmed by single crystal x-ray diffraction.

Characterization of Materials

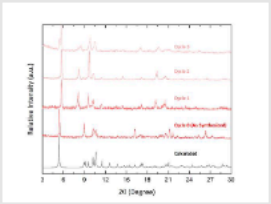


Figure 3. Powder x-ray diffraction plots. The relatively similar peaks of the material demonstrates retention of crystallinity and phase purity after three reaction cycles.

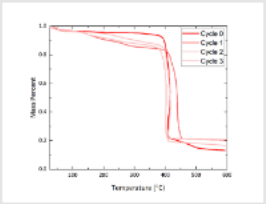


Figure 4. Thermogravimetric analysis plots. The material remains thermally stable up to >400 °C after three reaction cycles.

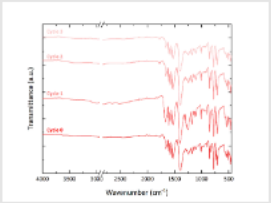


Figure 5. Fourier Transform IR spectra. All peaks remain the same after three reaction cycles demonstrating structural integrity of the MOF.

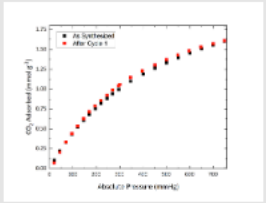


Figure 6. CO_2 isotherms of CeHTCPB before and after catalysis. The CO_2 uptake capacity remains the same at ~1.60 mmol g^{-1}.

Methods

- CeHTCPB and derivatives incorporating Nd, Sm, Eu, Tb, and Dy were synthesized and characterized to evaluate crystallinity, thermal stability and CO_2 uptake capacity.
- Propylene oxide to propylene carbonate conversion with MOFs was performed in 25 mL stainless steel autoclave reactors under 10 bars CO_2 and 100 °C for 12 hours.
- After the reaction, ^1H NMR was used to determine the yield of propylene carbonate.
- Catalysts were recovered after filtering, washing with DI H_2O/acetone, and drying.
- Additional studies were done with mixed gases, aqueous-rich solvent mixtures, and recycled materials.

Catalytic Performance

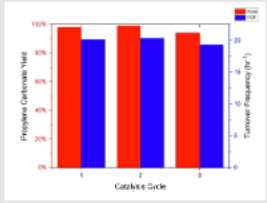


Figure 7. Yield of propylene carbonate for three conversion cycles. The conversion is nearly the same at ~94 - 99%.

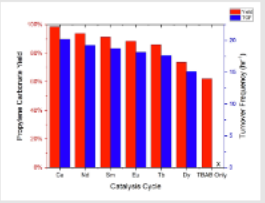


Figure 8. Yield of propylene carbonate for all materials synthesized. As the atomic number of the metal active site increases, the Lewis acidity of the catalyst increases which decreases the reaction rate.

References

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Oregon State University- Department of Integrative Biology

Qualitative Food Web Dynamics in Response to Extinction and Invasion

David J. Rockow

Oregon State University

Introduction

Predicting how species abundances will react to perturbations is fundamental to numerous applications of real-world ecology, including assessing the potential impacts of climate change and setting maximum yields on fishery harvests. However, due to the complexity of food webs, which contain many direct interactions and indirect effects, predicting the qualitative responses of food webs with certainty is an impossible task. The goal of this project was to determine the impact of interaction strength uncertainty on the predictability of species responses to press perturbations in webs of varying species richness due to extinction and invasion.

Methods

A ten species food web was constructed computationally in R, using the niche model. Thirteen variations were made on this original web. Ten webs were created that simulated species extinctions, each with one of the original ten species missing. Three webs were created with a species added, simulating species invasions. One web added a basal species, one an intermediate consumer, and one an apex predator. In each of these webs each species was assigned a trophic level. In the original web species 1-3 are basal, species 4 and 6 are primary consumers, species 5 and 7-9 are secondary consumers, and species 10 is an apex predator. Body masses were assigned based on trophic level. Basal species were assigned a body mass of 1/42 grams, primary consumers 1 gram, secondary consumers 42 grams, and apex predator 1,764 grams, reflecting a one step increase in trophic level is associated with a 42-fold increase in body mass, on average. The body masses of each species were used to determine the parameters as shown in the equations section. Using the calculated parameters, the Jacobian matrix was constructed for each web. The negative inverse of the Jacobian summarizes how species will react to press perturbations as direct effects cascade throughout the web. Each species was assigned an intraspecific link, which was made increasingly negative to achieve stability. The negative inverse Jacobian was subjected to increasingly higher levels of interaction strength uncertainty, which were compared to when the interaction strength uncertainty was zero, to analyze how many interactions underwent sign switches, and are thus considered mispredictions.

Equations

Parameter	Figure	Citation
Density ($\frac{g}{m^2}$)	$e^{-14.29}$ • (Body Mass)$^{-0.77}$	Brown et al., 2004
Biomass ($\frac{g^2}{m^2}$)	Body Mass • Density	Brown et al., 2004
Metabolic Losses ($\frac{\text{Joules}}{\text{Year}}$)	Basal: 0 Primary: 17.17 • (Body Mass)$^{-0.29}$ Secondary: 18.18 • (Body Mass)$^{-0.29}$ Apex: 19.50 • (Body Mass)$^{-0.29}$	Gauzens et al., 2018
Growth Rate ($\frac{\text{Joules}}{\text{Year}}$)	0.71 • (Body Mass)$^{-0.25}$ (If Basal, Zero for Non-Basals)	Gauzens et al., 2018
Feeding Efficiency (Unitless)	0.545 if Basal 0.906 if Non-Basal	Gauzens et al., 2018

Key Terms

Press Perturbation- Persistent disturbance on a food web that creates a new equilibrium

Species Richness- Number of species in a web

Connectance- Proportion of potential interactions that are realized ($\frac{\text{\# of Interactions}}{\text{Species Richness}^2}$)

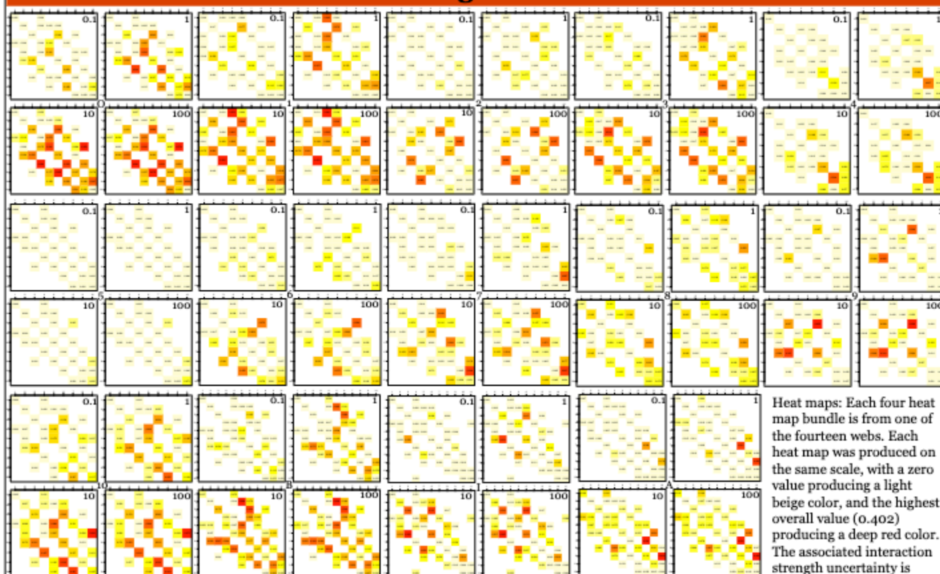
Diagonal Interactions- Impacts of a species on itself (intraspecific)

Upper Triangle Interactions- Impacts of a predator on its prey

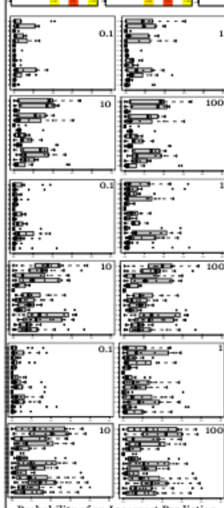
Lower Triangle Interactions- Impacts of prey on its predator

Trophic Level- The approximate hierarchical level of a species in a food web

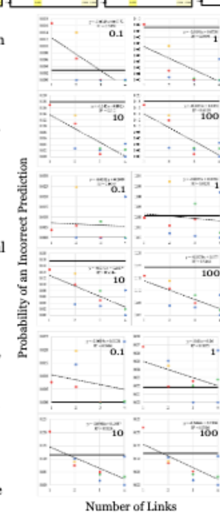
Figures



Heat maps: Each four heat map bundle is from one of the fourteen webs. Each heat map was produced on the same scale, with a zero value producing a light beige color, and the highest overall value (0.402) producing a deep red color. The associated interaction strength uncertainty is shown in the top right, and the associated web is shown in the middle of the bundle. The figures in each heat map are the percent of mispredicted interactions, when that interaction was misestimated.



Each of the three boxplot bundles is from one interaction type. Diagonal is on top, lower triangle in the middle, and upper triangle on the bottom. Interestingly, for the diagonal and lower triangle interactions, the largest median probability of an incorrect prediction belonged to the original web, but for the upper triangle interactions, the largest median belonged to the web where Sp. 1 was removed. In every plot, the web that was the easiest to correctly predict was when Sp. 5 was removed. Increasing the interaction strength uncertainty beyond 10 had little impact on the probabilities.



Each of the three scatterplot bundles is from one interaction type. Diagonal is on top, lower triangle in the middle, and upper triangle on the bottom. With the diagonal interactions, there existed a strong negative correlation between number of links had by a species and probability of an incorrect prediction of the remaining responses after it was removed, at all levels of interaction strength uncertainty. For the lower and upper triangle interactions, a strong correlation only existed at larger values of interaction strength uncertainty.

Results

1. Species extinctions and invasions both generally made species responses easier to predict.
2. Intraspecific interactions can be the least accurately estimated in real-world systems.
3. There exists a strong negative correlation between links had by a species, and probability of an incorrect prediction after that species was removed.

Discussion

I suspect that species extinctions generally made responses easier to predict because species extinctions simplified the network. By reducing the species richness, the number of direct interactions and indirect effects, also decrease. The only species extinction that produced harder to predict responses was removing species one. I suspect this occurred because, in the original web, species one was the least connected species, having just one direct interaction. In the original web it produced the smallest probability values. Thus, removing species one removed with it these easy to predict responses, increasing the average response value. Interestingly, in the original web lower triangle responses were harder to predict, but when species one was removed, upper triangle responses were harder to predict. Overall, whether webs had easier to predict upper or lower triangle responses varied, and was split evenly among which of these response types were easier to predict. Why this pattern occurred requires more attention and should be analyzed in future research. While lower and upper triangle responses were were relatively similar, diagonal responses were far easier to correctly predict. In future work intraspecific interactions do not have to be as accurately estimated as interspecific interactions, as misestimating intraspecific interactions least impacts the probability of accurately predicting species responses. Species invasions also made species responses easier to predict. I suspect this occurred because adding the additional species may have made the network unstable. Instability is positively correlated with network size. When networks become unstable the full interaction strength uncertainty cannot be applied. If the full interaction strength uncertainty was not applied than the webs created with an added species were simulated with systematically lower levels of error, causing their values to be systematically underestimated.

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College of Science | Department of Integrative Biology

TISSUE DEHYDRATION PREVENTION

Post-Preservation Maintenance of Cadavers to Prevent Tissue Dehydration in Educational Environments

Author: Alexandria Herrera Mentor: Dr. Devon Quick Committee Members: Dr. Brian Bay, Tamara Ostervoss

BACKGROUND

The human cadaver has held the spotlight in anatomy and physiology education for centuries, with dissection traced back to the 3rd century BC in Ancient Greece [1]. Medical and non-medical students rely on cadaver dissection and observation for a deeper understanding of the structural and functional anatomy that exists inside of us. It is imperative that the tissue hydration is maintained throughout the study [2]. Applying rehydrating solutions and evaporation-preventing materials to the tissues can prevent dehydration. Dehydration prevention is important for quality tissue presentation, to extend the study period of distinct anatomical structures, and to respect and honor those who choose to donate their bodies to science and education.

Current Oregon State University dehydration prevention protocols:

1. Re-covering the exposed tissues with the respective cadaver skin
2. Applying layers of plastic wrap, towels and shrouds soaked in a wetting solution to the external surfaces of the body

This study aims to identify ideal material coverings that outperform the current means.

It was hypothesized that an artificial material will maintain tissue hydration levels more effectively than the current methods deployed in the OSU Anatomy and Physiology labs.

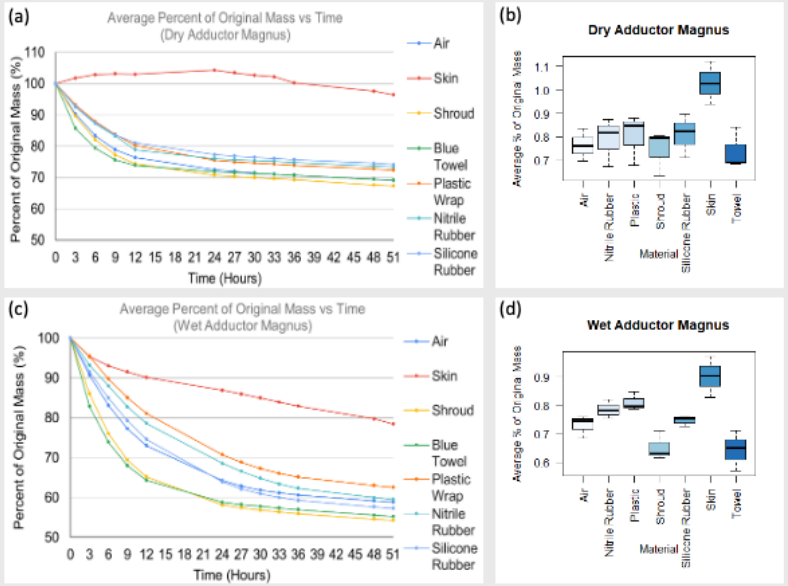

Figure 1. The average percentages of original mass of dry [top left] and wet [bottom left] adductor magnus samples generally exhibit an exponential decay over time. Samples covered in skin break this trend as mass tended to increase for several hours before any decrease. Results of an ANOVA & Multiple Comparisons Test reveal the average mass loss differences between samples of different coverings [top & bottom, right].

METHODS

- Candidate materials were selected based on mechanical and physical properties that are similar to human skin
- Tissue samples from the adductor magnus and oblique muscles were extracted using a 10mm punch biopsy (Figure 3)
- Each sample was covered by one of seven materials: skin, shroud, blue towel, plastic wrap, nitrile rubber, silicone rubber, or air (control) (Figure 3)
- Half of the samples were subjected to wetting solution, half were left dry
- The mass of each sample was recorded every few hours for a total of 51 hours (Figure 3)
- Material performance was determined from overall sample dehydration (mass loss)
- Temperature and humidity of the main sample environment were monitored
- ANOVA and Multiple Comparisons tests were performed on data from hour 12 (the longest storage period for an OSU lab cadaver, barring weekends and breaks)

Figure 2. Depiction of prone cadaver from a posterior view by Donovon Horst, featuring superficial back muscles and respective coloration just after skin removal.

RESULTS

- Skin is the only covering that demonstrated a statistically significant mass retainment when compared to samples exposed to air (Fig. 1b, 1d)
- Dry samples covered in skin gained mass before losing it again (Fig. 1a, 1c)
- By hour 12, samples covered in plastic wrap, nitrile rubber, and silicone rubber, generally retained more mass than the other samples (Fig. 1)
- Samples covered in towel or shroud experienced greater decreases in mass than the samples in air (Fig. 1)
- Wet oblique muscle samples covered in nitrile rubber and plastic wrap retained significantly more mass than those covered in towel
- Wet adductor magnus samples covered in plastic wrap preserved significantly more mass than those covered in towel and shroud (Fig. 1d)
- Samples of the same muscle and wetness that were covered in nitrile rubber retained significantly more mass than those covered in towel (Fig 1b, 1d)

DISCUSSION & RECOMMENDATION

Within twelve hours of tissue extraction, skin significantly outperformed all other material coverings, unexpectedly rehydrating the dry tissue samples for several hours before permitting dehydration to occur. This study provides concrete evidence that re-covering the cadavers in their respective skin is the superior method for hydration maintenance.

With respect to wet muscle samples, nitrile rubber and plastic wrap maintain tissue hydration better than those wrapped only in towels and shrouds.

Samples covered in towel and shroud experienced more dehydration than samples exposed to air. These findings suggest that dry towel and shroud coverings may be more harmful to the quality of muscles than neglecting to cover the tissues at all, likely absorbing moisture from the muscle samples faster than the moisture would have dried via unfacilitated evaporation.

Recommended dehydration prevention strategies for the OSU cadaver lab are as follows:

1. Primarily, skin should directly cover the cadaver for as long as possible (no intermediate material)
2. If skin is not available, cover the tissues with a combination of plastic wrap and nitrile rubber
3. Cover the cadaver with a shroud soaked in wetting solution or a black plastic tarp in order to maintain a respectful presentation of the donor

Figure 3. Mettler AT400 Digital Balance used to weigh tissue samples [top], 10mm punch biopsy used to extract skeletal muscle samples [middle], and materials used to cover samples, skin not depicted [bottom].

Figure 4. Lateral view of prone cadaver by Donovon Horst.

ABSTRACT

In anatomical education, clinicians, surgeons, and anatomists support the use of human cadavers over digital or artificial alternatives. Cadaver use raises the challenge of preventing tissue dehydration. The OSU cadaver labs attempt to maintain hydration by re-covering the exposed tissues with original skin, layers of plastic wrap, and/or towels and shrouds soaked in a wetting solution. It was hypothesized that an artificial material will maintain tissue hydration levels more effectively than the current methods deployed in the OSU labs. Candidate tissue coverings were selected based on their material properties and relative costs. Punch biopsies of skeletal muscles were subjected to different coverings, and half were soaked in the wetting solution. Changes in tissue masses over time were recorded and presented as dehydration trends. It was found that skin is the most effective covering to prevent tissue dehydration, followed by plastic wrap and nitrile rubber. Respective donor skin should directly cover the cadaver tissues during storage hours. Alternatively, a combination of plastic wrap and nitrile rubber coverings can prevent dehydration. In both cases, wetting solution should be applied to the tissues, and a shroud or black plastic cover should be placed over the cadaver to maintain donor dignity.

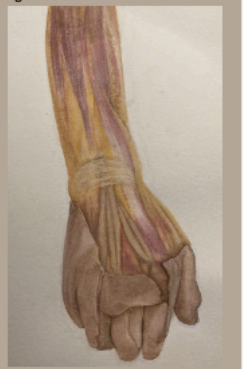

Figure 5. Posterior view of wrist and finger extensor muscles by Donovon Horst.

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Oregon State University

Effect of protein nitration on cell survival pathways in Schwann cells

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Oregon State University

Honor Thesis Research Project

CDMRP Neurofibromatosis Research Program

ABSTRACT

Neurofibromatosis type 2 (NF2) is a genetic disorder of the nervous system caused by inactivation of the merlin tumor suppressor gene. NF2 patients develop bilateral vestibular schwannomas (VS) and other nervous system tumors throughout their life for which there is no effective drug treatment. Production of the oxidant peroxynitrite occurs in pathological conditions and leads to tyrosine (Tyr) nitration of proteins. Although Tyr nitration is found in multiple tumor types, its role in tumorigenesis is unknown. We discovered that VS and human merlin-deficient (MD) Schwann cells show significantly increased levels of peroxynitrite and Tyr nitration compared to normal Schwann cells. Notably, prevention of tyrosine nitration selectively decreases MD-Schwann cell survival. Our goal is to identify nitrated proteins that support VS growth as new potential tumorspecific therapeutic targets, enabling the development of safe pharmacological strategies for NF2 schwannomas. This is the first focused effort to determine the signaling pathways regulated by tyrosine nitration in pathological conditions. By performing immunoprecipitations using a polyclonal anti-nitrotyrosine antibody followed by mass spectrometry analysis and western blot, we identified a limited number of proteins endogenously nitrated in VS from NF2 patients, and in mouse and human MD-Schwann cells. A group of the identified proteins play a role in pathways that are dysregulated in NF2. We then performed phosphorylation arrays for RTKs, and phospho-kinases, to further establish this relationship between protein nitration and NF2. While RTKs were unaffected, phospho-MAPK arrays demonstrated a role of nitration in supporting survival pathway activation in MD-Schwann cells, including the PI3K/Akt pathway, and the MEK/ERK pathway. The identification of the specific nitrated proteins that promote schwannoma growth could provide exceptional novel targets for the treatment of NF2 and possibly other tumors of the nervous system as well. Supported by DoD, NRP, NIA W81XWH-17-1-0409 (to MCF).

INTRODUCTION

Neurofibromatosis type 2

Single-gene disorder of the nervous system
1:25,000 people

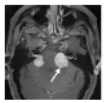
Mutations in the *NF2* gene that codes for merlin tumor suppressor

⇩

merlin loss of function

⇩

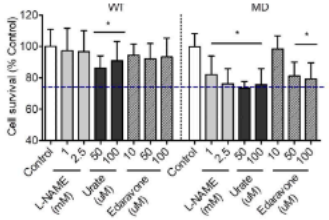
Development of benign, slow growing tumors: schwannomas, meningiomas, and ependymomas



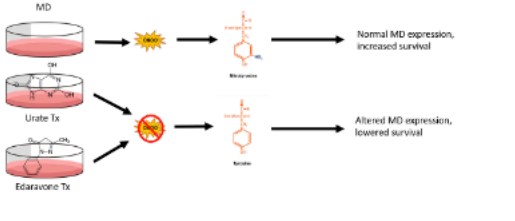
Bilateral vestibular schwannomas in NF2 patient (MRI) (http://www.mayoclinic.org)

BILATERAL VESTIBULAR SCHWANNOMAS are diagnosis of NF2

Prevention of Tyrosine Nitration Decreases MD-HSC Survival



Preventing Nitration may Impact Survival Pathway Expression



RESULTS

Mass Spectrometry Analysis

Nitrated proteins in vestibular schwannomas (VS) from NF2 patients

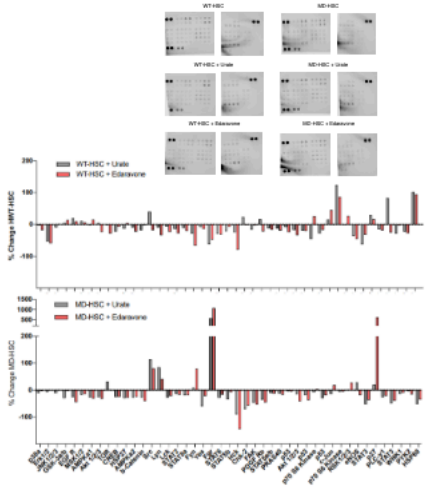
Function	Protein	Peptides (CI 95%)
Cytoskeleton	Vimentin	41
	β-actin	17
	β-tubulin	13
Structural (nucleus)	Histone H4	4
	Histone H2A	4
	Histone H2B	3
	Histone H3	1
Chaperones/ Protein folding	Hsp90	4
	Hsp70	3
	14-3-3	7
	Peptidyl-prolyl cis-trans isomerase A	1
Translation	Elongation factor 1-α 1	4
Metabolism (cytosol)	GAPDH	4
	L-lactate dehydrogenase	3
	Phosphoglycerate kinase	2
	Alpha enolase	1
	Pyruvate kinase	1
Metabolism (mitochondria)	ATP/ADP translocase	3
	ATP synthase subunit alpha	1
Antioxidant defense	Peroxiredoxin 1	1
	Peroxiredoxin 2	2
	Peroxiredoxin 6	1
Signaling	Galectin 1	2
	Ras-related protein Rab	2
	Moesin	2
	Ezrin	1
	Radixin	1
	Rho-related GTP-binding protein RhoC	1
	Transforming protein RhoA	1
	S100 protein A6	1
	S100 protein A9	1

Nitrated proteins in human merlin deficient Schwann cells (MD-HSC)

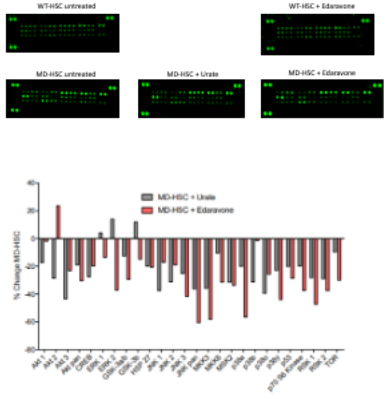
Function	Protein	Peptides (CI 95%)
Cytoskeleton	Vimentin	20
	β-actin	28
	β-tubulin	8
	cofilin-1	1
	cofilin-2	1
Structural (nucleus)	Histone H4	10
	Histone H2A	6
	Histone H2B	11
	Histone H3	3
Chaperones/ Protein folding	Hsp90	2
	Hsp70	6
	14-3-3	1
	SERPINH1(endoplasmic reticulum)	8
	Peptidyl-prolyl cis-trans isomerase B	3
	Stress-70 (mortalin) (mitochondrial)	2
	Hsp60 (mitochondrial)	2
Translation	Elongation factor 2	2
	Eukaryotic initiation factor-4A	2
	Eukaryotic initiation factor-5A	1
Metabolism (cytosol)	Pyruvate kinase	3
Metabolism (mitochondria)	ATP/ADP translocase	1
	ATP synthase subunit alpha	5
Antioxidant defense	Peroxiredoxin 1	1
	Peroxiredoxin 2	1
Signaling	Galectin 1	2
	Ras-related protein Rab	2

*Underlined proteins were also found in human wild type Schwann cells (WT-HSC)

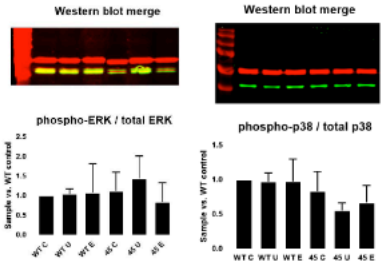
Phospho-kinase Arrays



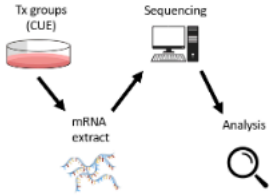
Phospho-MAPK Phosphorylation Arrays



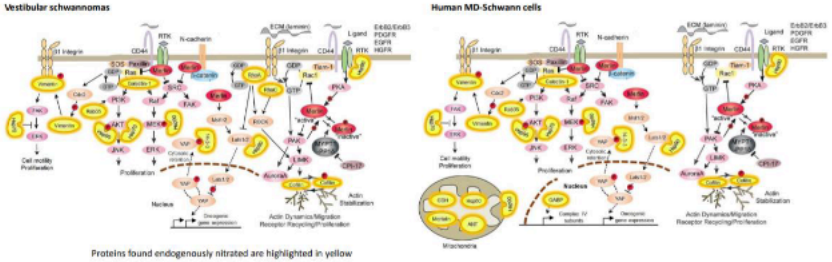
Survival Pathway Western Blots



Unbiased Transcriptome Analysis



Location of Endogenously Nitrated Proteins in Signaling Pathways Dysregulated in NF2 Vestibular Schwannomas and Human MD-Schwann Cells



Proteins found endogenously nitrated are highlighted in yellow

CONCLUSIONS

- We identified endogenously nitrated proteins in Vestibular Schwannomas from NF2 patients and MD-Schwann cells.
- Prevention of tyrosine nitration results in decreased pro-proliferative survival pathway activation in MD-Schwann cells, including Akt, JNK, and ERK.
- Prevention of tyrosine nitration does not result in significant changes in RTK activation.
- Western blots were inconsistent with array results, under identical cell conditions.

Together, these results suggest that nitrated protein regulate relevant signaling pathways in NF2 schwannoma cell culture models to promote cell survival and proliferation

FUTURE DIRECTIONS

- Repeat Western Blots for all proteins of interest.
- Repeat experiments for MTC10 cell model.
- Send out mRNA for unbiased transcriptome and pathway analysis.

ACKNOWLEDGEMENTS

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- Thank you to Carrie Marean-Reardon for assistance in cell culture.
- This work was supported by the Office of the Assistant Secretary of Defense for Health Affairs, through the Neurofibromatosis Research Program (NRP), New Investigator Award (NIA), under Award No. W81XWH-17-1-0409, and R01NS102479 from NINDS/NIH to MCF.

Oregon State University - Undergraduate Honors College Thesis

Janus-Type Microfluidic Devices for Separation and Collection of Plasma from Blood

Linus J. Unitan
Mentor: Dr. Vincent T. Remcho

Introduction

- Microfluidic analytical devices (μADs) are a simple, inexpensive, and easy to use device platform which enables chemical analysis at pL-μL sample and assay reagent volumes.
- Fabrication of μADs involves patterning of hydrophilic substrate material with hydrophobic barrier material.
- The combination of hydrophilic, chemically inert glass microfiber (GMF) membranes and biodegradable and biocompatible polycaprolactone (PCL) provides a unique substrate material for fabrication of wicking microfluidic devices.
- PCL-filled GMF substrate can be patterned into 2D and 3D microfluidic pathways by selective oxygen radical exposure [1].

Application and Significance

- μADs are a promising alternative for many resource-intensive analytical and sample preparation techniques.
- As the fluid transport mechanism of μADs is based on capillary action, it is challenging to separate the liquid phase from the device, limiting the use of the liquid phase for further analysis off of the wicking microfluidic platform.
- Enabling separation and collection of blood plasma on a single device expands the utility of these devices for use with any assay platform.
- We present a new Janus-type wicking-capillary μAD which bridges wicking and cut capillary-based microfluidics to enable fluid collection from wicking μADs. We also demonstrate the utility of this device as a blood plasma separation and collection device, and as a gated fluid control mechanism.

Optimization

Table 1: Wicking fluidic pattern resolution with 10 μL of 70% w/v BSA solution at different PCL thicknesses and molecular weights

w/v	25K Da Wicking	25K Da Control	37K Da Wicking	37K Da Control	50K Da Wicking	50K Da Control
5%						
7.5%						
10%						
15%						
20%						

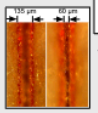


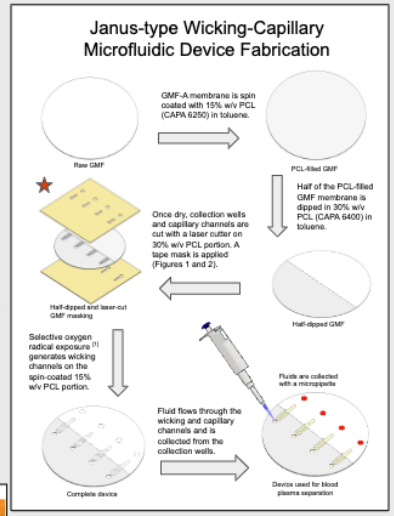
Figure 1: Maximum width (left image) and minimum width (right image) of laser-cut capillary channels

Figure 2: Complete device tested with BSA (top-surface view)

Experimental Results

- **With initial sample volume of 10 μL:**
  - Up to 3 μL of aqueous sample was collected
  - Up to 1.5 μL of 70 mg/dL BSA protein solution was collected (simulates human blood plasma) (Figure 2)
- **Preliminary results with real canine blood sample (EDTA treated)**
  - Demonstrated effective separation of plasma from whole blood
  - Plasma flows down cut capillary channels (Figure 3)
  - <1 μL of plasma collected out of 10 μL initial blood sample introduced (~20% yield)
- **Fluid yields can be improved**
  - Increase sample volume and optimize the pattern geometry
  - Optimization of PCL thickness and molecular weight for the wicking portion of the device (Table 1)

Janus-type Wicking-Capillary Microfluidic Device Fabrication



★Wicking channel geometries can be altered by changing the mask geometry (Figure 4, left and center) in order to create varied fluid flow features, such as dilution, separation, and mixing to assist different methods of sample preparation.

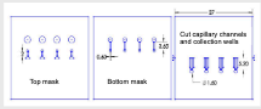


Figure 4: Wicking, cut-capillary, and collection well feature dimensions (mm)

Figure 3: Device used with EDTA-treated canine blood. Separated plasma is collected from rastered wells.

Gated Fluid Control

- Slight overlapping of the wicking and cut capillary features allows fluid to flow continuously between the two halves of the device, enabling fluid collection.
- Continuous fluid flow is prevented when wicking and cut capillary features are slight offset, introducing one-way gate functionality.
- Fluids introduced into the wicking portion of the device do not continue to flow into the cut capillaries, but solution introduced to the cut capillaries will reach the wicking portion of the device (Figure 5).
- This functionality enables integration of separate multi-step reactions on a single wicking microfluidic platform.

Figure 5: One-way gate mechanism. Red aqueous dye is introduced at the top of the wicking channel, and blue dye is introduced into the rastered well.

Remcho research group

Conclusions

- Treatment of GMF with PCL at different molecular weights allows for fabrication of wicking and cut capillary fluidic channels on a single device.
- The fabrication method consists of steps that are easily automated, such as membrane spin-coating and oxygen radical exposure.
- A Janus-type wicking-capillary μAD was developed that enables fluid collection after fluid has passed through the device substrate.
- These devices enable integration of on-device sample preparative and end assay chemistries using microscale reagent volumes, and are suitable for a broad range of detection methods, both on and off-membrane.
- Cut capillary channels can be used to guide fluid flow.
- Wicking and cut capillary features can be slightly offset to introduce one-way gate functionality.
- These devices successfully separate blood plasma from whole blood and enable collection of separated plasma for further analysis.
- On-device blood separation is achieved with minimum sample volume (~10 μL), minimizing patient discomfort and biohazard waste.

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1. G. C. Bandara, C. A. Heist, and V. T. Remcho, *Talanta* 2015, **176**, 589-594.

Acknowledgements

- Committee members: Dr. Vincent Remcho, Dr. Devon Quick, Dr. Gayan Chandima Bandara
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- Honors College, Oregon State University
- Department of Chemistry, Oregon State University

Oregon State University

Oregon State University Honors College

Linus Pauling Institute & Department of Biomedical Science, Carlson College of Veterinary Medicine

EFFECTS OF AGE MILITARY SERVICE ON MEMORY AND COGNITIVE FLEXIBILITY IN A VIRTUAL MORRIS WATER MAZE TASK.

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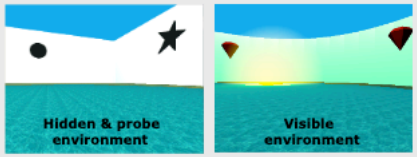
INTRODUCTION

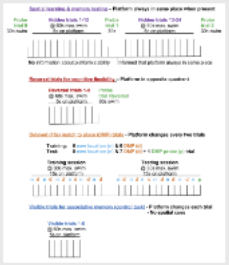
In order to enhance translation between rodent and human studies, we developed a virtual version of the Morris water maze (vMWM) and successfully applied it to show age-related differences in human males performing a spatial memory version of the task (Zhong et al., 2017, Behav. Neurosci. 131:470). This study expanded to examine the sensitivity of the vMWM tasks to aging of both genders on memory, reversal, and delayed matching to place performance in the vMWM as compared to standardized human cognitive tasks in the NIH Toolbox and the Wechsler Memory Scale (WMS) Logical Memory test. We also examined whether military service may be a hidden variable in cognitive aging studies.

METHODS

Participants: Young (21-31 years old) and older (60-86 years old) males and females were tested. Based on self-report, all participants were assessed to be in good health, without medications or conditions that could impact cognitive function. Older males could be divided into veterans from the Korean and Vietnam war eras or age-matched civilians. The veterans self-identified as performing non-combat service.

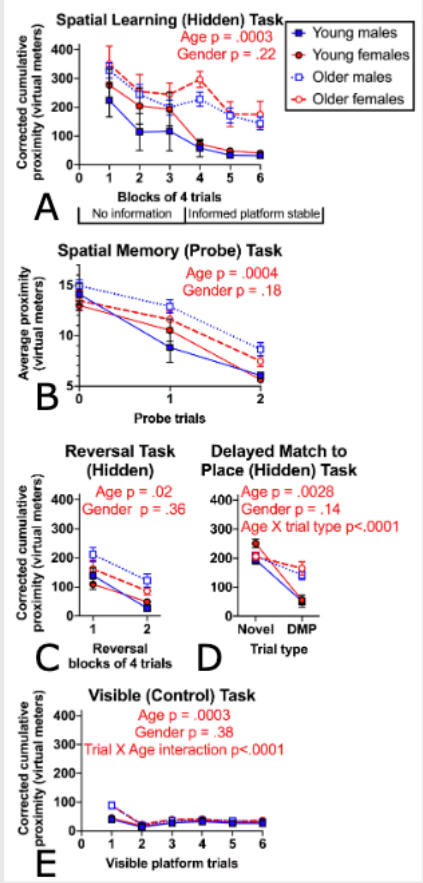


Testing: Participants were screened for visual acuity, color blindness & contrast sensitivity; MMSE for dementia, WMS Logical Memory (immediate and delayed (50 minutes) recall tasks, NIH Toolbox Cognitive Battery and vMWM tasks (see diagram to right).



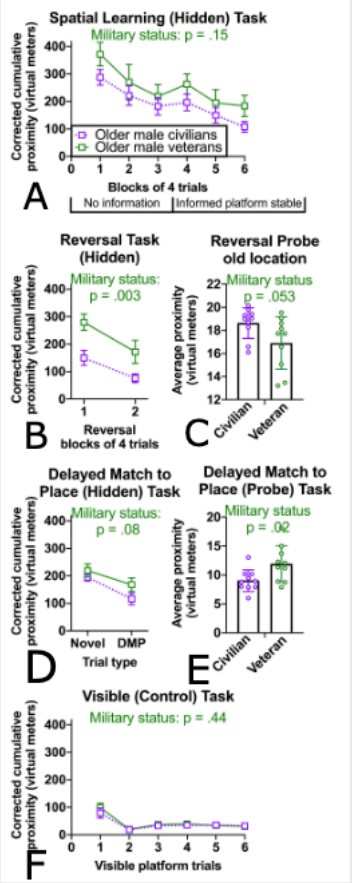
Analysis: vMWM tasks were analyzed by corrected cumulative proximity for hidden platform trials and average proximity for probe trials. Uncorrected Standard Scores were used for the NIH Toolbox tasks. Statistical analysis included ANOVA with the use of Statview software.

vMWM detected age differences, but not gender, in spatial learning & memory



There were significant effects of age in the vMWM tasks for spatial learning (A), spatial memory (B), cognitive flexibility (C), and working memory (D) with younger performing better than older, but no effects of gender. There was also a significant effect of age on visible trials (E). Mean ± SEM. N = 5-21.

vMWM detected effects of military service on working memory & flexibility



There were significant effects of former military service on some measures of cognitive flexibility (B,C), and working memory (D,E), with veterans performing worse than civilians. There was no significant effect of former military service on spatial learning (A) or visible platform trials (F). Mean ± SEM. N = 5-21.

NIH Toolbox Fluid Cognition tasks detected age-related differences, but no military service effects.

	Young males	Young females	Older males	Older females	Older male Civilians	Older male Veterans
N	5	11	21	8-9	11	10
Age (years) *	23 ± .4	24 ± 1	70 ± 1	70 ± 1	70 ± 2	72 ± 2
Education (years) *	16.8 ± 1.1	16.0 ± .6	19.0 ± .9	19.9 ± 1.2	20.8 ± 1.4	17.2 ± .9
Speed Maze (s) *	64 ± 2	62 ± 1	69 ± 2	72 ± 2	68 ± 2	69 ± 3
Picture vocabulary[1] *	114 ± 2	106 ± 2	123 ± 1	124 ± 2	126 ± 1	120 ± 2 #
Flanker inhibition[1] *	107 ± 3	108 ± 1	98 ± 1	96 ± 3	99 ± 2	97 ± 2
List sorting[1] * §	127 ± 3	114 ± 3	104 ± 2	106 ± 2	104 ± 3	103 ± 3
Discrimination card sort[1] *	114 ± 3	112 ± 2	102 ± 1	106 ± 2	101 ± 2	103 ± 1
Pattern comparison[1] *	128 ± 7	128 ± 4	93 ± 3	95 ± 3	93 ± 5	93 ± 4
Picture sequence memory[1] *	121 ± 5	121 ± 3	96 ± 2	104 ± 5	95 ± 3	97 ± 4
Oral reading[1] * §	116 ± 2	107 ± 2	114 ± 1	116 ± 1	116 ± 1	112 ± 2 #
Fluid cognition[1] *	126 ± 5	122 ± 3	97 ± 2	100 ± 3	97 ± 2	97 ± 3
Crystallized cognition[1] * §	115 ± 2	107 ± 2	120 ± 1	121 ± 1	122 ± 1	117 ± 2
Logical memory immediate *§	17 ± 2	15 ± 1	11 ± 1	17 ± 1	13 ± 1	9 ± 1 #
Logical memory delayed (50m) * §	20 ± 1	19 ± 1	15 ± 1	20 ± 1	15 ± 1	15 ± 1

[1]NIH Toolbox Cognitive Battery test – Uncorrected Standard Score
*Indicates p < .05 for effect of age.
§ Indicates p < .05 for effect of gender or age X gender interaction
#Indicates p < .05 for effect of military service.
Means ± SEM.

Table above indicates that older individuals had significantly more education (p<.0001) and took more time in the speed maze (p=.0002) than young. Older individuals had significantly lower scores in picture vocabulary (p<.0001), flanker inhibition (p<.0001), list sorting (p < .0001), discrimination card sort (p < .0001), pattern comparison (p<.0001), picture sequence memory (p<.0001), tasks and fluid cognition (p < .0001; A) than young. Veterans demonstrated a significantly worse performance in picture vocabulary (p=.02), oral reading (p=.02), and logical memory immediate (p=.0064), tasks and crystallized cognition (p=.026) than civilians.

CONCLUSIONS

- The vMWM tasks showed similar age related deficits as were demonstrated by the NIH Toolbox.
- Military service in the Korean and Vietnam war eras appeared to not be beneficial for working memory and cognitive flexibility later in life.
- The vMWM tasks were more sensitive to the effects of former military service on working memory and cognitive flexibility in older men than the NIH Toolbox tasks.
- Former military service may be a hidden variable in cognitive aging studies.

Oregon State University

Funding: Large Program Development Award, Research Office, Oregon State University

Presenter: Abbie Glickman | Advisor: Dr. Rebekah Elliott

CONNECTING COMPUTATIONAL THINKING AND MATHEMATICS:
AN EXAMINATION OF LINGUISTIC OPPORTUNITIES AND CHALLENGES

RESEARCH AIM

To examine opportunities for K-12 mathematics teacher candidates to learn and engage with computational thinking in their teacher education program.

RESEARCH QUESTIONS

1. What are the linguistic affordances and challenges that K-12 mathematics teacher candidates face as they engage in a computational thinking setting?
2. How do the teacher candidates navigate those affordances and challenges:
   a) With each other
   b) Using tools they are provided by computational thinking modules?

METHODS

The broader study was a five-day application of computational thinking. The participants were broken up into five groups of 2-3 teacher candidates each. Over the first two days they were learning basic coding concepts to address a simple money investment scenario on an interface called BlockPy. Throughout the module they were asked to respond to reflection questions about how computer science and mathematics are related. This study focused on a group of two teacher candidates, referred to as Rhoda and Ginny, over the course of the first day of the module.

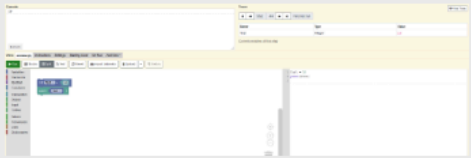


Figure 1. Screenshot depicts the BlockPy interface that the teacher candidates used in the modules. The left side shows the block-based code where they selected from puzzle pieces, and the right side shows the script-based code where they could type in Python.

BACKGROUNDS OF RHODA AND GINNY

Rhoda – computer science, mathematics, & mathematics teaching

Ginny – mathematics & mathematics teaching

DISCIPLINARY REPERTOIRES

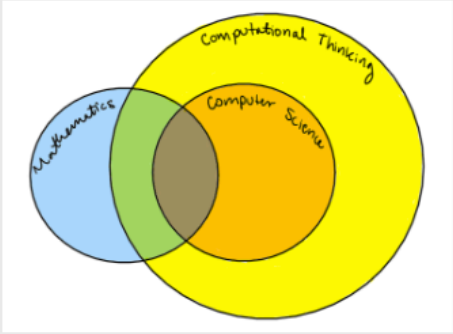


Figure 2. Venn diagram illustration of the overlapping disciplines drawn upon in the study and their connection to computational thinking.

CONCEPTUAL FRAMEWORK

Computational thinking

"A thought process involved in formulating a problem and expressing its solution(s) in such a way that a computer (human or machine) can effectively carry [them] out" (Wing, 2014, p.1).

- Abstraction
- Algorithmic thinking
- Developing models
- Designing efficient solutions

Translanguaging

"The development of a speaker's full linguistic repertoire without regard for the watchful adherence to the socially and politically defined boundaries of named (and usually national and state) languages" (Otheguy et al., 2015, p. 281).

Disciplinary repertoires

The full set of meaning-making and communicative devices that a person carries, specifically geared towards learning and problem solving within the various academic disciplines.

RESULTS

Theme 1: The disciplinary repertoires of the teacher candidates granted them both affordances and challenges.

Theme 2: The teacher candidates created meaning together by translanguaging and coordinating their disciplinary repertoires.

THEME 1

Rhoda

Affordances: familiarity with computer science concepts
Challenges: negotiating her knowledge of coding with the new BlockPy interface

Ginny

Affordances: mathematics knowledge acted as a base for new computer science knowledge
Challenges: negotiating how overlapping concepts differ between mathematics and computer science

THEME 2

Rhoda's explanations of variables developed through discussion

- "So variables are like if you're gonna have to use it in multiple lines and you don't wanna keep typing"
- Variables act as a placeholder
- Examples in code, further elaboration of placeholders

Ginny used similes and comparative reasoning in discussion

- "…because in Python that's how you'd let something go to x or something"
- Setting variables like "Let statements"
- Variables are varying quantities in mathematics

DISCUSSION

Computational thinking

While it has commonly been associated with computer science and STEM, computational thinking can be used both inside and outside this traditional setting. The definition provided by Wing (2014) hints at this relationship by adding "human or machine" to describe a computer, meaning that computational thinking can be separate of machines.

For instance, an example of algorithmic thinking, which is a type of computational thinking, is following a recipe. When someone follows a recipe to bake a cake, they are exemplifying the use of computational thinking in everyday life.

Translanguaging

What does translanguaging mean in context?

Think of someone who is bilingual in Spanish and English. Their linguistic repertoire would consist of elements of both languages such as their vocabulary, syntax, and other linguistic tools.

When this person uses their full linguistic repertoire to speak, an outside observer might assume that they are fluently switching between languages. However, the theory of translanguaging would contradict this to say that they are drawing upon their full linguistic repertoire to convey their message without actively deciding which language to speak from.

In this study, the term *translanguaging* is adapted to extend to a disciplinary context and describe mathematics teacher candidates.

[Linguistic repertoire: the set of linguistic and meaning-making tools that a person can draw upon when they communicate]

Disciplinary repertoires

The concept of a disciplinary repertoire is adapted from the linguistic repertoire.

In this study, the results showed that Rhoda and Ginny drew from their disciplinary repertoires and translanguaged to make meaning in a multidisciplinary setting.

As an example, Ginny's interactions with a new discipline, computer science, showed how she was able to use the mathematics in her disciplinary repertoire as a base for new information. She "tried on" computer science terminology as she spoke with Rhoda and weaved together her existing mathematics terminology with these concepts. This is a form of translanguaging in a disciplinary context.

CONCLUSIONS AND IMPLICATIONS

- Important to find ways to anticipate affordances and challenges such that teacher candidates can foreground their knowledge and address potential challenges
- Would the use of translanguaging in a disciplinary context hold if we were working with the overlaps of another set of disciplines?
  - e.g., physics & computer science

REFERENCES

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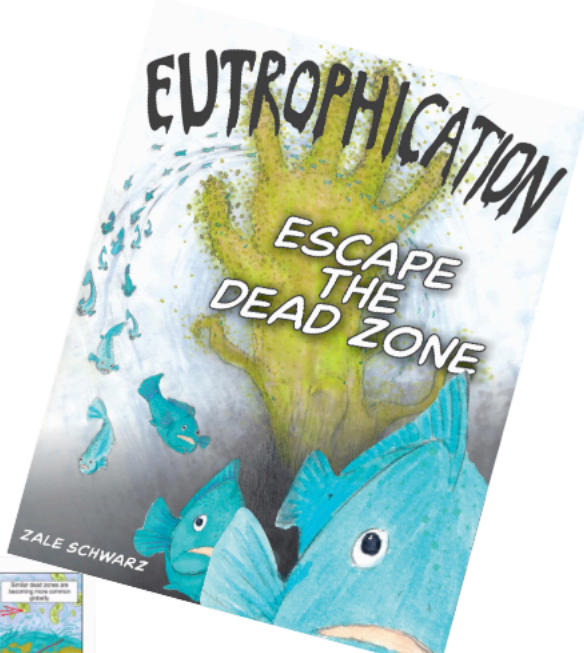
Oregon State University

Science Education Through Comics: Improving Understanding of Current Marine Environmental Issues

Zale Schwarz (Zoology), Dr. Stephen Atkinson (Microbiology), Matthew Schofield (*The Simpsons*)



Background/project summary

- We face multiple environmental challenges, yet public understanding of these is limited: for example: 52% of Americans would earn an F on a quiz on the basics about climate change (NEEF 2015).
- Lack of understanding prevents people from making informed decisions
- Scientific concepts can be explained and made accessible to a wider audience through *comics (*Munawwaroh et. al. 2018)
- Comics can easily communicate abstract or complicated concepts by combining text and art. Comics are quick and appealing to read.

For this project, I was driven by my concern for marine environmental issues, and an opportunity to build my comic-writing and science communication skills. I focused on **eutrophication** and **plastic pollution** because they are current, complex problems that can be improved by individual actions.

Methods

The **Workflow Diagram** shows the major parts of my process.

I did online research on the topics, and collected reference art for making concept sketches. The draft illustrations were created using pencil and alcohol ink markers on watercolor paper. When needed, I added detail with colored pencil and acrylic paint. Initially, I photographed colored illustrations using a phone app, but later digitized using a Canon CanoScan 9000F scanner. The comic panels were laid-out in CorelDraw X8, and text was added digitally. The complete draft was then critiqued by a professional illustrator, Matthew Schofield, who is animation director for *The Simpsons* and writes his own webcomic *Steamroller Man*. I created a custom webpage to host PDFs of the comics, and then posted links to Twitter and Instagram. I regularly monitored the social media feeds and collected feedback.

Workflow:

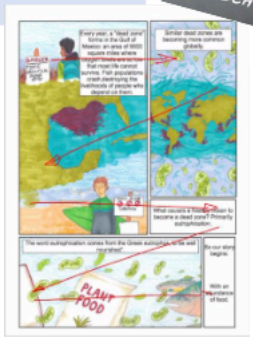
Research problem
Write story outline

Create concept sketches

Add color to art

Create initial panel layout

Scan art & combine



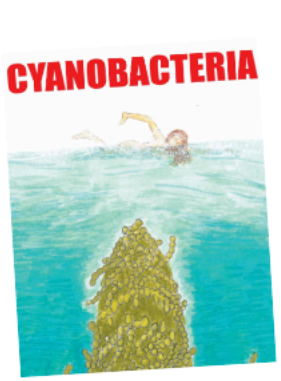
Critique by a comic artist

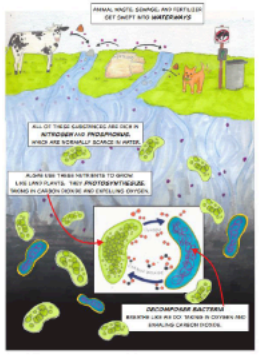
Publish final revised comic online. Share on social media.

Results & Discussion

- The final and draft comics can be read on **zschwarz.com/comics**

This process was developed during the creation of *Eutrophication: Escape the Dead Zone, and* adapted from previous projects I worked on to add illustration to explain scientific concepts. I found that digitizing the art using a scanner, rather than a document scanning phone app produced much clearer results.
The feedback I have so far showed me several important things: The comic format I used does effectively explain the concept. Layout and text density are essential to effective communication. If a page is too dense or unintuitive to read, the audience loses interest quickly.
Although the website and social media posts are being seen, the comics have not yet reached an audience as large as I had hoped. To reach a wider audience, I plan to partner with teachers and existing science communication organizations. There is clearly opportunities for reaching younger people, as two professors and one elementary school teacher have contacted me, asking to use my comic in their classes.





Conclusions and future directions

- In the future, I'd like to experiment with purely digital art, which will be easier to edit throughout the process.
- Other environmental issues that I am considering making into comics include **ocean acidification**, **overfishing**, and **deep sea mining**.
- I would like to partner with an aquarium or another organization to create focused projects with a larger audience.

Acknowledgements

Thank you so much to Dr. Atkinson and Mr. Schofield for their instruction and advice, Jay Edidin and Miles Stokes for inspiring my love of comics, and my family, for their constant support and encouragement.

Literature

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College of Science; College of Agricultural Sciences

Honey Bee (*Apis Mellifera*) Colony Strength And Its Effects On Pollination And Yield In Highbush Blueberries (*Vaccinium Corymbosum*)

Kennedy Grant, Mentored by Dr. Andony Melathopoulos

BACKGROUND

Beekeepers continuously report that their colonies are developing persistent diseases during the blueberry pollination season in the Pacific Northwest, while recent studies in Washington suggests there are benefits to increasing the number of rental colonies per hectare (also known as stocking rate). The relationship between demanding increased density and the deteriorating health of the hives could be problematic, as closer proximity may increase likelihood and intensity of brood infections.

We wanted to investigate whether there was an alternative approach to increasing yield that benefits both beekeepers and growers; not by adding additional colonies per hectare, but by supplying populous, higher-quality colonies. In addition, we also set out to confirm beekeepers' increased sightings of a concerning honey bee larval disease (European foulbrood; EFB; see image 1).

Therefore, the three main goals of this study were to:

1. Assess the effects of blueberry pollination on the health of honey bees
2. Define honey bee colony quality and strength parameters, and,
3. Determine whether colony strength influences blueberry yield.

Fig. 1 (right) **EFB severity before and after pollination.** The relative severity rating (0-3) of European foulbrood (EFB) found in individual colonies located at four blueberry fields, as colonies enter (Early) and leave (Late) pollination in (a) 2019 and (b) 2020. A rating of 0 indicated that no EFB was detected at the colony, and a rating of 3 was the most severe case a colony could be assigned. Dots indicate the rating assigned to an individual colony at the time of the assessment. One farm from 2019 was no longer available in the 2020 assessment, but the other three remain consistent across the two years. Data were collected in northwest Oregon within the Willamette Valley.

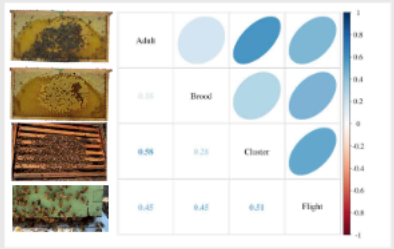
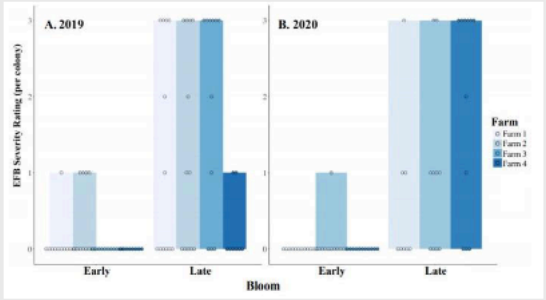


Fig. 2 (above) **Comparison of colony strength assessments.** Correlations among the adult and brood population (Liebefeld method) and two labor-saving assessment methods, cluster counts (Nasr et al. 1990) and returning flight counts (flight) (see text for description, n=49 colonies). Correlation coefficients (Kendall's tau) are listed in the lower cells and designated in strength by color as indicated by the scale. Ellipses indicate: (1) correlations with P-value < 0.05 (i.e., cells without an ellipse are ≥0.05) and (2) the color and eccentricity of the ellipse, which is scaled to the correlation value according to Friendly (2002).



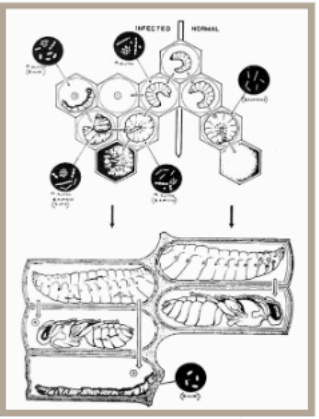
METHODS

Over the course of two years (2019/2020), we characterized the effect of variation in average colony strength on blueberry yield at 25 total commercial fields in Oregon stocked with identical densities (10 colonies/ha). Randomly selected colonies from each field were recorded to obtain colony strength measurements (n=50), as well as the presence and progression of any brood diseases from early to late blueberry bloom. Fruit set and yield data were provided by farmers from randomized bushes within each field. All fields used in the study were separated by at least two kilometers to maintain site independence.

RESULTS

- Both the presence and prevalence of EFB increased over the course of the pollination season in all fields, both years (Fig 1)
- "Flight" counts (the rate of returning forager bees per minute) is an accurate estimate of internal colony strength (Fig 2) that can be performed by blueberry growers, as it doesn't require the tools and skills needed to open a colony (beekeeper methods)
- The recommended flight count for adequate pollination services (100 bees per minute, Sagili and Burgett 2011) is an overestimate, and six frames of bees (typical minimum strength parameter) is equivalent to ~42 bees per minute (Fig 3)
- Using flight counts as a proxy for internal colony strength, there was a 62.6% difference in the estimates of blueberry yield (kg/ha) between colonies at the 25th percentile (6.4 adult frames; 42.6 bees per minute) and the 75th percentile (10.2 adult frames; 90.3 bees per minute).

Image 1 (right). A diagram showing the progression of EFB in a normal cell versus an infected cell (Bailey and Ball 1991). Note the bacterial cause of EFB (*Melissococcus plutonius)* persists in the gut and starves the larvae until they waste away.



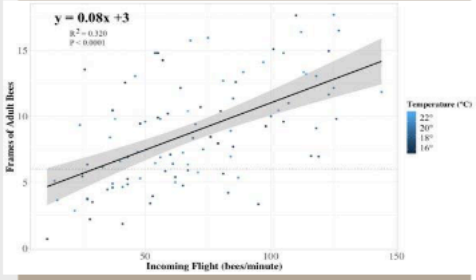


Fig. 2 **Colony strength linear model.** The relationship between the frames of adult bees in a colony to the rate of returning foragers at its entrance (n=84 colonies). Each dot represents a colony, and the color of the dots indicates the temperature at which the returning flight data were recorded. Our linear model prediction for the number of frames of adult bees (frames) depends on the number of 'x' returning bees to the colony ($R2 = 0.32$, $P<0.0001$). The trendline approximates that for every thirteen returning bees, the internal frame count of adult bees is predicted to increase by one, with a 95% confidence interval. The horizontal dotted line indicates the 6 minimum recommended frames of adult bees for adequate pollination as recommended by Sagili and Burgett (2011).

DISCUSSION

- Confirmed reports from Oregon beekeepers that colonies have increased prevalence of EFB following blueberry pollination
- Data suggests higher pollination benefits could be achieved without increasing hive density.
- This study is one of the first to demonstrate that yield benefits associated with crop pollination are also related to the strength of colonies brought into pollination
- The method used to assess honey bee colony strength, incoming flight entrance counts, is easy for growers to perform and a significant predictor of true internal bee counts
- An OSU extension publication is in production to train growers in measuring colony strength via flight counts
- Growers could realize a substantial yield increase by maintaining standard 10 colony/ha stocking rates, but selecting beekeepers who provide stronger colonies for pollination
- Further studies should track realized profit margins associated with colony strength to provide beekeepers with a way to competitively price their colonies (currently each $55, flat rate) and incentivize growers with increased yield.

Oregon State University

Hibernation Strategies to Improve Organ Preservation and Storage

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Oregon State University

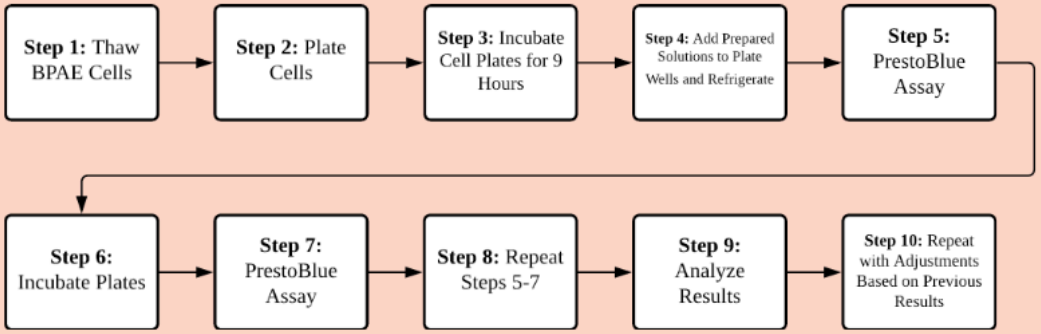
Introduction

The global shortage of organs and tissues needed for transplantation has become one of the leading crises in the biomedical community. According to the World Health Organization (WHO), it has been estimated that only ten percent of the worldwide need for organ transplantations is being met.[1] One potential solution to this humanitarian problem could be found in hibernating mammals.

Mammalian hibernation is a complex phenotype involving reduced metabolic rate, reduced oxygen intake, low body temperature, and a reliance on stored fat that allows the animal to survive 5-6 months without food.[2] These evolved mechanisms in hibernators provide a potential framework for new therapies targeting preservation of organs for transplantation.

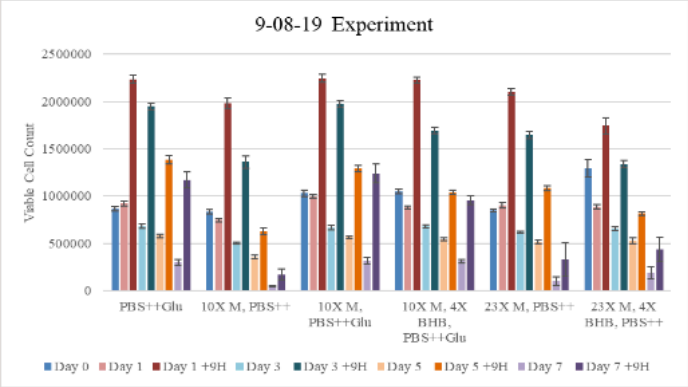
The goal of my thesis is to examine the effects of lipids in a hibernation-based solution for organ transplantation. Lipids play a large role in hibernation, from providing a source of energy to protecting the animal's heart from damage.

Methodology



Results

- Five experiments were conducted to test various solution components and concentrations in order to find the best combination for preserving cell cultures
- We hypothesized that the fatty acids would be utilized by the cells as energy sources during changes in conditions (refrigeration to incubation) in order to stay viable for longer periods of time
- Based on the results of the PrestoBlue assays, the addition of linoleic, linolenic, and oleic acids in the hibernation solution did not increase the longevity of the cell cultures. In fact, they seemed to decrease the longevity compared to the control solutions. The most successful preservation solution consisted of 10x melatonin and PBS++Glu



Results from the 9-08-19 experiment. Abbrevs: D-β-hydroxybutyrate (BHB); melatonin (M); intralipid (I); Phosphate Buffered Saline with Calcium, Magnesium (PBS++), and Phosphate Buffered Saline with Calcium, Magnesium and Glucose (PBS++Glu). The 10x melatonin and PBS++ glucose solution performed the best out of every solution tested throughout the five experiments, with record numbers of viable cells even on the seventh day.

Discussion

Conclusions

- Fatty acids may not be essential for cell preservation in a hibernation-based solution, and may instead hinder cell viability over long periods of time
- Melatonin may play a larger role than initially thought in increasing cell viability and preserving tissue cultures
- In the last experiment, we successfully preserved cell cultures for seven days

Future Directions

- These findings have the potential to increase the preservation time of human organs in a transplantation solution by up to seven days for abdominal organs and two days for thoracic organ, which is a drastic improvement to what is currently offered on the market[3]
- On a long-term timescale, placing humans in a state of suspended animation has been proposed as a futuristic means of conserving resources during long-term space travel

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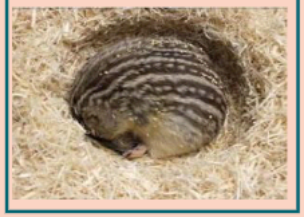


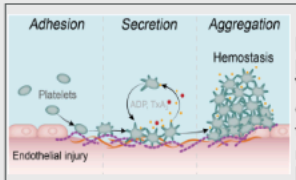
Effects of Inhibitors Against SYK-BTK-PI3K Signaling on Platelet Function

Liz Lofurno

Mentors: Dr. Joe Aslan & Dr. Skip Rochefort

College Of Science | Biochemistry and Molecular Biology | Honors College

Background



Platelets respond to physiological cues and stimuli present at the site of damage. Vascular injury, inflammation, and disruption of flow cause platelets to be activated and undergo morphological changes vital to hemostasis.

Tyrosine kinases mediate how blood cells respond to stimuli. Tyrosine kinase inhibitors (TKIs) serve important roles as drugs in managing hematological diseases, including several cancers.

In healthy B cells, the B cell receptor (BCR) activates a tyrosine kinase known as SYK, which then activates BTK, PI3K, PLCγ2 and protein kinase C (PKC) to mediate responses such as cell migration and proliferation. In many hematologic cancer cells, BCRs are overactive, causing an uncontrolled increase in cell survival and production. TKIs against SYK, as well as BTK can block highly active BCR, and restore normal cell function and turnover. In this regard, TKIs have been shown to successfully treat several blood cancers.

Unfortunately, many TKI drugs used to treat blood cancers also affect other healthy blood cells, including platelets, and bleeding complications are common to TKI therapy. To investigate these issues, the effect of various tyrosine kinase inhibitors on platelet activation was analyzed along with the protein-protein interactions.

Materials & Methods

- Platelets were isolated from anticoagulated blood from healthy adult human donors for assays of platelet adhesion responses.
- Purified platelets were incubated with TKIs or vehicle alone for 10 min, and then set to adhere to glass coverslips coated with fibrinogen protein for 45 minutes.
- Platelets were fixed with 4% paraformaldehyde and processed for light microscopy imaging. Other parallel: replicate sets of coverslips were stained with primary antibodies to visualize the localizations of PI3K and microtubule organization in adherent platelets under control and TKI conditions.
- Coverslips with adherent platelets were imaged using Kohler illuminated Nomarski differential interference contrast (DIC) optics as well as fluorescence imaging with a Zeiss 63x oil immersion 1.40 NA plan-apochromat lens on a Zeiss Axiovert 200M microscope.

Results & Discussion

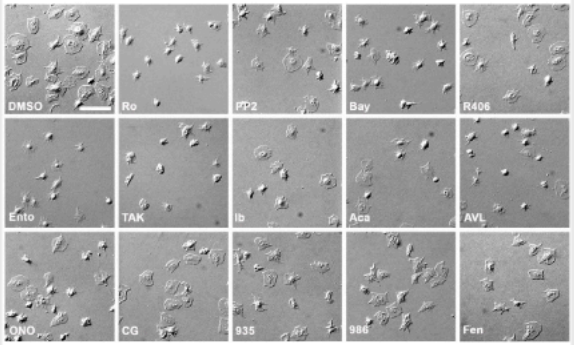


Fig. 1 (above) DIC images of DMSO and inhibited platelets spread on Fibrinogen. Platelet adhesion and spreading were quantified to determine a distribution of individual platelet surface areas per experimental condition (Fig. 2).

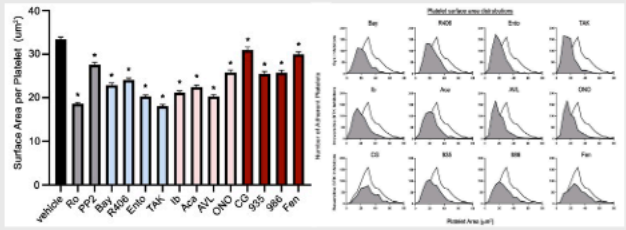


Fig. 2 (above) Average platelet area among inhibited platelets and frequency distribution curve of spread platelets.

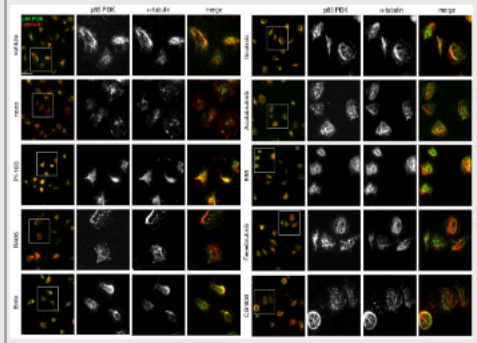


Fig. 3 (left) Fluorescence images show protein localization of p85 PI3K (green) and α-tubulin (red). This shows that pretreatment of platelets with Syk and BTK inhibitors affect intracellular distributions of p85 PI3K and α-tubulin in adherent platelets and alters localization of these proteins in a differential manner.

(Fig. 1) Results demonstrate that all Syk and BTK inhibitors examined have inhibitory effects on platelet spreading. In general, while inhibitors to Syk and inhibitors that irreversibly target BTK uniformly inhibit platelet spreading on fibrinogen, inhibitors that reversibly target BTK were less potent in inhibiting platelet spreading, which lead to an increase variability in surface area as noted by the frequency distribution analysis (Fig. 2).

(Fig. 3) These results show a relationship between microtubule organization and localized PI3K and other signaling systems in platelets, suggesting that Syk and BTK inhibitors differentially alter platelet PI3K signaling in a manner related to the inhibitor's pharmacology.

Conclusions

- Tyrosine kinase inhibitors which target components of the same SYK-BTK-PI3K signaling pathway, have varying effects on platelet responses.
- Differential effects may be attributed to altered protein-protein interactions within platelets with SYK and BTK specific inhibitors, and not others in a manner that may be relevant to better understanding off-target effects and the toxicity of therapeutic TKIs in different contexts.
- Inhibiting platelets causes similarities and differences in the tubulin and PI3K interactions.

The results from this study may help to understand "off target" or undesired effects of TKIs on platelets. This will help to address a growing need to better understand the effects of such compounds on essential molecular machinery around Syk-BTK signaling in platelets and other physiologically relevant cell types.

Acknowledgements

Thank you to my mentors, Dr. Joe Aslan and Dr. Skip Rochefort, as well as Dr. Owen McCarty and Tony Zheng. I was honored to be a part of the CBEE Johnson Internship Program funded by Pete and Rosalie Johnson, which provided me with the opportunity and guidance to conduct the research for this project.

Social Context Affects Response to an Acute Stressor in Captive Red Crossbills (*Loxia curvirostra*)

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Integrative Biology, Oregon State University

Introduction

- Social animals can use available information to inform behavioral and physiological responses to situations, but the way that information is processed can be influenced by the social context in which an event occurs.
- Consequently, the presence or absence of conspecifics can alter stress perception.
- In a recent experiment, red crossbills – a social songbird – that were housed in pairs (i.e., doubly-housed) lost mass when moved to a new room and presented with a neighbor cage, whereas individually-housed birds did not lose mass in the same situation.

- We tested the following non-mutually exclusive hypotheses in relation to how social grouping affects responses to a change in environment and the presentation of a neighbor.

Dominance Hypothesis: The weight loss observed in doubly-housed birds is due to the introduction of a neighbor, which may have temporarily disrupted the pre-existing dominance hierarchy.

Social Buffering Hypothesis: The maintenance of weight observed in previously isolated, singly-housed birds is due to social interactions mitigating the stress of moving rooms.

Methods

- 96 adult red crossbills were placed into cages based on their treatment group:

  Alone Control – Individually-housed birds on a natural photoperiod, not paired on move day
  Singly-housed – Individually-housed birds on a natural photoperiod, paired with a neighbor cage on move day
  Doubly-housed – Doubly-housed birds on a natural photoperiod, paired with a neighbor cage on move day

- Visual barriers were put up during the pre-pairing period (8 weeks) such that individually-housed birds in each cage could hear, but not see, the birds in other cages. Doubly-housed birds could see and interact with their cage-mate.
- Birds experienced a change in environment (i.e., a new room) on move day, which is known to be a stressor, and were allowed visual access to a second cage of the opposite housing type (i.e., singles were paired next to doubles). Alone controls remained visually isolated in their new room.
- Mass and food intake was measured pre and post pairing to the nearest 0.01 gram using an electronic scale. Activity was recorded continuously pre and post pair using an infrared activity monitoring system by Starr Life Sciences. Activity was summed per hour and averaged across 24-hour periods for daily activity estimates.
- A least squares method was used to test for the impact of food intake, activity, treatment group, and interactions on the change in mass.

Results and Discussion

- The mean change in mass was significantly different by treatment (Figure 1, ANOVA – $F_{2,92} = 70.3$; $P < 0.0001$; $R^2 = 0.61$), with doubly-housed birds losing significantly more mass than singly-housed birds and alone controls (Tukey Comparison of Means, $P < 0.05$).
- These data suggest that treatment group (i.e., social context) affects whether mass was retained or lost after exposure to a stressor.

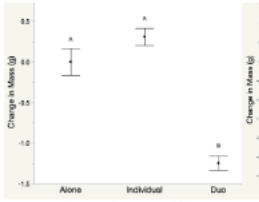

Figure 1. The mean change in mass (g) by treatment group, where the mean change in mass of doubly-housed birds (B) differs significantly from singly-housed birds (A). Error bars denote S.E.M. Groups with different letters are significantly different.

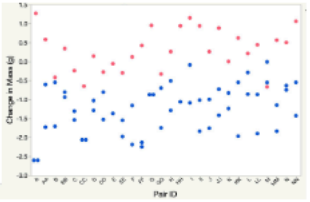

Figure 2: Changes in mass (g) by social pairing, where red represents individually-housed birds (individuals) and blue represents doubly-housed birds (duos). The change in mass is often similar within doubly-housed pairs (e.g., pairs A, BB, C, CC, D, etc).

Predictions and Results

1. If pairing induces stress in doubly-housed birds by upsetting dominance hierarchies, then we predicted that we would see increased activity, reduced food intake, and asymmetric weight loss in doubly-housed partners given that one bird of the duo would be excluded from food.

- For social pairings, there was almost no overlap between the change in mass for individually-housed birds and doubly-housed birds (Figure 2).
- Individually-housed birds consistently maintained or gained weight, while doubly-housed birds consistently lost weight.
- Pair ID was a nearly significant random effect ($P = 0.06$) in explaining mass loss in doubly-housed birds.
- These data suggest that doubly-housed birds may have responded similarly to their partner in response to the stressor.
- Because changes in mass were not asymmetric between duos, we can conclude that one bird was not being excluded from food. Therefore, we can reject the dominance hypothesis.

2. If social buffering mitigates stress in previously isolated, singly-housed birds, then we predicted that we would see a reduction in activity levels and/or an increase in food intake following pairing in singly-housed birds relative to those that remained in social isolation.

- The difference in the change in mass between singly-housed birds and alone controls was not statistically significant (Figure 1).
- There was no significant interaction between food intake and treatment group on the change in mass (LMM, $P = 0.88$; Figure 3b).
- There was a weak trend suggesting the interaction between activity and treatment group impacted the change in mass (Figure 3a, $P = 0.11$). In doubles, there was an inconsistent relationship between change in activity and change in mass (Regression, $P = 0.34$), but singles that had an increase in activity tended to lose more mass (Regression, $P = 0.08$).
- Treatment group significantly predicted change in food intake (Figure 4a; LMM, $R^2 = 0.2$; $t_{1,55} = 3.6$; $P = 0.0006$), but not change in activity (LMM, Figure 4b).
- Across all birds, treatment group was the only significant factor driving change in mass (LMM; $p < .0001$).

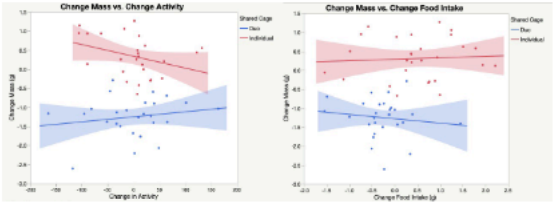

Figure 3a (left): Change in mass (g) versus change in food intake (g) by treatment group./ Figure 3b (right) Bottom – Change in mass (g) versus change in activity by treatment group, with "duo" (blue) representing doubly-housed birds and "individual" (red) representing singly-housed birds. Best fit line and 95% confidence intervals shown.

- These data do not provide any direct support for the social buffering hypothesis, and it appears that social buffering did not occur in any context.
- However, stress physiology may still be playing an important role in our observations. Singly-housed birds and alone controls may have had an altered physiological state from being in long-term isolation, which affected their ability to activate the hypothalamic-pituitary adrenal axis in response to an acute stressor (i.e., moving to a new room).

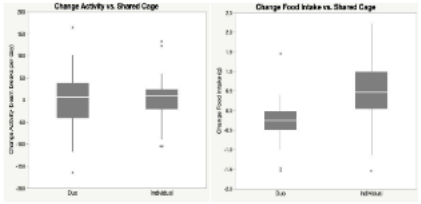

Figure 4a (left): Change in mass (g) versus change in activity (left) and Figure 4b (right): Change in mass (g) versus change in food intake by treatment group. Box plots denote first quartiles from median and whiskers denote 95% CI. Outliers shown as points.

Conclusions

- The changes in mass in doubly-housed birds did not seem to be due to dominance by any one individual and the social buffering hypothesis was not directly supported by these data.
- These data suggest that the mechanism driving this phenomenon may be a more complex intersection between social context and physiological decision-making in response to stress.

Inclusion development in cells infected with *Chlamydia abortus*

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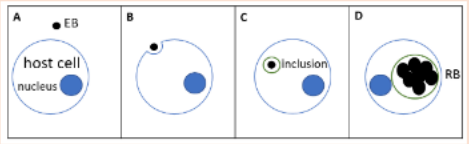
ABSTRACT

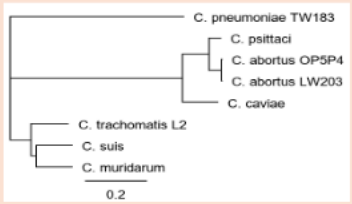
Background Chlamydia is a genus of bacteria that include many species of host-specific intracellular pathogens. Elementary bodies (EBs) are brought into host cells through endocytosis for lysosomal destruction (1). Chlamydiae transform membrane into inclusion, hospitable environment for replication. The inclusion membranes of the species vary, with *C. trachomatis* always forming one inclusion and *C. caviae* always forming a multi-lobed inclusion (2,3). *C. abortus*, an important sheep and goat pathogen that causes miscarriages, sometimes forms one inclusion and sometimes forms multiple (1). This project is examining how different chlamydial species inclusions fuse of do not fuse during coinfection. We also examined the properties of *C. abortus* homotypic inclusion fusion.



Methods McCoy cells (a murine fibroblast immortalized cell line) was singly or coinfected with chlamydial species. Immunofluorescence labeling and microscopy were used to photograph and count instances of fusion in singly or doubly infected cells.

Results *C. trachomatis* did not fuse with *C. caviae* or *C. abortus*. *C. pneumoniae* did not fuse with *C. caviae* or *C. abortus*. *C. abortus* and *C. caviae* fused in greater than 80% of coinfected cells. In singly infected cells, *C. abortus* about 25% of bacteria produced multiple inclusions, a number that increased with increasing MOI (multiplicity of infection).

Figure 1. Phylogenetic tree of chlamydial species made from whole genome alignment



***C. caviae* and *C. abortus* are more closely related to each other than to *C. pneumoniae* or *C. trachomatis*.** C. suis *and* C. muridarum *fuse inclusions in vitro (4).* C. psittaci and C. pneumoniae *do not fuse with* C. trachomatis (5). *Neighbor-joining HKY tree made with Geneious Prime from MAFFT nucleotide alignment.*

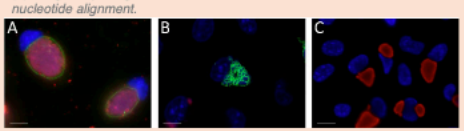
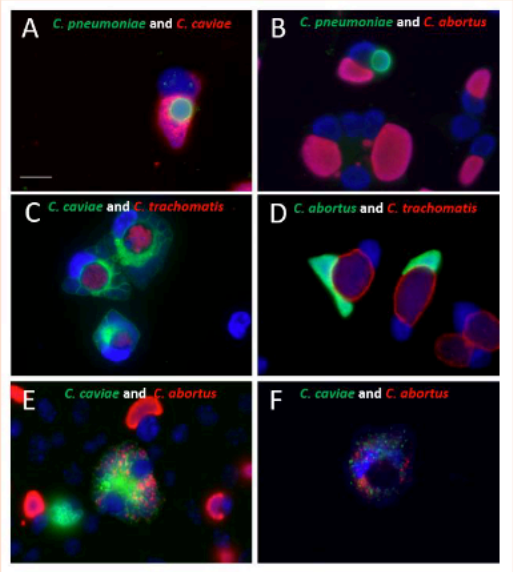


Figure 2. *Number of inclusions varies between species.* *Singly infected McCoy cells with nucleic acid dyed blue (DAPI).* **A.** C. trachomatis *IncA protein (green) and MOMP (red).* **B.** C. caviae *inclusion membrane (IncA) green.* **C.** C. abortus *inclusions in red (MOMP).*

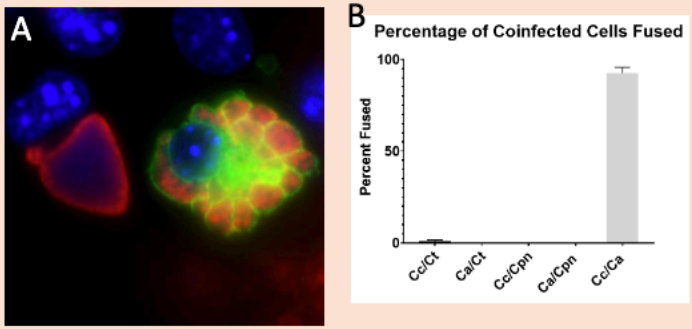
RESULTS

Figure 3. *C. abortus* and *C. caviae* inclusions fuse; no other combination of species tested does.



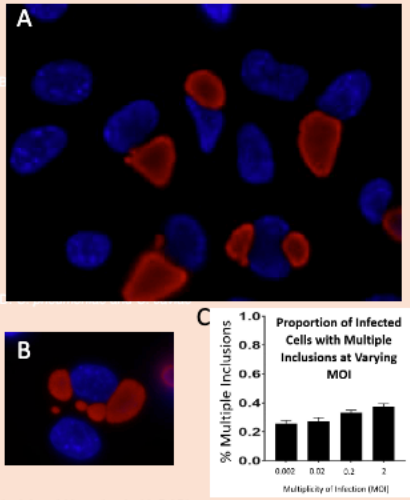
A. C. pnuemoniae *(green, IncA) and* C. caviae *(red, IncA) do not fuse in coinfected cell. Scale bar is10 µm.*
B. C. pneumoniae *(green, IncA) and* C. abortus *(red, MOMP) do not fuse inclusions*
C. C. caviae *(green, IncA) and* C. trachomatis *(red, MOMP) do not fuse inclusions.*
D. C. abortus *(green, MOMP) and* C. trachomatis *(red, IncA) inclusions do not fuse*
E. C. caviae *(green, IncA) and* C. abortus *(red, MOMP) inclusions fuse (see center cell)*
F. C. caviae *(green MOMP and blue IncA) inclusion surrounds* C. abortus *bacteria (red MOMP).*

Figure 4. *Chlamydia abortus* and *Chlamydia caviae* inclusions fuse



Coinfection with C. abortus *and* C. caviae. *A) The inclusion membrane of* C. caviae *is labeled in green. The* C. abortus *bacteria are labeled in red. The inclusion on the left is a cell infected only with* C. abortus. *The inclusion on the right contains* C. abortus *and* C. caviae. *B) In greater than 80% of counted coinfected cells,* C. caviae *and* C. abortus *fused, which was not seen in other coinfections.*

Figure 5. *C. abortus* form one or multiple inclusions, and number of inclusions increases with MOI



Nucleic acid is stained with DAPI.
A and B. C. abortus *bacteria is labeled in red* C. abortus *forms either one inclusion or multiple. C. abortus fixed at 48 hpi.*
C. *When multiplicity of infection (MOI) increased, the proportion of infected cells with multiple inclusions also increased in a statistically significant manner. However, even at MOI far below 1, some infected cells continued to have multiple inclusions.*

NEXT STEPS

- Analyze recombination and lateral gene transfer in *C. caviae* and *C. abortus*
- Determine mechanism of fusion and division of *C. abortus* inclusions
- Test fusion between *C. abortus* and *C. psittaci*

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