2021 Rhode Island Summer Undergraduate Research Conference

Friday, July 30, 2021

University of Rhode Island

Center for Biotechnology & Life Sciences
Paramaz Avedisian '54 Hall, College of Pharmacy
Fascitelli Center for Advanced Engineering

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2021 Rhode Island Summer Undergraduate Research Conference

8:00 – 9:00 AM

Check-in & Continental Breakfast

- Center for Biotechnology & Life Science

Poster Set-up

- Paramaz Avedisian '54 Hall, College of Pharmacy
- Fascitelli Center for Advanced Engineering

9:00 – 9:30 AM

Welcoming Remarks

- Center for Biotechnology & Life Science

Donald H DeHayes, PhD, Provost, University of Rhode Island

Peter J Snyder, PhD, Vice President for Research & Economic Development, University of Rhode Island

Kelli J Armstrong, PhD, President, Salve Regina University

9:30 – 11:00 AM

Poster Session A

- Paramaz Avedisian '54 Hall, College of Pharmacy
- Fascitelli Center for Advanced Engineering

11 AM - 12:30 PM

Poster Session B

- Paramaz Avedisian '54 Hall, College of Pharmacy
- Fascitelli Center for Advanced Engineering

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POSTER PRESENTATION SCHEDULE

*** PLEASE NOTE: Posters are to be set up prior to the welcoming remarks and should remain up until 12:30 PM. Posters are to be presented according to the schedule below.*

Session	Presentation Times
A	9:30 – 11:00
B	11:00 – 12:30

Poster numbers	Location
1-46 (A & B)	Paramaz Avedisian ’54 Hall College of Pharmacy
47-72 (A & B)	Fascitelli Center for Advanced Engineering

PARAMAZ AVEDISIAN '54 HALL
COLLEGE OF PHARMACY

Posters

A-01 to A-46

and

B-01 to B-44

SESSION A: 9:30 – 11:00 AM

SESSION B: 11:00 AM – 12:30 PM

Enzyme catalyzed degradation by the AAA+ protease Lon

Ashley Tai, Ben Piraino & Jodi Camberg

Cell and Molecular Biology, University of Rhode Island, Kingston, RI

Lon protease is widely conserved in bacterial and mitochondrial genomes and is a member of the large AAA+ superfamily of ATPases. The enzyme is thought to processively unfold specific client proteins and use associated serine protease activity to degrade the client protein by peptide bond cleavage. In Escherichia coli, Lon degrades many proteins, including several that regulate cell growth. One key substrate that is degraded by Lon is MqsA, the antitoxin component of the MqsR/MqsA toxin-antitoxin (TA) pair in *E. coli*. TA systems are prevalent across bacterial genomes and function in the cellular stress response. The MqsR/MqsA TA system includes the MqsR toxin, which degrades mRNA to halt cell metabolism, and the cognate antitoxin, MqsA, which functions to neutralize the toxin in the absence of stress. When the cell is under stress, cellular proteases like Lon degrade the antitoxin, diminishing cellular levels of antitoxin, thus releasing the toxin. Here, we purified Lon protease by column chromatography and monitored enzymatic activity in ATP hydrolysis assays and protein degradation assays *in vitro*. To detect ATP hydrolysis, we measured the conversion of ATP to ADP and phosphate under a range of buffer conditions and temperatures in the presence and absence of client proteins. To monitor proteolysis activity of purified Lon, we measured the ATP-dependent turnover of a fluorescent casein substrate (FITC-casein). Once we have optimized the enzyme activity of Lon *in vitro*, we will monitor the degradation of purified MqsA to further understand how Lon regulates TA system activity by degradation of antitoxin. These studies will help us understand how cells respond to stress through proteolysis of regulatory proteins.

This work was supported by the URI MARC U*STAR program

Alzheimer's disease-related protein expression following perinatal PFOS exposure in wild-type mice

Anya Sondhi[1], Jaunetta Hill[2] & Nasser Zawia[2]

[1]College of the Environment and Life Sciences, University of Rhode Island, Kingston, RI
[2]College of Pharmacy, University of Rhode Island, Kingston, RI

Perfluorooctane sulfonic acid (PFOS) is a man-made persistent organic pollutant found in everyday products from non-stick pans to firefighting foam. PFOS has been studied for its potentially neurotoxic properties and has been shown to cross the placental and blood-brain barriers, indicating that each person is exposed to this pollutant before they are born. Studies have shown that PFOS exposure can cause neurodegenerative and neurobehavioral deficits. In the last year, our lab has published data on the biochemical changes resulting from the perinatal PFOS exposure in PND20 CD-1 mouse pups. Additionally, we have examined the behavioral and biochemical changes at PND 388. Using the western blot technique, we found that the mice exposed to PFOS demonstrated a trend ($p=0.0556$) of increase in total tau compared to the vehicle mice. Additionally, from an enzyme-linked immunoassay (ELISA), we identified a strong trend ($p=0.0583$) of increase in p-tau ser404 in the PFOS-exposed mice. Our next steps include investigating the effects of developmental PFOS exposure through the western blot technique on total APOE, AMPK, p-tau, and APP, as well as performing ELISAs for p-tau thr181 to further examine the changes in these proteins.

This work was supported by the URI MARC U*STAR program

Evaluation of nebulized lipid coated nanoparticles for sustained pulmonary delivery of chemotherapeutics

Sabrina Delva[1], Andrea Gonsalves[2] & Jyothi Menon[2]

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[2]Biomedical & Pharmaceutical Sciences, University of Rhode Island, Kingston, RI

Nanoparticles (NPs) are becoming more popular as a potential solution to site-specific and long-term treatment for chronic disorders. Biodegradable NPs are appropriate for this application as they can deliver prolonged drug release at a pace corresponding to polymer degradation and their ability to degrade inside the body without accumulating hazardous by-products. Due to its non-invasive and localized manner of providing medication, pulmonary drug delivery through nebulization has been an attractive application. However, the physico-chemical characteristics and in-vitro behavior of the particles post-nebulization has not been thoroughly studied to our knowledge. Multiple polymer or lipid layers and components are integrated into a single NP system but little studies have been carried out to prove the preservation of structural and chemical integrity of the NPs after nebulization. The research goal was to design and assess an inhalable core-shell NP formulation for its physico-chemical properties and in-vitro therapeutic success after passing through a commercial nebulizer, Aeroneb®. Prior to this, we developed a core-shell NP formulation containing a poly lactic-co-glycolic acid (PLGA) polymer core and a lung surfactant (Infasurf shell). Infasurf, a commercially available surfactant, prevents macrophages from phagocytosing NPs. Paclitaxel, an anti-cancer agent, was encapsulated within PLGA_NPs. Using Dynamic Light Scattering (DLS), the NPs were analyzed for their hydrodynamic diameters and surface charge both before and after nebulization. The particle size of the NPs were 148.3 ± 3.66 nm, polydispersity index of 0.261 and zeta potential of -31.4 ± 1.60 mV and they demonstrated a fairly constant DLS characteristics post nebulization. The morphology of the NPs was examined by using Scanning Electron Microscopy. The drug loaded particles were analyzed for its encapsulation efficiency and in-vitro drug release. Previously, we obtained encouraging in-vitro results showing a reduced cellular uptake of coated NPs by macrophages. These particles were also tested for coating retention and stability in bodily fluids after nebulization. Post nebulization, these NPs are being tested for both their in-vitro and therapeutic efficacy.

This work was supported by the URI MARC U*STAR program

Targeting of hepatic macrophages with dual-functional nanoparticles to mitigate chronic inflammation

Heather Bettencourt, Kalindu Perera & Jyothi Menon

Biomedical and Pharmaceutical Sciences, University of Rhode Island, Kingston, RI

Liver cancer is one of the fastest-growing causes of cancer-related mortality. Inflammation in the liver, when left untreated, can lead to cancer of the liver. During inflammation, liver-resident macrophages, Kupffer cells (KCs), overexpress TGR5, a G-protein-coupled bile acid receptor with anti-inflammatory activity downstream. INT-777 is a synthetic bile acid and TGR5 agonist. INT-777-coated nanoparticles (NPs) therefore have the potential to target hepatic macrophages to decrease chronic inflammation of the liver. Furthermore, encapsulating dexamethasone, an anti-inflammatory drug, creates a targeted dual-functional, anti-inflammatory nanoparticle platform. THP-1 cells were used as model KCs, as these cells are monocytes that differentiate into macrophages and express TGR5 during, much like Kupffer cells. THP-1 cells were differentiated with 50ng/ml phorbol 12-myristate 13-acetate and Inflammation was induced using 50ng/ml or 100ng/ml of lipopolysaccharide (LPS). The average size of the poly-lactic-co-glycolic acid NPs was 188 ± 19.6 nm, as measured by dynamic light scattering (confirmed by scanning electron microscopy), and Zeta potential was determined to be -25.3 ± 1.1 mV using a Zetasizer. Dose-dependent uptake of NPs was observed in differentiated THP-1 cells. Significantly higher (9.9-fold) uptake of INT-conjugated NPs was observed in the inflamed KC model relative to nonconjugated NPs at 1.0mg/ml NP concentration. The encapsulation efficiency was 42.7 ± 4.9%. Studies are ongoing to measure uptake of NPs in a non-KC model cell line (HepG2) to examine NP targeting specificity. Future studies will include the analysis of the effect of dexamethasone encapsulated NPs on pro- and anti-inflammatory signaling molecules in both KC- and non-KC model cell lines, as well as in *in vivo* models.

This work was supported by RI INBRE

Formulation optimization and characterization of clindamycin phosphate vaginal creams

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Clindamycin phosphate vaginal cream (2%, brand name: Clindesse®) is an FDA approved vaginal cream to treat bacterial vaginosis in non-pregnant women. Currently no generic version of the drug product is available to the general population. The main objective of our research is to utilize reverse-engineering principles to produce a laboratory-made cream with similar quality to the marketed Clindesse® cream. Following formulation optimization, we were able to identify which components of the cream had a great impact on the physicochemical properties (e.g., rheological properties, droplet sizes, pH) of this cream product. Drug content was analyzed using an established High-Performance Liquid Chromatography (HPLC) method. The results indicated that rheological properties, pH and drug content of our laboratory-made cream are similar to that of the marketed product. However, it was observed that the laboratory-made cream had a different mean droplet size compared to Clindesse® due to manufacturing differences. Future studies will be conducted to identify critical manufacturing parameters in order to produce a vaginal cream product that is equivalent to the marketed product.

This work was supported by RI INBRE

Identifying manganese reducing bacteria in Rhode Island soils

Ailyn Mendoza, Karen Figueroa, Ethan Dionne & Brett Pellock

Biology, Providence College, Providence, RI

Dissimilatory metal-reducing bacteria can use oxidized transition metals such as iron or manganese as a terminal electron acceptor during anaerobic respiration. These organisms are frequently found in anaerobic sediment environments. For example, *Shewanella oneidensis* strain MR-1 was isolated in the anaerobic sediments of Oneida Lake in upstate New York and demonstrates a robust reduction of the oxidized manganese species in lake sediments. *Shewanella* species are widely distributed in the environment, but they are not the only bacteria capable of dissimilatory metal reduction. We used indicator of reduction in soils (IRIS) films, which are coated in an oxidized manganese paint, to detect the presence of bacterial manganese-reducing activity in soils. Under anaerobic conditions, manganese-reducing bacteria transfer electrons to the oxidized manganese on these films, which solubilizes and thus removes the paint. We sampled a variety of soil types across Rhode Island and found that manganese reduction by bacteria is ubiquitous in local soils. Using enrichment culturing methods, we isolated manganese-reducing bacteria from our Rhode Island soil samples. We have characterized these isolates using Gram staining and 16S rRNA gene sequencing analyses.

This work was supported by the Verizon Foundation and the Walsh Student Research program

The role of tmbi-4 in ER stress in *C. elegans*

Kevin Ly, Zach Medeiros, Edy Pineda, Michael Bittner & Melissa Silvestrini

Biology, Providence College, Providence, RI

The Bax inhibitor-1 (BI-1) gene is regulated by IRE-1, a transmembrane protein in the ER. Notably levels of BI-1 expression are altered in a myriad of human cancers and neurological diseases. Therefore, elucidating the cellular functions of BI-1 may reveal potential therapeutic targets to treat human disease. IRE-1 is one of the three pathways responsible for the detection of ER-induced stress caused by an accumulation of unfolded proteins. In response to ER stress, IRE-1 activates downstream targets involved in the unfolded protein response (UPR) to alleviate stress. Our objective is to determine whether the *C. elegans* Bax inhibitor-1 homolog, tmbi-4, is involved in ER stress in *C. elegans*. To determine if tmbi-4 is sensitive to ER stress similar to ire-1 we performed an ER stress assay. To induce ER stress, we treated animals with tunicamycin and measured their development. As expected, fewer ire-1 mutants developed into adults than wildtype animals. Our preliminary data suggests that tmbi-4 mutants exhibit similar developmental defects and increased sensitivity to ER stress as observed in organisms with impaired IRE-1 function. Past studies showed that ire-1 mutants are more sensitive to heat than wildtype animals. Hence, we performed a heat tolerance assay to investigate if tmbi-4 has similar sensitivity to heat as ire-1 mutants. Notably, tmbi-4 mutants were more sensitive to heat than wildtype animals. Overall, our studies suggest that tmbi-4 may be involved in ER stress.

This work was supported by Providence College

Development of a fluorescent based assay for fungal transglycosylases

Jett DuVal

Chemistry, Bryant University, Smithfield, RI

Pathogenic fungi are fungi that cause disease in humans and other organisms. Approximately 300 species are known to be pathogenic to humans. *Candida parapsilosis* is an emerging fungal pathogen associated with hospital-based infections and premature infants. *C. parapsilosis* can invade the bloodstream of neonatal infants because of their immature and easily injured skin and mucosal immune systems. In previous studies, the β-(1,3)-glucan transglycosylase Phr1 has been identified as an important virulence factor for adhesion to host tissues, making it a potential target for antifungal development. The overall objective of this project is to develop a robust enzyme assay for fungal transglycosylases using *Saccharomyces cerevisiae* β-(1,3)-glucan transglycosylase Gas2 as a model. Here we present the development of a fluorescence based assay for transglycosylase activity. A sulforhodamine derivative of laminarihexose (Lam6-SR) was synthesized via reductive amination of the reducing end to form the corresponding 1-amino-laminarihexose. This was followed by addition of sulforhodamine (SR) acid chloride to give Lam6-SR. Lam6-SR was obtained in 97% overall yield. This assay will allow for the screening of potential inhibitors to this class of enzyme and biochemical characterization of Phr1 from *Candida* sp.. Enzyme reactions were monitored by thin layer chromatography and mass spectrometry. Preliminary results show that Lam6-SR can serve as a substrate for Gas2. Gas2 hydrolyzes Lam6-SR giving rise to a trisaccharide product (Lam3-SR). Production of Lam3-SR was confirmed by mass spectrometry by direct analysis of TLC plates. This is an advantage over existing assays, as conventional assays give rise to multiple hydrolysis products. Preliminary results suggest that Lam6-SR can serve as a glycosyl acceptor when Gas2 is in presence of purified β-(1,3)beta-1,3-glucan. Transglycosylation products up to Lam12 were observed by mass spectrometry. Optimization of reaction conditions will be presented.

This work was supported by RI INBRE

Antagonism assays to map interactions of autolysin inhibitor masarimycin

Monica Thoma, Mika Gallati & Christopher Reid

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Peptidoglycan (PG) is the major polymer contributing to the structure of the cell wall of most bacteria, offering shape and protection to the cell. It is made up of alternating chains of N-acetylglucosamine (GlcNAc) and N-acetylmuramic acid (MurNAc) residues connected by a β-(1,4)- glycosidic bond, attached to the C-3 of MurNAc, is a pentapeptide. PG metabolism involves a system of biosynthetic and degradative enzymes, including those that regulate the cell wall by destroying old material and incorporating new material when necessary. Autolysins are degradative enzymes that break down components of the PG layer to enable normal cell wall turnover, among a multitude of other functions, which allows for growth and replication of a bacterial cell. Autolysins contribute to cell growth, division, motility, PG maturation, protein secretion, and genetic competence. When acted upon by antibiotic agents, autolysins are inhibited and can no longer produce chemical signals that communicate changes in the PG hydrolysis to the cytoplasmic biosynthetic steps. Masarimycin is a chemical probe developed to study PG metabolism, and has been shown to inhibit the autolysin, LytG, of the Gram-positive bacteria, Bacillus subtilis. Masarimycin inhibits the N-acetylglucosaminidase (GlcNAcase) LytG, the major active GlcNAcase during vegetative growth. The purpose of this study is to further characterize interactions between masarimycin and other antibiotics that have an already known mode of action and to uncover interacting pathways and further evaluate the mode of action. This was done through antagonism assays of masarimycin and cell wall targeting antibiotics on B. subtilis. Results from this study suggest that the relationship between fosfomycin and masarimycin is antagonistic (FIC index value 4.25), the relationship between cefuroxime and masarimycin is indifferent (FIC index value 3.4), and the relationships between masarimycin and bacitracin, ampicillin, and cefoxitin are synergistic (FIC index values 0.5, 0, 0.375, respectively).

This work was supported by the NSF

Neural circuits for moderate alcohol responses in *Drosophila melanogaster*

Anh Nguyen[1], Sam Pollack[1], Sophia Song[2], Nishell Savory[2] & Kristin Scaplen[1]

[1]Psychology, Bryant University, Smithfield, RI
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Drugs of abuse such as alcohol disrupt dopaminergic reward pathways, leading to maladaptive goal-directed behaviors. In both mammals and *Drosophila*, evidence suggests that dopamine also mediates ethanol-induced locomotor activity, however, the complex underlying neural circuitry remains poorly understood. Using flyGrAM, an automated group activity monitor, we investigated whether distinct populations of dopamine neurons (DANs) and their outputs are recruited to support ethanol-induced activity. Subsets of DANs and mushroom body output neurons (MBONs) were blocked using thermogenetic techniques and group activity was measure upon acute ethanol exposure. We performed high-content behavioral analysis of behavioral features in response to ethanol exposure using FlyTracker. Here we report changes in velocity, angular velocity, and distance to wall during baseline, early ethanol exposure, late ethanol exposure and recovery. We found that subsets of DANs and their corresponding output neurons are involved in behavioral responses to ethanol. Additionally, we discovered these neuronal microcircuits have temporally specific dynamic roles in modulating ethanol-induced activity. Overall, this study clarifies our understanding of the role of DANs in modulating ethanol-induced activity and serves as a starting point for more detailed circuit analyses.

This work was supported by RI INBRE

Cancer-derived human DNA polymerase θ variant L2538R shows altered DNA polymerase activity.

Naisha Rodrigues, Sarah Ebirim, Morgan Andrews, Corey Thomas, Lisbeth Avalos-Irving, Jorge Victorino & Jamie Towle-Weicksel

Physical Sciences, Rhode Island College, Providence, RI

UV radiation is an environmental factor known to damage DNA. Cells use specialized enzymes, such as DNA polymerases, to maintain genomic stability. Human DNA polymerase Theta (Pol θ, POLQ) is involved in translesion bypass repair of UV induced DNA lesions. However, overexpression of POLQ has been associated with poor survival rates amongst cancer patients, while POLQ deficient cells have shown increased UV radiation sensitivity in comparison to normal cells, indicating its crucial role in DNA repair. To date, there have been no biochemical functional studies on patient-derived variants of Pol θ. Through a collaboration with the Yale SPORE in Skin Cancer, we have identified variants of Pol θ in various melanoma tumor samples suggesting its potential involvement in cancer. By comparing DNA repair activities of mutant Pol θ to wild-type, we aim to elucidate the mechanism of UV-repair by Pol θ. Initial studies of the variants suggest decreased DNA polymerase activity as well as decreased bypass of UV-induced DNA lesions and altered DNA repair capabilities. Taken together, this first of its kind, biochemical study, may provide evidence as to the greater role of Pol θ in DNA repair.

This work was supported by RI INBRE

Detection of the anti-MRSA compound laurenobiolide from different parts of the tulip tree (*Liriodendron tulipifera*)

Nana Oblie, Riley Kirk, Christopher Via, David Rowley & Matthew Bertin

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The tulip tree (*Liriodendron tulipifera*) has a long history in Native American medicine and was also used during the Civil War to treat sick or wounded soldiers. Scientists later investigated these medicinal claims with modern chemical and biological tools and found that a tulip tree extract showed antibacterial activities against methicillin-resistant *Staphylococcus aureus* (MRSA). MRSA is a bacterium that causes infections in different parts of the body and can be lethal if left untreated. Because of its resistance to commonly used antibiotics, these infections are becoming increasingly difficult to treat, and new small molecule agents are necessary to overcome resistant strains. The University of Rhode Island (URI) Heber W. Youngken Jr. Medicinal Garden has over 200 medicinal plants. As part of URI education and research, the plants were extracted and added to the Principle Rhode Island Secondary Metabolite (PRISM) library. The PRISM library was recently screened for potential antibacterial agents against E.coli and MRSA. The tulip tree organic extract from the PRISM Library showed an inhibitory effect in MRSA growth, and subsequent bioassay-guided isolation workflows using high-performance liquid chromatography (HPLC) and mass spectrometry (MS) highlighted the known compound laurenobiolide, a sesquiterpene lactone, as the active antibacterial agent. The goal of my SURF project was to determine the abundance of laurenobiolide in different parts of the tulip tree, including the flowers, leaves, petioles, branches, and bark. All the plant parts collected showed the presence of the active compound except for the bark, and the branches showed the highest quantity of the metabolite. Further examination of the branches showed that the molecule was sequestered to the outer layers of the branches, which provokes intriguing questions with respect to the ecological role of the molecule for the plant. Additionally, this information will create more efficient extraction and isolation workflows for continued accumulation of this compound for future biological evaluation.

This work was supported by RI INBRE

Initial characterization of drug metabolizing enzymes found in human gut bacteria

Lily Lockhart[1], Kailey Paar[1], Jackson DeMartino[1], Matt Ciesla[2] & Tyler Stack[1]

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Individuals have varied responses to drug therapies, which can be costly and dangerous due to treatment delays and adverse side effects. Personalized medicine aims to address this issue, which will depend on an individual's genome. However, the specific molecular mechanisms by which gut microbes metabolize drugs receive much less attention, and the heterogeneity between individual gut microbiomes further complicates the matter. To address this problem, we have targeted several proteins in the human gut microbes Eggerthella lenta, Clostridium scindens, and Bacteroides thetaiotaomicron to metabolize and alter drug structures. Some of these enzymes include hydroxysteroid dehydrogenases, which alter the structure of host-produced steroids such as cortisol. Thus, we hypothesized that these enzymes affect drug metabolism on therapeutics designed to mimic cortisol structurally. Other enzymes from protein family PF13472 are generally lipolytic and have been previously shown to hydrolyze acetyl groups from drugs. In this project, we have cloned, produced, and tested the metabolizing activity of some steroid dehydrogenases and acetylesterases. Our future goal is to clone more genes and begin to quantify the efficiency of these drug-metabolizing enzymes. This investigation serves as a springboard into how a unique community of gut microbes modifies therapeutics, which can help predict drug metabolism based on an individual's microbiome.

This work was supported by RI INBRE

Tachykinin increases acute cold temperature tolerance in *Drosophila melanogaster*

Ana Martinez, Tallya Maciel, Renalison Farias-Pereira & Belinda Barbagallo

Biology and Biomedical Sciences, Salve Regina University, Newport, RI

Temperature, as a universal environmental stressor, threatens the homeostatic physiology of all organisms and alters their survival long-term. Survival needs to be ensured; thus, it is important to understand the neural circuits and neuropeptides involved in temperature regulation. Our main goal is to determine which neuropeptides and target tissues modulate acute cold stress response. Preliminary work from our lab has identified a novel cold stress circuit suggesting that the mushroom body (MB) prime lobes and corazonin (Crz) neuropeptide target the heart to provide an acute cold stress response. However, silencing the MB prime lobes results in a greater behavioral change than silencing Crz cells. Therefore, we hypothesized that additional neuropeptides, such as tachykinin (TK), play a role in acute cold stress response downstream of the MB. To screen for these potential neuropeptides, we utilize the fruit fly, *Drosophila melanogaster*, as a model organism, as they enter a chill coma state when their thermosensory systems get affected by cold temperatures. Our results indicate that the TK-like receptor and the TK ligand mutant had an increased chill coma entry at 8°C in comparison to the wild-type flies. These findings collaborate with those of Crz suggesting that the TK neuropeptide also moderates acute cold temperature toleration.

This work was supported by RI INBRE

Developmental perfluorooctanesulfonic acid (PFOS) exposure induces metabolic dysfunction in *Drosophila melanogaster*

Tegan Tanner, Renalison Farias-Pereira & Belinda Barbagallo

Biology and Biomedical Sciences, Salve Regina University, Newport, RI

Perfluorooctanesulfonic acid (PFOS) is a synthetic fluorinated hydrocarbon that is ubiquitous and persistent in the environment and bioaccumulates in humans. These characteristics have led to an increased concern about its health risks, however, little is known about the biological impacts of PFOS exposure. Previous studies performed by our lab showed preconception PFOS exposure resulted in metabolic dysfunction in *Drosophila melanogaster* when only the mother was exposed to PFOS. To further understand the biological effects of PFOS, the present study developmentally exposed D. melanogaster to PFOS throughout its life cycle and measured metabolic changes after exposure. Our results indicate that PFOS exposure significantly reduced the number of viable adults in the F1 generation. Cholesterol content was significantly increased due to PFOS, which could be correlated to the steroid ecdysone receptor (EcR) known to regulate developmental growth. PFOS exposure significantly upregulated EcR gene expression in both males and females. Total sugar levels were significantly decreased in females; this led to testing the expression levels of *Drosophila* insulin-like peptides which were significantly upregulated in males and females. Additionally, supplementation with alpha lipoic acid (ALA) showed promising results to mitigate the observed effect of decreased viability following developmental PFOS exposure of the F1 generation.

This work was supported by RI INBRE

Investigating the metal binding affinities of KmtR mutants

Avery Arbuckle, Gregory Labrie, Sebastian Santos & Khadine Higgins

Chemistry, Salve Regina University, Newport, RI

Mycobacterium tuberculosis (*M. tuberculosis*) is the causative agent of tuberculosis, which kills up to 2 million people per year. There is an increase in number drug resistant strains of this bacteria, and therefore targeting other pathways to kill these bacteria is important. The bacteria contain several metal transport systems which are necessary for its survival. The metalloregulator KmtR aids in regulating the concentrations of Ni (II) and Co (II) in the cell. Our goal is to identify the protein residues that are responsible for binding Ni (II) and Co (II). The E101Q mutant protein was expressed and purified. Metal binding studies of this mutant protein are being pursued.

This work was supported by RI INBRE

Understanding how N-terminal acetylation of microtubule associated protein tau affects oligomerization

Miguel Martinez Guzman & William Holmes

Biology, Rhode Island College, Providence, RI

Microtubule associated protein tau is a protein that is involved in strengthening and promoting assembly of the microtubules a structural component of cells. Especially being abundant in the neurons of the central nervous system. Various neurodegenerative diseases such as Alzheimer's and Parkinson's can be linked to insoluble aggregates of tau and it remains an area of intense study. Key in these studies are the effects of post-translational modifications on tau structure and fucntion. Tau's affinity for microtubules is regulated by phosphorylation, so these modifications are clearly important for regulation of function and structure. In order to better understand what causes these aggregates, work has focused on the binding domain and its C-terminus (and alterations to these areas), but little research on the N-terminus. Our goal is to look at N-terminal acetylation, a nearly ubiquitous modification to the N-terminus in tau and view its effects on aggregation and structure.. To better understand the disease causing pathways that lead to tauopathies, we will produce, modify, and purify tau for further study. Through *Escherichia coli*, we can both produce tau and the NatA complex which allows us to produce the wild-type tau and in a co-translational manner acetylate its N-terminus. Using methods of detection such as native gel electrophoresis allow us to assess oligomerization and aggregation of the protein in both its native and acetylated state and view acetylation's effect on stability and structure. Further characterization of the N-terminus may allow for prophylactic therapies to protect against neurodegenerative diseases in the future.

This work was supported by RI INBRE

Determining the effects of N-terminal acetylation on tau-microtubule binding affinity

Sara Palombo & William Holmes

Biology, Rhode Island College, RI

Tau, a protein known for its role in causing neurodegenerative diseases such as Alzheimer's, also works to stabilize neuronal microtubules, an essential structure in the function of the human brain and cytoskeleton. There is abundant research currently being done on the protein since it has a very complex structure and functionality. Tau is an intrinsically disordered protein that lacks any tertiary structure, making it difficult to determine the structure-function connection. This is complicated by the fact that Tau undergoes post-translational modifications (PTMs), like phosphorylation, which affects the structure of the protein and in some cases causes aggregates to form which contributes to the synaptic degeneration that is seen in many neurodegenerative diseases. Another PTM that affects the structure Tau is N-terminal acetylation, a process in which the positive N-terminus region of the protein is changed to a neutral one by adding an acetyl functional group which alters the stability of the protein. Our focus is to determine whether N-terminal acetylation alters the structure and function of Tau and whether it affects Tau's ability to bind to microtubules. To accomplish this, our first step was to successfully complete the process of purifying Tau with and without the acetyl group. We co-expressed Tau with an N-terminal acetyltransferase (NAT) and subsequently isolated Tau to a high degree of purity. Then, we were able to carry out a microtubule binding assay which would allow us to learn more about how N-terminal acetylation affects the affinity of Tau and microtubules. This work will further our understanding of how post-translational modifications can lead to changes in Tau's structure and function.

This work was supported by RI INBRE

Antioxidant and tyrosinase inhibitory activities of oleanolic acid indole derivatives

Madeline Melchiori[1], Kara Torrey[2], Hang Ma[2] & Navindra Seeram[2]

[1]College of Engineering, University of Rhode Island, Kingston, RI
[2]College of Pharmacy, University of Rhode Island, Kingston, RI

Triterpenes are a class of bioactive natural products with various pharmacological properties including antioxidant, anti-cancer, and anti-inflammation. Oleanolic acid (OA), a pentacyclic triterpenoid, is a bioactive compound found in a variety of medicinal plants and functional foods. It has been reported that the biological activity of OA can be enhanced by structural modifications of the OA skeleton. Our laboratory synthesized a series of new OA indole derivatives with improved skin permeability, but their potential skin beneficial effects are unknown. Herein, the OA indole derivatives were evaluated for antioxidant and skin lightening activities using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical bioassay and tyrosinase inhibition assay, respectively. The results showed that three OA derivatives, namely, HH-OA-62, HH-OA-63, and HH-OA-64 had promising antioxidant capacities with IC_{50} values of 616.6, 630.9, and 741.3 µM, respectively. In addition, OA derivatives HH-OA-62 and HH-OA-63 (between 15.625-62.5µM) showed inhibitory effects on the activity of tyrosinase enzyme with an inhibition rates of 29.8-82.7 and 16.0-56.8%, respectively. The anti-tyrosinase activities of OA derivatives were supported by data obtained from molecular docking experiments showing that these OA derivatives were able to bind to tyrosine protein. Findings from this study support the antioxidant and anti-tyrosinase effects of OA and its derivatives. Therefore, further studies are warranted to explore their mechanisms of action.

This work was supported by RI INBRE

Expression of eukaryotic and archaeal ribosomal proteins in *Thermus thermophilus*

Neelam Ahmed, Kelly McManus, Erin Killeavy & Steven Gregory

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Ribosomes are the macromolecular machine responsible for protein synthesis. They are assembled from large RNA molecules that are folded into their active conformation by the binding of numerous ribosomal proteins. Our laboratory has explored the role of one ribosomal protein, uS17, in ribosome assembly. Mutants of the bacterium *Thermus thermophilus* engineered to lack the gene for uS17 exhibit a severe ribosome assembly defect and a temperature-sensitive phenotype. Both the assembly defect and temperature sensitivity can be rescued by expression of uS17 from other bacterial species. In this study I have tested the ability of uS17 from archaea and eukaryotes to rescue these defects. My results show that *T. thermophilus* can be transformed with 5 of the 6 plasmids. *Pyrcoccus furiosus* showed little to no growth in comparison to the “No uS17” plate, indicating that its expression may be toxic.

This work was supported by RI INBRE

Formation of triplex DNA as a probe of conformational heterogeneity of bulky DNA adducts

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Our genome is constantly under attack from internal and environmental factors, such as UV radiation and DNA-damaging chemicals found in tobacco smoke, air pollution, and contaminated foods. Cells can deal with DNA damage in one of three ways: 1) repair mechanisms can recruit polymerases and other enzymes to the damage site to remove the damage and re-synthesize the DNA; 2) lesions unable to be bypassed or repaired can trigger cell death; or 3) lesions that can be bypassed may cause mutations to accumulate, leading to cancer. There are three major conformational motifs the bulky aminofluorene (AF) lesion can adopt, namely major groove (B), stacked (S), and minor groove (W). The S/B/W-population ratios are sequence-dependent, which influence lesion repair, replication, and mutagenesis. The present study seeks to use the formation of triplex DNA to probe the dynamics of AF-induced conformational heterogeneity and the nature of DNA-damage-protein interactions.

This work was supported by RI INBRE

Innervation of the spiny dorsal fins in bluegill (*Lepomis macrochirus*)

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Throughout evolution, appendages have adapted to meet specialized needs such as flying, swimming, and walking. Despite differences in form and function, appendages share similar mechanisms. For most animals, the musculoskeletal and nervous system work in tandem to produce voluntary movement. Neuromuscular pathologies that affect humans, such as multiple sclerosis and muscular dystrophy, severely limit mobility and reduce quality of life. Current prosthetics lack neural integration to provide sensory feedback for correct proprioception. By studying the innervation of spiny dorsal fins in bluegill, an important appendage used to stabilize perturbations, we hope to improve the design of prosthetics. We hypothesize that the spines are heavily innervated by motor and sensory neurons, with greater sensory innervation proximally and motor innervation up to the point of muscle attachment. Because methods to visualize muscle innervation are challenging, we used a combination of traditional histology and immuno-histochemistry. Specimens were stained with Sudan Black B, a myelin sheath stain, after digestion with trypsin, which has been shown to not alter nervous tissue, and we were able to identify peripheral nerves branching distally into the dorsal fin spines. In conjunction, we used immuno-histochemistry to visualize neuronal morphology. We used anti-acetylated tubulin and anti-calcitonin gene-related peptide primary antibodies to differentiate between motor and sensory neurons, respectively. Acetylated α-tubulin has been shown to be a structural protein used in neurons, and α-calcitonin gene-related peptide has been implicated as a pre-synaptic neurotransmitter in only sensory neurons. Neurons tagged by acetylated α-tubulin were shown to be denser near the point of spiny fin ray attachment. Motor innervation was absent in the fin ray whereas sensory neurons were present. On average, sensory innervation was found to be higher caudally in the body of the spiny fin rays. Future investigations should aim to create a 3D reconstruction of the spiny dorsal fins, as a model for understanding normal and abnormal limb proprioception.

This work was supported by RI INBRE

Motor control of the spiny dorsal fin in swimming bluegill

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Studying motor control in a simple system, such as that found in the Bluegill (*Lepomis macrochirus*) spiny dorsal fin, can inform how human systems control motor function. When the system loses its ability to receive feedback or produce muscle contraction, for instance Parkinson's disease or in prosthetics users, an individual's quality of life is reduced. Understanding how simple systems respond to lack of sensory or motor control will help us to understand how humans are affected. Bluegill were injected with the following solutions: Lidocaine, a nerve blocker; Flaxedil, a non-depolarizing muscle relaxant; or saline, used as the control treatment. Electrodes were implanted bilaterally in the erector and epaxial muscles. Dorsal and lateral video streams were recorded while fish swam in both turbulent and non-turbulent conditions. Particle image velocimetry was used to quantify the turbulence and to determine fluid displacement by the fins. The electromyography data were analyzed for duration, magnitude, frequency, duty factor, and cycle duration. The kinematic data were analyzed to see if, while under the different treatments, the fish had a significantly different fin displacement or velocity. When sensory and motor control are affected, we expect that fish will display a decrease in magnitude and duration of active muscles than seen in the control group. When turbulence is introduced to the fish, we expect the experimental group to show higher magnitude and duration than the trials run without turbulence. Under control conditions, turbulence caused higher fin displacement. Under sensory nerve impairment, there was no difference. While under muscle relaxants, this pattern is reversed. Under turbulence, fin displacement was higher in the control group. Activity of fin muscles was shorter under muscle relaxant than in the control group while under turbulence. In fish with impaired sensory system, the fin muscles were unable to correct for perturbations. Overall, lack of muscle control and sensory deprivation greatly impact fin deployment and the ability to correct fish position in the water column.

This work was supported by RI INBRE

The role of alx4b in embryonic zebrafish pigment cell specification

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Tropical fish have a variety of extravagant patterns and colors. These diverse patterns are produced by thousands of individual pigment cells. In fish and other vertebrates, pigment cells originate in the neural crest (NC), an embryonic population of pluripotent cells that differentiate into a plethora of cell types including muscle, bone and cartilage, pigment, and nerve cells. Prior to differentiation, neural crest cells undergo an epithelial to mesenchymal transition (EMT) transforming from stationary cells to invasive, migratory cells. As they travel to their final locations in the embryo, the neural crest receives signals from the cellular environment that direct both their migration and their differentiation. Data from zebrafish suggests that many neural crest cells undergo progressive fate restriction, but the complex gene regulatory networks underlying these restrictions are not fully characterized. Zebrafish have three types of neural crest-derived pigment cells: black melanophores, silvery shiny iridophores, and yellow xanthophores. The mechanisms of lineage restriction that generate three distinct differentiated pigment cells from multipotent or bipotent precursors are still not well understood. Recent work suggests that ALX homeobox transcription factors may play roles in promoting iridophore fate and repressing melanophore fate from a bipotent precursor. In zebrafish, both alx4a and alx4b are expressed by iridophores and alx4a mutants lack all body iridophores. The role of alx4b in zebrafish pigment cell development is currently unknown. Our lab previously used CRISPR/Cas9 to generate mutations in alx4b and isolated two alleles with premature stop codons. The goal of this project was to determine the role of alx4b during pigment cell differentiation and fate restriction. To do this, I crossed carriers of alx4b mutations and examined the phenotype of the offspring. I also counted iridophores and collected genomic DNA to genotype individual offspring. Finally, to investigate the possibility that alx4a can compensate for the loss of alx4b, we used Alt- R CRISPR technology to knockout alx4b in alx4a mutants. Together these experiments will provide a clue as to the role of alx4b during zebrafish development and fate specification of undifferentiated NC cells.

This work was supported by RI INBRE

The rotifer *Brachionus plicatilis* as a model for aging research

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Rotifers have been a subject of aging research for nearly a century. Many notable scientists, including Dr. Matthew Meselson, famed for discovering the semi-conservative model of DNA replication, have worked to understand links between stress, reproduction, and life span in rotifers. Rotifers have many characteristics that make them suitable for aging research, including parthenogenic reproduction, a natural life span of only two weeks, and somatic cell mitosis is complete before the adult emerges from the egg. Of the >2,000 species of rotifers documented, *Brachionus plicatilis* is commercially useful as a nutritional food source for larval fish. The *B. plicatilis* annotated genome was published in 2019. Here, we establish culture conditions for life span analysis of *B. plicatilis* and test the hypothesis that the drug rapamycin, a specific inhibitor of the TORC1 complex known to increase longevity in yeast, *C. elegans*, fruit flies, and mice, also increases the life span of *B. plicatilis*. We find that rapamycin at concentrations as low as 5□M can extend the median life span by as much as 50% without a significant reduction in fecundity.

The recent genome publication opens the doors for targeted genetic mutation in rotifers. We have begun studies to make a targeted mutation in the rotifer insulin-like growth factor receptor-1 (IGFR1) locus, a gene whose deletion leads to extended longevity in *C. elegans* and fruit flies. We have designed three guide RNAs to target the CRISPR-Cas12a endonuclease to the *B. plicatilis* IGFR1 locus and demonstrate efficient double-strand breaks (DSB) *in vitro*. We have also cloned a repair template to use in homology-driven repair that contains 500bp of complementary sequence flanking each side of the targeted DSB, with enhanced green fluorescent protein (eGFP) expressed by the *B. plicatilis* GAPDH promoter. DSB repair by homologous recombination with a fluorescent reporter allows for selection of targeted mutants. We are exploring methods of transfection utilizing electroporation and lipofectamine to introduce the Cas12a-gRNA complex into eggs. GFP+ rotifers may also be more enticing to larval fish as a food source, and genetic modification of rotifers may be advantageous to the commercial aquaculture trade.

This work was supported by RI INBRE

Rapamycin increases the life span of a *C. elegans* model of Huntington's disease but fails to improve a motility defect late in life

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Huntington's disease is a neurodegenerative condition caused by a dominant mutation in the human protein Huntingtin (HTT). The mutation is the result of a polyglutamine (CAG nucleotide) expansion in exon 1 of HTT that results in protein aggregation and progressive proteotoxicity in the basal ganglia. There is currently no cure for Huntington's disease.

Rapamycin is a drug that inhibits the mechanistic target of rapamycin complex 1 (mTORC1) and has been shown to increase the life span of many model organisms, including yeast, roundworms, fruit flies, and mice, partially due to the associated upregulation of cellular autophagy (molecular recycling). Rapamycin has also been demonstrated to increase markers of health in various transgenic animals expressing diseased alleles. Here, we investigate whether rapamycin provides a benefit to a *Caenorhabditis elegans* (roundworm) strain expressing a polyglutamine tract in frame with yellow fluorescent protein in the body wall muscle (Q35::YFP), a proteotoxic fusion protein that mimics diseased alleles of HTT. First, we demonstrate that rapamycin increases the life span of both wild-type (N2) and Q35::YFP animals and that the life span increase is dependent upon autophagy. Further, we show that aged (9-day old) Q35 animals have a significantly decreased thrashing rate compared to age-matched N2 animals in liquid medium. While rapamycin increased the thrashing rate in young N2 worms, rapamycin failed to rescue the decreased thrashing rate in aged Q35::YFP worms. Interestingly, the thrashing benefit observed in young N2 worms on rapamycin was found to be independent of autophagy, as demonstrated by RNA interference (RNAi) against two homologous genes required for the initiation of autophagy, lgg-1 and lgg-2.

This work was supported by RI INBRE

Isolation and identification of antibacterial-producing bacteria from the local temperate coral, *Astrangia poculata*

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Temperature changes are known to disturb the health and composition of coral's microbiome. The native temperate coral, *Astrangia poculata*, is a useful model organism for exploring these microbial shifts, because of the drastic temperature fluctuations it endures during the cold winters and warm summers in New England. Previous research demonstrates that the *Astrangia poculata* microbiome during its winter quiescence, a period of dormancy, is relatively unpredictable and variable, and its composition resembles that of diseased tropical corals. In spring, it recovers to a state that is consistent with healthy tropical corals and is less variable. This project aims to add to an existing culture library of bacteria isolated from *Astrangia poculata* mucus throughout seasonal timepoints. This library, the *Astrangia* Culture Collection (ACC), currently consists of 192 bacterial strains isolated from *A. poculata* mucus in Winter 2018 and Spring 2018. Mucus from wild colonies will be collected across four seasonal timepoints spanning 2021-2022. New additions to the ACC will be screened for antibacterial activity against surrounding seawater bacteria. Previously isolated active strains from winter 2018 and spring 2018 were re-isolated for purification, and identified via 16S rRNA sequencing. Results from screening these libraries will provide insight into the involvement of antibacterial compounds in regulation of the microbiome, and its relationship with seasonal shifts. This study may help in better understanding the impact that climate change is having on the microbiome of tropical coral, as well as the importance and function of the antibacterial isolates for overall coral health. Identifying these bacterial strains may also prove useful in probiotic, aquaculture, and other biotechnology applications.

This work was supported by RI INBRE

Effects of *Ulva compressa* on juvenile mussels

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In the summer months, sea lettuce blooms (also known as *Ulva* blooms) are all too familiar to those that live in the northeast regions of the coastal United States. These green algae are found on nearly all coastlines and are known to have growth-inhibiting effects on marine invertebrates; species of *Ulva* can produce compounds that influence the growth, development, and survival of other organisms. This study focused on the allelopathic effects of *Ulva* on juvenile mussels. Mussels have a significant ecological value as natural filters, continually feeding on plankton, algae, silt, and through this purifying the ocean ecosystem. As filter feeders that primarily reside on the bottom of the ocean, attached to other mussels or rocks, where they constantly filter water and particles through their siphons. To study these effects, twenty-five mesocosms were arranged under artificial lighting, provided air, and placed in divided mesocosms along with 20 mussels. There were five different treatments, 3.5g/L of *Ulva compressa* (n=5), 5.0 g/L of *Ulva compressa* (n=5), 3.5 g/L of *Gracilaria* (n=5), 5.0 g/L of *Gracilaria* (n=5), and mesocosm control (no algae; n=5). *Gracilaria* was used since it is not known to produce allelochemicals. We recorded the growth and mortality of the mussels weekly by examining them under a microscope; growth was determined by measuring the shell height and width. We documented an unexpected but rather strong effect of *Gracilaria* on mussel growth. Mussel growth was the same in both *Gracilaria* treatments and the *U. compressa* 3.5 g/L treatment and all were less than in the mesocosm control. Mussels grown with 5.0 g/L of *Ulva* had both the most mortality and the lowest growth. The reasons behind the decreased growth rate observed in Gracilaria treatments may be linked to the fluctuating pH of the algae mesocosms or other factors such as the location the seaweed was collected or cross-contamination of seaweeds. In the future, additional trials will be conducted to determine the specific causes for these patterns.

This work was supported by RI INBRE

Impacts of sea lettuce compounds on survival and growth of marine isopods

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With the changing climate we have seen an increase in macroalgal blooms across Narragansett Bay and surrounding areas. Macroalgal blooms occur when there is intense growth in free floating bloom-forming species of macroalgae that is often driven by an abundance of excess nutrients in the water and a reduction of herbivory. Blooms have the potential to alter seawater chemistry and soil biogeochemistry as well as harm the communities of organisms that reside in affected waters. These blooms include *Ulva*, a green macroalgae that releases certain compounds that have been linked to the inhibition of other macroalgal growth, as well as mortality of larval oysters. Based on this previous evidence, we investigated the lethal and sublethal effects of compounds released from both *U. compressa* and *U. lacinulata* on *Idotea balthica*, a marine isopod. *Idotea balthica* is essential to the health of intertidal ecosystems as they are an important prey species for larger invertebrates and juvenile fish. Using divided co-culture mesocosms *I. balthica* was observed for changes in growth and survival over the course of 4 weeks while being exposed to *Ulva* (either 3.5 g/L or 5.0 g/L) or *Gracilaria* (either 3.5 g/L or 5.0 g/L), a red macroalgae that is not known to release inhibitory compounds. We also included mesocosm controls that had isopods but no seaweed. Separate trials were conducted for *U. compressa* and *U. lacinulata*. *Idotea balthica* length was measured weekly via photographs to track if *Ulva* did inhibit growth and mortality was tracked daily. This presentation will discuss the results regarding inhibition of growth and mortality, as well as the implications of our findings.

This work was supported by RI INBRE

Elucidating glucocorticoid stimulated glucoregulatory changes in Hepa1-6 and Neuro 2a cells

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Metabolic syndrome is a pervasive public health issue in developing and developed countries due to overconsumption of obesogenic diets and a sedentary lifestyle. Human and animal findings support the notion that excessive and chronic exposure to GCs is sufficient to promote obesity and insulin resistance, which are 2 features of metabolic syndrome. Insulin is an anabolic peptide hormone produced by β-cells of the pancreas and it stimulates the uptake of glucose into cells. For example insulin stimulates glucose uptake in neurons, liver and adipose cells via an insulin-regulated glucose transporter (i.e, GLUT4). Glucocorticoids (GC) belong to a class of steroid hormones that play a key role in stress adaptation as well as basal glycemia. GCs stimulate central and peripheral tissue to promote hyperglycemia, in part via its antagonism of insulin signaling. For example in hepatocytes, GCs induce hepatic gluconeogenesis and glycogen catabolism functioning opposingly to insulin. Our prior findings revealed, similarly to others, an upregulation of Sirt1 protein and a corresponding downregulation of FoxO1 activity in hepatocytes exposed to insulin. Importantly, pharmacological induction of ER stress did not alter Sirt1 nor FoxO1 activity; yet ER stress was sufficient to mitigate the expression of GLUT1 upregulated due to insulin treatment. This suggests an insulin-ER stress pathway- dependent effect of glucoregulation stimulated via excessive GCs.

The goal of the current study was to investigate glucocorticoid stimulated changes to key glucoregulatory mechanisms in an in-vitro hepatic and neuronal cell line system. Hepa 1-6 and neuro 2a cells were treated with DEX (0, 10, or 100 uM) for 2 or 4 hours to test the hypothesis that chronic exposure to supraphysiological levels of GC impairs intracellular insulin signaling or insulin-independent GLUT expression via a Sirt1-dependent mechanism. Overall, findings reveal DEX-dose and length of exposure effects related to glucocorticoid receptor expression and GLUT expression.

This work was supported by RI INBRE

Elucidation of sex-specific hedonic and homeostatic disturbances caused by an obesogenic diet in Sprague Dawley (*Rattus norvegicus*) rats

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Chronic overconsumption of hyperpalatable, hypercaloric foods is a growing concern in modern society, despite increased awareness and mitigation efforts to reduce its prevalence. It is a significant contributing factor to metabolic syndrome, a cluster of symptoms that includes hypertension, hyperglycemia, abdominal obesity, and abnormal cholesterol levels. Unresolved metabolic syndrome can paradoxically result from or lead to insulin resistance and the development of type 2 diabetes, among other diseases. Dysregulation in both central (brain) and peripheral tissue (i.e., liver and adipose tissues) contributes to the development of metabolic syndrome. In this study, male and female subjects were fed a high-fat diet (HFD) or a standard control diet for 12 weeks. Findings revealed that males are prone to development of metabolic dyshomeostasis while females are resilient to the effects of chronic HFD exposure. While no differences in anhedonia were discerned, HFD males displayed behaviors suggestive of enhanced anxiety. We posit that glucoregulatory and anxiety-related effects are likely due to changes in stress hormone physiology in both central and peripheral tissues, in tandem with dysregulated hepatic glucose metabolism.

This work was supported by RI INBRE

Stress and eating behavior

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Background:
Cortisol is a glucocorticosteroid synthesized by the adrenal glands and is associated with several precursors to chronic disease including hyperglycemia, nerve damage, digestive issues and weakened immune system. Cortisol is also directly involved in blood pressure maintenance. Elevated blood pressure, or hypertension, is among the most important risk factors associated with cardiovascular disease (CVD), the leading cause of mortality in the US and worldwide. Investigation of the factors associated with elevated cortisol may help researchers and clinicians better understand and prevent disease associated with chronic stress.

Objective:
The goal of this study is to determine if there is an association between salivary cortisol concentration with eating behavior, supplement use or body mass index (BMI).

Methods:
To test our hypotheses, we interviewed 20 healthy, non-pregnant women between the ages of 18-49 in the Kingston, RI area using the Intuitive Eating Scale and a food diary to assess eating behavior. Late night eating was defined as eating a meal or snack after 8:00 pm. Supplement use was determined by survey and weight was measured by the participants, who were asked to submit a photo of a scale recording their weight. Participants were also asked to collect a total of 8 saliva samples over 2 days (4 per day) upon waking up, 30 minutes after waking up, 1 hour after waking up and 1 hour before going to bed for analysis by competitive enzyme-linked immunosorbent assay (ELISA).

Results:
The results indicate that there is not a significant association between salivary cortisol concentration with eating behavior, supplement use or BMI. Mean salivary cortisol concentration was 1.051 ug/dL in the early eaters' group and 1.103 ug/dL in the late-night eaters' group ($p = 0.696$). Mean salivary cortisol concentration was 1.228 ug/dL in the non-supplement users' group and 1.013 ug/dL in the supplement users' group ($p = 0.121$). Lastly, mean salivary cortisol concentration was 1.079 ug/dL in the normal weight group, 1.032 ug/dL in the overweight group, and 1.283 in the obese group ($p = 0.707$).

Conclusion:
More research is needed to conclude whether there is an association between salivary cortisol concentration with eating behavior, supplement use or BMI. Specifically, an adequately powered and more heterogenous study population would be needed to impact our understanding of health and healthcare.

This work was supported by RI INBRE

Chromosome conformation capture (4C) on BA clusters

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DNA and histone proteins are complexed to form nucleosomes, the basic unit for chromatin structure. The organization of chromatin, specific folding and looped regions, regulate the expression levels of genes which determine whether genes are expressed (on) or repressed (off). In order to identify the regulatory interactions that mediate these changes in gene expression, we used circular chromosome capture conformation techniques (4C). This chromosome conformation capture method produces a snapshot of the three-dimensional organization of the chromosomal folding found within the nuclei of a cell. Analysis of this information can serve as a depiction of the structure chromatin develops in order to efficiently place regulatory sequences between, for example, enhancers and promoters necessary for proper control of the gene. Overall, the 4C methodology is advantageous, for it results in less bias in region identification since most other chromosome capture techniques require known primers and interactions in order to amplify the capture sequence.

The following study focuses on the HOX gene family of regulatory genes. This family controls cellular mechanisms such as differentiation and embryonic development important for producing the proper spatial body plan along with correct functionality of tissues and organs. The developmental processes of the HOX gene that regulate gene expression, which corresponds to cellular growth, coincide with pathways commonly manipulated by cancer to metastasize beyond natural borders and bypass apoptotic signals. Studies have illustrated that molecular events that result in chromatin modification is a crucial factor to the transcription of regulatory genes, such as the HOX genes. Although the full extent of how HOX genes are regulated by chromatin structure is unknown, many studies have demonstrated that alterations in the spatial architecture of chromatin results in improper regulation. In addition, HOX genes have emerged as a factor identified to be mis-regulated in various cancer types (such as colon, lung, and prostate), though their role in disease is unknown. Therefore, to provide more insight on this field of molecular biology and understand the mechanisms that regulate HOX gene expression, we mapped chromatin interactions through the HOX clusters of zebrafish embryos using 4C followed by high-throughput sequencing technologies.

This work was supported by RI INBRE

Activation of the retinoic acid signaling pathway during embryogenesis in Fanconi anemia

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Fanconi Anemia (FA) is a rare genetic disorder characterized by developmental abnormalities, increased risk for bone marrow failure, and heightened susceptibility to various cancers. There are currently 23 known FA genes. Functional FANC genes regulate and repair interstrand crosslinked (ICLs), but defects in FANC genes give patients a reduced ability to repair DNA damage making them vulnerable to issues involving genetic instability. Recent RNA-seq analysis has demonstrated major alterations in the expression of retinoic acid (RA) signaling genes in cells from an FA patient. During RA signaling, retinol is converted to retinaldehyde by the RDH10 enzyme and retinaldehyde is metabolized by ALDH1A1 into retinoic acid. Retinoic acid is transported to the nucleus by the CRABP2 protein, where it binds to the RAR/RXR transcription factor and regulates the transcriptional activation or repression of many genes. Genes regulated by this network are associated with cell growth and differentiation. RNA-seq analysis revealed highly significant increases in the expression of ALDH1A1 and RDH10 in an FA patient line. The primary goal of this research project was to validate the increased expression of the ALDH1A1 and RDH10 enzymes in these cells. Using immunoblotting analysis with appropriate positive and negative controls, we have confirmed increased expression of both the ALDH1A1 and RDH10 enzyme in the FA patient line. This mis-regulation of the retinoic acid signaling pathway is critical as the pathway is responsible for vertebral development and limb patterning regulation. Developmental abnormalities are prevalent in FA patients in which the importance of this pathway should be explored further.

This work was supported by RI INBRE

Biofilm resistance: The role of antimicrobial combination therapy in device related infections

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Background:
Antibiotic concentrations needed to eradicate biofilm require further research as infections caused by biofilm-forming bacteria result in antibiotic failure and recurrent infections. We aim to simulate a prosthetic joint infection (PJI) to elucidate high dose (HD) localized concentrations of antibiotics ability to eradicate varying *Staphylococcus epidermidis* biofilms.

Methods:
We utilized a *Staphylococcus epidermidis* high biofilm-forming strain (ATCC 35984™) and a low biofilm-forming strain (ATCC 12228TM). The Centers for Disease Control (CDC) Biofilm Reactor with chromium cobalt coupons was utilized to simulate a PJI. The reactor underwent a 24-hr growth phase and 16-hr conditioning phase to form an established biofilm. Then a 48-hr PK-PD phase was run. We modeled growth control, systemic regimens, monotherapy HD localized of 1000x MIC, and HD combination therapy with each monotherapy drug run with 1000x MIC rifampin. Coupons were sonicated, vortexed, then plated on Tryptic Soy Agar for colony counts. Samples were taken from these to determine shifts in susceptibility.

Results:
In both isolates, HD localized daptomycin followed by levofloxacin with and without rifampin, were the most effective antibiotics. In the formed biofilm, HD localized vancomycin alone or in combination with rifampin did not eradicate biofilm, and all systemic treatments failed to eradicate biofilm. Only rifampin treatments experienced MIC shifts (0.015 to >32mcg/mL).

Conclusion:
The failure of systemic and vancomycin treatments validates clinical recurrent infections. High concentrations of daptomycin and levofloxacin with and without rifampin are needed at the site of action to eradicate biofilm. Further evaluating these concentrations and their PK-PD indices will provide a foundation for altered clinical therapy.

This work was supported by RI INBRE

Exploring the effect of quorum sensing inhibitors on bacterial biofilm

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Antibiotic resistant bacteria pose a significant threat to public health. Bacteria such as *Chromobacterium violaceum*, a Gram-negative bacterium found in soil and water in tropical regions, form a biofilm on surfaces and once formed it is almost impossible to remove. This biofilm mode of growth is known to protect bacteria against the host immune defense and enable tolerance against conventional antibiotics. The quorum sensing (QS) pathway in *C. violaceum* and other antibiotic resistant bacteria regulates the formation of biofilm. Autoinducers like N-acetylated homoserine lactones (AHLs) bind to the LuxR receptor in the QS pathway in Gram-negative bacteria, which signals for virulence factors and the formation of biofilm. At this time, not many compounds have been identified that inhibit biofilm formation. Our laboratory has designed and synthesized several compounds that inhibit quorum sensing in *C. violaceum*. We are currently testing these compounds in *C. violaceum* to determine if they have the ability to inhibit the formation of biofilm without affecting growth. The discovery of such compounds would enhance the understanding of small-molecule disruption of QS pathways and provide potential new leads in the development of new drugs against antibiotic-resistant pathogens.

This work was supported by RI INBRE

Targeting quiescent *E. coli* for prevention of recurrent urinary tract infections

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Urinary Tract Infections account for nearly 25% of all bacterial infections and more than 50% of women will be diagnosed with at least one UTI. Of these infections, 27% of them will reoccur within the following 12 months even after successful antibiotic treatment. One of the underlying causes of this recurrence is the ability of uropathogenic *Escherichia coli* (UPEC) to enter a dormant, quiescent state within the epithelial cells of the bladder. This state allows the bacteria to survive antibiotic treatment and continue growth after "successful" treatment. It was recently discovered that certain peptidodglycan stem peptides can prevent or reverse the quiescent state in UPEC. The goal of this research is to synthesize various peptides using the manual solid phase synthesis method in order to determine the molecular characteristics required to reverse quiescence. This research will help to discover peptides that could lead to new treatments for recurrent UTIs and reversal of quiescence.

This work was supported by RI INBRE

Studying the function of heterogeneous ribosomes using antibiotics

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Antibiotic resistance is an ever-growing global threat to our ability to treat bacterial infection. A potential bioweapon, the Gram negative bacteria *Francisella tularensis* is highly infectious and has the potential to cause lethal disease. The ribosomal protein bS21 is important for translation initiation and the *F. tularensis* genome encodes three homologs: rpsU1, rpsU2, and rpsU3. We are interested in bS21 as we suspect that differential use of these homologs may impact translation initiation by altering ribosome structure. These three bS21 homologs were investigated individually using isogenic strains in which all three native rpsU genes were deleted but contain a single homolog at a neutral location. These strains, named Tn7::rpsU1-V, Tn7::rpsU2-V, Tn7::rpsU3-V, respectively, were used to determine if ribosomes incorporating different bS21 proteins have different susceptibilities to ribosome-targeting antibiotics. The only antibiotic found with altered efficacy among these strains is kasugamycin, a drug that inhibits translation initiation. We were able to confirm differences in kasugamycin resistance among the strains with different bS21 content and also discovered resistant mutants during the process. We determined that all the kasugamycin-resistant mutants have predicted inactivating mutations in the ksgA gene. In this project, we validated the differences in susceptibility to kasugamycin among *F. tularensis* strains with altered bS21 content and identified mutants with increased resistance in a known kasugamycin resistance-determining gene. The differences we identified in kasugamycin sensitivity suggest structural differences among ribosomes with altered bS21 content, suggesting that we may be able to exploit heterogeneity in ribosomes to develop antibiotics that target different ribosome populations.

This work was supported by RI INBRE

Cafeteria experiments with ants: Testing hypotheses about the nutritional ecology of frass

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Caterpillars can eat a significant amount of leaves and therefore make an abundant amount of waste, which is disposed of to the ground in granular pieces called frass. A species of ant called *Trachymyrmex septentrionalis* is known to actively forage for these pieces of frass to feed to a fungus garden that it cultivates. It has not been determined if other species of ants also, including any in our region, forage for frass. Are ants attracted to caterpillar frass and if so, would the ants eat the frass? Ants are known to eat the waste of other insects, such as aphids which eat vegetation. Plants are high in carbon and low in nitrogen and phosphorus, which means aphids have to eat a substantial amount of plant tissue to receive the important elements needed to grow. This causes aphid's fluid excrements to be a sweet delicacy for the ants who drink it and in turn the ants protect the aphids in a mutualistic relationship. Caterpillars can be in a similar ecological niche as the aphids, so we predicted that, if given the opportunity, ants would tend them (and feed on their waste) similarly. We collected frass from *Danaus* and *Cecropia* caterpillars, presented the pieces to ant colonies reared in the lab, and recorded observations over the following month for the following species: vampire ants (*Stigmatomma pallipes*), needle ants (*Brachyponera chinensis*), acorn ants (*Temnothorax curvispinosus*), and seed-harvester ants (*Aphaenogaster rudis*).

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The role of Copine2 in brain homeostasis

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Mitochondrial dysfunction is a hallmark of aging and has been implicated in many neurodegenerative diseases, such as Parkinson's and Alzheimer's disease, and in promoting neurocognitive deficits. Mitochondria play a crucial role in biological processes that maintain cellular energetics and homeostasis. A genetic screen that focused on genes associated with mitochondrial membrane potential found Copine2 (Cpne2) to be a positive regulator of mitochondrial function. CPNE2 is a member of the Copine family, which are a group of calcium dependent membrane binding proteins conserved in all eukaryotes. Our *in vitro* data indicate that the removal of Cpne2 increased mitochondrial membrane potential and stability. Since mitochondrial transcription was not found to be impacted, this would suggest that CPNE2 has a role in mitochondrial turnover. We found that Cpne2 is expressed in brain, liver, and spleen. In brain, high expression of Cpne2 has been detected in ependymal cells and choroid plexus as well as in amygdala and in cerebellum.

Whether Cpne2 regulates mitochondrial function *in vivo* and how it contributes to brain homeostasis is currently unknown. We have found that the removal of Cpne2 in mice results in increased anxiety and aggression as well as cognitive deficits. Here, we investigated whether *in vivo* levels of mitochondrial proteins are affected by removing Cpne2 in mice, as indicated in our *in vitro* studies, by measuring succinate dehydrogenase protein B (SDHB) and propionyl-CoA carboxylase (PCCA) with western blot in cerebellum and liver from Cpne2 Knockout (KO) and wild-type (WT) mice. Additionally, we performed immunohistochemistry to assess the amount of gliosis in brain from Cpne2 KO and WT mice by immunolabeling glial fibrillary acidic protein (GFAP). Our results suggest that SDHB levels decrease in cerebellum from female, but not in male, Cpne2 KO mice as compared to WT mice. Preliminary immunohistochemistry data suggest decreased GFAP expression in several Cpne2 KO brain regions, including cerebellum, hippocampus, and ventricular regions, as compared to WT. Ongoing experiments aim to better understand the biological function of CPNE2 and its role in brain homeostasis, which could lead to treatments to ameliorate brain health.

This work was supported by RI INBRE

Role of flagellin methylation in plant-human pathogen interactions

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Salmonella enterica is a Gram-negative bacterium that causes gastroenteritis. It is mainly associated with contaminated poultry and meat products but can also be found on fruits and vegetables. Motility of this pathogen is mediated by thin appendages called flagella, which are made up of the flagellin proteins FliC and FljB. Flagellin proteins in *S. enterica* are post-translationally modified by methylation on lysine residues via the methylase, FliB. When a *S. enterica* bacterium enters a host, these proteins are targeted by the host's immune system. The methylation of FliC and FljB may help this pathogen to bypass immunogenic attacks. Flagellin methylation was also found to be influential in adhesion to hydrophobic host cells and colonization of host epithelial tissue. In previous studies, residual methylation was found in an *S.* Typhimurium fliB deletion mutant, signifying that there may be other enzymes may be capable of methylating this protein. The main hypothesis of this project predicts that residual methylation in a *S. enterica* serovar Typhimurium fliB deletion mutant occurs preferentially at certain sites and is not influenced by growth phase. It is also predicted that loss of the fliB gene in any serovar will result in decreased levels of plant-bacterium interactions due to reduced hydrophobicity of these flagellin proteins. To determine whether *S.* Typhimurium's flagellar methylation influences adherence to surfaces, a series of experiments were performed using a fliB deletion mutant strain and a wild-type strain. These included biofilm assays, plant cell wall attachment assays, motility assays, and methylation mapping of flagellin proteins by mass spectrometry of an SDS Page gel. Preliminary data suggests the fliB deletion mutant in *S.* Typhimurium adheres more strongly than a corresponding mutant in *S.* Thompson to a plant cell wall model comprised of cellulose. Data from the motility assay has revealed that the *S.* Thompson fliB mutant is more motile than the *S.* Typhimurium mutant. Both mutants yielded strong biofilm formation, signifying that the deletion of fliB did not influence the bacterium's ability to form a biofilm. Methylation mapping is also underway, with early data suggesting that both mutants preferentially express the FliC flagellin during stationary phase. From these data, it appears that flagellin methylation can influence many aspects of *S. enterica*'s physiology.

This work was supported by RI INBRE

Methylation patterns of phase locked flagellar mutants of *Salmonella enterica* and their effect on plant-human pathogen interactions

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Salmonella enterica, a Gram-negative bacterium, is the most common causative agent of food-related illness in the United States. Although customarily associated with eggs and poultry, 12% of *S. enterica* outbreaks are linked to fruits and vegetables, which is why studying plant-*Salmonella* interactions is crucial. Wild-type *S. enterica* serovar Typhimurium cells express two different types of flagellar filaments, made up of either FliC or FljB flagellin proteins. The phase-locked OFF cells produce only FliC while the phase-locked ON cells produce only FljB. An enzyme FliB modifies the flagellin proteins following translation by adding methyl groups to lysine residues, however, it is unknown if methylation is dependent on the accessibility of lysine residues or the amount of FliB available. The objective of this research project is to map the relative methylation levels of the FliC and FljB proteins in *S.* Typhimurium and to determine whether selective expression of one flagellin type influences motility, biofilm formation and adherence to a bacterial plant cell wall model. Previous data from our lab suggests that the FljB flagellin is more methylated than FliC. These differing methylation levels are expected to affect attachment and adhesion due to their influence on surface hydrophobicity of the flagella, such that flagellin proteins with high levels of methylation would colonize the artificial cell wall more efficiently and be more likely to form strong biofilms. SDS-PAGE analysis of purified flagellin proteins confirmed the expression of FliC and FljB in the phase-locked OFF and ON strains, respectively, and methylation analyses by mass spectrometry are currently underway. Initial biofilm data revealed that these strains are both strong biofilm formers, suggesting that expression of a single flagellin type does not impact this phenotype. Preliminary results of a bacterial plant cell wall attachment assay imply that phase-locked ON (FljB+) cells adhere more strongly to cellulose, the hydrophilic portion of the cell wall. This is not consistent with higher methylation levels leading to increased hydrophobic interactions and suggests that additional cell surface components may be mediating the observed interactions.

This work was supported by RI INBRE

Investigation of *Pseudoalteromonas* sp. strain JC3 as a putative probiotic for shrimp aquaculture

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Early Mortality Syndrome (EMS) is a disease affecting many species of Penaeus shrimp in aquaculture systems. In 2013 the causative agent was identified as *Vibrio parahaemolyticus* causing Acute Hepatopancreatic Necrosis Disease (AHPND). This Gram-negative bacterium utilizes a Type 6 Secretion System (T6SS) to inject binary toxins PirA and PirB, causing deterioration of epithelial cells in the shrimp's key organs. Current forms of disease mitigation, including antibiotics, vaccines and immunostimulatory methods, either fuel antibiotic resistance, are impractical, or are costly. Probiotics hold a promising future for pathogenic bacterial elimination because they may boost host survival using multiple modes of action. *Pseudoalteromonas* sp. strain JC3 is a Gram-negative marine bacterium that has shown protective effects against the AHPND-causing strain *V. parahaemolyticus* PSU5579; however, the mechanism of action for this host protection is currently unknown. Prior to conducting this research, genomic analysis of JC3 suggested the biosynthetic potential to produce secondary metabolites, such as alterochromides: highly conjugated, cyclic peptides that exert cytotoxic effects. In this project, we've investigated alterochromides and other possible antimicrobial compounds produced by JC3, such as quinolones and the Gram-negative antimicrobial, Darobactin. We hypothesize that these compounds decrease *V. parahaemolyticus* pathogenicity by limiting growth and interfering with cell-cell signaling.

This work was supported by

Evaluations of minor phytocannabinoids' antioxidant activity and inhibitory effects on the tyrosinase enzyme

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Apart from the major cannabinoids including cannabidiol (CBD) and delta-9-tetrahydrocannabinol (Δ-9-THC), minor phytocannabinoids may also contribute to the overall biological effects of the *Cannabis* species. However, the biological effects of minor phytocannabinoids remain largely unknown. Herein, we evaluated the antioxidant activities of a series of phytocannabinoids including Cannabigerolic acid (CBGA), Cannabidivarin (CBDV), Cannabigerol (CBG), Cannabinol (CBN), Cannabicitran (CBT), Cannabichromene (CBC), and delta-8-tetrahydrocannabinol (Δ-8-THC) using the 2,2-Diphenyl-1-picrylhydrazyl (DPPH) free radical bioassay. The results showed that these minor phytocannabinoids exerted minor antioxidant capacities, with the lowest IC50 value reported being Δ-8-THC at 0.955 mM. In addition, the anti-tyrosinase enzymatic assay was used to evaluate the skin beneficial effects of the minor phytocannabinoids. Δ-8-THC, and CBG exhibited strong inhibitory effects against tyrosinase, with IC50 values of 0.24 µM, and 0.276 µM, respectively. The inhibitory effects of each minor phytocannabinoid on tyrosinase were supported by data from computational docking studies. Molecular docking revealed that CBN, CBT, and Δ-8-THC showed favorable binding affinity with tyrosinase enzyme protein (PDB ID: 2Y9X) with free binding energies of -8.76, -9.55, and -8.37 kcal/mol, respectively. Taken together, findings from our study suggested that some minor phytocannabinoids are weak antioxidants with promising anti-tyrosinase activities. Further studies are warranted to evaluate phytocannabinoids as active ingredients for dermatological applications.

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Synthesis of a novel water-soluble noria and its binding properties

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A macrocycle is a chain of repeating molecules that forms a ring. Depending on the size of the ring, other molecules can fit inside it and bind to it. Noria is a macrocycle that forms six outer pockets in addition to the center pocket. These pockets can be employed to bind additional molecules in them. If a drug was bound in noria's outer pockets, then it could be used to deliver seven drug molecules versus one per macrocycle. However, noria is not soluble in water, posing an issue in pharmaceutical applications as water is a primary component in the human body. Therefore, the purpose of the project was to modify noria so that it becomes water-soluble.

A methylene sulfonate group was successfully added to noria to form a water-soluble version, dodecasulfonatomethylnoria (SMN). After synthesizing the molecule, nuclear magnetic resonance (NMR) titrations were performed with SMN as a host and amino acids as the guest binding into the pockets of SMN. Future work on this project, which has started, includes making a rotaxane with SMN as the host and performing binding studies with the rotaxane.

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The use of cyclodextrins to enhance the potency and solubility of anti-cancer agents

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N-(4-Hydroxyphenyl)-2-benzofurancarboxamide (NHBI) was the single benzofuran derivative out of 50 assayed by that showed substantial cytotoxic anti-tumor cell activity and was the most efficient at inhibiting transcription factor NF-κB activity.1 With a favorably placed alcohol group opposite to the binding site, the idea of this project was to target this alcohol to create a linear molecule composed of two NCBI molecules linked by a central linker molecule. One of the goals of this project was to use the cationic 4,4 Bipyridine central linker, which is well known to form inclusion complexes, with 2 NCBI molecules. Cyclodextrins (CDs) are cyclic structures that consist of multiple glucose subunits covalently linked by glycosidic bonds. These naturally occurring cyclic sugar-based molecules are known for their ability to form inclusion complexes with small linear molecules, especially those with a cationic center. Cyclodextrins are known to increase the solubility of their guest molecules, and by having 2 NCBI molecules to be phagocytosed by cancerous tissue, this work also shows a potential to increase the potency of the drug delivery. In this research, we demonstrate the potential to create a new linear guest molecule that contains two active NCBI molecules per inclusion complex. The driving force of this phenomenon relies heavily upon hydrophobic effect and electrostatic interactions between host and guest. The potential mechanism by which we hoped to synthesize the thread molecule was by reacting the NCBI with dibromoethane in excess, creating a monosubstituted product of NCBI with a bromoethane attached to the alcohol on the amine adjacent ring. This monosubstituted product is then reacted with 4,4 Bipyridine, a cation, to form a favorable pseudorotaxane guest molecule. This is then titrated into cyclodextrin at varying concentrations to show how the peaks change at varying levels of host to guest.

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Looking for the signal: Determining how uropathogenic *Escherichia coli* exit dormancy

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Uropathogenic *E. coli* (UPEC) cause the majority of urinary tract infections (UTIs) and many of these infections are recurrent within six months. This recurrence may be attributed to UPEC entering a dormant state termed quiescence *in vivo*. At a low cell density, UPEC are unable to grow on a minimal medium containing glucose as the sole carbon source. This *in vitro* system for quiescence can be used to provide mechanistic insight into UPEC quiescence. UPEC cells are stimulated to grow when supplemented with fragments of the essential bacterial cell wall component peptidoglycan (PG) but it is unclear how PG triggers UPEC exit from quiescence. Using a mini-Tn5 transposon screen to generate random mutants of CFT073, the classic ST73 endemic lineage of UPEC, that are unable to respond to PG fragments. We have thus far developed a system that can screen a high quantity of mutants at a time and an arbitrarily primed polymerase chain reaction (AP-PCR) can then be performed to identify which gene(s) are responsible for utilizing PG fragments to exit quiescence. This will provide insight into how UPEC exits the quiescent state which will help guide the design of novel therapeutics.

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MinD oscillation in *Escherichia coli*: Investigating MinE mutant proteins *in vivo*

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In the United States, nearly two million people are infected by antibiotic resistant bacteria each year; thus, novel antibiotic targets are constantly being investigated. The cell division pathway is widely conserved across bacterial taxa, and many proteins that make up the cell replication machinery are favorable targets for these novel antibiotics. During division, the protein FtsZ polymerizes to form a ring at the mid-cell position that is thought to constrict, resulting in septation and two identical daughter cells. FtsZ-ring assembly is regulated by the Min system, which consists of three proteins: MinC, MinD, and MinE that work together to allow FtsZ polymerization at the mid-cell region and restrict it at the poles. This is regulated by MinD oscillation. MinD binds to the membrane with ATP. MinE stimulates MinD ATP hydrolysis, causing MinD's release from the membrane and for MinD to move from cell pole to cell pole with MinC through regulated membrane association, where MinE continually binds and releases MinD, driving oscillation forward. This regulated binary fission event is essential to creating healthy daughter cells and preventing minicell formation. We plan to investigate MinE's interactions with the rest of the Min system in promoting normal cell division. This will specifically focus on MinE's function of removing MinD from the cytoplasmic membrane to establish the oscillation of MinD. To observe the effect on oscillation we will monitor the cellular location of a fluorescent MinD chimeric protein, fused to green fluorescent protein (GFP) in the absence and presence of MinE wild type and mutant proteins expressed from the chromosome.

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Development of GMCSF encapsulated Cell-Membrane Coated Nanoparticles for Cancer Immunotherapy

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Granulocyte-macrophage colony-stimulating factor, GM-CSF, a cytokine, is known to be an excellent immunotherapeutic agent, as it is a dendritic cell chemoattractant, and is a critical factor for dendritic cell development. However, it has a very short half life of about 50-85 minutes resulting in low therapeutic efficacy [1]. As a result, GM-CSF is usually used only in the treatment of palpable tumors (e.g. melanoma). Encapsulating GM-CSF in nanoparticles will facilitate a more controlled and prolonged release of the cytokine thus improving the therapeutic efficacy. We were able to successfully encapsulate GM-CSF inside a PLGA nanoparticle having a particle size of around 130 d.nm and a good polydispersity index (PDI) of 0.232. The drug release study performed for 21 days in PBS at 37°C demonstrated a burst release of GM-CSF over a 24 hour period. Although the concentration of GM-CSF drastically dropped after 24 hours owing to the degradation of the released GM-CSF, the presence of GM-CSF throughout the study proved the sustained release of the cytokine from the nanoparticle system. Encapsulating GM-CSF in cancer cell membrane coated nanoparticles (CCNP) can enhance the delivery of GM-CSF to the tumor site, as the homotypic properties of cancer cell membranes can interact with the tumor [2]. CCNPs were synthesized using an extruder, with a promising average size of 164.2 d.nm and PDI of 0.281. EVOS images after loading fluorescent dyes in both the cell membrane and the PLGA layers confirmed the core-shell nature of the formulation. Once the ratio of cell membrane to nanoparticles is optimized, GM-CSF will then be encapsulated inside CCNP and studies will be performed to analyze physicochemical characterization of the particles and to confirm it's immunotherapeutic efficacy.

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Understanding the molecular connection between stroke and neurodegenerative disease

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Hypoxia, the sudden loss of oxygenated blood supply to the brain that occurs during stroke, is associated with an increased risk of developing a neurodegenerative disorder later in life. However, it is still unknown what early molecular and cellular changes occur after an hypoxic event that lead to the long-term negative impacts on neuronal survival. It has been shown that hypoxia triggers the rearrangement of the actin cytoskeleton and cytoplasmic accumulation of nuclear RNA-binding proteins(RBPs). Our previous studies have shown that actin plays a significant role in modulating the stability of the nuclear lamina and of the nuclear pore, the gateway for RBPs shuttling in and out of the nucleus. Therefore, we hypothesize that the hypoxia-induced rearrangement of the actin cytoskeleton compromises the integrity of the nuclear membrane, leading to changes in the structure and function of the nuclear pore complex. To test this hypothesis, we exposed differentiated neuroblastoma SH-SY5Y cells to hypoxic stress and examined changes to the nuclear pore stability through immunofluorescence and image analysis. Our results provide a greater understanding into the early pathogenic events that follow hypoxia and could give insight into novel therapeutic targets to prevent or slow down neurodegeneration.

This work was supported by RI INBRE

Characterization of LytG-ligand interactions by differential scanning fluorimetry and protein foldedness ratio

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Peptidoglycan (PG), the major structural polymer in most bacterial cell walls, is a heteropolymer composed of a carbohydrate backbone cross-linked via short peptides. Bacterial autolysins are enzymes that break down the PG and are required for cell growth and separation of daughter cells following cell division. PG metabolism is a tightly coordinated process between synthetic and degradative enzymes. As chemical biology approaches to study eukaryotic glycobiology continues to develop rapidly, chemical biology approaches to study microbial glycobiology (in particular PG metabolism) lags behind. LytG, from *Bacillus subtilis*, is a novel 32 kDa autolysin, and is the major active N-acetylglucosaminidase during vegetative growth. While there are several inhibitors that target PG synthesis, there are very few (if any) inhibitors that target enzymes involved in PG degradation. To bridge this gap, the diamide inhibitor masarimycin has been identified as a bacteriostatic inhibitor of *B. subtilis* growth, targeting LytG.
The purpose of this study is to characterize the LytG-masarimycin and LytG-ligand interactions using differential scanning fluorimetry (DSF), foldedness ratio assay, and circular dichroism. By characterizing the inhibitor-LytG and ligand-LytG interactions using biophysical techniques, we can better understand the glycosyl hydrolase family 73 cluster 2 enzymes and the key features they recognize in a substrate. Previous studies have shown that Mg^{2+} is required for maximal LytG activity. Based on this, Mg^{2+} was investigated for its ability to stabilize LytG structure. The calculated unfolding constant (ku) of masarimycin from DSF experiments was found to be 63 µM, and the kd of magnesium was found to be 53.7 mM. The ku value derived from the foldedness assay of masarimycin was found to be 21 µM, which is in good agreement with DSF calculated values. The analysis of peptidoglycan partial substrates (chitooligosaccharides, muramyl-dipeptide, stem peptides) did not identify any stabilizing ligands suggesting that LytG has an absolute requirement for the specific carbohydrate backbone and stem peptide present for binding.

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Determining the mechanism through which sulforaphane reduces α-synuclein in a Parkinson's yeast model

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Parkinson's Disease (PD) is a human neurodegenerative disease characterized by Lewy body formation, known as α-Synuclein aggregates, in the brain. These aggregates disrupt dopamine flow in neurons, resulting in neurological abnormalities. Our lab uses the Budding Yeast, *Saccharomyces cerevisiae*, to express human α-Synuclein and serve as a model for PD. Yeast cells treated with Sulforaphane (SFN), exhibited a significant reduction in the amount of α-Synuclein aggregates. However, the mechanism behind SFN's effectiveness at reducing α-Synuclein aggregation is still unknown. Studies have shown that a reduction in oxidative stress is directly correlated with a reduction of α-synuclein phosphorylation and aggregation. Thus, SFN may reduce α-synuclein aggregation through reducing oxidative stress. SFN is known to act on the mammalian Nrf2 transcription factor, which induces the expression of antioxidant genes. The Nrf2 transcription factor is very similar to the YAP1 transcription factor in yeast, which also induces antioxidant gene expression. Thus, SFN may reduce α-Synuclein aggregation in yeast through regulating the YAP1 pathway. The goal of my research is to determine how SFN reduces α-Synuclein aggregation and to discover the role, if any, YAP1 has in reducing the amount of aggregates. To determine if SFN regulates YAP1, I will use immunofluorescence with confocal microscopy to see if SFN induces YAP1 localization in the nucleus. Thus far, I have used DAPI to visualize the nucleus and am optimizing my antibody staining to visualize YAP1. If the reduction in α-Synuclein aggregation is due to SFN's impact on YAP1, I expect YAP1 to be localized in the nuclei of cells treated with SFN. The results from this study may reveal a mechanism by which we can treat PD using SFN.

This work was supported by Providence College

Lipid metabolism and autophagy

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Autophagy is a cellular process in which cytosolic components are degraded through the lysosome. This process maintains homeostasis through the breakdown of worn-out or damaged proteins and organelles. We are interested in the complex relationship between autophagy and lipid metabolism. It has previously been shown that autophagy is required for lipid storage in *C. elegans*. Autophagy-deficient nematodes have normal nutrient uptake and can synthesize and store lipids. It was also found that short-term starvation in autophagy gene mutant animals had no effect on normal lipid breakdown. These findings suggest that the low-fat phenotype is not a result of decreased lipid synthesis, storage, or defective utilization of lipids. We identified ATGL-1 in a candidate screen to identify a lipase that may drive lipid-loss upon autophagy deficiency. Furthermore, we found that ATGL-1::GFP expression was increased in several autophagy gene mutant animals. ATGL-1 is degraded by the proteasomal pathway during a fed state. Under nutrient deprivation, increased cAMP levels activate phosphofructokinase A (PKA), which phosphorylates ATGL-1 and stabilizes it. We hypothesized that an increase in cAMP levels and activation of the PKA pathway may be responsible for the increased lipid hydrolysis observed in autophagy-deficient organisms. Hence, we quantified cAMP levels in adult WT and atg-7(bp411) mutant animals; however, we did not detect a significant difference between the two. Our lab is now interested in exploring other mechanisms by which autophagy deficiency alters normal lipid levels in *C. elegans*.

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Stressed Out: The *B. subtilis* autolysin inhibitor masarimycin induces cell wall stress response

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In many respects, peptidoglycan remains as mysterious as it was some 8,000 odd publications ago. Peptidoglycan (PG) is the major structural heteropolymer found in most bacterial cell walls, providing both structure and shape to the cells. PG is a complex structure composed of polysaccharide chains cross-linked via peptide side chains. The synthesis of PG requires complex coordination between biosynthetic and degradative processes. A key component of these cell wall remodeling processes are autolysin enzymes that function by cleaving polymeric PG bonds to enable cell division and growth. The diamide inhibitor masarimycin operates as the single micromolar inhibitor of *Bacillus subtilis* growth, via inhibition of the N-acetylglucosaminidase (GlcNAcase) LytG. LytG is the major active GlcNAcase during exponential growth. The objective of this investigation is to confirm the mode-of-action of masarimycin in *B. subtilis* as cell wall metabolism. Targeting of the cell wall was determined by monitoring the induction of the cell wall stress response in *B. subtilis*. Induction of cell wall stress was measured by changes in the gene expression of relA, a guanosine tetraphosphate synthase, responsible for production of the alarmone ppGpp. Initial experiments focused on quantifying the alteration in expression levels of relA in *B. subtilis* at early exponential phase growth via qPCR. Preliminary data suggests an 8-fold increase in relA expression upon treatment with masarimycin. Thus, suggesting that masarimycin appears to induce cell wall stress response.

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Unraveling the the role of Phr1 in *Candida parapsilosis* adhesion

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Candida parapsilosis is a pathogenic fungus of growing concern particularly in premature babies. *C. parapsilosis* demonstrates intrinsic antibiotic resistance. According to the CDC non-*albicans* species are a serious global health threat, due in part to their intrinsic antibiotic resistance. Structurally, the *Candida parapsilosis* cell wall contains polysaccharides, proteins, lipids, and pigments. Previous data has indicated the transglycosylase Phr1 was significantly upregulated under host adhesion conditions. Another finding reflected a knockout of Phr1 results in impaired adhesion of *C. parapsilosis* to host tissue. These findings allow for the proper further analysis on where to focus. These findings allow for the proper further analysis on where to focus directly for the next portion of the project. The overall goal of this project was to characterize the changes to the cell surface of *C. parapisilosis* under conditions that impair adhesion (Phr1 downregulated) and promote adhesion (Phr1 upregulated). . The cell surface of *C. parapsilosis* JMB77 wildtype and Δphr1 mutant under Phr1 repressed/induced conditions were subject to limited trypsinization of the cell surface. These tryptic peptide mixtures were desalted by RP-HPLC and analyzed by mass spectrometry at the Arkansas INBRE proteomics facility. In addition to analyzing the cell surface proteome, changes to the surface N-glycan profile was investigated. Glycans were analyzed as their 2-aminobenzamide derivatives via mass spectrometry and structures identified via the Glycomod database. Certain proteomics that was analyzed in which represented a specific difference between the conditions would be that of the Certain findings that were solely present in uninduced was that of a single oligosaccharide containing three deoxyhexose compounds. There does not suspect to be a change in the N-glycans found of the surface since this mannose component in M199 was a part of the oligosaccharide, not an addition.

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Connectivity patterns of mushroom body neurons

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Neural circuits are essential for basic brain function as well as the extensive repertoire of complex behaviors. However, our limited ability to map anatomical connectivity of neural circuits has precluded us from gaining an understanding of their functional connectivity. The *Drosophila melanogaster* is a powerful model organism for mapping the fundamental structure of neural circuits due to its small yet complex and tractable brain. Specifically, the mushroom body (MB) is a prominent neuropil structure within the *Drosophila* brain with an established role in learning and memory. The MB integrates inputs from multiple sensory modalities, receives organized valence related inputs from dopamine neurons (DANs) and projects to specific MB output neurons (MBONs) to bias behavior. Thus, it is an ideal structure for mapping anatomical connectivity and inferring functional architecture of neural circuits. Using the trans-Tango anterograde transsynaptic tracing tool, we recently mapped the neurons to which MBONs project and identified three regions of convergence: other MBONs, the fan-shaped body, and the lateral accessory lobe This architecture enables the brain to update and integrate information with previous experience before executing appropriate behavioral responses. These data are largely consistent with the recent E.M. dataset obtained from a single adult female fly. However, there were a number of postsynaptic connections present in the E.M. that were not identified in the trans-Tango dataset. We hypothesized that the threshold for trans-Tango signal was dependent on the number of postsynaptic connections between neurons. Here we compare the number of synaptic connections identified in the E.M. dataset with the trans-Tango dataset. We conclude that the number of synapses between neurons does not correlate with the trans-Tango signal and that discrepancies are likely a consequence of synapse strength, sexual dimorphism, or variation across subjects.

This work was supported by RI INBRE

Examining the DNA repair mechanism of human DNA polymerase theta via 2-aminopurine fluorescence

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DNA Polymerases are highly dynamic enzymes involved in DNA replication and repair to help maintain genomic information. There are numerous DNA polymerases that have unique activities depending on specific function. Some replicate DNA at high speeds with great accuracy, while others preserve the genome during DNA damage repair despite misincorporating incorrect nucleotides. DNA Polymerase Theta (Pol θ) is a DNA repair enzyme known to be error-prone but has been shown to protect cells against damaging agents. How Pol θ chooses dNTPs for nucleotide incorporation is crucial for understanding genomic stability. Furthermore, uncovering the mechanism of Pol θ might provide insight into disease including mutagenesis and cancer. To study this mechanism, previous studies of high-fidelity DNA polymerases have utilized FRET techniques in which a site-specific dye is placed on the enzyme with its corresponding dye on the DNA substrate to allow for real-time analysis of the Polymerase/DNA complex during nucleotide incorporation using stopped-flow fluorescence. Previous attempts to fluorescently label Pol θ have been unsuccessful due to its unusually large size and amino acid content. Thus, we pivoted to using unlabeled Pol θ with a labeled DNA substrate containing the natural fluorescent nucleotide analog 2-aminopurine (2-AP). This technique has been used to study other DNA polymerases and we would expect the fluorescent signal from the 2-AP labeled DNA to be reduced by pi-stacking from adjacent bases especially during correct nucleotide incorporation. For Pol θ, we hypothesize that these fluorescence changes will be indistinguishable between correct and incorrect nucleotide incorporation due to Pol θ's low fidelity and that other non-covalent interactions drive nucleotide selection. Our preliminary fluorescence data taken together with biochemical studies reveal a unique nucleotide selection pathway that mechanistically define genomic integrity with the goal of designing new inhibitors against Pol θ.

This work was supported by RI INBRE

Metabolite profiling of medicinal plants using LC-MS/MS-based molecular networking

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In this work, we explore the utility of LC-MS/MS-based molecular networking (LC-MS MN) to systematically detect and identify potential medicinal compounds from plant extracts. The LC-MS MN approach organizes metabolites together into “clusters” based on similarities in the MS/MS fragmentation patterns. While the functionality of this approach initially appears promising, there are several important questions to answer in the validation of the approach. The questions we posed were: 1) Can LC-MS MN show metabolite differences from plant parts? 2) Will the analysis of organic and aqueous extracts together contain more analytes than either alone? 3) Are more metabolites detected from rarefied chromatography fractions versus crude extracts? 4) Can LC-MS MN be used to show metabolite differences in related species? Using the Global Natural Products Social (GNPS) molecular networking platform for post LC-MS/MS acquisition analysis, we provided answers to the questions posed above and highlight the utility of this approach as a metabolite profiling tool for medicinal plants such as black cohosh, which is a well-known medicinal plant for women’s health. Furthermore, we analyzed metabolite differences in species of swallow-wort, an invasive plant in the New England region. Overall, this method can additionally be used to analyze components in dietary supplements, herbals, and botanicals.

This work was supported by RI INBRE

Investigating metabolic reprogramming in frontotemporal dementia

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Metabolic reprogramming is a common hallmark of many diseases. In recent years the focus on metabolic change in cancerous tissues has increased. However, fewer studies have investigated the metabolic shifts in neurodegenerative diseases. Metabolic reprogramming in neurodegenerative disease has been well documented and glucose uptake is even used as a key diagnostic indicator for some of these diseases. We are utilizing an established *Drosophila* model of the neurodegenerative disease, Frontotemporal Dementia (FTD), to investigate metabolic changes using a whole brain energy utilization assay. We are also studying the underlying molecular mechanism by using quantitative PCR to measure expression of metabolic enzymes in the brain. Lastly, we are analyzing changes in cellular morphology of the learning and memory regions of the brain by immunostaining. The goal of this project is to determine if metabolism could be a potential target for treatment of FTD.

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Corazonin and tachykinin as modulators in metabolic response to starvation in *Drosophila melanogaster*

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Metabolism consists of chemical reactions that produce necessary products for organisms to properly function, but what happens when metabolism is challenged by an environmental stressor such as starvation? Physiological responses are generated using neurocircuits in response to metabolic stress; however, little is known of what neuropeptides aid in signaling within these circuits. Our lab previously characterized a neuronal circuit consisting of signaling neuropeptides, corazonin (Crz) and tachykinin (TK), in *Drosophila melanogaster* in response to cold stress, further work indicated that the neuropeptides might also signal a metabolic response to starvation. To better characterize the probable neuronal circuits used in response to metabolic stress, total food consumption, triglycerides, and glucose contents were measured within fed and starved mutants with either Crz receptor or TK ligand absent within *Drosophila melanogaster*. Crz mutants died faster, had a smaller food intake, and stored less sugar and lipids than the wildtype when starved. In comparison to the wildtype, TK mutants had significantly less food intake but stored higher contents of sugar and lipids when starved, suggesting multiple pathways are involved in response to starvation. With this understanding, Crz and TK play a vital role in the metabolic signaling in response to starvation.

This work was supported by RI INBRE

Metal binding studies of KmtR mutants

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Mycobacterium tuberculosis is the bacteria responsible for the disease tuberculosis, which kills about 1.6 million people yearly, making it one of the leading causes of deaths around the world. The bacteria contain several metal transport systems that are essential for its survival. The metalloregulator KmtR is one of two nickel and cobalt regulators in the bacteria. KmtR is responsible for regulating the transcription of genes involved in the export of cobalt and nickel. The focus of this research is to determine the residues involved in the binding of KmtR to both cognate metals, nickel and cobalt, and the noncognate metal, zinc. To do this, various residues were mutated and are being studied. One of these residues was H102, which was mutated to obtain Q102. The mutant protein was expressed and purified. Metal binding experiments are being conducted to determine the respective affinities of each metal to the mutant protein.

This work was supported by RI INBRE

Determining the binding affinity of nickel(II), cobalt(II) and zinc(II) to the H111Q KmtR mutant

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Tuberculosis is a disease caused by *Mycobacterium tuberculosis*, it has caused 1.5 million deaths in 2018 and the bacteria is hard to kill off. *Mycobacterium tuberculosis* is able to survive in a human host by changing the pH of its environment. To do this, the bacteria utilizes a nickel enzyme, in the urease, which produces ammonia. KmtR is a metallosenor protein that aids in regulating the concentrations of nickel and cobalt in the bacteria. The purpose of this research is to look at the KmtR metal sensor family that is also present in *Mycobacterium tuberculosis*, specifically the H111Q protein, a mutated variant of KmtR

This work was supported by RI INBRE

Purifying N-terminally acetylated Tau and determining alterations to phosphorylation *in vitro*

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Neurodegenerative diseases cause the cells of the central nervous system to lose function and die and they are one of the top leading causes of death in the world today. The microtubule associated protein Tau forms aggregates that cause nerve cell death, ultimately leading to neurodegeneration. Tau's structure and function is affected by post-translational modifications, most notably through the addition of phosphate groups via the process of phosphorylation. For this project, an acetyl group is added to the N-terminus of Tau to represent its physiological state more accurately. Our overall goal is to determine how the addition of the N-terminal acetyl group affects Tau structure and function. Our first goal was to establish a purification method of full-length Tau and N-terminally acetylated Tau to conduct further experiments on the structure and function of the protein. N-terminally acetylated Tau could have an impact on the formation of Tau aggregates. To test this aggregation, the project focuses on improving the purification and long-term storage of Tau and acetylated Tau in order to begin experiments with a variety of structural and functional parts of the protein. Testing these two proteins can help to better understand why aggregates form, and potentially lead to how to stop them from forming. Our second goal focuses on how N-terminal acetylation alters the phosphorylation of Tau and how this leads to other changes in structure and function. Acetylated Tau will be compared to unacetylated Tau's phosphorylation properties using a kinase assay. The results from this project will provide insight into how the combination of post-translational modifications can alter Tau's structure and function and possibly how these modifications contribute to the disease state.

This work was supported by RI INBRE

Computational analysis of functionalized monomers in ring-opening polymerization for disease treatment applications

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Functionalized polymers are promising new drug delivery vehicles. By appending a drug to a polymer containing targeting vectors, the drug can enter the cell, overcoming drug resistance. Upon hydrolysis of the polymer in the cellular environment, the drug will be released. The aim of this study was to computationally study the polymerizability of our monomer targets. One potential issue is that monomers containing substituents are often more difficult to polymerize than unfunctionalized monomers. We examined the position of functional groups on monomers targets and the relationship to the enthalpy of polymerization (ΔH_p). We also examined another potential monomer variation, sulfur containing monomers (thionolactones), due to the possibility of an improved hydrolysis profile and increased biological compatibility.

We used Spartan 18 to calculate ΔH_p, ΔG_p and energy values using the density functional B3LYP 6-31G* data set. All molecule geometries were optimized prior to calculations. By comparing the ΔH_p of a variety of methylated caprolactones, we found that the ΔH_p was similar regardless of the position of substitution. This finding suggests that the position of substitution on caprolactone does not affect polymerization of the monomer. This opens a diverse range of synthetic targets. The lactone and thionolactone data was found to have similar ΔH_p varying by about 2-3 kcal/mol, with lactones being more stable. This was attributed to a hydrogen bond seen in the lactone but not present in the thionolactone in the lowest energy conformers. These computations suggest that the target monomers will perform favorably in ring opening polymerization, and potentially allow the incorporation of drug and targeting vectors into these polymers.

This work was supported by RI INBRE

Evaluation of the antioxidant and anti-tyrosinase activity for pyrazole derivatives

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Pyrazoles, a class of organic compounds with a five membered and two-nitrogen containing heterocyclic ring, have been studied as leading compounds for the development of therapeutic agents with a variety of pharmacological activities including antioxidant, anti-inflammatory, anti-viral, and antitumor activities. Herein, a group of new pyrazole compounds were chemically synthesized and their antioxidant activities were evaluated using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical assay. The results demonstrated that several pyrazole derivatives showed promising free radical scavenging effect with IC50 values ranging from 30.56-444.4 μM. In addition, pyrazole compounds were evaluated using the tyrosinase inhibition assay for skin lightening activities. Pyrazole compound 9 also showed promising anti-tyrosinase effect with inhibition rates ranging from 47%-97% at a concentration range of 4-500 μM. Further assays using biophysical tools will be used to elucidate the mechanisms of pyrazole compounds' antioxidant activities.

This work was supported by RI INBRE

Multicopy suppression of ribosomal protein deletion mutant

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To suppress the ribosome assembly defective phenotype of a ribosomal protein uS17-deficient mutant, various ribosomal proteins were used to act as multicopy suppressors. Ribosomal protein uS17 is a highly conserved component of the 30S (small) ribosomal subunit and is important for 30S subunit assembly. Mutants with a deletion of rpsQ (the gene encoding uS17) are viable but have a severe 30S subunit assembly defect and a temperature-sensitive (ts) phenotype. While fully assembled 30S subunits do form, incomplete ("20S") particles lacking several proteins accumulate. We hypothesized that increasing the intracellular concentration of one or more of the proteins missing from the 20S particles could drive the binding assembly process forward, thereby partially suppressing the temperature sensitive phenotype.

This work was supported by RI INBRE

Outreach for the supplemental nutrition assistance program in Rhode Island older adults

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Over 4,275,000 households with older adults in the United States experience food insecurity (FI), meaning they lack access to enough food to support a healthy and active lifestyle (Coleman-Jensen et al., 2019; FRAC, 2019). FI among older adults can lead to lower nutrient intake, increased risk of depression, and diabetes (FRAC, 2019). Among physical health issues, food insecurity may force older adults to choose between buying food and medicine, postpone medical care, and forgo foods required for medical conditions (FRAC, 2019). Implemented in 1939 to combat hunger in America, the Supplemental Nutrition Assistance Program (SNAP) is a program administered through Rhode Island's Department of Human Services (DHS) to provide benefits to supplement the food budget of eligible people. While SNAP can help older adults prioritize medical care and is associated with reduced hospitalizations, there are many challenges, including the long application, other required reports or DHS, being misinformed, or having preconceptions about eligibility (Samuel et al., 2018; Srinivasan & Pooler, 2018). The URI SNAP Outreach Project operates out of the Feinstein Center for a Hunger Free America. Over the summer of 2021, the SNAP Outreach Project managed a hotline that community members can call for help with any SNAP related questions. Outreach workers also conducted in-person outreach throughout RI communities by visiting locations where people could approach with applications, questions, and other documents related to SNAP. Through the project's hotline and events, including food pantries, bingo, and information tables at low-income housing, we were able to provide information and assistance to clients, and collect and interpret data. So far, between June and July 2021, we have helped 67 older adults,13 of whom screened eligible for the program. Common issues and questions included questions about their benefits or EBT card, requesting assistance with an application and other documents. Additionally, we were able to help 35 individuals below the age of 60. Discussions with clients revealed that many barriers that older adults face with SNAP could be removed by implementing a shorter application for older adults, known as the Elderly Simplified Application or ESAP. In addition, shorter wait times when contacting DHS would help make DHS more accessible and generally approachable for older adults, ensuring they have all of the information needed (Walker & Lee, 2020).

Uncovering the neuromuscular anatomy of the Bluegill spiny dorsal fin

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The spiny dorsal fin is essential in recovering fish stability after perturbations and the loss of sensory information or motor control causes erratic swimming behavior. Yet, little is known about the innervation (neuromuscular anatomy) of the spiny dorsal fin. To understand the control input to fin motion, we examined the spiny dorsal fin of the bluegill, *Lepomis macrochirus*, in terms of afferent and efferent fin innervation. The dorsal fin is composed of a spiny (cranial) and a soft (caudal) portion. We used histological techniques such as whole fin staining with cresyl violet, and immunohistochemistry (IHC) with anti-acetylated tubulin and calcitonin gene regulated protein (CGRP) as primary antibodies. The best staining protocol for macro identification of nerves was cresyl violet. We found branching innervation of the descending tracks into the erector muscles of the spiny dorsal fins. Anti-acetylated tubulin staining of dorsal fin rays 2-3 and 6-7 and the surrounding muscle showed heavy innervation suggesting fine motor control, as well as sensory neurons present in the fin rays and on the adjacent fin web. Motor innervation was denser closer to the joint at the base of each spine. The nerve diameter was also smaller in the posterior section. In the fin base, the nerve diameter is larger, then decreases in size as the nerves branch to the tip of the fin ray. Information on the delivery of motor control and sensory feedback will help propose a mechanism for how spiny dorsal fin deployment is fine-tuned. We plan to continue with immunohistochemistry and serial histology to obtain more resolution towards the neuronal network so we can improve on the design of prosthetic prototypes that modulate motor function with local sensory input.

This work was supported by RI INBRE

Investigating the role of alx4a in iridophore development

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The cells that give rise to melanoma, melanocytes, originate from a pluripotent population of embryonic cells called the neural crest (NC). During embryogenesis, neural crest cells undergo an epithelial to mesenchymal transition (EMT) allowing them to leave the neuroepithelium, invade underlying tissues and migrate to their terminal locations. The microphthalmia-associated transcription factor (MITF) has been identified as the master regulator of melanocyte fate and as being repressed in other neural crest cell lineages. Previous studies suggest that varying levels of mitfa drive cell invasion and the progression of melanoma. Understanding the ways in which mitfa expression is regulated during development may further elucidate the mechanisms that promote oncogenesis. In zebrafish, melanocytes share a bipotent precursor with iridescent iridophores, another NC derived pigment cell. Aristaless-like transcription factors (ALX), alx4a and alx4b, have been recently implicated in promoting iridophore fate, possibly by repressing mitfa expression. Our lab used CRISPR/Cas9 to knockout alx4a in zebrafish and found that alx4a mutants did not develop iridophores. Based on these findings, the goal of my research this summer was to confirm that the resulting mutant phenotype is in fact due to the loss of alx4a and further investigate the role of alx4a in promoting iridophore fate. To confirm that the loss of iridophores was due to the loss of alx4a, we performed rescue experiments using a sox10 promoter to drive alx4a cDNA in neural crest cells. To determine if alx4a is sufficient to drive iridophore fate, we additionally performed overexpression experiments using an hsp70l promoter to drive alx4a cDNA throughout the embryo at 25 hpf. Finally, whole mount in situ hybridization (WISH) was used to examine and locate where alx4a is expressed at different developmental stages (24 and 48 hpf). Understanding the underlying mechanisms that promote iridophore specification and repress melanocyte fate, may potentially highlight significant future therapeutic treatments for neural-crest derived diseases like malignant melanoma.

This work was supported by RI INBRE

Identification of genes required for pigment cell migration in *Danio rerio* via CRISPR/Cas9 microinjections

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Melanoma, a common form of skin cancer, can rapidly spread if left untreated. It is triggered by mutations caused by UV radiation. Melanocytes, the cells responsible for melanoma formation, originate in the embryonic neural crest of Danio rerio. The cells differentiate, detach, and migrate toward their final destination within the embryo via epithelial-to-mesenchymal transition (EMT). It is a tightly regulated and controlled mechanism that permits polarized epithelial cells to undergo a variety of changes in gene expression and cytoskeletal rearrangement, allowing them to take on a mesenchymal phenotype which includes, but is not limited to, higher resistance to apoptosis and enhanced migratory capacity. When EMT is complete, the underlying basement membrane degrades as a mesenchymal cell with migratory capabilities is formed. While the process of EMT is similar in humans, melanomas metastasize via unregulated EMT, where healthy cells are permeated by neural crest derived pigment cells.

Identification of signals that initiate and terminate embryonic pigment cell migration can provide pertinent information to controlling melanoma metastasis. By conducting a CRISPR/Cas9 genetic screen on zebrafish, we are able to identify the genes required for pigment cell migration. By inducing mutations via CRISPR/Cas9 injections, we are able to examine the effects on pigment cell migration as well as stripe formation, furthering research on the manner in which melanomas metastasize.

Candidate genes expressed by melanocytes (prickle1b, cdc42ep3, atp6v1f, plk3, arhgap1, ptk7a, tlcd5 and stk17a) were selected due to known functions in cell signaling, cellular component organization, response to stimuli, cell differentiation, establishment of localization, cell population proliferation and neural crest migration.

To perform knockouts for our targeted genes, solutions containing CRISPR/Cas9 and guide RNAs were prepared for microinjections in 1-cell embryonic zebrafish. Observations were recorded at distinct intervals post fertilization to determine if phenotypical changes were present. Embryos which exhibited these changes and possessed successful survival rates were harvested for genomic DNA extraction and amplification. Verification of genomic mutations were observed in several of the candidate genes selected for knockout and sent out for sequencing. Results from these experiments will allow us to further understand the mechanisms by which pigment cells migrate.

This work was supported by RI INBRE

Evidence for the conservation of aging pathways among evolutionarily divergent species

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Genetic screens have been highly informative in mapping the cellular pathways that promote the aging of cells and organisms. One important question in the field is whether the underlying molecular mechanisms that cause aging are similar across various species. We distinguish mechanisms of aging as being either private (specific to one organism) or public (a mechanism that is shared among various species). A comparative genomic approach can quantitatively address the degree to which aging pathways are publicly shared. *Saccharomyces cerevisiae* (baker's yeast) and *Caenorhabditis elegans* (roundworm) shared a common ancestor ~1.1 billion years ago, making these two model organisms exceptionally well suited for a comparative analysis of aging factors (for comparison, human and roundworm are distanced by ~700 million years).

Loss-of-function screens have historically been used in studies of aging because deletion of a gene that promotes cellular aging could be identified by a resultant increase of organismal life span. Collections of yeast deletion strains, as well as bacterial collections of RNA interference (RNAi) for *C. elegans*, are commercially available and provide a relatively accessible way of performing comparative life span analysis. From a list of 61 published chronologically long-lived yeast strains, we identified 13 *C. elegans* orthologs that BLAST to 9 of the long-lived yeast genes and performed life span analysis using the RNAi collection. From the preliminary screen of 13 *C. elegans* orthologs, we identified 2 previously unknown genes involved in C. elegans aging: gpa-7 (ortholog of yeast GPA1) and M05D6.2 (ortholog of yeast SOK1). Our data indicates that a comparative analysis of aging pathways represents a viable scientific approach for the identification of novel aging pathways in multicellular organisms.

This work was supported by RI INBRE

Designing an experimental aquarium system to explore the microbiology of quiescence in the coral *Astrangia poculata*

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During winter months, the local temperate coral *Astrangia poculata* undergoes a period of dormancy known as quiescence in which the polyps fully retract, colonies become unresponsive to stimuli, and they cease feeding. Previous studies demonstrated that the *A. poculata* microbiome in quiescence resembles that of diseased tropical corals, but by springtime, it restructures to resemble a healthy tropical coral microbiome. We propose that changes in the *A. poculata* microbiome during the winter-spring transition may be a model for coral microbiome recovery from general disturbance. Here we present an experiment in which an aquarium system was designed to induce quiescence in *A. poculata*. Replicate aposymbiotic *A. poculata* colonies from Rhode Island and Massachusetts were held in individual tri-pour beakers and divided evenly into two tanks: one held at an ambient temperature of 19^{o}C, and one tank with temperatures starting at 19^{o}C, ramped down to 5^{o}C over 1 week, held at 5^{o}C for 4 weeks, and then ramped up to 19^{o}C over 1 week. We designed the aquarium system to support independent seawater flow to each replicate colony and minimal cross-contamination of seawater around replicate colonies (n=10 per geographic origin, per tank). Seawater temperature was maintained with precision within 1^{o}C. Visual assessment of polyp extension and response was performed every day for the 57-day experiment. Replicate seawater and coral mucus samples were collected every other day during ramp-down and ramp-up periods; weekly during the four weeks of quiescence; and weekly for two weeks post-quiescence. Seawater and mucus samples are currently being processed for 16S amplicon sequence analysis to create a high-resolution time series of microbiome profiles throughout the experiment. Additionally, microbiome data from tank specimens will be compared to wild collections of *A. poculata* colonies collected before, during, and after natural quiescence, to determine whether quiescence-related microbiome shifts can be accurately modeled in aquarium manipulations. Analysis will be performed to determine whether population (Rhode Island, Massachusetts) influences the patterns of microbiome dynamics throughout quiescence. Exploration of community succession of the *A. poculata* microbiome before, during, and after quiescence using this high-resolution time series will likely identify key microbial players involved in coral recovery from disturbance.

This work was supported by RI INBRE

Tracking the fate of polyethylene microbeads and microbead-associated microbes in *Astrangia poculata* exposure assays

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Microplastics (<5mm diameter) are detrimental to marine life and are found in even the most remote oceanic regions. Microplastics are consumed, often unintentionally, by a variety of marine organisms, including *Astrangia poculata*, a temperate heterotrophic coral. *A. poculata*, a suspension feeder, feeds on zooplankton and other particles in the water column making it vulnerable to incidental microplastics ingestion, either directly from the water column, or via contaminated prey. We aim to use *A. poculata* and its copepod prey, *Pseudodiaptomus pelagicus*, as an experimental system to track the fate of microplastics and microplastics-associated microbes. In this study, *P. pelagicus* was fed GFP-*E. coli* biofilmed UV-fluorescent polyethylene microbeads, and the copepods were subsequently fed to *A. poculata*. Fluorescence imaging was used to image the microbeads and *E. coli* cells. Data demonstrated that *A. poculata* ingests microplastics via contaminated *P. pelagicus*. Imaging is ongoing to localize GFP-*E.coli* cells resulting from this ingestion. These findings will provide important insight and new methods for exploration of the fate of microplastics and their associated microbes throughout food webs, specifically via indirect ingestion of microplastics in marine organisms.

This work was supported by RI INBRE

The impact of sea lettuce on grass shrimp behavior and growth

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Macroalgal blooms are large accumulations of floating algae that are deposited on beaches at low tide. In Rhode Island, blooms are generally dominated by green macroalgae in the genus *Ulva*, commonly referred to as sea lettuce. The effects of *Ulva*, a macroalgae found along the coast of Rhode Island, have been suggested to be harmful to certain species in the area, resulting in decreased growth or survival. These effects have been hypothesized to be due to the production and release of compounds from *Ulva* that have allelopathic effects. The objective of this study was to identify if *Ulva lacinulata* had a negative impact on grass shrimp, *Palaemontes pugio*, behavior or growth. We conducted co-culture laboratory assays with *U. lacinulata* and *Gracilaria*, a red macroalgae not known to produce allelopathic compounds and grass shrimp. Two shrimp and a portion of macroalgae were placed on opposite sides of a divided mesocosm and grown for 4 weeks. For each macroalga, we had treatments with 3.5 g/L (n=5) and 5.0 g/L (n=5); we also had a set of mesocosm controls that contained shrimp but no macroalgae (n=5). Each week, shrimp were videotaped to determine pleopod beating rate, a measure of stress in shrimp, and photographed to determine growth rates. This presentation will discuss the results of our analysis of shrimp behavior and growth and implications for continued blooms of *Ulva* in the coastal environment.

This work was supported by RI INBRE

Microfluidic immunosensor based on poly-horseradish peroxidase for electrochemical detection of cancer biomarkers proteins, interleukins IL-22 and IL-6 in serum

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Simple, accurate, low-cost biosensor arrays for clinical measurements of biomarker for early detection and monitoring of cancer are critically important and successful development of such arrays will lead to reliable on-the-spot cancer diagnosis, improved therapeutic outcomes at lower cost, decreased patients stress and new targeted therapies. Herein, we report on the development of a microfluidic immunosensor via the use of streptavidin polymerized horseradish peroxidase (poly-HRP), coupled to nanostructured electrode arrays. Capture antibodies were bound to a chemically modified surface on the nanostructured working 8 - electrode array and placed into a microfluidic device. A full sandwich immunoassay was constructed following a simultaneous injection of target proteins, biotinylated antibodies, and polymerized horseradish peroxide labels into the microfluidic device housing the working the working electrode. The electrochemical signal was generated upon injection of a mixture hydrogen peroxide and hydroquinone charge mediator via HRP-enzyme catalyzed reaction. Concentrations of the antibodies in the sandwich immunosensor and BSA concentrations in the blocking step were optimized to minimize non-specific binding effect (NSB) that often control the sensitivity and detection limits of the immunosensor. The optimized concentrations of Ab1 and Ab2 for IL-6 were determined as 50 ug/mL and 1 ug/mL, respectively. Work is in progress to develop the calibration curve and assess the selectivity and accuracy of the multiplex immunosensor.

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In vitro examination of GC stimulated glucoregulatory effects in a hepatic and neuronal cell line

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The overconsumption of high caloric food and reduced physical activity increasingly leads to numerous health problems in the United States. Specific lifestyle choices (i.e., diet selection) contribute to the high prevalence of metabolic syndrome (MetS), a spectrum of disease characterized by unregulated hyperglycemia, hypertension, dyslipidemia, and obesity. The dysregulation of neuroendocrine systems (i.e., the hypothalamus pituitary adrenal (HPA) axis) and peripheral metabolic mechanisms (i.e., systemic insulin resistance, ER-stress) mediates the development of MetS. The HPA axis is responsible for the body's homeostatic response to stress and also critically regulates basal glycemia, in part via the production and release of glucocorticoids (GC; i.e., cortisol and corticosterone) produced from the adrenal cortex. While initially adaptive, chronically elevated levels of GC can result in metabolic dysfunctions that promote excessive bodyweight gain and glucose dyshomeostasis. At the molecular level Sirtuin 1 is a master metabolic sensor with a multifunctional role in glucose homeostasis. This NAD+ dependent deacetylase is a molecular transducer of stress stimuli resulting in adaptive transcriptional and cellular responses. In the liver, diminished Sirt1 activity was correlated to enhanced hepatic glucose production; while in brain, Sirt1 is cell-type specifically involved in both central and peripheral effects on energy balance. We show that ER stress induction ablated insulin stimulated GLUT1 expression, while having no effect on Sirt1 protein levels and FoxO1 activity in hepatocytes. Additionally, dexamethasone (DEX; a synthetic glucocorticoid) increased hepatic pEIF2α expression, a marker of ER stress; yet this effect was muted in cells pre-treated with a Sirtuin1 specific inhibitor. The goal of the current study was to elucidate glucocorticoid stimulated effects on glucoregulatory mechanisms using both hepatic and neuronal cell lines. Immortalized hepatic (hepa 1-6) and neuronal (neuro 2a) cells were subjected to a time (2 vs 4 hours) and DEX dose (0, 10, or 100 uM) treatment strategy to test the hypothesis that chronic exposure to supraphysiological levels of GC negatively effects hepatic glucose production and glucose transporter expression via a Sirt1-ER-stress pathway in hepatic and neuronal cells, respectively. Overall, findings revealed cell-type specific glucoregulatory effects occurring as a consequence of DEX dose or period of incubation.

This work was supported by RI INBRE

pH effects on oxidative modifications of alkyl DNA adducts by AlkB-family enzymes

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The AlkB-family are naturally occurring, DNA repair enzymes in the body that reverse the effects of DNA alkyl lesions, a form of DNA damage. They perform oxidative dealkylation of the lesions to restore the proper structure of the DNA bases. Our research will show that AlkB-family DNA repair enzymes can oxidize alkylated DNA adducts to different extents dependent upon pH conditions. To test our hypothesis, we synthesized oligonucleotides containing the DNA lesion 3-methylcytosine (m3C) and subsequently purified them using Reverse Phase and Anion Exchange HPLC. We then ran *in vitro* enzymatic reactions with *E. coli* AlkB and m3C to evaluate the repair activity in different buffer systems and ranging pH conditions. Finally, we evaluated the repair reactions with Anion Exchange HPLC and formulated our results. Our findings support the conclusion that buffer salts and pH conditions influence the repair activity of *E. coli* AlkB, and that AlkB is most efficient, in regard to m3C repair, under pH conditions of 7.0 and 7.5. These results may be relevant to human cancer cells that exhibit the Warburg Effect, as they differ from normal cells in their intracellular pH. Under these conditions, reduced repair activity of enzymes such as ABH2 and ABH3, the human homologs of *E. coli* AlkB, could contribute to the accumulation of cytotoxic and mutagenic DNA adducts in the body.

This work was supported by RI INBRE

Mechanical allodynia in cerebral palsy and the role of astrocytes

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The most common comorbidity of cerebral palsy (CP) is pain, but the impact of prenatal injuries on pain sensation (nociception) is completely unknown. As glial cells are implicated in both previous studies on CP and studies on pain and neuroinflammation, we hypothesized that glial cells could contribute to heightened nociception. To accomplish this, we utilized a model of CP in New Zealand White rabbits with prenatal hypoxia-ischemia (HI) compared with control and sham groups. Uterine blood flow was occluded for forty minutes to induce HI in fetuses at 70-80% gestation. Rabbits were born naturally at term and behavioral testing indicated increased mechanical sensitivity present in HI kits, but not in sham or control kits. Spinal cord tissue was harvested at postnatal day 5 and fixed in paraformaldehyde. Tissue from cervical and lumbar enlargements was cryosectioned at 30-40um and immunohistochemistry was performed to detect activated astrocytes which express Glial Fibrillary Acidic Protein (GFAP). Labeled tissue was quantified using densitometry in the dorsal horn and spinothalamic tracts (spinal cord regions important for nociception). There were no differences between the control, sham, and HI groups. The results do not validate the hypothesis that astrocytes have an impact on sensory abnormalities in CP. Future studies will explore other mechanisms, including the excitability of nociceptors and nociceptor outgrowth into the dorsal horn. The ultimate goal of our work is to prevent heightened pain in individuals with CP.

This work was supported by RI INBRE

Connecting the dots: Deep learning and its application in identifying chromatin regulatory loops

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Within the nucleus the genome is organized in a well-ordered three-dimensional (3D) structure consisting of loops created by protein-DNA interactions. For any cell type the 3D organization of chromatin controls gene expression and is important for normal cell function. As such, changes in wild type chromatin organization have been observed to correlate with diseases states including cancer. However, though much is known about the general factors that make up the mechanisms that control global chromatin organization (at the chromosomal level) less is understood about the mechanisms of organization that control local groups of genes within a chromosome. The major limitation to our understanding these mechanisms is identifying the complex set of factors that in combination regulate chromatin organization and gene expression within the large data sets generated in chromatin mapping studies. To address this limitation, we have deployed a computational deep learning system with the goal of uncovering chromatin connections that regulate gene expression that have previously gone unidentified. This study has the potential to help us better understand the mechanisms of chromatin organization important in gene regulation and could impact our understanding of cellular pathways important for organismal development, and cellular aspects of disease.

This work was supported by RI INBRE

Discovery of a novel link between retinoic acid signaling and Fanconi anemia

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Fanconi Anemia (FA) is a rare genetic disease with an incidence rate of 1:120,000 live births. There are currently 23 known FANC genes. Patients' manifest FA by carrying biallelic mutations in key one or more FANC genes. FA is clinically characterized by congenital defects, e.g., limb abnormalities, and increased risk for bone marrow failure and cancer. FA patients have a reduced ability to repair DNA interstrand crosslinks (ICLs) which can lead to DNA mutation. Our lab recently conducted RNA-seq analysis of mutant and complemented FA patient cells. Results from this analysis indicated highly significant alterations of the retinoic acid signaling pathway. This study describes the discovery of a novel connection between the retinoic acid signaling pathway - a pathway that plays a critical role in embryonal development - and FA. Specifically, we have established that several components of the retinoic acid signaling pathway are misregulated in an FA patient line. To validate RNA-seq results, we immunoblotted for FANCD2, RDH10, ALDH1A1, and ALDH2 in FA patient cells. These results align with RNA-seq data and validate a misregulated retinoic acid signaling pathway. Our results are significant because limb abnormalities are a poorly understood aspect of FA. Associating an embryonal development pathway with FA provides further insight into the pathogenesis of this disease.

This work was supported by RI INBRE

Understanding stages of biofilm growth in *S. aureus*

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BACKGROUND: Staphylococcal biofilms are communities of bacteria that adhere to surfaces. Upon maturation, cells are secured by an extracellular polymer matrix (EPS-matrix). The aim of this study was to assess the time points of initial bacterial attachment, biofilm growth, and permanent biofilm attachment.

METHODS: Utilizing a high-biofilm forming S. aureus strain (ATCC 35556TM), a 6.0 log10 CFU/mL bacteria inoculum was added to 96 well polystyrene tissue cultured treated plates. Biofilms were grown to specific time points in tryptic soy broth supplemented with 1.0% dextrose (TSB-Dex) only or with additional supplementation of 12.5mg/mL magnesium and 25mg/mL calcium (TSB-DMC) which are necessary for antibiotic stability. Each time point was rinsed one, two, or three times with distilled sterile water and dried overnight to fix the biofilm. Plates were stained with 0.1% crystal violet for 15 minutes then rinsed three times. Glacial acetic acid (33%) was added to resolubilize crystal violet inside the biofilm then read on a BioTek plate reader at 570nm.

RESULTS: Initial attachment was observed at 4hrs and reached cell density limit by 7hrs with no increase by 24hours. Biofilms experienced increasing detachment from multiple rinses at 7hrs; with percent differences from one to three rinses being 77% (TSB-DEX) to 108% (TSB-DMC). For 24hour plates, there was no change in biofilm as the number of rinses increased.

CONCLUSION: Biofilm grew exponentially between 4-7¬hrs but easily detached until maturation at 24hrs, which enabled EPS-matrix development and enabled the biofilm to endure the shear stress of rinses. Different broths caused no major alterations in biofilm growth/stability.

This work was supported by RI INBRE

Investigating synergistic interactions of quorum sensing inhibitors with antibiotics against *Staphyloccocus aureus*

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The rise of antibiotic resistant bacterial infections is a major public health threat and has rendered many conventional antibiotics ineffective. This is particularly problematic for multidrug-resistant strains of the opportunistic human pathogen, *Staphylococcus aureus*. Thus, there is an increased demand to create new therapies against resistant infections. Combination therapies or alternatives to antibiotics are being investigated as new treatments against drug-resistant bacterial infections. Quorum sensing (QS) is the way that bacterial cells communicate to regulate the expression of genes involved in virulence and pathogenicity and recently has been shown to be an ideal target for the development of anti-virulence agents which do not lead to resistance. In this study we explore the potential of combining QS inhibitor compounds developed in our lab with conventional antibiotics against *S. aureus*. Checkerboard assays were performed to determine whether the QS inhibitors could exert a synergistic effect with vancomycin and lower the MIC. Such combination therapies have the potential to be both effective in treating infections and in limiting the spread of antibiotic resistance.

This work was supported by RI INBRE

Pre-conception exposure of imidacloprid arrests development in *Drosophila melanogaster*

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Imidacloprid (IMI) is a widely used neonicotinoid insecticide that targets post-synaptic nicotinic acetylcholine receptors (nAChRs) within the central nervous system (CNS). When IMI binds to nAChRs it causes a sustained flux of Ca^{2+} and Na^{+} ions into the neuron, thereby causing prolonged neuroexcitability and other downstream effects in insects, such as tetanic contractions and paralysis. While the acute effect of IMI is evident when used agriculturally to control pests, the long-term, chronic effects of low doses of IMI towards non-target insects, as well as humans, has not been completely elucidated. The purpose of this study is to explore the effects of pre-conception and chronic exposure of IMI on the lifecycle development of the insect model system, *Drosophila melanogaster*. Pre-conception exposure of IMI in the presence and absence of sucrose was evaluated on *D. melanogaster* development in a standard mating assay and larvae development was observed. Mating experiments were evaluated with the first appearance of each *Drosophila* development stages over a two-week period. The results indicated that sucrose and IMI-containing diets cause developmental delay in *D. melanogaster*, with IMI arresting development at the 3rd instar stage. Furthermore, while it was found that there was a statistically significant difference between the development of *D. melanogaster* on normal diet versus the 0.1M sucrose diet, there was no significant difference in the adult weights subsequently obtained between the two diets. Future experiments will investigate the concentration-response relationship of IMI in the presence and absence of sucrose to better elucidate the mechanism of chronic exposure and low-dose IMI and if the effects are altered by diet.

This work was supported by Salve Regina University

Investigating regulation of ribosomal protein production in *Francisella tularensis*

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Francisella tularensis is a Gram-negative pathogenic bacterium which causes tularemia, an infectious disease that can cause serious illness and death in animals and people. *F. tularensis* is considered a potential bioweapon due to its ease of aerosolization and extraordinary infectivity; inhalation of as few as ten organisms can cause disease. Accordingly, we work with the live vaccine strain, which does not cause disease in humans. There is an incomplete understanding of gene regulation in *F. tularensis*, however, this organism is notable because it encodes three distinct homologs of the small ribosome protein bS21. Although known to play a role in translation initiation, the molecular role of bS21 in translation is poorly understood. In other organisms, bS21 homologs appear to control gene expression and we have found that one *F. tularensis* bS21 homolog, bS21-2, regulates expression of virulence genes. To date, there are no studies examining the regulation of rpsU3, which encodes bS21-3, and there is no information regarding when it is expressed. In this study, our goal is to investigate how expression of the rpsU3 gene is controlled in *F. tularensis*. To examine bS21-3 expression, we used a reporter strain in which the lacZ gene is transcriptionally fused downstream of rpsU3. The production and activity of the protein encoded by lacZ, beta-galactosidase, serves as a reporter for rpsU3 transcription. The *F. tularensis* rpsU3-lacZ reporter strain was mutagenized by transposon mutagenesis and a library of mutants, which appeared to have changes in beta-galactosidase production, had been created. This summer, we have screened this library to identify and validate mutants with changes in rpsU3 expression. These studies will help us understand the regulation of rpsU3 and possibly provide insights into the conditions that lead to bS21-3 production and subsequent bS21-3-mediated gene regulation.

This work was supported by RI INBRE

Hot flies and needle ants: Using thermolimit respirometry and high-throughput phenotyping to test hypotheses about the evolution and comparative physiology of metabolic rates

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Metabolic rates represent the fire of life, the rate at which living things can harness chemical energy and transform it into the work that powers everything from communication to reproduction. Major unanswered questions in the comparative physiology of metabolic rates include understanding the tradeoffs associated with body size, thermal sensitivity, phenotypic plasticity, encephalization, and social evolution. Previous studies that have measured the metabolic rates of flies have produced results that vary greatly between papers due to variations in technique, time of day, temperature, species, prandial state, sex, etc. Using flow-through respirometry and high-throughput metabolic phenotyping, we quantified the metabolic rates of fruit flies (*Drosophila melanogaster*) and needle ants (*Brachyponera chinensis*) across a series of three main collaborative experiments: 1) we tested for a effect of group size on the metabolic rates of flies, 2) we determined the thermal sensitivity (Q10) and upper critical thermal limit (CTmax) for a set of ten genetically distinct *Drosophila* lines, and 3) we determined the metabolic encephalization quotient (MEQ) to determine the relative costs of brain and whole body metabolic rates in both ants and flies.

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Development and characterization of BLZ-loaded liposomes for glioblastoma treatment

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Glioblastoma (GBM) is the most common and malignant primary brain tumor with an average overall survival of 15 to 21 months after first diagnosis and a 5-year survival rate of less than 5%. Currently, there is an urgent need for better therapeutic strategies against GBM. Glioma-associated microglia/macrophages, play a pivotal role in tumor growth, cancer immunosuppression, and drug resistance, thus bleak prognosis. The main objective of the present research was to develop a novel immunotherapeutic strategy based on BLZ-loaded liposomes to reprogram tumor-associated macrophages into tumor-inhibiting macrophages to block glioma progress. Following the formulation optimization, BLZ-loaded liposomes were prepared and characterized. The prepared BLZ-loaded liposomes had a particle size around 140 nm and with a zeta potential of -5 mV. In addition, the liposomes were monodispersed and had a more than 70% encapsulation efficiency. Future studies will be conducted to evaluate the immunotherapeutic effect of the prepared BLZ-loaded liposomes.

This work was supported by RI INBRE

The Role of the O-antigen capsule of *Salmonella enterica* serovar Typhimurium in flagellin methylation and cell-to-cell interactions

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Salmonella enterica serovar Typhimurium can be found on a wide variety of produce and meat products, making it one of the most common culprits of foodborne illness in the US. The flagella that surround this rod-shaped bacterium enable motility and influence interactions between this bacterium and its environment. Most *S. enterica* serovars have the ability to express two types of flagellin proteins, FliC and FljB. Methylation of the lysine residues located on the flagellar filaments increases the hydrophobicity of this structure, which may impact the ability of *S.* Typhimurium to interact with other hydrophobic surfaces, such as pectin in plant cell walls. The yihO gene is required for surface expression of the O-antigen capsule, a polysaccharide structure that surrounds the cell and plays a role in evasion of host immune responses. Deletion of the yihO gene results in a lack of expression of the O-Antigen capsule and the exclusive expression of the FliC flagellin, whereas the wild-type strain preferentially expresses the FljB flagellin. The objective of this research project is to determine whether the lack of expression of the O-antigen capsule impacts flagellar methylation and cell-to-cell interactions in *S.* Typhimurium. If methylation levels increase in the absence of the yihO gene, this is expected to lead to increased hydrophobic bacterium-host cell interactions. The influence of yihO on flagellin methylation was determined by methylation site mapping by mass spectrometry, while the influence of yihO on bacterial interactions was assessed using assays to measure motility, biofilm formation, and adherence to a bacterial plant cell wall model. Preliminary results suggest that deletion of the yihO gene does not affect the ability of *S.* Typhimurium to form a biofilm. The yihO mutant strain also appears to be slightly more motile and less adherent to a bacterial plant cell wall model containing cellulose than the wild-type. Analysis of purified flagellin proteins by SDS-PAGE confirmed the expression of the FliC flagellin in the yihO mutant and the FljB flagellin in the parent strain, and mass spectrometry analysis is underway to determine whether the methylation sites and levels of these proteins differ. As this project continues to investigate a relationship between flagellar methylation and *S.* Typhimurium interactions with surfaces, we strive for a better understanding of how this foodborne pathogen can adhere to and invade host cells.

This work was supported by RI INBRE

Development of an RNA-seq pipeline through Snakemake

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RNA is the essential molecule in living organisms responsible for the coding, expression, and regulation of genes. RNA-seq is the combination of experimental and computational methods that analyze the abundance of certain RNA sequences in biological samples. Through many generations of sequencing technology, the ability to produce high throughput sequences used for experiments has become not only more readily available and accessible but streamlined for efficiency and accuracy. The RNA is isolated from samples, is sequenced into its individual nucleic acids, and is then processed through the RNA-seq workflow for analysis. The processing of the samples through the RNA-seq pipeline generally consists of quality control, read mapping and alignment, and transcript assembly and quantification. After this is completed, differential expression analyses can be performed with the data. This project developed an RNA-seq pipeline by using Snakemake, a workflow engine that aims to reduce the complexity of creating pipelines. The pipeline utilizes several RNA-Seq tools to process the sequences and produce data used in many types of biological experiments.

This work was supported by RI INBRE

Modulating the tumor microenvironment through Wnt/β-Catenin to improve immunotherapy treatments

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Advances in immunotherapy are revolutionizing the treatments of certain cancers, especially those in the blood. However, wider applications for eradicating solid tumors have been slow to emerge. A likely reason is the presence of myeloid-derived suppressor cells (MDSCs) in the tumor microenvironment that secrete immunosuppressive molecules which attenuate the attack of immune cells. We hypothesize that small molecule Wnt/Beta-Catenin inhibitors can be used to modulate the immunosuppressive actions of MDSCs and increase the effectiveness of immunotherapy treatments, such as with chimeric antigen receptor (CAR) T-cells. The Wnt/Beta-Catenin Pathway has been shown to be a crucial driving force behind the immunosuppressive effects exerted by various cells within the tumor microenvironment (TME), including MDSCs, regulatory T-cells, and cancer cells. Our project goal is to test if inhibitors of the Wnt/Beta-Catenin Pathway can increase the infiltration, proliferation, and effectiveness of anticancer immune cells in the TME. To date, we have developed a workflow for identifying lead compounds that alter the immunosuppressive actions of MDSCs without toxicity to human T-cells. Peripheral Blood Mononuclear Cells (PBMCs) are isolated from healthy human donors and differentiated into MDSCs using pro-inflammatory cytokines commonly found within the TME. Fluorescence Activated Cell Sorting (FACS) and antibody-based magnetic cell sorting are used to prepare and validate human MDSCs for use in assays. Compounds are tested for toxicity against donor T-cells to determine non-toxic concentrations for future assays and filter for MDSC specific compounds. The workflow also includes Sequential Windowed Acquisition of All Theoretical Mass Spectra (SWATH-MS) proteomics analysis to characterize the proteome of each donor's PBMC and MDSC fractions. A future direction is to use proteomics in tandem with 3D spheroid cultures to help elucidate the mechanisms of MDSC modulation by test compounds. This workflow is now poised to identify novel compounds that modulate the immunosuppressive actions of MDSCs via Wnt/Beta-Catenin inhibition. This work is part of an ongoing collaboration with the laboratories of Dr. Steven Katz at the Roger Williams Medical Center and Dr. Jyothi Menon at the University of Rhode Island.

This work was supported by RI INBRE

Skin permeability properties of various phytocannabinoids leading to potential transdermal drug delivery

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Throughout the ages, mankind has turned to nature's bounty for medical purposes. It is then up to scientists to identify the most efficient way to transform those natural compounds into actual medicine, whether that be in pill form, a syrup, or even an ointment for topical use. Phytocannabanoids are compounds found in the cannabis plant that can be extracted and used in a therapeutic setting, all of which having differing abilities to be absorbed through the skin. A drug that is applied through the skin in a transdermal manner must penetrate the initial layer of skin, then continue to absorb deeper until reaching the systemic region to elicit medical benefits. This study focuses on the effect of various phytocannabinoids on skin permeability and the ability of those compounds to penetrate the skin. Once the compounds have made their way through the skin, they may elicit some sort of localized medicinal effect. Transdermal delivery applied directly to the skin has many advantages, including a noninvasive nature and improving the efficacy of drug delivery by avoiding any reduced concentration of the drug that occurs when taken orally. The ability for phytocannabinoids to penetrate the skin was tested using a Parallel Artificial Membrane Permeability Assay (PAMPA). In this assay, samples of each compound are put into a specific chamber where specific body conditions are demonstrated to mimic the skin. Overtime, the samples pass through an artificial skin membrane, in which the ability of a compound to diffuse across the membrane correlates to its permeation ability. Although the results from the assay conducted are out of acceptable range in comparison to the standards, both compounds tested, CBD and CBGA, indicate a high permeability reading in accordance with the data collected. Further testing must be conducted to perform another assay in which the standards are within range to determine proper results. This study is important because it offers insight into the possibilities of phytocannabinoids being used medically without having to orally consume any medication, thus increasing the drug's bioavailability, or rate of absorption.

In the future, further evaluation of a larger number of phytocannabinoids using the PAMPA Skin Permeability Assay could prove to be beneficial in determining the best potential compound to be used in a transdermal drug application.

This work was supported by RI INBRE

FASCITELLI CENTER FOR ADVANCED ENGINEERING

Posters

A-47 to A-72

and

B-47 to B-70

SESSION A: 9:30 – 11:00 AM

SESSION B: 11:00 AM – 12:30 PM

Next-gen smart motor-assisted therapy bike for patients with motor disability

Demetrios Petrou, Kellen Waters, Anna Cetera, Emma Lokey, Dhaval Solanki, Gozde Cay & Kunal Mankodiya

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Patients who have suffered a stroke, have Parkinson's disease, or other conditions that impact motor function require regular therapy to slow down the disease progression and regain mobility. Forced exercise aids this process by facilitating motion for patients during their workouts. Exercise bikes with forced therapy offer a convenient way to conduct productive rehabilitation sessions on one's own.

Most physical therapy devices currently lack advanced data collection methods and user interfaces. Our aims this summer were to understand how the current model of the therapy bike works, convert this functionality to a digital format, and add new features. Overall, we wanted to test the feasibility of using the updated bike as a tool for physical therapists to more effectively monitor their patients' recovery progress.

In the future, we hope to incorporate more sensors to gather further physical therapy data and add virtual reality to make workout sessions more enjoyable.

This work was supported by RI INBRE

Development of particle-based therapeutics for the treatment of pulmonary diseases

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Nanotechnology is defined as the design, production, and application of structures, devices, and systems by manipulation of size and shape at the nanometer scale (<200 nm). The work done in our lab focuses on the development of nanoparticle-based systems as a drug delivery method for the treatment of pulmonary diseases. It is preferred to release therapeutic agents under slightly acidic conditions, as those conditions can be found in targeted places in the pulmonary system. Polymers that are hydrophilic can be modified to become hydrophobic in water, yet dissolvable in organic solvents. The pH-tunable polymer acetalated dextran (AcDex) was synthesized in order to create polyvinyl alcohol (PVA)-coated nanoparticles loaded with curcumin. Curcumin was used as the model drug in the PVA nanoparticles (NP) as its fluorescent nature makes it easy to detect and image. Ac-Dex allows NP to degrade at a faster rate and release curcumin under acidic conditions. Nanocomposite microparticles (nCmP) were developed using spray drying technology as a way to deliver therapeutics to the lungs via a dry powder inhaler. Dynamic light scattering was used to ensure that the size of the nanoparticles remained consistent and below 200 nm. Ongoing research will examine the development of nCmP aerosol technology for their use as drug loading systems to treat pulmonary diseases. In particular, we will design nCmP systems capable of being delivered to different regions of the lungs based on their aerodynamic diameter while also exhibiting low water content.

This work was supported by RI INBRE

Characterization and applications of acetalated dextran on various delivery mechanisms

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Biodegradable polymers are often used in drug delivery applications owing to their ability to allow for controlled release of therapeutics and targeting abilities. Unfortunately, some polymers are not ideal to use for drug delivery due to their slow degradation rates, inability to degrade over time, and the potential for toxic bioaccumulation in tissue. However, acetalated dextran (Ac-Dex) is biocompatible, biodegradable polymer that degrades via hydrolysis of its acetal groups, thereby releasing acetone, methanol, and-water soluble dextran, all of which are non-toxic and will not affect the drug that is being delivered. Ac-Dex has also been proven to be a tunable degradable material based on synthesis reaction time, which allows for the design of drug delivery materials capable of different degradation rates. Furthermore, Ac-Dex undergoes acid-mediated degradation in environments such as diseased tissue with low pH, allowing for targeted fast delivery of therapeutics at these sites. To date, there have been no comprehensive studies on Ac-Dex itself or drug delivery-based materials, thus driving this work. We synthesized and characterized Ac-Dex with varying reaction times in addition to fabricating and characterizing Ac-Dex-based nanoparticles (NP) and microparticles (MP). The size (diameter), polydispersity index, and zeta potential of the NP and MP systems were determined through dynamic light scattering, the morphology of the NP and MP was visualized through scanning electron microscopy, and the release profiles of the particle systems were shown with a release study. The varying release profiles demonstrate that release of the drug depends on the reaction time of Ac-Dex and the pH of the environment.

This work was supported by RI INBRE

Using atomic force microscopy to delineate the interaction of plastics with marine bacteria

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About 8 million tons of plastic are dumped into the ocean each year, carried by flowing water in rivers, storm drains, and through air. Much of this plastic sinks. As this problem progresses, it is important to learn about the effects plastics might have on the marine ecosystem and its organisms. Cyanobacteria are ubiquitous in the ocean, as they control dissolved oxygen levels, important for phytoplankton growth. These bacteria are a critical component of the delicately balanced ocean ecosystem. In this study, the physical interaction of cyanobacteria and a common plastic, polyethylene terephthalate (PET) is analyzed using atomic force microscopy. Cyanobacteria were cultured using previously frozen samples, A+ Media, and a vitamin mix. The substrate was prepared by taping a piece of plastic water bottle, made of PET, to a magnetic disk that can be inserted into the AFM. To attach bacteria to the cantilever tip, the tip was coated with poly-L-lysine (PLL) a cationic polyelectrolyte. The gram negative bacteria were then deposited on this cantilever, and fixed using glutaraldehyde. Scanning electron microscopy confirmed bacterial adhesion to the cantilever tip. Data on force versus distance (in air) between the uncoated, PLL- and bacteria-coated AFM tips and a PET surface are presented. The uncoated tip showed no attractive interactions with PET. The PLL-coated tip showed a distinct attraction to the PET surface. Experiments on the force between bacteria-coated tips and the PET are being conducted currently. Liquid cell measurements will be conducted shortly, as they replicate realistic conditions experienced by the bacteria.

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Recovery of osprey in Narragansett Bay: The importance of human structures

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Beginning in the 1950's Osprey (*Pandion haliaetus*) US populations began to decline due to use of DDT, resulting in Osprey being listed as an Endangered Species in 1976. Since the ban of DDT, Osprey nest sites have increased from a total of 13 in 1978 to a total of 239 in 2020; however, they are still listed as a species of Special Concern. Our research seeks to monitor the nesting success of Osprey throughout different regions of RI using citizen science data collection managed by the Audubon Society of RI. These regions include West Bay, East Bay, Bristol-Barrington-Warren estuary, Providence, and the Islands. Working closely with the Audubon Society's Osprey monitoring project, we addressed whether or not Osprey nest success is contingent upon coastal location, region, and/or structure. For the purpose of this project a successful nest is a nest with at least one fledgling. A total of 2,133 nests were monitored between 2008 (n=117) and 2020 (n=240), the majority of which were located in Bristol-Barrington-Warren estuary (n=615), West Bay (n=543) and Islands in the bay (n=543). The coastal area of Narragansett Bay has been critical to the nesting success of Osprey in 2008-2020. Despite the high number of large freshwater lakes and ponds in the state, 83% of nests are located within one mile of the bay and coastal nests fledge significantly more young than interior nests. Regionally, around the bay, Osprey fledge more young in the East Bay (mean 1.9 young per nest, n=18) Bristol-Barrington-Warren estuaries (1.7, n=384), and fewest along the West Bay (1.3, n=356). Osprey use a variety of structures for nesting. 68% of Osprey nests built on Osprey platforms (n=83 in 2020) have been active nests across the focal years of study, which is lower than active nests in trees (75%, n=12), lights (84%, n=28), cell towers (86%, n = 40), marker buoys (100%, n=4). Nests on human structures such as osprey platforms (85.27%), lights (89%), cell towers (75%), and marker buoys (90%) are more likely to be successful than tree nests (73.21%). From successful nests, there are significantly more Osprey young fledged from marker buoys (mean 2.0 young, n=30), Osprey platforms (1.7, n=436) and utility poles (1.8, n=113) than from cell towers (1.2 young, n=191). The coastal communities of Narragansett Bay remain important to reproductive success of these top piscivores, especially by placement and maintenance of Osprey platforms as well as secure human structures.

This work was supported by RI C-AIM

This year's data visualization workshop

Jovan Dias

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Quality is necessary, we can have solid information, but it can be misunderstood or might not be received the way we attended if some aspects of quality is not met. It is important because an example of what I am saying is this: If we have a bad interface but our tools would help on our website, the users might need a longer learning curve making the task harder. It should be easy so they can focus on when to use it in their lessons.

As each time we have these workshops, we need to improve each time. This will help us bring more participants who are interested to know more. One way to do this is to make workshops and get feedback. “Feedback can improve a student's confidence, self-awareness and enthusiasm for learning. Effective feedback during the first year in university can aid the transition to higher education and may support student retention.” This is a fact for students, and in general everyone always is learning. We ensure that our surveys offer a way to gain feedback so we ourselves can learn too. The more quality we ensure, the better everyone receives the benefit of learning. The previous workshop surveys I worked on so far did not have as much feedback questions as now, so hopefully this will make this year’s workshop more suitable than last years for improvement.

The website itself is being created for a better interface and better qualities than the old one. Hopefully, this will help all inexperienced users in understanding. Having the website having more improvements than the one that was present in the previous year’s workshop will guide us on how to improve it again in next times workshop. There is always an adaption to be considered.

New methods and updated approaches must be taken to create more efficiency. This year's workshop is different than last year's workshop. We are working hard to make sure improvements are being made and exploring latest ideas to see if it works. This is necessary to achieve a better new workshop.

This year's workshop has more participants, that is a good sign. It is important to gain interest from others so they can hopefully use this data as a valuable tool. It is important for this year’s workshop to be different so we can have a new and better experience. Even if it is the smallest change like sending 3D graph models with their activi

This work was supported by RI C-AIM

Continuous culturing of MnOx producing bacteria to biosynthesize manganese oxide nanoparticles

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Manganese oxide nanoparticles (MnONPs) with tunable properties can be biosynthesized by *Pseudomonas putida* GB-1 growing under controlled conditions. MnONPs have a high redox potential and can oxidize organic compounds and adsorb heavy metals found in contaminated water. Here, *P. putida* was cultured using a batch culture technique with the goal of performing a continuous culture in the future. *P. putida* was chosen because they have been found to naturally oxidize Mn and produce MnONPs. Batch cultures were inoculated with bacteria to a fixed volume of fresh media under constant temperature, pH, and aeration. The bacteria complete their growth cycle and start the stationary phase as the nutrients in the media are depleted. The specific growth rate (μmax) of *P. putida* was calculated from the growth curve recorded during the batch cultures as a first step toward developing a continuous culture. Inductively coupled plasma mass spectrometry (ICP MS) was used to quantify MnONP production. Leucoberbelin blue (LBB) reagent was used to confirm the presence of manganese oxides. The MnONPs produced by *P. putida* were then characterized for their physicochemical properties including size, morphology, and oxidation state using techniques such as scanning electron microscopy (SEM), X-ray photoelectron spectroscopy (XPS), X-ray fluorescence spectrometry (XRF), and transmission electron microscopy (TEM). The structure and properties of MnONPs were dependent on the culture conditions of the MnONP producing bacteria (MnOBs). This method was able to generate biosynthesized MnONPs, calculate an accurate μmax, and characterize nanoparticles in preparation to begin a continuous culture.

This work was supported by RI C-AIM

Native and non-native macro-algae's response to grazing by the snails *Littorina obtusata* and *Littorina littorea*

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Macro-algae in the shallow intertidal zone are prone to grazing by macro invertebrates. This research investigated macro-algae's response to this grazing. Algae have different strategies to cope with herbivory, including induction of chemical defenses and compensatory growth. The study investigated the change in tissue quality, the alga's ability to utilize photosynthesis and measured the stress response before and after grazing. This study serves as preliminary research to understand macro-alga's inducible defenses and whether these defenses are herbivore specific or general defenses.

The snails *Littorina obtusata* and *Littorina littorea* were collected at Conimicut Point in Warwick, RI and Pier 5 in Narragansett, RI and then acclimated to lab conditions. Three different macro-algae were investigated: the native *Chondrus crispus* and *Ascophyllum nodosum* and the non-native *Grateloupia turuturu* was collected at Mackerel cove in Jamestown, RI and Pier 5 in Narragansett, RI. Each seaweed and snail trial was run for ten days and light curves and fv/fm were measured before and after exposing the macro-algae to grazing by either *L. obtusata* or *L. littorea*. In addition, the snails were measured, and the seaweed was weighed at the beginning and end of each trial. Tissue samples were dehydrated to compare change in tissue quality after exposure to grazing. A pulse amplitude modulated fluorometer (PAM2500) was used to measure the stress response. This research will provide insight on the alga's stress response by measuring how much of the light they receive can be used for photosynthesis. Previous research suggests that when a plant is stressed, energy is allocated to mount a stress response and therefore photosynthesis is less productive.

This work was supported by RI C-AIM

eDNA shows fish diversity may not increase along a salinity gradient

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The project aimed to see if there was a correlation between salinity and fish diversity in Narragansett Bay using eDNA. Water samples were collected Summer and Winter of 2019 and Winter of 2020 using sterile whirl packs and filtered using funnels. DNA was extracted using the Qiagen DNeasy Kit and sequenced (Schedl 2019). We hypothesize that as salinity increases along a longitudinal gradient from North to South in the bay (Hess 1976), the diversity of fish species will also increase. Upon analysis of the data, there is no correlation between the salinity gradient and fish diversity. A t-test in RStudio showed that there is no significant relationship between diversity and North or South sample location ($p = 0.050875$) or diversity and salinity ($p = 0.555$). Some possible explanations for the lack of correlation are low sample size or that other factors such as chlorophyll and human activity have a stronger effect on fish diversity. One weakness of the study is that Shannon and Simpson indexes only measure diversity and cannot account for how the species communities may change over the salinity gradient. Future studies should attempt to look at community assemblage along these gradients, focus on other factors that could affect diversity and include eDNA samples from a wider variety of taxa. The study highlights the importance of quantifying limiting factors for diversity within Narragansett Bay so the ecosystem can be better protected, and the potential for eDNA as a noninvasive sampling method to quantify species diversity in aquatic ecosystems.

This work was supported by RI C-AIM

Mercury bioaccumulation and stable nitrogen isotope analysis of commercially important fish in Rhode Island coastal waters

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Mercury (Hg) is a toxic, heavy metal that bioaccumulates in fish tissues at concentrations that may exceed human consumption thresholds. Rates of Hg bioaccumulation differ across fish species, however, and are affected by the fish's feeding ecology. Therefore, it is important to interpret Hg contamination in a variety of fish species and analyze the results in the context of a fish's trophic position in the food web. In this study, Hg concentrations and stable nitrogen isotope (δ15N) signatures, a proxy for trophic status were measured in the muscle tissue (ppm dry weight) of four commercially important fishes found in Rhode Island waters. The target species monkfish (*Lophius americanus*; n = 20), silver hake (*Merluccius bilinearis*; n = 50), yellowtail flounder (*Pleuronectes ferruginea*; n = 34), and haddock (*Melanogrammus aeglefinus*; n = 29) all experienced increased Hg levels at larger body sizes. Monkfish had the highest mean Hg concentration (mean ± SE = 0.52 ± 0.09 ppm), followed by yellowtail flounder (0.25 ± 0.03 ppm), silver hake (0.13 ± 0.01 ppm), and haddock (0.07 ± 0.01 ppm). The low Hg content of haddock is partly explained by only sub-legal individuals being analyzed in this study. The mean δ15N signature of monkfish (14.09 ± 0.17) was significantly greater than the values observed in the other target species (12.44-12.88), thus confirming that species occupying a higher trophic position tend to bioaccumulate Hg at a greater rate. Finally, among all tissue samples examined, < 1% (1 monkfish) exceeded the U.S. Environmental Protection Agency's recommended Hg limit (~1.36 ppm), indicating that the target species pose minimal risk to human consumers.

This work was supported by RIDEM Department of Marine Fisheries

Predicting natural habitat trends of frosted elfin butterflies

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The frosted elfin (*Callophrys irus*) is a rare New England butterfly. As a host plant specialist, we know frosted elfins only lay their eggs and develop on a few plant species, including small yellow wild indigo (*Baptisia australis*). However, these rare butterflies are hard to find. Therefore, we mapped locations of frosted elfin sightings from iNaturalist, a database for biodiversity observations. We chose a site, Gavins Pond (Sharon/Foxboro, MA), with suitable metrics such as canopy cover, indigo abundance, and a history of frosted elfin sightings. Gavins Pond had numerous areas of wild indigo where we called the areas "patches" to track the 22 areas. We measured host plant density in two ways: 1) qualitatively at the patch level, and 2) quantitatively at the quadrat level. Patches with "abundant" indigo had an average of 5 stems/square meter, while patches with "common" indigo had an average of 3 stems, and "uncommon" indigo's average was 2 stems/square meter. As predicted, qualitative density significantly correlated with quantitative measures. These results from Gavins Pond inform trends of the frosted elfin habitat and compare them to other frosted elfin sightings for future research.

This work was supported by Providence College and the Southeastern New England Educational and Charitable Fund

Assessing plant-pollinator relationships between native insect pollinators and flowering plants in Providence and Westerly, Rhode Island

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Insect pollinators are crucial for producing food and maintaining the health of ecosystems. We know native insect pollinators are under threat from the use of pesticides, fragmented habitats, and the effects of climate change. Conserving native pollinator species is critical but we lack knowledge on which plant species are preferred by these insects. Therefore, we used various sampling methods and observational data to record plant-pollinator interactions in Rhode Island at both inland Providence College (Providence, RI) and coastal Westerly Land Trust sites (Westerly, RI). Data were collected at a total of six sites: two at Providence College and four at Westerly Land Trust sites. To record plant-pollinator interactions plant species are observed within defined quadrats along with the species of insect pollinators that forage on each flowering unit. To create a reference collection of all bee and other insect pollinator species at our research sites, bee bowls are used to trap insects that can later be pinned and identified to species. This collection can be used to help identify live insects in the field and record the species that call Rhode Island home over time. In addition to these methods, bee hotels were installed at each site to provide solitary bees with nests. In addition to investigating plant-pollinator interactions, these nests can then be dissected to gain information on solitary bee life cycles and fill the gap of knowledge on lesser-known native bee species. Few longitudinal studies have been conducted on plant-pollinator preferences. These methods can be replicated for years to come to better understand which native pollinator species inhabit Rhode Island and what plants we can incorporate into our landscapes to help native species thrive. Based on the data collected so far, Providence College and the Westerly Land Trust provide diverse flowering plant species throughout each season that provide native pollinators with the resources they need to continue to thrive.

This work was supported by RI C-AIM

Synthesis of biodegradable polythionoesters

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Polythionoesters possess tunable degradation through the concentration of thionoester in the polymer backbone and polymer molecular weight. Our goal is to create biodegradable thionoester-co-ester polymers to be used as slow-release capsules for medicinal drug delivery applications. This study focuses on the polymerization of thionolactone and lactone monomers from polyethylene glycol macroinitiators. Organic catalysts were used, and the reaction conditions leading to polymerization are highly sensitive to the monomers/initiator being employed. Our reaction conditions were then used to make the copolymers PEG-CL-Lactide and PEG-Lactide-CL.

This work was supported by RI C-AIM

Functional and mechanistic traits in wild caught *Drosophila* from distinct thermal regimes in New England

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Climate change poses a serious threat to ecosystem predictability, with increases in severe weather, precipitation, and extreme temperatures projected by climate models. Temperature is a major determinant of organism niche, affecting viability from cellular to organismal levels. The climate variability hypothesis specific to temperature suggests that adaptations of organisms native to a thermally variable environment will be more likely to have mechanisms that allow them to have increased survivorship under extreme temperatures compared to those originating from a thermally stable environment. Here we investigate the functional and mechanistic traits of wild caught *Drosophila* from thermally variable (Mount Mansfield, VT) and stable (Newport, RI) environments. Functionally we will assess critical thermal ability (CTmax/min) and changes in expression of genes known to be associated with environmental stressors (heat shock proteins). We predict that if evolutionary background effects an organism's ability to withstand extreme temperatures, then organisms adapted to thermally variable environments will be better equipped to persist in the conditions predicted by climate models than those from a thermally stable environment.

This work was supported by VT NSF EPSCoR Track 2

How plastic and microplastic toxins effect the development of aquatic vertebrates

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It is well known that plastic pollutants are an ongoing problem in our oceans and waterways. Though the physical disruption plastics have on ecosystems are well established, less is known about how chemicals leeching from plastics disrupt cellular function and processes in organisms that encounter the pollutants. In particular, microplastics, which are fragments of plastic that do not exceed five millimeters in diameter, present an emerging threat to aquatic ecosystems. Microplastics can not only be created by toxic chemicals that can then be released into the environment, but also absorb chemicals that can then be released. The impact of plastics on the environment starts at the lower trophic levels with primary consumers, and inevitably works its way up the pyramid to larger marine mammals. Understanding how the chemicals leeched from plastics into the environment affect tissues of organisms which ingest plastics are disrupting cellular function at molecular levels is important because of the implications on the health of upper trophic levels – including humans. Previous findings suggest that toxins from plastics can have fatal impacts on developing embryos, in some cases reducing survivability and driving developmental abnormalities. Polyethylene, for example, has shown great detriment to the physical development and survivability of zebrafish. However, few studies delve into how molecular mechanisms, such as gene regulation and transcription, are affected by Polyethylene and the chemical toxins that come along with it.

Using zebrafish embryos as a model organism, we will treat embryos with Polyethylene to observe how they affect hindbrain development. Using in situ hybridization and qPCR we will monitor correct formation of early formation of tissues in the developing hindbrain of zebrafish and gene expression in the embryo. Together these observations will provide us with a better understanding on how microplastics, and the chemicals associated with them, affect vertebrate development in aquatic environments, and give us insight into the affects that they will have on the entire ecosystem.

This work was supported by RI C-AIM

Developing a web-based platform for the visualization of microbial community functions in Narragansett Bay

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One of the most common techniques used for understanding the ecological role of a microbial community is to measure the diversity of its constituents. Previously, we developed a web portal that visualizes the taxonomic profiles of bacteria in Narragansett Bay. As an extension to this project, we built a web-based platform for the visualization and analysis of the functional potentials represented by the genomes composing the bacterial populations within Narragansett Bay. This platform, called PSAMMvis, utilizes the Portable System for the Analysis of Metabolic Models (PSAMM) to visualize and interact with metabolic models. In particular, this platform enables the user to visualize subsets of metabolic models based on pathway information, compounds of interest, and element transfer networks (e.g. examining the flow of carbon, nitrogen, phosphorus, or sulfur through the network). This web app was applied to visualize the genome scale metabolic reconstruction of a Reinekea species identified from the Narragansett Bay. Through the development of the PSAMMvis platform, we have provided a proof of concept for the utilization of this web app to examine the functional diversity of microbial communities. Further, the online deployment of this platform permits access to this platform by a broad community of researchers.

This work was supported by RI C-AIM

Paired appendage turning in fluids

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Swimming organisms have different methods of propelling themselves forward, along with different mechanisms of turning. Multiple species are examined because organisms move in different fluids, including water and air, which may be accompanied by different mechanisms of turning. We will evaluate the similarities and differences between organisms of different phylogenetic backgrounds based on the movement of the inside and outside appendage in relation to each other and in relation to the body centroid. From the centroid data we can find out if the center of mass stays linear during a turn, which will give us insight into how these organisms turn.

This work was supported by RI C-AIM

What's the story? Developing engaging narratives to communicate scientific research

Ciara French & Shaun Kirby

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From development of nano-sensors to detect nutrients in Narragansett Bay to creating computer models that predict future changes in fish populations, the scope of RI C-AIM research is broad in order to answer scientific questions regarding climate change in the Ocean State. There is thus a critical need to effectively communicate this research in narrative ways to multiple audiences that hold a social, cultural and/or economic stake in the health of Narragansett Bay.

Storytelling is one of the most powerful ways to teach, influence and inspire. Our personal stories can give readers an opportunity to learn from another person's experience. Developing personal narratives allows for the audience to build a trustworthy relationship with the author while understanding science communication can offer new insight into its relevance to society.

The project entailed engaging with other SURF students to create journalistic pieces highlighting their research, interests and experiences. I established necessary background information to generate interview questions, conducted interviews and developed narrative stories that effectively communicate the science of a given research topic, as well as the experiences of investigators engaging in that scientific research.

To conclude, I assembled a website including all of the completed narrative stories utilizing Adobe Spark.

This work was supported by RI C-AIM

Academic success and perception of academic success differences amongst students with visible and non-visible disabilities

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Federal guidelines require colleges and universities across the country to provide reasonable accommodations to students with disabilities. Many institutions of higher education provide these accommodations through their offices of disabilities. Students with disabilities are expected to voluntarily seek their accommodation within their respective institutions and disclose their disabilities. However, there is often stigma, lack of knowledge, and other barriers that prevent this from happening. The term Disability refers to a range of possible impairments of the body and or the mind, which result in added difficulty or impairment to performing some or all activities. Not all disabilities manifest in the same ways for everyone and as such, it can be challenging for colleges and universities to find adequate accommodations for all students. This can result in inconsistencies in accommodations. Challenges in finding appropriate accommodation for all types of disabilities have the potential to disproportionately affect academic success by creating additional barriers. We are particularly interested in visible (e.g. Paraplegia, Blindness with assistive cane or dog) and non-visible disabilities (e.g. Dyslexia, Attention Deficit Disorder, ADD; Post-Traumatic Stress Disorder, PTSD). If we compare the academic success of students with visible disabilities and their perceived academic success to that of students without visible disabilities, would we find any differences? And what factors are potentially contributing to it? This research aims to answer these questions by distinguishing how visible versus non-visible disabilities impact academic success. We also discuss best practices aimed at improving the perceived academic success of students with disabilities. We first establish what the perceived academic success is for students with both visible and non-visible disabilities at colleges and universities and then, explore how the unique experiences by both groups shape their perceptions of academic success.

This work was supported by RI C-AIM

Microplastic pollution: sampling, identifying, and quantifying

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Microplastic pollution is a relatively new and rapidly developing field of scientific inquiry. This global environmental issue is being investigated in countless forms. The research being conducted by the Ocean State Initiative for Marine Plastics (OSIMAP) team aims to improve our understanding of the presence and impacts of microplastic pollution in Narragansett Bay. This team employs a number of methods to collect and analyze microplastic pollution. One of the main sampling techniques used is a Manta Trawl net system that sieves large volumes of surface water to collect debris down to a minimum size range of 330um. Using this sampling technique, the OSIMAP team conducts an ongoing survey repeatedly samples six sites distributed throughout the bay at 3-time points in the year. Following the collection process, samples are processed in order to isolate individual microplastic particles from organic debris in preparation for further analysis. Microplastic particles are then picked from solution, mounted on slides and imaged to allow for measurement and morphological categorization, before the polymer type is identified using a confocal Raman microscope. Only 20% of each sample is analyzed using Raman due to time constraints. Because of this, a hot needle test is employed to test if unanalyzed particles are plastic or not. As this research is ongoing, definitive conclusions cannot yet be drawn as to the full extent of microplastic pollution in the Narragansett Bay, but current findings suggest plastics are present in various forms throughout all sampled sites and time points. Such sample collections and data can be used to feed into determining the presence, composition, and spatial and temporal distribution of microplastics in Narragansett Bay. Ultimately, this team seeks to provide quantitative research that can better inform other coastal communities concerning this global environmental issue of microplastic pollution.

This work was supported by RI C-AIM

What microbes grow on polyethylene in Narragansett Bay, and can biofilmed microplastics alter the *Astrangia poculata* microbiome?

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Microplastics pollution is prevalent and increasing in abundance in marine ecosystems across the globe. Microplastics, plastics pieces <5mm long, are often ingested by marine organisms, especially suspension feeders such as the native stony coral *Astrangia poculata*. Identifying bacterial communities that colonize marine microplastics is essential for understanding microplastics pollution and its impact on marine organisms and ecosystems. The aims of this study are to 1) identify microbes that colonize polyethylene microbeads in coastal Narragansett Bay, and 2) to determine whether microplastics exposure can alter the microbiome composition of benthic organisms, using the local coral *Astrangia poculata* as an experimental system. Polyethylene (PE) microbeads were deployed in seawater at Fort Wetherill State Park for three weeks. Throughout the summer, methods were adapted and developed for high-quantity DNA extraction from the PE microbead biofilms. The resulting DNA will be used for next-generation 16S rRNA amplicon sequencing to characterize the microbial community composition of PE microbead biofilms that have incubated in wild seawater and in aquarium tank (RWU) seawater. Additionally, a laboratory assay has been developed to expose *A. poculata* colonies to ecologically relevant concentrations of microplastics. A sampling scheme is being developed to test whether microplastics exposure results in alterations to the coral microbiome, and to identify potential impacts on the coral physiology. These methods will be adapted in the coming months to include *A. poculata* colony exposure to PE microbeads treated in a variety of ways, including non-biofilmed microbeads, and microbeads incubated in wild seawater for different durations. This study has established methods that will be used in continued research on identification of microbes that colonize polyethylene in Narragansett Bay, and to determine whether microplastics and their associated microbes can alter microbiomes and health of marine organisms.

This work was supported by RI C-AIM

Synthesis and application of metal complexes as nitrate detectors

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Nitrate, NO^{3-}, is a common contaminant ion. In high concentrations, it has negative impacts upon human health and the environment, including the Narragansett Bay ecosystem. Dipodal and tripodal pyrrole-based ligands were synthesized and coordinated with iron or manganese triflate. These colored complexes were incubated with a nitrate source and assessed through UV-vis spectroscopy. Dose dependent responses were observed for some organometallic complexes upon nitrate exposure. Future investigations will explore extended conjugated ligands and assessing other ions.

This work was supported by RI C-AIM

Nanostructured sensor for electrochemical detection of phosphate based on carbon black decorated with gold nanoparticle (CB-AuNP)

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Phosphate is an essential nutrient for plants, however, at high concentrations, it leads to a condition called eutrophication, a rapid growth of plant populations in aquatic environments (algal blooms), eutrophication which results in a reduction or elimination of dissolved oxygen that is crucial for fish and other aquatic life. Herein, we report on a highly sensitive electrochemical sensor for detection of phosphate levels in seawater samples. The method is based on measuring phosphomolybdate complex formed by a reaction between phosphate and molybdate which is subsequently detected on the electrode surface. To enhance the sensitivity of the sensor and lower the detection limit, a modified screen-printed electrode was used. Screen-printed electrodes modified with carbon black decorated with gold nanoparticles (CB-AuNP) successfully increased the detection of phosphomolybdate complex reduction at + 64 mV vs. Ag/AgCl. Analytical figures of merit including reagent concentration, working potential, flow rate and concentration of CB-AuNP on electrode surface were optimized. Results show a linear range at low phosphate concentrations from 0.05 – 50 uM with a detection limit of 0.05 uM phosphate, calculated as three times the standard deviation of the blank divided by the slope of calibration curve. Work is in progress to assess accuracy and selectivity.

This work was supported by RI C-AIM

Revealing the complex growth of anaerobic ciliates and their methanogenic endosymbionts

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Ciliated eukaryotic protists and their interactions with methanogenic endosymbionts remain understudied despite their abundance in a wide range of environments, including some that are considered extreme for most eukaryotes, such as anoxic sediments. Even though nearly every anaerobic ciliate described to date likely hosts archaea as intracellular symbionts, a unique partnership among eukaryotes, little is known about the effects of these relationships on the growth and life cycle of anaerobic ciliates. In this study, the effects of temperature and population growth phase on the growth rate of the anaerobic ciliate *Heterometopus palaeformis* (Metopida) and its archaeal endosymbiont *Methanobacterium* sp. (Methanobacteriales) is examined. Using cultivation and cell-counting methods, the growth and life cycle were monitored under nine different temperatures. In addition, changes in ciliate cell shapes were observed and symbiont numbers were counted at various points of the growth cycle. Our collected data show that *Heterometopus palaeformis* appears to have a dynamic growth cycle depending on the temperature while the intracellular numbers of *Methanobacterium* sp. do not seem to be significantly affected by the population phase of its ciliate host. The knowledge gained from this study will better inform cultivation techniques for anaerobic ciliates and has expanded our understanding of their endosymbiont growth.

This work was supported by RI C-AIM

Using drones to map current vectors in Narragansett Bay

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The OSOM (Ocean State Ocean Model) simulates the Narragansett Bay by utilizing local ocean models and forcing data taken from the environment. To further constrain the OSOM's current velocity readings, vector maps are created using the CopterCurrents approach developed at HZG as an alternative to Acoustic Doppler Current Profilers and other methods of data collection. A DJI Mavic drone was flown over the shore of the Save the Bay Center and Phillipsdale with the attached camera pointed in the nadir direction. Short duration footage of the waves is recorded at a variety of nearby sites, then run through the CopterCurrents program to create a vector map of wave direction, wave length, phase velocity, and current velocity. Footage was taken of the two locations at high, low, ebb, and flow tides to account for a full tidal cycle. In total, 4 vector maps are produced for each location at each site. The vector data has been made available through the Rhode Island Data Discover Center (RIDDC).

This work was supported by RI C-AIM

Observing wave energy attenuation in sea ice

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The region in which sea ice and ocean waves interact, known as the marginal ice zone, has been a subject of great attention, as the propagation of waves in sea ice often creates fractures in the sea ice pack and alters the sea ice pack's thermodynamic properties. Competing theories and frameworks have been proposed to understand the attenuation of waves in ice, however developments in the field are challenged by the scarcity of data. In this work, we study the utilization of NASA's ICESat-2 satellite as a remote sensing method for observing the propagation of ocean surface waves. Specifically, we examine the use of spectral unmixing to distinguish wave spectra from those related to sea ice surface roughness, leading to detection of wave energy attenuation within sea ice. We anticipate that these methods could be useful for the analysis of wave propagation and mixing in coastal systems and estuaries.

This work was supported by RI C-AIM

Controlling the droplet size of cyclohexane-in-water emulsions

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Emulsions are mixtures of two immiscible fluids where one fluid is dispersed in the continuous phase of the other fluid and are used throughout the food and medical industries. One significant drawback, however, is that emulsions are thermodynamically unstable and naturally separate over time to reduce the interfacial area between the two fluids. Surfactants prolong this separation by lowering the interfacial tension between the water and oil phases in emulsions, and the emulsions take longer to separate. The goal of this research is to use cyclohexane-in-water emulsions bridged by a triblock copolymer to generate cell-like structures that mimic the mechanical properties of biological tissues. This study aims to control the size of cyclohexane droplets to promote bridging between the droplets by the triblock polymer and improve mechanical properties. Here, surfactant mixtures of Tween 20 and Span 20 are used to stabilize cyclohexane-in-water emulsions prepared by dropwise addition of cyclohexane to water under ultrasonication. The droplet size is then characterized by dynamic light scattering (DLS). Results indicate that the sonication amplitude and the size of the sonicator probe do not have major impacts on the cyclohexane droplet size, but the droplets prepared with the Span 20 surfactant are more stable and smaller. These findings will make it easier to control the overall structure of a suspension of emulsion droplets and to achieve the polymer bridging between droplets required to replicate tissue-like mechanics.

This work was supported by RI INBRE

Uptake and cytotoxic affects of aggregation state of DNA-wrapped single-walled carbon nanotubes in mammalian cells

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Previous studies have shown that DNA-wrapped single-walled carbon nanotubes (SWCNTs) are actively internalized by mammalian cells. An important aspect that is often overlooked when considering DNA-SWCNT internalization is the aggregation state of the nanotubes, how the state of aggregation affects the cell's uptake of the nanotubes, and their cytotoxic effects. Upon internalization of SWCNTs and their subsequent interactions with cellular bodies and proteins *in vivo*, SWCNTs are more likely to become aggregated, so it becomes important to study the differences in how singly-dispersed versus aggregated SWCNTs are processed by cells. This study aims to characterize the differential uptake, intracellular processing, and cytotoxic effects of aggregated DNA-SWCNTs as compared to a singly-dispersed sample in mammalian cells. The two samples are compared through *in vitro* investigation of the fluorescence intensity of SWCNTs in cells, analysis of hyperspectral cubes, confocal Raman microscopy, label-free cellular proliferation, and cytotoxicity assays. Analysis of hyperspectral cubes leads to the ability to conduct "spectral counting," where regions of interest (ROIs) are processed to relate number of fluorescence peaks to number of nanotubes in each ROI, thus quantifying the state of aggregation. Further analysis of hyperspectral cubes shows that samples that are aggregated prior to dosing display quenched fluorescence in cells. More studies in the near future are to be conducted to investigate the cytotoxic effects of the singly-dispersed DNA-SWCNTs as compared to aggregated ones.

This work was supported by RI INBRE

A novel method of preparing DNA wrapped SWCNTs via lyophilization and subsequent resuspension

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Single-walled carbon nanotubes (SWCNTs) have been found to have unique optical properties, fluorescing when exposed to near-IR light, which allow them to act as biomarkers. Current techniques of dispersing SWCNTs used specialty equipment that is required by institutions to break up aggregates and remove contaminants from raw SWCNTs. Additionally, most of the weight from the sample was due to the large volume of water. A novel method was found that can make chemically pure and singly dispersed SWCNTs without the need for specialty equipment and no weight attributed to water. An ultra-centrifuged sample of SWCNTs underwent lyophilization and was investigated whether the powder form could be resuspended and behave in the same way as a non-lyophilized sample. A powder that could be resuspended would allow manufacturers to make their products chemically pure first and then ship the product to institutions with limited equipment and a maximized reduction in weight. In this study, both the powder and resuspended liquid of the lyophilized sample were characterized and compared against a non-lyophilized sample. We found that the lyophilization process produced aggregates, contradicting the goal of creating a singly dispersed sample. To mitigate aggregation a filler was introduced, in our case 6K PEG, which to our surprise increased aggregation instead of decreasing aggregation. Instead of adding a filler, we were able to achieve our goals by centrifuging our resuspended liquid at low speeds and removing the supernatant, providing us with our desired sample.

This work was supported by RI INBRE

Detecting enzymes and reaction products when plastics are exposed to marine cyanobacteria using liquid chromatography-mass spectroscopy (LC-MS)

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Plastic pollution in the ocean continues to be a prevalent issue in the world. It is important to discover and understand the effects of plastic pollution on the marine ecosystems and organisms. Synechococcus sp. strain PCC 7002 is a cyanobacterium species that resides in Narragansett Bay. This bacterium helps maintain the dissolved oxygen levels and are thus a critical part of the ocean ecosystem. A recent study has shown that when PCC 7002 was exposed to PET microparticles there was an upregulation of genes that code for PEThydrolase, a PET degrading enzyme. A key goal of this study is to identify and quantify enzymes and degradation products when bacteria is exposed to plastics. Liquid chromatography coupled with mass spectrometry (LC-MS) is used to identify these products. One known product of the reaction of the enzyme-PET reaction is terephthalic acid (TPA). We have successfully detected and quantified TPA using our LC-MS equipment. Our current work involves optimizing the column material and operating parameters to detect enzymes and other reaction products being produced by the cyanobacteria after exposure to PET.

This work was supported by RI C-AIM

Songbirds in refugia on uninhabited islands in Narragansett Bay

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What is the value of the many uninhabited islands in Narragansett Bay to biological diversity? Small island habitats are known to be predator-free refuges for many species and provide valuable breeding habitats for many colonial nesting herons, egrets, and ibis (Families Ardeidae and Threskiornithidae). In Narragansett Bay, many such islands are protected from human contact during the breeding season by RI DEM. However, the value of these small islands for breeding songbird populations is assumed but not quantified. In the summers of 2020 and 2021, we monitored the survivorship and productivity of songbirds using standardized mist-netting procedures (MAPS, Institute for Bird Populations). Data were collected on Rose Island in Newport Harbor, as well as an adjacent site, Sweetflag, in the lower Bailey Brook watershed of Middletown. Both sites share similar maritime shrubland environments dominated by *Rosa multiflora* with scattered low-growing canopy trees of *Quercus*, *Amelanchier*, *Rhus*, and *Prunus*. Consistent with the theory of Island Biogeography, the island had fewer breeding species: 14 different species of songbirds were captured on Rose Island and 18 different species were captured at Sweetflag. Common Yellowthroat (*Geothlypis trichas*), Yellow Warbler (*Setophaga petechia*), Song Sparrow (*Melospiza melodia*), and Gray Catbird (*Dumetella carolinensis*) were the most abundant species at both sites and focal species for comparative measures of productivity and survival. In 2020, there were significantly more hatch-year Common Yellowthroats and Song Sparrows on Rose Island than Sweetflag, while there was no significant difference between sites for other species and as of this report there are no significant differences for 2021. Survivorship, as measured by the percent of adults captured in 2020 that were recaptured in 2021, is respectively higher on Rose Island for Song Sparrows (11.36%, 4%), Yellow Warblers (14.29%, 5.88%), and Gray Catbirds (15.79%, 14.81%). Common Yellowthroats had higher survivorship rates at Sweetflag than Rose Island (14.81%, 33.33%). Due to high seasonal variation, this five-year study is only near completion of year 2 and will undoubtedly benefit from continued data collection. Preliminary conclusions suggest that Rose Island has lower species richness, equal or higher species abundance, and in some cases higher species productivity and survivorship than nearby conserved habitats on large islands or the mainland.

This work was supported by RI C-AIM

Once Upon a Data Visualization: Visual datasets for SimpleChartsRI

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Data visualization is not widely used in the classroom. Teachers struggle with tools that are too complex for everyday use. With the help of visualizations, teachers can reinforce their verbal teachings and increase the engagement and interest of students. This paper aims to create a tool that is simple yet provides features that support the everyday needs of teachers. Through our tool, SimpleChartsRI, more features were added, such as a sample selection page and downloading raw data from samples to allow teachers and students to create quick visualizations while also inspiring them to collect data and create their own. This tool can also help introduce high-school students to the data science field. Many companies seek out people who have the skills to interpret data, create graphs and charts, and understand patterns. With these skills under their belts, it puts students at an advantage when looking for careers, whether in finance, management, or data analytics. Being exposed to data visualizations both in the classroom and out, students gain data skills to carry on into their future careers. They also learn to create narratives by picking apart data, choosing which data are displayed, and creating their own data visualization story.

This work was supported by RI C-AIM

Use of x-ray photoelectron spectroscopy for the analysis of early biofilms

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The formation of biofilms on materials immersed in seawater hinders economic productivity and obstructs efforts to monitor the health of marine ecosystems via sensors. Early biofilms are crucial to the biofilm formation process as they prime the surface of immersed materials for subsequent adhesion and colonization of marine organisms. Studies of early biofilms can help inform the biofilm formation process, and ultimately, prevention of biofilm genesis. Nonetheless, few techniques can adequately detect the biomolecules which adhere to surfaces in early biofilm formation. Techniques that are well-suited for characterization of more developed biofilms, such as FTIR, are unable to provide the same utility for the analysis of very early biofilms. Contrastingly, X-Ray Photoelectron Spectroscopy (XPS) is a quantitative, highly sensitive surface analysis method well-suited for the analysis of early biofilms due to its ability to identify the chemical state and elemental composition of the surface of a material. In this study we performed a literature review of XPS studies of biofilms. We identified a notable gap in the literature-- most XPS studies of biofilms are limited to mature, laboratory-grown biofilms and few evaluate early biofilms sampled in the field. To examine the kinetics of early biofilm formation we used XPS to analyze samples of glass deployed into Narragansett Bay at the University of Rhode Island Graduate School of Oceanography dock at 1 hour, 1 day, 1 week and 1 month intervals. XPS analysis revealed increasing organic matter adsorption with time and fluid attachment/detachment at the 1 day interval.

This work was supported by RI C-AIM

The effects of *Ulva compressa* and eutrophication on *Fucus*

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Rockweeds and specifically *Fucus* spp. are brown seaweeds that grow in rocky intertidal zones and have historically been prevalent in Narragansett Bay. Rockweeds form important ecosystems that provide a home to many animals, in addition to serving as a food source. The changing ocean environment means that *Fucus* spp. is exposed to more and more excess nutrients, known as eutrophication. *Ulva compressa* is a green macroalga that has become more prevalent with excess nutrients. Other work has shown that *Ulva compressa* produces compounds that can negatively impact the growth of other organisms. The first objective of this study was the determine if *U. compressa* inhibited *Fucus* spp. growth in co-culture laboratory assays. We found that *U. compressa* did not significantly impact the growth of *Fucus* spp. The second objective was to determine if the addition of excess nutrients has any impact on the interaction between *U. compressa* and *Fucus* spp. There was also no significant impact of *U. compressa* on the growth of *Fucus* spp. under eutrophication. However, *Fucus* spp. grown with excess growth was observed to have lower tissue quality. Our results show that *Ulva* does not impact the growth of *Fucus* spp. with or without the presence of excess nutrients.

This work was supported by RI C-AIM

Investigating genetic variation in queen snapper (*Etelis oculatus*) in Puerto Rico

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Commercial fisheries support the livelihoods of many US Caribbean families. Like many other Caribbean countries, snappers are one of the most important finfish landed by weight. On the Puerto Rican west and east coast, a vast majority of the snappers targeted by recreational and commercial fishers are in deep waters (>200m). One of the main fish species found within these depth ranges is the queen snapper (*Etelis oculatus*) (Valenciennes, 1828). They are abundant near oceanic islands. Even though the queen snapper is a central part of the US Caribbean's commercial fishing industry, much is unknown about the general biology of this species (life history, habitat preferences, prey, etc.). The lack of information has made it challenging to manage this species. A key aspect for successful management is understanding whether populations of the queen snapper represent different units of management or behave as a single population. This project looks at genetic variability in populations of the queen snapper throughout Puerto Rico. It evaluates if the two morpho-types identified by commercial fishermen on the island represent different populations that deserve independent management. We collected 40 specimens representing all morphotypes across five sampling sites around Puerto Rico. We measured standard length and length in the dorsal and ventral lobes of the caudal fin. Collaborators estimated gonad variation and sex with these same samples. To determine if different morphotypes are genetically isolated, we amplified and sequenced the mitochondrial control region. We found extensive variation in the fish sizes and caudal fin sizes and shapes. We also found genetic variation in the control region in populations around Puerto Rico.

This work was supported by RI C-AIM

Spatial and temporal variations in mercury contamination in recreational fisheries from southern New England estuarine and coastal waters

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Fish provide many dietary benefits to human consumers due to their high concentrations of proteins and omega-3 fatty acids. However, persistent contaminants in fish tissue can also adversely affect human health when eaten in high concentrations. For example, mercury (Hg) is a toxic environmental contaminant that bioaccumulates in fish tissue and bioconcentrates across successive trophic levels. To reduce Hg exposure, federal and state government agencies issue consumption advisories to minimize the potential health risks associated with eating fish. Fish consumption advisories, however, often lack species-specific detail, and rarely account for small-scale spatial and temporal variations in fish Hg contamination. This study examined Hg contamination in recreational fisheries of southern New England estuarine and coastal waters, with a focus on the effects of habitat (inshore and offshore waters) and time (months: May to October; years: 2006-2020) on intra-specific Hg bioaccumulation rates. Mean Hg concentrations differed across species and were highest in striped bass and bluefish (mean ± SD Hg = 1.41 ± 0.65 and 1.23 ± 0.61 ppm dry weight, respectively), followed by summer flounder and black sea bass (0.99 ± 0.45 and 0.99 ± 0.49 ppm, respectively), scup (0.78 ± 0.49 ppm), and winter flounder (0.26 ± 0.12 ppm). All target fish experienced Hg bioaccumulation, i.e., positive Hg-size relationship. Intra-specific Hg contamination varied by habitat with offshore fish consistently having lower Hg levels or slower accumulation rates relative to conspecifics from other locations. Summer flounder, scup, black sea bass, and bluefish had maximal Hg concentrations in June, July, and August, and Hg content also increased significantly over years in summer flounder, scup, and striped bass. The results from this study support more effective and efficacious state consumption advisories for marine fish.

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Rare elfin abundance correlates with host plant density

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It is understood that the rare frosted elfin butterfly (*Callophrys irus*) uses small yellow indigo (*Baptisia tinctoria*) as one of its host plants. However, it is unclear how host plant abundance, density, and nutrition can affect the frosted elfin population abundance and population trends. Using indigo leaf samples and butterfly survey data we examined the potential effect indigo has on the total adult elfin count in various host plant patches at one field site, Gavins Pond (Sharon/Foxboro, MA). Analysis was done by collecting leaf samples from five indigo plants in each of the 22 indigo patches at Gavins Pond. Samples were freeze-dried to preserve carbon and nitrogen levels for later elemental analysis. While the butterflies were flying, we did Pollard walks to estimate abundance at both the path and site-level. We found that indigo density significantly affects the adult elfin count. Patches were assigned host plant density as abundant, common, uncommon, or rare. “Abundant” patches had significantly more butterflies in comparison to “common” and “uncommon” patches. In the "common" and "uncommon" patches of indigo elfin numbers had means of less than two butterflies throughout the season while in the "abundant" patches the mean was around seven. Our results display that frosted elfins have a higher chance of being spotted in high density or abundant patches of indigo. The frosted elfin is listed as vulnerable due to habitat fragmentation. While nutritional analysis is ongoing, the current findings suggest that augmenting host plant density may help the frosted elfin population.

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Exploring indicator displacement assays for phosphate detection

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Indicator displacement assays are based on the optical signal modulation of a noncovalently bound indicator upon dissociation by an analyte species. Our interest is displacement assays for inorganic phosphate using complex ions containing two di(2-picolyl)amine (also called DPA or bis(2-pyridylmethyl)amine) ligands, each binding a metal cation. We have prepared three ligands by covalently attaching two DPA moieties 2,6-bis(chloromethyl) benzene, and 2,6-bis(chloromethyl)-4-methylphenol, and 1,2-phenylenedimethylamine. We are exploring absorption assays with Alizarin Red S, Eriochrome Black T, Murexide, and Pyrocatechol Violet as well as fluorescence assays with 6,7-dihydroxy-4-methylsulfonate coumarin in neutral pH buffers with and without salt. Our work with absorption assays has at present only involved Zn^{2+} - DPA complexes while our fluorescence assays have been extended to coordinated Cr^{3+}, Cu^{2+}, and Fe^{3+}ions. To date, our best limits of detection for absorption assays are approximately 100 ppb both with and without the presence of 0.1 M salt. Our best limits of detection for fluorescence assays are approximately 10 ppb in the presence of 0.1 M salt.

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Simulated environmental transformations of consumer plastics

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Plastic debris from industrial and consumer sources represents a large portion of marine pollution in the environment. After prolonged exposure to environmental forces, large plastics can continue to break down into microplastics and nanoplastics. While large plastic particles can be identified visually, smaller particles require further study through various microscopy methods. More research is needed on the impact of nanoplastics in a marine environment. Commercial microplastics available for lab usage are typically spherical in shape and do not have the irregularities found in microplastic pollution from a marine environment. In this study, we replicate the mechanical and photochemical degradation of consumer plastics- e.g. expanded polystyrene, polypropylene, and polyethylene terephthalate- in the lab setting at the University of Rhode Island. The plastics are first subjected to accelerated UV-Weathering, then further processed using a commercial immersion blender to simulate mechanical degradation via repeated wave action. After exposing the macroplastic sample to UV irradiation, physical changes such as embrittlement and discoloration are clearly visible. We characterize the representative particles using three different methods (Field-Emission Scanning Electron Microscope, Confocal Raman Microscope, and Dynamic Light Scattering Machine) and found nanosized polystyrene and polypropylene particles with irregular shapes and neutral surface charge. By creating representative microplastics with a variety of sizes and surface characteristics, we can further study the behavior and impact of nanoplastics in the environment.

This work was supported by RI C-AIM

Evolutionary background and thermal tolerance in wild caught *Drosophila* from elevational gradients across the eastern slope of the Colorado Rocky Mountains

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The ability to cope with substantial fluctuations in environment variables is a major determinant of ability to persist in the face of climate change. Adaptation to some local environments may select for increased ability to persist under fluctuations in temperature due to climate change. Habitats with extreme temperature variation, both annually and over shorter periods, may select to enhance or preserve mechanisms associated with thermal stress, while those with little variation may not. Here we present updates on our study investigating the role of evolutionary background on thermal tolerance in wild caught *Drosophila* species from elevational gradients in the eastern slope of the Rocky Mountains in Colorado.

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Changes of N-linked glycans expressed on the cell surface of *Oxyrrhis marina* during resource deprivation

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Dinoflagellates are single celled organisms that are essential in sustaining a healthy marine environment. The marine dinoflagellate, *Oxyrrhis marina*, are significant members of oceanic plankton that cycle organic matter and serve as an energy source for higher level consumers. Their biomass and cellular composition play an important role in the efficiency of this energy transfer. Carbohydrates attached to larger macromolecules like lipids and proteins found on the cell surface of eukaryotic cells are referred to as glycans and serve many biological functions including cell-to-cell recognition and communication. Surface glycans on marine dinoflagellates have been found to play a specific role in recognition and communication with marine coral through symbiotic relationships that are vital to the health of coral reef systems and an overall prosperous marine environment.

The purpose of this study is to investigate the changes in N-linked glycans expressed on the surface of *O. marina* after active feeding, and prolonged starvation. N-glycans were released from the cell surface by PNGaseF digestion, and labeled with 2-aminobenzamide (2-AB) via reductive amination prior to analysis by mass spectrometry. When comparing the N-linked glycan profiles of actively fed and starved *O.marina*, the data suggested a shift from predominately hybrid glycans to more complex structures along with an increase in sulfation and sialylation and a reduction of fucosylation as starvation progressed to 21-days. Little is known regarding the function of these changing glycan trends in dinoflagellates, however, increased sulfation and overall negative charge on human cells are often associated with biological abnormalities such as cancer. We suspect these changes in *O.marina* may play a similar role in communicating with other cells that something is wrong. Upon further investigation of dinoflagellate surface carbohydrates, analysis of N-glycans could potentially serve as a biomarker for assessing the health of a marine ecosystem.

This work was supported by RI C-AIM

Characterization of bacteriophage diversity in Narragansett Bay, RI during a winter-spring algal bloom

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Bacteriophages (viruses that infect bacteria) play important roles in regulating community dynamics of ecologically important bacteria in marine ecosystems. However, little is known about the dynamics of bacteriophage communities in Narragansett Bay (NBay), RI. Previous analysis of NBay bacterial communities during a historically large winter-spring phytoplankton bloom in 2018 revealed decreased bacterial community diversity in response to the bloom, but little is known about the bacteriophage dynamics during the same time. In order to evaluate the structure of bacteriophage communities, VirFinder was applied to a shotgun metagenomic co-assembly from the 0.2 -5 µm fraction of surface waters during a winter-spring algal bloom in 2018. Of all of the assembled contigs, 22% (over 300,000) were predicted to be from bacteriophages. Across all 12 samples in the co-assembly, there was a mean bacteriophage relative abundance of 16.2 ± 2.1%. Similar to what was observed in bacterial communities, decreased alpha diversity, measured with Shannon and Simpson diversity indices, was observed during the bloom. Further work to profile the taxonomic identity and functional potential of these bacteriophages will help to reveal their significance to the ecologically and economically important NBay ecosystem.

This work was supported by RI C-AIM

Population genetics of the evolution of pollution resistance in the Atlantic killifish (*Fundulus heteroclitus*)

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Our research studies the population genetics of Atlantic Killifish (*Fundulus heteroclitus*) to comprehend more about their resistance to pollution and their ability to flourish in polluted environments such as New Bedford Harbor. This ability is known to be a result of rapid evolutionary change, and by looking for genetic variations present in killifish populations, we attempt to identify loci that could explain the mechanisms of this rapid evolution event. The genetic markers used are called SNPs or Single Nucleotide Polymorphisms, and represent a locus in the genome that has two possible sequences. To examine the SNPs, we collected 500+ fin clips from killifish present along the Rhode Island and Massachusetts coast at 11 different locations. The fin clips were turned into purified DNA so they could undergo genotyping using Fluidigm technology, which performs PCR on 96 different samples at 96 different SNP loci per plate, to complete a total of 64,512 PCR reactions over several plates. DNA barcoding was also used to confirm the identity of the killifish we collected at the various locations. Once all the samples were genotyped, we observed two patterns. The first being the expected pattern of genetic isolation by distance when all loci were examined as a group. The second pattern is that genotypes at some loci are consistent with selection at polluted locations around New Bedford Harbor. This comes together to allow us to develop new hypotheses about which loci may be involved in the rapid evolution that allowed for the Atlantic Killifish to survive in an unfavorable habitat.

This work was supported by RI C-AIM & Providence College

Real-time monitoring of Narragansett Bay utilizing remote sensing and sampling platforms

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Narragansett Bay is a very popular spot for locals and tourists alike to partake in recreational activities and to enjoy all the sea has to offer. The maintenance of a healthy bay is paramount in sustaining the region's economy and health as so much is dependent on the ocean here in Rhode Island, highlighting the importance of understanding the environmental processes and changes the bay experiences on a day to day basis. As a result, the goal of this project is to monitor the overall health of Narragansett Bay, providing a snapshot of the conditions of the Bay, and high-resolution real-time data for the prediction of events of interest such as harmful algal blooms (HAB), or environmental hypoxia, through the use of three remote data collection sites: the first being offshore of the Castle Hill lighthouse in Newport operating with an onshore pumphouse setup, one buoy off the west coast of Jamestown at the URI GSO Long Term Sampling (LTS) site, and a second buoy at the mouth of Greenwich Bay. These sites collect data including biological, chemical, and physical measurements, through a suite of on-board sensors. This data is collected autonomously and is publicly accessible in real time for anyone to view including researchers, Narragansett Bay stakeholders, fishermen, and anyone else who wishes to view and observe how the bay is changing over time. Additionally these buoys can support remote samplers, capable of collecting filtered and whole water samples upon command at a time of interest. The combination of plankton community composition data, from samples collected by remote samplers, and co-located measurements of the current water conditions of the Bay, will provide further insight into how these organisms, and ultimately the Bay, responds to ever changing environmental factors. An improved understanding of these biological responses to environmental events can be implemented into the prediction of events of interest which may occur in the bay ,including harmful algal blooms that can impact Rhode Island shellfisheries, and affect human health if infected seafood is consumed.

This work was supported by RI C-AIM

Work-related mistreatment in higher education: characteristics and consequences

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For decades now, researchers have studied the effect bullying has on employees in the private sector. The same, however, cannot be said about work-related mistreatment in higher education. Due to this lack of empirical research on this subject, many areas of abuse often studied in the private industries--and specifically the role race and position status play in leading to mistreatment--have rarely been studied by scholars. This paper explores the role that racial and enthic identities play in workplace bullying in higher education, and it discusses how this abuse affects the performance and the wellbeing of the victims.

This work was supported by RI C-AIM

Plastic pollution: Do microplastics pose a risk to the eastern oyster: *Crassostrea virginica*?

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Microplastic research is a new and limited field littered with challenges and roadblocks such as how to keep plastics suspended in the water column and contamination concerns. Over the course of the summer, we designed and tested a range of methodologies in order to determine the most effective way to conduct microplastic experiments on the Eastern oyster, *Crassostrea virginica*. Pilot trials included: 1) how to reliably dose microplastics to oysters, 2) how to run controlled upper lethal temperature trials as a tool to determine how microplastics impact thermal tolerance, and 3) algae clearing tests to determine whether microplastics impact abilities to effectively feed. These pilot trials are important to ensure that research can be done in a controlled manner and yield accurate data needed to address research hypotheses. These experiences have highlighted the importance of preparation for research, often not reported in scientific literature, and provided transferrable skill sets to be used in future scientific research work.

This work was supported by RI C-AIM

Investigating staining methods to identify neoplasia in hard clams, *Mercenaria mercenaria*

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Mercenaria mercenaria, (hard clam) is a marine bivalve whose population is being negatively affected by hemotic neoplasia (HN). HN is a contagious leukemic-like disease that is thought to be transmitted to naïve animals of the same species via filtration of water with contaminated cells. A common way to diagnose leukemia-like diseases is sample the hemolymph (blood) of an organism and examine a stained smear of the cells under light microscopy. Hemocytes (blood cell) of bivalves are fragile and are crushed in traditional cytological smears. This research project aimed to investigate different hemocyte preparation methods combined with various staining methods with the aim of developing effective staining methods and then cytologically describe these unusual cells.

HN infected animals are primarily causing disease in Wellfleet, MA. Clams were sampled from this location and hemolymph was sampled with a syringe and needle. Three methods have been used to evaluate the hemolymph from each animal: a Protocol-stained blood smear slide, a Poly-L-lysine coated Protocol-stained slide and a Poly-L-lysine coated Giemsa-stained slide. Because of minimal time, only two staining methods were able to be compared. Under light microscopy, the measurements and staining evaluations of the cells were taken to determine the size and descriptions of the normal hemocytes vs. HN cells. The Giemsa Stain provided a method that preserves cell structure better than the Protocol method. Our next steps are to collect more diseased animals and use the new cytological preparations with Giemsa stains to describe the HN cells and compare them to normal hemocytes.

This work was supported by RI C-AIM

Design of a smart underwater camera system utilizing visual odometry

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An underwater smart camera system was designed to fuse visual odometry and inertial measurements to provide an affordable navigation solution for underwater vehicles. The system consist of a Arducam 16MP wide angle camera and a miniature attitude heading reference system (AHRS) from Yostlab. A standalone version is created during the SURF program for developing algorithms before the AUV integration. On this standalone system, the sensor processing algorithm is implemented on a Raspberry Pi-4 single-board computer. The program could be easily adapted to other computers onboard the AUV. Our development leverages open-source software, ROS and OpenCV. This allows for onboard processing of images to provide useful data such as Feature Detection, Image-Stitching, and Optical Flow. The work will continue after the SURF program and the system will be integrated into Autonomous Underwater Vehicles that are available in the Smart Ocean Systems Laboratory.

This work was supported by RI C-AIM

Characterization of bacterial community composition and biofilm formation of marine bacteria from Narragansett Bay

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Biofilms are a community of microorganisms adhered to a surface and encased in a protective extracellular matrix composed mostly of exopolysaccharides. The formation of these multi-species communities on underwater sensors is known as biofouling and leads to inaccurate readings. One objective of this study was to identify strong biofilm formers among bacterial strains previously isolated from Narragansett Bay for subsequent use in *in vitro* biofouling studies. Bacteria contained in water samples collected from various locations around Narragansett Bay were recovered and cultured on low-nutrient media. The 55 isolates recovered in pure culture were subjected to biofilm and pellicle assays to determine their potential for forming multicellular communities. Select isolates belonging to the genera *Serratia*, *Pseudomonas*, and *Acinetobacter* were observed to form strong biofilms, while isolates belonging to the genera *Bacillus*, *Exiguobacterium*, and *Providencia* predominantly formed weak biofilms. This was observed in assays measuring biofilm formation in the wells of 96-well plates as well as on peg lids submerged in bacterial culture. These weak and strong biofilm producers were then cultured in the presence of polydimethylsiloxane (PDMS) coupons separately and in pairs to determine if their behavior on this material mimicked that seen on polystyrene and if there was synergy between isolates.

Given that standard plating techniques recovers approximately 0.01 to 0.1% of marine bacteria, an understanding of bacterial community composition and dynamics are not possible using this approach. To supplement the study of individual bacteria, total genomic DNA was extracted from seawater samples and subjected to metagenomic analysis using 16S rRNA amplification and Next Generation Sequencing. Metagenomic analyses showed a majority of microbes from each water sample belonged to the phylum Proteobacteria, which is consistent with the fact that 78.2% of the isolates recovered from these samples belonged to this group. In contrast, while isolates belonging to the Firmicutes phylum account for 16.4% of the bacteria recovered in pure culture, these represent less than 2% of the bacterial community at each location, further illustrating the limitations of bacterial culture. These data will be further analyzed to compare community composition at various sites and to identify microbes of interest for targeted recovery in pure culture.

This work was supported by RI C-AIM

Isolation, growth, and toxicity of *Pseudo-nitzschia* strains from the seasonal June bloom in Narragansett Bay

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Diatoms are unicellular algae that make up a significant portion of phytoplankton biomass at the base of marine food webs. Narragansett Bay, RI is home to several species of the diatom genus *Pseudo-nitzschia*, some of which produce the potent neurotoxin domoic acid (DA), which causes harmful algal blooms and can lead to amnesic shellfish poisoning through human consumption of affected shellfish. Although the presence of *Pseudo-nitzschia* in Narragansett Bay has been well established for over 50 years, levels of DA have only recently become a problem, with shellfish harvest closures in 2016 and 2017. Weekly DA samples from 2017 - present show recurring seasonal peaks in toxin in the fall and summer, although well below levels of concern for harvest closures. During summer 2021, we continued to monitor Pseudo-nitzschia cell counts and DA at the GSO Time Series and West Passage mouth (Whale Rock) sites. We observed the anticipated DA increase in June and used this opportunity to isolate two *Pseudo-nitzschia* species into laboratory monocultures in addition to a previous isolate from December 2020. We genetically identified these additional isolates as *P. pungens* and *P. multiseries* through Sanger sequencing. Growth curves of these monocultures were generated by measuring *in vivo* fluorescence and DA was measured (via LC-MS/MS with multiple reaction monitoring) during the mid-log and stationary growth phases. This data will aid in understanding the biological factors behind these harmful algal blooms by directing future experimentation and manipulation of these cultures. The implications of this work are important to the economic stability of Narragansett Bay shellfisheries and to public health through safe consumption of shellfish

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