RI SURF

12TH ANNUAL
RHODE ISLAND SUMMER UNDERGRADUATE RESEARCH FELLOWS CONFERENCE

Friday, July 26, 2019
8:00 AM

UNIVERSITY OF RHODE ISLAND

BEAUPRE CENTER FOR CHEMICAL & FORENSIC SCIENCES
AND
CENTER FOR BIOTECHNOLOGY & LIFE SCIENCES

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RHODE ISLAND INBRE
Biomedical Research Excellence

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RHODE ISLAND CONSORTIUM FOR
Coastal Ecology Assessment Innovation & Modeling

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12TH ANNUAL RHODE ISLAND SUMMER UNDERGRADUATE RESEARCH FELLOWS CONFERENCE

FRIDAY, July 26, 2019
BEUPRE CENTER FOR CHEMICAL & FORENSIC SCIENCES
AND CENTER FOR BIOTECHNOLOGY & LIFE SCIENCES
UNIVERSITY OF RHODE ISLAND
KINGSTON, RI

8:00 – 9:00 AM	**CHECK-IN, CONTINENTAL BREAKFAST AND POSTER SET-UP**
9:00 – 9:30 AM	**WELCOMING REMARKS**

- DONALD DEHAYES, PHD, PROVOST, UNIVERSITY OF RHODE ISLAND
- PETER SNYDER, PHD, VICE PRESIDENT FOR RESEARCH & ECONOMIC DEVELOPMENT, UNIVERSITY OF RHODE ISLAND
- STEFAN PRYOR, RHODE ISLAND STATE SECRETARY OF COMMERCE

9:30 – 11:00 AM	**POSTER SESSION A (ODD-NUMBERED POSTERS)**
11 AM - 12:30 PM	**POSTER SESSION B (EVEN-NUMBERED POSTERS)**

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EXHIBITORS

Located on the Lower Floor of the Beaupre Center

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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	Posters
A	9:30 – 11:00	Odd-numbered
B	11:00 – 12:30	Even-numbered

Research Theme	Location
Cell Biology (CB)	CBLS, Lower Atrium
Chemistry (CHEM)	Beaupre, 2nd Floor Lobby
Environmental Science (ES)	CBLS, 1st Floor, South Lobby
Marine Systems (MS)	CBLS, 1st Floor Hallway
Microbiology (MICRO)	Beaupre, Room 130
Molecular Biology (MB)	Beaupre, Rooms 135 & 140
Neuroscience (NEURO)	Beaupre , Room 120
Pharmaceutical Sciences (PHARM)	CBLS, 1st Floor, North Lobby

CELL BIOLOGY

CENTER FOR BIOTECHNOLOGY & LIFE SCIENCES
LOWER ATRIUM

ODD -NUMBERED POSTERS ARE TO BE PRESENTED FROM 9:30 – 11:00 AM
EVEN-NUMBERED POSTERS ARE TO B PRESENTED FROM 11:00 AM – 12:30 PM

Endoplasmic Reticulum Stress Induction of Yeast Bax Inhibitor (BXI1) Gene Expression Does Not Require IRE1

John Finn[1], Wesley Parker[2] & Nicanor Austriaco[1]

[1]Biology, Providence College, Providence, RI
[2]Biology, Brown University, Providence, RI

Bax Inhibitor (BI-1/ TMBIM6) is a regulator protein within the Unfolded Protein Response (UPR) that influences rates of apoptosis within cells. Studies have shown that BI-1 is upregulated in human cancer cells like prostate and breast, therefore it is important to study the factors that control BI-1 within a cell. In this paper, the *Saccharomyces cerevisiae* homolog of BI-1, BXI1, is studied to better understand the signaling pathway that influences concentrations of BXI1, specifically the Inositol Requiring Enzyme 1 (IRE1) pathway. IRE1 is an Endoplasmic Reticulum (ER) bound transmembrane ligand receptor which detects the UPR and activates downstream targets which play further roles in regaining homeostasis. Here, our lab used two genetically modified *S. cerevisiae* strains, both with Green Fluorescent Protein (GFP) bound to BXI1 (BXI1p-GFP) and one lacking genetic information to code for IRE1 (Δire1) to test the effect the IRE1 pathway has on the expression of BXI1. Using Tunicamycin, DDT, and Rapamycin, our lab increased the expression of BXI1, based off earlier INBRE funded research. Our preliminary data suggests that the expression of BXI1 is not dependent on the IRE1 pathway, suggesting that other signaling pathways influence the expression of Bax Inhibitor.

this work was supported by RI INBRE

Inhibition of Alpha-Synuclein as a Parkinson's Model by Metformin in *Saccharomyces cerevisiae*

Stephen Moss & Nicanor Austriaco

Biology, Providence College, Providence, RI

Parkinson's disease is a neurodegenerative disorder experienced as a result of protein aggregation in the nervous system, called Lewy bodies. These are made up of the proteins alpha-synuclein and Tau. We used *Saccharomyces cerevisiae* to model the effects of Metformin on the progression of alpha-synuclein aggregation. Metformin has possibly been shown to inhibit alpha-synuclein aggregation without interfering with the production of the protein.

this work was supported by RI INBRE

Mannitol Reduces α-Synuclein Aggregation in Yeast Modeling Parkinson's Disease

Alissa Pacheco & Nicanor Austriaco

Biology, Providence College, Providence, RI

Parkinson's Disease (PD) is an incurable, neurodegenerative disorder. This disorder is the second most common of its type and occurs by the accumulation of abnormal proteins called Lewy Bodies (LBs). LBs are found within the neurons of PD patients and trigger cell death. As a result of both damages and decreased neurons, dopamine levels decrease triggering common symptoms of the disease such as muscle stiffness, tremors, and slowed movements. Previous studies have revealed that the human protein alpha-synuclein is one of the major components of Lewy Bodies. In the effort to better understand the relationship between both protein aggregation and cell death, a mechanism that overexpresses human alpha-synuclein fused to GFP in the budding yeast Saccharomyces was derived. Our preliminary data suggests that the localization of the alpha-synuclein protein is found in the cell membrane. Based upon other data, mannitol may alleviate the aggregation of alpha-synuclein within the cell membrane of the yeast cells, suggesting a possible pharmacological intercession that may lower the amount of protein aggregation in PD.

this work was supported by RI INBRE

Modeling Parkinson's Disease in the Budding Yeast, *Saccharomyces cerevisiae*

Yuri Takenaka

Biology, Providence College, Providence, RI

Parkinson's Disease (PD) is the second most common, incurable neurodegenerative disorder worldwide. PD patients have accumulations of abnormal proteins called Lewy Bodies (LBs) within their neurons that trigger cell death. Damaged and dead neurons lead to decline in dopamine levels, triggering the symptoms of Parkinson's such as muscle stiffness, tremors, and slowed movement.

Numerous studies have shown that the human protein, alpha-synuclein and tau, are two major components of Lewy Bodies. To study the relationship between protein aggregation and cell death, I have created a system to co-overexpress human alpha-synuclein fused to GFP and human tau fused to mCherry in the budding yeast, Saccharomyces. As shown in the accompanying figure, my preliminary data suggests that low levels of alpha-synuclein-GFP appear to localize to the yeast cell membrane while tau-mCherry localizes to the vacuole. This summer, we would like to confirm this localization with vital dyes that localize to different organelles in the cell. We are also in the process of expressing high levels of both proteins in the cell.

Our preliminary data from the lab also suggest that the drugs mannitol and sulforaphane may alleviate the protein aggregation of alpha-synuclein in the yeast cell. In this proposal, I describe experiments to determine if these drugs would also alleviate the aggregation of both PD proteins when they are co-expressed in the same cells. Hopefully, these studies and experiments will lead to new treatments of PD.

this work was supported by Providence College

The Developmental Effects of PFOS on the Metabolic Maturation in *Drosophila melanogaster*

Rachael Aresco, Cailin McVey, Megan Johnstone, Cole Tindall & Belinda Barbagallo

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

Essential preconception factors including, maternal nutrition loading, plays an essential role in oocyte maturation and disruption of this factor specifically is associated with significant adverse health effects for offspring. One suspected disruptor of these preconception factors is the chemical, perfluorooctanesulfonic acid (PFOS). This industrial chemical has infiltrated the environment through drinking water, food packaging and cooking surfaces and poses as a major health exposure risk due to its 5 ½ year half-life in humans. While the mechanism is well understudied, it is understood that during preconception, PFCs may be deposited into the oocyte causing a significant reduction in nutrient loading and maturation leading to metabolic disease and type II diabetes in offspring. Therefore, we hypothesis, that preconception material exposure to Perfluorooctanesulfonate (PFOS) will alter nutrient loading in oocytes to produce offspring with altered tissue development, resulting in high levels of triglyceride, cholesterol, glucose, and fatty acid concentration as an indication of metabolic disease. To test this, virgin female *Drosophila melanogaster* were dosed with PFOS and mated with CS males. Offspring were collect at 9 hour, 5 days and 14 days into adulthood and run on four biochemical assays to observe, triglyceride, glucose, cholesterol and fatty acid concentrations at each time point. Results showed female flies dosed with PFOS produced offspring with shifts in tradition markets for metabolic disease including triglyceride, glucose, cholesterol concentrations. These results indicate that preconception exposure to PFOS is sufficient in showing an increase of markers in metabolic disease. Therefore, the data collected provides a mechanistic understanding of reproductive biology extended to preconception nutritional modulation by environmental containments and informs an adverse outcome pathway for metabolic syndrome. Future research aims to further study the mechanism and contribute to nutrition intervention targets for preconception counseling approaches.

this work was supported by the NIH

Preconception Exposure to PFOS Alters Insulin Peptide Expression Profiles in *Drosophila melanogaster*

Megan Johnstone, Cole Tindall, Rachael Aresco, Cailin McVey & Belinda Barbagallo

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

Perfluorooctanesulfonate (PFOS) is an industrial compound that has infiltrated water and air systems. Due to the long half-life, bioaccumulation potential, and current omnipresence in human serum, it is important to gain an understanding of the exact mechanism of PFOS exposure and its impact on human health. Preliminary work has shown that PFOS alters oocyte nutrient loading and tissue morphogenesis. We hypothesize that altered tissue morphogenesis will lead to long term health problems, including an increased prevalence of type 2 diabetes. Using *Drosophila melanogaster* as a model system, we tested whether maternal exposure to PFOS will result in progeny with altered insulin peptide expression levels reflecting profiles seen in insulin resistance models. Preliminary results using qPCR show upregulation of *Drosophila* insulin like peptides, suggesting that preconception exposure to PFOS is sufficient to induce altered insulin expression profiles in adult progeny. This study will provide insights into how exposure to environmental toxins are contributing to the increased prevalence of type 2 diabetes and further studies the aim to understand the contribution of diet to this effect.

this work was supported by the NIH

Developmental Effects of PFOS on the Metabolic Maturation in RNAi Megalin Knockdown *Drosophila melanogaster*

Cailin McVey, Rachael Aresco, Megan Johnstone, Cole Tindall & Belinda Barbagallo

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

Perfluorooctane Sulfonate (PFOS) is an industrial chemical that has infiltrated the environment. Found in drinking water, food, and cooking surfaces, PFOS can be deposited into the oocyte prior to conception and preliminary studies indicate that PFOS exposure alters oocyte nutrient content leading to altered tissue development. Nutrient loading occurs through receptor mediated endocytosis using the Multifunction Endocytosis Receptor Complex (MERC), which is made up of three receptor proteins: Megalin, Amnoinless, and Cubilin. We hypothesize that preconception exposure to PFOS alters oocyte nutrient loading that causes embryonic nutritional stress and disrupted tissue development leading to metabolic dysfunction at later life stages and that knockdown of MERC will enhance this effect. We used *Drosophila melanogaster* as a model system to knockdown MERC and investigate how maternal exposure to PFOS during oocyte maturation the prevalence of metabolic disease onset. This study will describe the interplay between environmental toxins and the onset of metabolic disorder, implicating the highly conserved MERC as a key mechanism in metabolic disease onset and provide a potential therapeutic target for future study.

this work was supported by the NIH

Preconception Exposure to PFOS alters development and morphology of *Drosophila melanogaster*

Cole Tindall, Cailin McVey, Rachael Aresco, Megan Johnstone & Belinda Barbagallo

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

Perfluorooctane Sulfonate (PFOS), a fluorinated organic compound that has been used in the manufacturing of a wide array of consumer products, has recently been shown to have adverse effects on human health. A large portion of the population has been exposed to PFOS and related chemicals, making it essential to understand the impact of PFOS exposure on physiology. Preliminary work has shown that PFOS is able to alter oocyte nutrient loading by disrupting receptor-mediated endocytosis. In this study, I hypothesize that preconception exposure to PFOS will result in developmental delays and changes in final adult morphology due to alterations in oocyte nutrient composition. Using the *Drosophila melanogaster* model system, virgin females were treated with PFOS and the developmental time course and final adult weights of their progeny were assessed. These experiments show that preconception exposure to PFOS is significant to delay development and that disruption of the MERC complex leads to further developmental delay. Additionally, progeny of PFOS exposed animals show an overall decrease in final adult size in otherwise wild type animals. These results provide a model for future studies of the mechanisms underlying developmental disorder in PFOS exposed animals which can be translated to the study of human PFOS exposures

this work was supported by the NIH

Investigating the Life Span and Motility of Neurodegenerative Models of *Caenorhabditis elegans*

Cassandra Faria, Alexa Larson & Christopher Burtner

Biology, Marine Biology & Environmental Science, Roger Williams University, Bristol, RI

As organisms age, they develop pathologies that lead to disease states, such as neurodegeneration, which is defined as the age-associated progressive deterioration of neuronal structures and functions leading to cognitive and motor dysfunction. Here, we report the life span of the soil nematode *Caenorhabditis elegans* under normal and dietary-restricted conditions. We also investigated the life span of genetically-modified nematodes expressing genes associated with neurodegeneration in humans, including Huntington's, Alzheimer's, and Parkinson's Disease. Furthermore, we quantified the effect of age on motility of *C. elegans* expressing these transgenic proteins. These investigations set the groundwork for exploring interventions which may delay the age-associated onset of neurodegeneration in a genetically-tractable model system.

this work was supported by RI INBRE

ZapE is a Novel Cell Division Associated ATPase in *Escherichia coli*

Rebecca Dickinson, Eric DiBiasio & Jodi Camberg

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

During early septal phase of cell division in bacteria, a large protein structure called the Z-ring assembles at the division site. The major protein that comprises the Z-ring is FtsZ, a tubulin homolog that hydrolyzes GTP and assembles into polymers. In *Escherichia coli*, many cell division proteins interact with FtsZ and direct Z-ring assembly, while others may modulate constriction or direct cell wall insertion and remodeling. Several accessory proteins that interact with FtsZ are called Z-ring associated proteins (Zaps). The Zaps (ZapA, ZapB, ZapC, ZapD, and ZapE) are recruited to the division site and are predicted to influence Z-ring assembly and/or stability. ZapE was recently identified to be an ATPase that accumulates during late constriction in *E. coli* and is important for bacterial growth under low-oxygen conditions and high temperatures (Marteyn, *et al.*, 2014). *In vitro*, ZapE destabilizes FtsZ polymers suggesting that it may promote Z-ring disassembly *in vivo* (Marteyn, *et al.*, 2014). To evaluate ZapE function and interactions we cloned ZapE with a N-terminal hexahistidine tag (H6-ZapE) into a high-copy inducible expression vector and purified via metal affinity chromatography. We determined that H6-ZapE hydrolyzes ATP with an average rate of 1.25 Pi/min/pmol. ZapE is predicted to contain an ATP binding pocket with putative Walker A (GGVGRGK84T) and Walker B nucleotide-binding sites. We performed site directed mutagenesis on the Walker A nucleotide-binding site at position K84. H6-ZapE(K84A) was defective for ATP hydrolysis. Since ZapE has been shown to interact with FtsZ we decided to further probe this interaction using a colorimetric assay detecting free phosphate released by H6-ZapE in the presence of ATP and FtsZ and later GTP and FtsZ. Under the conditions tested, H6-ZapE had no impact on ATP or GTP hydrolysis in the presence of FtsZ. We also tested the oligomeric status of H6-ZapE using size exclusion chromatography. Under these conditions, H6-ZapE eluted as a monomer with and without the presence of ATP. Overall, these experiments will help to further understand the role of ZapE during prokaryotic cell division.

this work was supported by RI INBRE

Determining the Relationship Between Swi/Snf and H3T11

Jacob Ullom, Kathleen Tran & Arnob Dutta

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

The Swi/Snf complex is a family of chromatin remodelers in mammals and yeast and mutations in subunits of the complex are associated with ~20% of all human cancers. Elevated aerobic glycolysis, termed the Warburg effect, is also a major contributor to tumor proliferation. Pyruvate kinase M2 (PKM2) plays a major role in the Warburg effect by not only catalyzing the conversion of phosphoenolpyruvate to pyruvate, but also inducing chromatin remodeling at MYC and CCND1 via the phosphorylation of histone H3T11. In yeast homolog of PKM2, Pyk1, and casein kinase 2 catalytic subunit Cka1 have been shown to phosphorylate H3T11. Interestingly, genes with high levels of H3T11 phosphorylation are also targets for regulation by SWI/SNF. Given the co-occupancy of H3T11p and SWI/SNF at the same genes, we wanted to ask if the phosphorylation of H3T11 regulated SWI/SNF occupancy using *in vitro* reconstitution biochemistry. We found that increase in H3T11 phosphorylation negatively impacted *in vitro* remodeling of nucleosomes by SWI/SNF. Our results are a first to show the impact of H3T11 phosphorylation on regulation of a chromatin remodeler complex.

this work was supported by RI INBRE

Mixexpression of Alx4a and Alx4b in the Zebrafish, *Danio rerio*

Samuel Restrepo

Biology, Rhode Island College, Providence, RI

In vertebrates, neural crest cells originate in the dorsal neural tube and produce a vast array of cell types including glia, neurons, smooth muscle and pigment cells. In the zebrafish, *Danio rerio*, the neural crest generates three pigment cell types: Black melanophores, iridescent iridophores and yellow xanthophores. Previous studies suggest that some iridophores and melanophores share a bipotent precursor and that an unknown factor is required to promote iridophore fate. Aristaless-like homeobox proteins (ALX) are known to play roles in neural crest migration, craniofacial development and tumor suppression. Recent work showed that alx4a and alx4b are highly expressed in committed iridophore precursors but not in melanocyte precursors. These results led us to hypothesize that alx4a and/or alx4b act to inhibit melanophore fate and promote iridophore fate in a bipotent precursor. Previously, we used CRISPR/Cas9 to knockout alx4a and alx4b and observed fewer iridophores and more melanophores in F0 injected zebrafish. To further test the roles of alx4a and alx4b in pigment cell fate, we designed and constructed six plasmids to drive the expression of alx4a or alx4b in neural crest cells. Plasmids were injected into WT zebrafish embryos at the 1-cell stage and embryos were later examined for fluorescent reporters and alterations in pigment cell development. Identifying and understanding the genes required for iridophore fate specification may provide insight into mechanisms of cell fate specification and differentiation of other neural crest cell types.

this work was supported by RI INBRE

The Diffusion Patterns and Effect Carbon Nanotubes Have on Tumor Cells

Julie Phin, Mohammadmoein Safaee & Daniel Roxbury

Chemical Engineering, University of Rhode Island, Kingston, RI

Due to the stable and fluorescent nature single-walled carbon nanotubes have (SWCNTs), they are a rather reliable source for usage in bio-sensing and imaging applications. During the course of this experiment, cancer cells were grown into tumor cells. These tumor cells were later exposed to two different types of SWCNT: a DNA-SWCNT and a PEG-SWCNT. The manners in which the tumor cells were introduced to these SWCNTs also varied. The first was by adding the SWCNT into the same well as the tumor cell and monitoring the diffusion patterns overtime while the second was allowing the SWCNT to grow alongside the cells as the tumor was forming. A hyperspectral microscope captured the images over a period of 24 hour in order to observe the SWCNTs and how they interact with these cells.

this work was supported by RI INBRE

Visualizing Soluble and Insoluble SOD1 Protein in a *Drosophila melanogaster* Model of Amyotrophic Lateral Sclerosis

Helen Magana & Geoff Stilwell

Biology, Rhode Island College, Providence, RI

Amyotrophic lateral sclerosis (ALS) is an adult-onset, neurodegenerative disease characterized by loss of motor neuron function. Superoxide dismutase 1 (SOD1) is a ubiquitously expressed free radical scavenger and over 170-point mutations in the sod1 gene cause familial ALS. All ALS-associated sod1 mutations produce misfolding protein leading to the formation of protein aggregates. More broadly, SOD1 aggregates found in patients and model organisms are a hallmark molecular feature of ALS. Here, we present methods to visualize the wildtype and aggregated conformations of SOD1 in a *Drosophila melanogaster* model of ALS using mono-specific polyclonal antibodies. Immunocytochemistry (ICC) techniques were used to evaluate aggregation in wildtype, sod1G85R, and sod1H48R homozygotes. Staining in salivary glands was used as an initial model tissue because the cells are large and aggregates can be readily discerned. Our preliminary results suggest that aggregate-specific antibodies (56-71 and 111-126) detect cytoplasmic aggregates in sod1H48R homozygotes. In a *Drosophila melanogaster* model of ALS, the characterization of molecular behaviors of mutant alleles such as sod1G85R and sod1H48R facilitates our understanding of disease pathogenesis in human models.

this work was supported by RI INBRE

Characterization of Enhancers in a *Drosophila melanogaster* ALS Model

Raquel Villot & Geoff Stilwell

Biology, Rhode Island College, Providence, RI

Amyotrophic Lateral Sclerosis (ALS) is an adult onset, neurodegenerative disease characterized by a loss of motor neurons. Mutations in the superoxide dismutase (sod1) gene cause ALS, and we have previously created and characterized *Drosophila* models in which mutant ALS-causing sod alleles were inserted into the genome through homologous recombination. In flies, the most severe mutation is SODG85R, which causes recessive adult lethality because the molecular pathways leading to neurodegeneration are not understood. We conducted an enhancer screen to identify genes that produced early lethality in SODG85R heterozygotes. RNA interference (RNAi) was used to knock down gene expression throughout development and lethality was assessed. From this screen, we identified 14 enhancers that produced pupal lethality. Our current work further characterizes enhancer phenotypes in adults. Heat-shocked induced knock-down of selected enhancers produced selectively shortened lifespans in SODG85R heterozygotes. Possible molecular mechanisms by which Ebp1 pathways influence mutant SOD1 toxicity will be presented.

this work was supported by RI INBRE

Characterization of SOD1 Staining Patterns in a Humanized *Drosophila* Model of Amyotrophic Lateral Sclerosis

Anastasia Welch & Geoff Stilwell

Biology, Rhode Island College, Providence, RI

Amyotrophic Lateral Sclerosis (ALS) is a neurodegenerative disease that causes degeneration of motor neurons leading to muscle weakness and death 3-5 years after onset. Inherited forms of ALS are caused by missense mutations within the superoxide dismutase 1 (sod1) gene. More than 170 pathogenic mutations in sod1 cause ALS, and all mutant forms produce misfolded protein leading to cytoplasmic aggregates, the presumptive toxic species. To develop a better model of ALS, *Drosophila* expressing human sod1 (hsod1) were created through a gene replacement strategy. This technique generated a humanized model of ALS. We monitored cellular phenotypes in flies expressing hsod1 in wildtype and mutant forms. Using immunocytochemistry, aggregation potential of mutant hsod1 alleles were evaluated in larval salivary glands. Our results indicate that hsod1+ shows a similar pattern to wild type *Drosophila*. Patterns of expression and aggregate potential in mutant hsod1 will be presented.

this work was supported by RI INBRE

CHEMISTRY

BEAUPRE CENTER FOR CHEMICAL & FORENSIC SCIENCES
2ND FLOOR LOBBY

ODD -NUMBERED POSTERS ARE TO BE PRESENTED FROM 9:30 – 11:00 AM
EVEN-NUMBERED POSTERS ARE TO BE PRESENTED FROM 11:00 AM – 12:30 PM

Screening the Youngken Medicinal Plant Garden Extract Library for Antibacterial Activity Against Methicillin Resistant *Staphylococcus aureus* (MRSA) and *E. coli*

Marina Carro[1], Riley Kirk[2], Elizabeth Leibovitz[3], Margaret Rosario[2], David Rowley[2] & Matthew Bertin[2]

[1]Cell & Molecular Biology, University of Rhode Island, Kingston, RI
[2]Biomedical & Pharmaceutical Sciences, University of Rhode Island, Kingston, RI
[3]College of Pharmacy, University of Rhode Island, Kingston, RI

The development of new therapeutic lead molecules depends on access to new molecular diversity. The University of Rhode Island's Youngken Medicinal Plant Garden is a collection of over 200 medicinal plants that may contain previously overlooked therapeutic compounds. Collections from various plants in the Youngken Medicinal Plant Garden were dried and extracted. Aerial and root sections of plants were extracted separately in both water and methanol with the goal of replicating traditional teas and tinctures. The extracts, after being filtered and dried in micronic vials, were stored at -4°C constituting an extract library. Extracts were analyzed via high pressure liquid chromatography (HPLC) and mass spectrometry to determine complexity and composition. The extracts were also screened for antibacterial activity using *Escherichia coli* and methicillin-resistant *Staphylococcus aureus* (MRSA). Our results identified extracts with positive results, which will be subjected to continued investigation to isolate and characterize pure active components.

this work was supported by RI INBRE

Lab-to-Field: Nanostructured Sensors for Monitoring Pollutants in Coastal Ecosystems

Tanner Wildfong, Timo Kuester & Geoffrey Bothun

Chemical Engineering, University of Rhode Island, Kingston, RI

Excessive presence of nitrate and phosphate in ocean water can lead to large algae blooms and eutrophication – or dense growth of plant life which causes the death of animal life due to lack of oxygen – which can in extreme cases destroy coastal habitats. By detecting these harmful pollutants, the coastal community is afforded the opportunity to respond effectively to the threat at hand and protect aquatic ecosystems. Surface Enhanced Raman Spectroscopy (SERS) can be used to magnify what would otherwise be weak Raman signals. SERS works by using the nanostructured surface of a substrate to intensify the electromagnetic field. By using SERS, it is possible to more precisely detect otherwise difficult to read concentrations of nitrate and phosphate. More conventional procedures include Colorimetric sensing methods or UV-VIS Spectroscopy, which may be more labor-intensive processes. By using known concentrations of nitrate and phosphate we will record the SERS data for several substrates and establish a relationship between signal intensity and concentration of analyte. These results will demonstrate that it is both feasible and realistic to develop a microfluidic sensor capable of continuous detection in a coastal ecosystem. The application of such a device would be critical for ensuring the health of shoreside habitats by allowing timely and accurate pollutant measurements with relative ease. Therefore, the goal of our project is to establish a suitable substrate to detect pollutants within a microfluidic device capable of continuous readings in an *in situ* environment.

this work was supported by RI C-AIM

Towards the Development of a Low-Cost and Easily-Deployable Sensing Platforms for Phosphate and Nitrate

Maureen Pontarelli, Thomas Koch, Jared DiBella, Emma Fink & John Breen

Chemistry & Biochemistry, Providence College, Providence, RI

We will present results from our experiments leading to the development of a low-cost and easily-deployable sensing platforms for phosphate ($H_2PO_4^-$ or HPO_4^{2-}) and nitrate (NO_3^-) based on fluorescence methods. The phosphate sensor is based on carboxylic acid functionalized carbon nanodots complexed with europium (III) ions.(1) Upon excitation at 370 nm, the native carbon nanodot fluorescence at 450 – 560 nm and quenched by the complexation with the europium (III) ions is restored upon interaction with phosphate. The nitrate sensor is based on a potential sensitive lipophilic dye, diA [4-(4-dihexadecylaminostyryl)-N-methylpyridinium iodide], that is incorporated with a nitrate ionophore and a plasticizer in a PVC membrane.(2) The diffusion of nitrate into the membrane leads to formation of an ion pair with the cationic diA that partitions into micro-droplets of the plasticizer resulting in an increase in diA's fluorescence at 590 nm following excitation at 460 nm. At present our LOD for phosphate at near neutral pH is approximately 1 PPM and we will detail our attempts to incorporate the europium adjusted carbon nanodots in a suitable matrix for a dip-stick like sensor. On the nitrate project we are working to improve reproducibility in the preparation of mounted membrane sensing elements and improve the sensitivity through optimization of the composition of the membrane cocktail.

(1) H X. Zhao, L. Q. Liu, Z. D. Liu, Y. Wang, X. J. Zhao, and C. Z. Huang. Chem Comm. 47, 2604-2606, 2011.
(2) G. Kim, K. A. Sudduth, S. A. Grant and N. R. Kitchen. J. Biosystems Eng, 37, 209-213, 2012.

this work was supported by RI C-AIM

Structural, Electrochemical, and Catalytic Properties of Zinc and Iron Complexes of Redox Active α–Diimine Ligands

Alexandra Chaparro, Leah McCarthy, Erin Ostrowski & Maria Carroll

Chemistry & Biochemistry, Providence College, Providence, RI

The purpose of our project is to synthesize multiple α –diimine ligands that have the potential to be redox active in transition metal complexes. We propose that iron complexes of these various ligands can be tuned electronically to be selective for the reduction of either protons or carbon dioxide. Specifically, complexes in which reduction occurs at the ligand will be more prone to bind carbon dioxide, whereas, complexes in which the iron center is reduced will favor reaction with protons. In order to analyze each reduction potentials of the various ligands, we synthesized zinc complexes of the general formula Zn(α –diimine)Cl_2 because redox activity will most likely occur at the ligand, as zinc will remain in the 2+ oxidation state. The zinc complexes were synthesized and characterized by 1H NMR spectroscopy and X-ray crystallography, and we determined the reduction potentials of the complexes using cyclic voltammetry. Based on these reduction potentials, we synthesized iron carbonyl complexes of the form Fe(α –diimine)$(CO)_3$, in which the α –diimine ligands have a range of reduction potentials. We will present the characterization of these complexes by NMR and IR spectroscopies, as well as X-ray crystallography. Additionally, we explored the electrochemical properties of the iron carbonyl complexes and their reactivity with acid and carbon dioxide.

this work was supported by the Walsh Summer Research program at Providence College, the Dreyfus Foundation, and the Providence College Committee to Aid Faculty Research

Synthesis and Functionalization of Pillar[5]Arenes

Shealyn Davey[1], Lauren Seveney[2] & Brenton DeBoef[2]

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Easily synthesized water-soluble pillar[5]arenes are a potential host for use in HyperCEST 129Xe NMR imaging. This imaging platform has a multitude of applications in the medical field. Various pillar[5]arenes were successfully synthesized and functionalized with cationic and anionic moieties. Through the use of our molecular hosts, along with the incorporation of affinity tags, the goal is to image xenon atoms encapsulated in the hydrophobic cavity of the host within a whole animal. The affinity tag, as the name implies, will bind to a disease biomarker. These hosts, tags, and xenon guest will be able to selectively diagnose and image diseases through a revolutionary non-invasive method of MRI imaging.

this work was supported by RI INBRE

Mutating Residues in KmtR from *Mycobacterium tuberculosis*

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Mycobacterium tuberculosis is the bacteria that is 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 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, the amino acids E101 and H102 were mutated to obtain E101Q and H102A. The mutant protein will be expressed and purified. Metal binding experiments will be done to determine the respective affinities.

this work was supported by RI INBRE

Assessing the Effects of His88 on Metal Binding in KmtR

Brianna Mayoka

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Killing 1.6 million people each year, the disease tuberculosis caused by the bacterium *Mycobacterium tuberculosis* (Mtb), remains a public health crisis. New strategies must be developed due to the increasing drug resistance of this bacterium. The metal transport systems involved in Mtb are important for its pathogenicity. KmtR is a metalloregulator identified in Mtb involved with the export of nickel and cobalt in the cell. Our goal is to identify the residues in KmtR involved in binding nickel, cobalt, and zinc to the protein. In a previous study performed by Campbell *et al.* KmtR was shown to contain six residues that affect the expression of the export protein when mutated. This study aims to look the His88 residue and its ability to bind metals.

this work was supported by RI INBRE

Exploring Metal Binding to the Metallosensor, KmtR Through the H111Q and H102Q Mutations

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Mycobacterium tuberculosis (*M. tuberculosis*), the causative agent of tuberculosis, infects one in four people on Earth and was involved in the deaths of 1.3 million people in 2017. The percentage of drug resistant *M. tuberculosis* cases has not decreased in the last 20 years, creating a need for new ways to combat the bacteria. In order to survive in its host, *M. tuberculosis* must efficiently regulate metal concentrations. In *M. tuberculosis*, the concentrations of Ni(II) and Co(II) are regulated in the cell by the transcriptional regulators NmtR and KmtR. This research focuses on KmtR, which controls the expression of the gene, cdf, encoding an efflux protein. This research aims to understand how KmtR, binds to both the cognate metals, Ni(II) and Co(II), and the noncognate metal, Zn(II). Six residues on KmtR including His102 and His111 have been identified in previous studies to be important for sensing Ni(II) and Co(II). In this study, the His102 and His111 residues were mutated in KmtR and the effects on metal binding are being assessed.

this work was supported by RI INBRE

Functionalizable and Degradable Multi-Use Polymers: From Sustainable Packaging to Disease Treatment

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Plastics (polymers) are an indispensable convenience of modern life that have transformed fields such as food storage, medicine, packing materials, electronics, agriculture and construction. A simultaneous advantage and disadvantage of plastics is that they are extremely durable and are designed to last for hundreds or even thousands of years. Plastics pose a threat to our environment, entering the ocean at alarming rates and causing considerable damage to points of entry, like Narragansett Bay. Recycling rates are very low, and conventional recycling technology often results in downgraded material whose journey to the landfill is only delayed. This research aims to develop alternatives to existing plastics that are biodegradable under aqueous conditions. In addition to addressing environmental concerns, polymers that are susceptible to hydrolysis are ideally suited for drug delivery applications. Polymers for these applications would feature the ability to target a site such as cancer cells, deliver a therapeutic agent upon hydrolysis of the polymer, and mitigate the shortcomings associated with existing polymeric drug delivery systems including poor solubility, off-target effects and (multi)drug resistance.

Our approach involved the development of modular synthetic routes to polycarbonates and polyesters. Initial synthetic steps toward functionalized cyclic carbonate and cyclic ester monomers were screened to determine optimal conditions and maximize conversion to product.

this work was supported by RI C-AIM & RI INBRE

Synthesis of Thiocarbamate and Thiourea Based Catalysts for ROP

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Polymeric materials have become increasingly significant over the past few decades, and a substantial number of consumer products and biomedical devices are created using polymers. Significant research has been conducted in the polymerization of lactones through ring-opening polymerization (ROP), as they can have comparable materials properties to commercial polymers. Catalysis, and specifically catalyst development, is the enabling science of polymer synthesis. Traditional metal-containing catalysts, if left in the polymer, may leach out into the environment and contribute to metal contamination. Organocatalysts, on the other hand, do not contain metals, and they can be used to catalyze these polymerizations. This study focuses on the synthesis of a new class of thiocarbamate and thiourea-based catalysts for the ROP of lactones, especially lactide.

this work was supported by RI C-AIM

Paper-Based Devices for Phosphate Detection in Real-World Environments

Alexander Olivelli, Joan Racicot, Teresa Mako & Mindy Levine

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High concentrations of certain nutrients such as phosphate lead to undesired algal growth and can be detrimental to organisms in marine ecosystems. The rapid and robust detection of these nutrients using a colorimetric, paper-based system that can be applied on-site is of high interest to individuals monitoring marine environments. The molybdenum blue method is a well-established method for detecting phosphate that involves the formation of molybdophosphoric acid from ortho-phosphate and an excess of molybdate in acidic solution followed by a reduction to give a molybdenum blue complex. Phosphate detection systems that use the molybdenum blue method have already been developed, but most of them suffer from high detection limits, reagent instability and require the user to handle highly toxic reagents. For these reasons, the development of a new and improved detection system is necessary. Reported herein is the optimization of a paper-based, colorimetric detection system for phosphate with improved stabilization of the molybdenum blue reagent. The colorimetric response was analyzed and quantified using RGB analyses (ImageJ), allowing for more precise analysis than naked-eye permits. The device was further implemented to test a broad variety of water samples at locations throughout Rhode Island, to validate the device performance and obtain important information about the ecosystem health.

this work was supported by RI C-AIM

Colorimetric Detection Method for Nitrate and Phosphate Using Squaraine Macrocycle

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There are a number of nutrients in marine environments, including nitrate and phosphate, that are harmful to environmental health when in significant concentrations, and thus need to be monitored and controlled in order to promote ecosystem wellbeing. Commercially available methods to detect nitrate and phosphate exist, yet can be tedious to use and/or suffer from other serious drawbacks, including low sensitivities and selectivities, the use of large sample volumes, and the lack of rigorous quantitation. The rapid and robust detection of these nutrients using a colorimetric system that can be applied on-site with fully quantitative detection schemes is of high interest to individuals monitoring marine environments. To address this need, we are developing new colorimetric and fluorometric methods for detection that will be highly sensitive, selective toward only one analyte of interest, and paired with cell phone applications for quantitative analysis, with an end goal of integrating these systems into highly portable and used friendly paper-based microfluidic devices. Reported herein is the development of new colorimetric and fluorometric methods for the detection of these nutrients using a fluorescent blue dye, 2,4-bis[4-(N,N-dihydroxyethylamino)-phenyl] squaraine and a new squaraine-based macrocycle. The addition of certain ions to these highly fluorescent and highly colored species caused near-full quenching of emission, leading to no discernable fluorescent or colorimetric readout. The addition of other ions that sequester the quenching ions leads to a restoration of both fluorescence and color of these species, leading to a discernable and easily quantified change in signal. Anions of interest, including nitrate and phosphate were explored using this “turn on/off” system and can be further integrated into paper-based microfluidic devices and applied towards detecting these nutrients in marine environments.

this work was supported by RI C-AIM

Textile Strain Sensor Utilizing Electrospun Polyvinylidene Fluoride

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Electronic textiles (e-textiles) combine the functionality of electronics with the comfort of fabrics, allowing for technology to be embedded into everyday clothing. E-textiles serve applications far and wide, from health monitors to controls for virtual reality. This technology overcomes the limitations of traditional wearable electronic devices such as heart rate monitors, as conventional electronic components are stiff and can be uncomfortable during physical activities. The goal of this research is to develop truly wearable e-textiles by fabricating thread that is capable of sensing stress and pressure through the physical and chemical properties of the material.

The thread must be capable of fine grain motor monitoring such that it can accurately sense small variations in strain. Along with being sensitive it must also portray a large linear response range with minimal hysteresis. Accomplishing this will require a three layer thread. The thread is to consist of a non-conductive fiber coated in piezoelectric polymer nanofibers and silver nanowires. The base fiber provides the conventional fiber properties (flexibility, stretchability, washability etc.) needed in order to incorporate this thread into textiles. The piezoelectric polymer enables the thread to convert mechanical stress into an electrical signal. Finally, the silver nanowire coating makes the thread conductive, and adds antibacterial properties to the final textile.

The polymer used is Polyvinylidene fluoride (PVDF), which has excellent piezoelectric properties. However, in order to harness the piezoelectricity of PVDF, the polymer must crystallize in a specific chain conformation. While the alpha-phase of PVDF is the lowest energy conformation, it exhibits poor piezoelectricity; the beta-phase, however, is the most prominent piezoelectric polymer in literature. In order to obtain beta-phase PVDF, electrospinning is employed. Electrospinning exposes a liquid polymer solution to a high voltage electric field, which charges the solution and draws out a nanofiber of polymer. Electrospinning of PVDF is the most common processing technique used in order to increase it’s beta-phase content.

This three layer method shows promising results for the fabrication of a conductive thread strain sensor. With further refinement of the electrospinning process, this technique can produce entirely textile strain sensors with incredible

this work was supported by RI INBRE

Inhibition of Quorum Sensing by a New Panel of Beta-Keto Ester Compounds

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Antibiotic resistant bacteria is an epidemic that continues to grow throughout the world with around two million infections yearly. Traditional medical treatments focus on killing or inhibiting the growth of bacteria, which promotes antibiotic resistance. An alternative to these traditional methods is the inhibition of quorum sensing (QS), a process known to be the cell to cell communication mechanism among bacteria. Quorum sensing capable bacteria produce auto-inducer molecules which chemically signal to other bacteria to express genes for biofilm formation, virulence factor production, and swarming. Our laboratory has previously demonstrated that beta-keto esters are a new chemo-type capable of inhibiting quorum sensing. This research focuses on synthesizing additional beta-keto ester derivatives and testing their ability to inhibit the production of luminescence, a quorum sensing regulated phenotype, by *Vibrio harveyi*.

this work was supported by RI INBRE

Optimization of the Crystal Violet Assay as a Method to Measure Inhibition of Biofilm Formation in *Pseudomonas aeruginosa*

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Quorum sensing is a cell-cell communication mechanism by which bacteria communicate via releasing and accepting autoinducers. It has been established that quorum sensing plays a role in the production of biofilm in many pathogenic bacteria. Biofilm is created when planktonic bacteria adhere to a surface and produce an extracellular polymeric substance after accepting the autoinducer. It is difficult to treat pathogenic biofilm with antibiotics, because they do not readily pass through the extracellular substance produced by biofilm, thus rendering the infection resistant to treatment. The overarching goal of our laboratory is the inhibition of the quorum sensing mechanism that leads to the production of biofilm. The crystal violet assay is commonly used for the quantification of biofilm, but the repeatability of this assay is often low. The focus of this project was to determine the optimal conditions for conducting this assay using the opportunistic human pathogen *Pseudomonas aeruginosa*. Coumarin, a known quorum sensing inhibitor, was used as a positive control. Once established, the assay will be used to test a variety of compounds synthesized in our lab that have demonstrated the ability to inhibit quorum sensing in several different bioassays.

this work was supported by RI INBRE

Synthesis of Alpha- and Beta-Carbolines via Transition Metal Catalysis

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Our research focuses on developing new methodology in synthesizing nitrogen-containing heterocycles. The first project centers on synthesizing annulated α-carbolines using two different schemes for the addition of the substituent and cyclization of the cyanamide precursor. The one-pot process uses a palladium (0) catalyst, which through two mechanistically unique catalytic cycles, allows for addition and cyclization to occur in the same reaction vessel under the same conditions. The stepwise process first utilizes a palladium (0) catalyst for Sonogashira coupling of the substrate to the cyanamide precursor, which is isolated, then a rhodium (I) catalyst is used to perform a [2+2+2] cyclization in different reaction conditions. Eleven different annulated α-carboline derivatives have been successfully synthesized using both synthetic pathways, with varying yields. By comparing the two synthetic schemes, we can determine which process is more efficient to synthesize the desired annulated α-carbolines for further analysis. The second project is centralized on developing new ways to synthesize beta-carboline atropisomers that can be separated into their enantiomers. The current pathway involves two main substrates in a 6-7 step sequence. This research is focused on a successful synthesis that will allow the initial substrates to be successfully coupled. The product from this coupling would then be cyclized under conditions that involve a rhodium (I) catalyst to perform a [2+2+2] cyclization. Currently, two pathways to synthesize these annulated pyrido(3-4-b) compounds are in trial.

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Highly Sensitive and Selective Amperometric Nitrite Sensing Based on Multi-Wall Carbon Nantotube Screen Printed Electrode

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Nitrite salts are widely used in industrial manufacturing and overfertilization can lead to the contamination of surface water and groundwater. For example, nitrites can cause the transformation of normal hemoglobin to methemoglobin, leading to loss of hemoglobin's ability to transport oxygen and can also lead to accelerated algae growth and eutrophication of large bodies of water. There is an unmet need for portable, reliable and economical sensor for nitrites due to its ubiquitous nature and toxicity. Herein, we demonstrate the use of multi-wall carbon nanotubes screen printed electrodes (MWCNT-SPE) that have the capability for individual determination of nitrite anions at micromolar concentrations in aqueous solutions. MWCNT-SPE electrodes were used to determine the concentration of nitrite at low micromolar concentrations using amperometry in a microfluidic system. Analytical figures of merit were optimized including applied voltage, pH and flow rate to provide the best conditions for nitrite detection. Results gave a detection limit of 0.02 uM nitrite and a wide linear range from 0.02 to 400 µM at mild pH= 4.00 condition and an applied potential of 0.65V versus Ag/AgCl. The disposable MWCNT-SPE offers a low cost, portable and economic approach for nitrite detection in sea water samples.

this work was supported by RI C-AIM

Microfluidic Based Immunosensor Array for Simultaneous Electrochemical Measurements of 2 Cancer Biomarker Proteins in Serum Samples

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Multiplex electrochemical detection of cancer biomarkers is a major challenge, however, when realized it will lead to personalized approaches to early cancer detection, disease monitoring and patients' response to therapy. Interleukin 17A (IL-17A) and interleukin 17F (IL-17F) cancer biomarkers are found in high levels in patients with Cutaneous T cell lymphoma (CTCL) and can be measured as a means to detect cancer. Herein, we report on a microfluidic Immunosensor array fabricated using functionalized gold nanoparticles on printed carbon electrodes for simultaneous electrochemical detection of cancer biomarker proteins, IL-17A, and IL-17F in serum samples. The sandwich Immunosensor features capture antibodies, anti-human IL-17A and anti-human IL-17F that were attached on to the electrode arrays, followed by a flow of offline captured antigens on to a polyethylene glycol (PEG) modified HRP multi-enzyme labeled magnetic bead bioconjugate (PEG-MB/HRP/(Ab2)n-Ag) in the microfluidic channel. The electrochemical signal which correlated with the antigen concentration is generated by injection of a mixture of hydroquinone charge mediator and hydrogen peroxide which triggers a peroxidase iron-heme catalytic reduction of hydrogen peroxide under an applied voltage. Results indicate a detection limit of 100 fg mL-1 for both IL-17A and IL-17 F in diluted serum samples with a linear range from 100 fg mL-1 to 1000 fg mL-1. Furthermore, results showed minimal crosstalk between different antibodies indicating good selectivity and specificity. As a simple detection system for fast measurement of interleukin 17A and 17F this disposable immunosensor array shows significant promise of clinical value for application in point–of-care (POC) cancer screening and disease monitoring.

this work was supported by RI INBRE

Design and Fabrication of a Prototype Microfluidic Device for Nanopore Detection of Marine Biopolymers

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The goal of this project is to lower operational and commercial barriers to nanofluidic profiling of dissolved organic matter (DOM), polysaccharides using nanopore sensors integrated in to microfluidics. DOM is an abundant global reservoir of carbon, rivalling atmospheric carbon dioxide, that plays an important role in large-scale environmental events and trends affected by carbon cycling. The ability to chemically profile the various polysaccharides in DOM is limited by the difficulties of chemically characterizing glycans in general. Herein, we fabricated a prototype microfluidic interphase for the nanopore sensor element. Soft lithographic method was used to fabricate a dual 500 um width by 40 um height channel in Polydimethylsiloxane (PDMS) allowing hydrodynamic focusing of fluids on both sides of the nanopore sensor surface. The dual channel is mounted on 2 slabs of 5.9 x 3.6 cm Poly(methyl methacrylate (PMMA) housing each fitted with an inlet/outlet PEEK tubing connected with M10-32 flat bottom flangeless fitting. The microfluidic nanopore device is undergoing testing at Dr. Dwyer's group (URI). Nanopore sensors integrated in a microfluidic interphase will dramatically simplify sample processing and improve analysis reliability and performance

this work was supported by RI C-AIM

Mars Global Simulants Compared to Marine Sediments CEC Organo-Halogen Chemistry

Lyndsay Marlowe, Sean Nugent, Madeline Mitchell & Stephen O'Shea

Chemistry, Roger Williams University, Bristol, RI

Mars global simulants are mineralogical standards designed to replicate basaltic soils on Mars and were developed based on quantitative mineralogy from the Curiosity Rover. The bulk composition and surface XRF spectroscopic analysis of dried, decarboned, and decarbonated mars simulant was compared to surface marine sediment. After each treatment, both substrate forms were accessed for metal ion capacity extraction in an aqueous solutions by the presence of buffered EDTA and for all potential bioavailable metal ions in 1M HCl acid by ICP. The changing metal surface composition determined by XRF combined with ICP analysis eludes to potential surface reactions with halogenated organic compounds (HCs). This work has important implications from both a climate perspective and for what it tells us about the HCs biogeochemical cycling and signature molecules within the Martian sediment. The mechanistic HCs degradation pathway can be confounded by oxidation-reduction potential (ORP) of the environment and its pH, clarifying in-situ metal oxidation states. Measuring a site's in-situ capacity (soil/water) for transformation directly by treating it with a ^{13}C labelled HC substrate that can undergo the fundamental processes of oxidation, reduction, and substitution allows the chemistry that occurs to characterize the site. Both the nature and rates of these transformations can be assessed utilizing carbon labeled ^{13}C substrates, ^{13}C nuclear magnetic resonance spectroscopy analysis and head space gas chromatography/mass spectroscopy.

In Field XRF of *Mercenaria mercenaria*: a Bioindicator of Heavy Metal Legacy Pollution

Madeleine Mitchell, Sean Nugent, Lyndsay Marlowe & Stephen K. O'Shea

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The release and leaching of metal pollutants into coastal and estuarine environments has been greatly curtailed, but capped deposits can still reenter the ecosystem by natural perturbation or human activity. The potential use of Mercenaria mercenaria exterior shells as a heavy metal biosorbent that contain the polymorphs of $CaCO_3$ (aragonite and calcite) was investigated in conjunction with the development of an in-field X-ray fluorescence (XRF) as a bio indicator analysis protocol. XRF assessed the shell surface adsorption sensitivity to metal ions (Zn^{2+}, Cd^{2+}, Cu^{2+} and Pb^{2+}) in various saline ocean waters and at differing pHs. Shells were washed with spiked sediment and non-treated sediment slurries from the harvest location to assess the potential rerelease of metal to its surroundings and metal ion exchange capacities. The cation exchange capacity of the metal ions between sediment, pore water, and shell at varying substrate habitats from high carbon and sandy sediment reflect the sedentary borrowing of the quahog through sediment strata (1 meter depth). Further application of waste shells is the remediation within contaminated aqueous waste settling ponds. The ponds are designed remediation through two mechanisms, precipitation and absorption. With application of this technology, precipitation is enhanced by raising the pH to 8 on addition of the carbonate based shells forming insoluble metal hydroxides. The metal ions remaining in the water column were removed by the CEC of the shell substrate. The conditions allowing flocculation clarification of the water column is the solid deposit removed by dredging reducing volume of contamination greatly.

Anion Augmentation of Seawater and Sediment Microcosms on the Production of Oxygen Enhancing Predictive Modelling of Ecological Pollution Impacts

Sean Nugent, Lyndsay Marlowe, Madeleine Mitchell & Stephen O'Shea

Chemistry, Roger Williams University, Bristol, RI

To elicit the amount of nutrients in the aquatic environment by remote sensing is a challenging goal, notably in a marine setting with high saline content. To validate new technologies, a series of classic spectrocolormetric tests and spectroscopic analysis were used to characterize phosphorus and nitrogen speciation in these bodies of water with correlation to surface bound nutrients at the sediment-water interface. Simple light dark biochemical oxygen demand bottles allow the creation of reproducible microcosms of the natural seawater column with or without underlying sediment. The variation of oxygen production through photosynthesis is greatly impacted by augmentation of phosphorus and nitrogen anion species. These ions are key limiting nutrients in marine and terrestrial waters integral to primary production, understanding their impact will allow better modelling of ecological impacts from anthropogenic releases. The variation of anion composition, pH, and oxygen under varying experimental conditions were accessed over a one-month period. Bioavailable labile pore water and microwavable optimal permanently bound anion release capacity from under lying sediment were characterized for the potential flux between the two phases to determine the impact on primary production . Aqueous spectrocolormetric spot tests for phosphorous and nitrogen species anions determination were preformed following the HACH® procedure. These color spot tests were compared to instrumental analysis by HPLC-IC with inline electrochemical detector RI, UV/Vis phosphorus lines in ICP [214 and 217 nm], and vacuum XRF. Analysis of sea and terrestrial water evaporates by ATR-FTIR, diffuse FTIR, XRF and Raman spectroscopy were used to illicit spectroscopic anionic signatures and their detection limits were determined.

this work was supported by RI C-AIM

Synthesis of an Analog of the Bacteriostatic Antibiotic Fgkc

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Previously, the lab has shown that the diamide fgkc is an effective bacteriostatic antibiotic. The fgkc compound is effective against Gram-positive bacteria. The discovery has been a starting point to future development of more potent inhibitors. The primary goal of this project is to synthesize an analog of the lead compound fgkc with a modification at the aldehyde position. The modification of the compound is expected to alter the physico-chemical properties, specificity and potency of fgkc to be effective against Gram-negative bacteria. It's expected that those properties will be observed by the alteration of the hydrophobic interactions and hydrogen bond acceptors of the compound.

The analog synthesis was achieved by doing a microwave-assisted Ugi reaction. Then the compound was purified by an aqueous workup. The compound was further purified by flash chromatography and characterized by NMR. In order to test the antimicrobial efficacy of the fgkc compound, modified fgkc compounds were screened against *Bacillus subtilis* and *Escherichia coli* in an MIC assay.

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Lighting Up LytG with Differential Scanning Fluorimetry: Elucidating the LytG-Diamide Interaction

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Peptidoglycan (PG) is a polymer consisting of a polysaccharide backbone cross linked by stem peptides to form a rigid three-dimensional structure. PG acts as a physical barrier between the bacterial cell and the outer surface, making it essential to the cellular functioning. PG and its associated metabolism are one of the most successful targets for antibiotic development. Currently there are few modern chemical biology tools for studying PG metabolism and microbial glycobiology. This is partially due to the unique sugars employed by microbes in the assembly of polysaccharides and glycoconjugates. To address this shortfall, small molecule inhibitors of bacterial autolysins have been developed in our laboratory. Autolysins are a broad class of enzymes that are important for cell wall remodeling by cleaving bonds in the polymeric PG and are an intriguing target for developing chemical biology probes for PG metabolism. The focus is on developing inhibitors of the exo-acting N-acetylglucosaminidases LytG, the major active autolysin in the model organism *Bacillus subtilis*. Previous *in vitro* findings indicate that diamide inhibitors inhibit LytG with an IC50 of 60 μM. To further develop fgkc as a chemical probe, the target-probe interaction must be characterized. Target-probe interactions were characterized by differential scanning fluorimetry (DSF) and circular dichroism (CD). Baseline melt curves for LytG were obtained in the absence of ligand using the fluorescent dye Sypro Orange to monitor unfolding. DSF melt curves were then obtained in the presence of the ligands magnesium, chitotriose, and fgkc. LytG in the presence of magnesium and chitotriose positively shifted the melting temperature, indicating that LytG is further stabilized when forming a complex with these two ligands. Surprisingly, LytG in the presence fgkc had a negative shift in melting temperature, indicating that fgkc may favor binding the unfolded state rather than the native LytG. To confirm the destabilization of LytG upon fgkc binding was not an artifact of the assay, DSF experiments with fgkc in the presence of BSA and lysozyme were performed. BSA and lysozyme had no shift in their baseline melt curves, indicating that the destabilization by fgkc was specific to LytG. Destabilization of LytG by fgkc was further supported by CD spectroscopy which indicated that LytG in the presence of fgkc has a loss in structure, reinforcing the assumption that fgkc favors the unfolded LytG.

this work was supported by RI INBRE

Piece by Piece - Synthesis of Analogs of the Diamide Antibiotic Fgkc with Improved Physico-Chemical Properties

Joseph Prete[1], Robin Fidel[1], Amit Basu[2] & Christopher Reid[1]

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Previously, our lab has demonstrated the effectiveness of diamides as an antibiotic scaffold. We have demonstrated that our lead compound fgkc is a single micromolar bacteriostatic inhibitor of *Bacillus subtilis* and *Streptococcus pneumoniae* growth. By selectively modifying structural features in fgkc, new and more potent analogs with broader spectrum may be identified. The goal of this project was to explore the chemical space provided by the aldehyde and amine components of the Ugi reaction used in fgkc synthesis. These structural changes will be investigated for broadened specificity and increased potency through varying hydrophobicity and hydrogen bond donors/acceptors.

In looking to modify the aldehyde and amine position, selected aldehydes were either not commercially available or cost prohibitive. Desired aldehydes were synthesized through oxidizing their alcohol counterparts with pyridinium chlorochromate, with yields up to 35%. Modified fgkc diamide synthesis was conducted by a microwave assisted Ugi reaction in which an aldehyde, amine, isocyanide and acid were reacted with each other, one by one in that order, with a yield range of 40-50%. All diamides were purified by flash chromatography and characterized by proton and carbon NMR before and after deprotection. Modified compounds were run in an MIC assay against *Bacillus subtilis* and *Escherichia coli* to test antimicrobial effectiveness. Note, compounds are part of intellectual property filing.

this work was supported by RI INBRE

Evaluating the Active Form of the Diamide Antibiotic Fgkc

Caroline Williams, Michael Pepin & Christopher Reid

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Many antibiotics are inhibitors acting upon bacterial cells to limit or stop cell growth. However, a large number of bacteria are becoming antibiotic resistant. Every year in the U.S. more than 2 million infections and 23,000 deaths occur from antibiotic resistance. It is essential that new antibiotics with novel modes-of-action be developed in order to help combat this global challenge. In the development of antibiotics, the active form of the inhibitor must be determined in order to facilitate biophysical characterization of the target-inhibitor complex. Identification of the active form relies on results that show the presence of a specific compound or a derivative of it. Many of our most successful antibiotics target the cell wall of bacteria, specifically the synthesis of peptidoglycan. Peptidoglycan is a heteropolymer that provides structure and shape to the cell. Recent studies have shown that diamide antibiotics exhibit bacteriostatic action in Gram-positive bacteria. Specifically, the compound fgkc exhibits antimicrobial activity against *Bacillus subtilis* and *Streptococcus pneumoniae* in the micromolar range. It acts as a small molecule autolysin inhibitor in order to stop the metabolism of peptidoglycan in bacterial cells.

The purpose of this study is to analyze the lead compound fgkc, a single micromolar inhibitor of *B. subtilis* and *S. pneumoniae* growth under different conditions that may lead to a modified form of the inhibitor. RP-HPLC separation of diamides was established with a 5µ, (250 x 4.6mm) C-18 column along with an isocratic gradient of acetonitrile and water (50:50) with 0.5% formic acid. The diamide fgkc was tested under varying conditions to investigate whether the compound was modified by the cells. Fgkc was extracted from complex media with dichloromethane followed by RP-HPLC and analysis mass spectrometry. After incubation in two- and four-hour periods in complex media, it was shown that the compound remained intact in the presence of *B. subtilis* and *S. pneumoniae*. Thus, the data suggests that the active form of fgkc is the intact form.

this work was supported by RI INBRE

Synthesis of Metal-Ligand Complexes and Evaluation as Detectors of Pollutant Ion

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The assessment of nutrient and pollutant ions within aqueous solutions is an important goal aimed toward determining the health of freshwater/ marine environments and impacted communities of the Narragansett Bay ecosystem. Various bioinspired dipodal and tripodal Schiff base proligands were synthesized and reacted with metal salts, to form organometallic complexes. Spectroscopically, these complexes were evaluated as ion (nitrate, phosphate) binding/ detecting compounds.

this work was supported by RI C-AIM

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Too Hot To Handle?: How Changing Environmental Conditions Affect Mysid Populations

Daniel Clark & Elisabeth Arevalo

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Americamysis (Peracarida, Mysida) is a genus of opossum shrimp that is found along the Atlantic coast of the Americas and the Gulf of Mexico. The current taxonomical classification divides *Americamysis* into six separate species, five of which are found off the coast of the United States. *A. bahia* is believed to be the most widely distributed of these five species, spanning from Florida to southern Rhode Island. All mysids constitute an integral part of the trophic food chain; however, *A. bahia* is the only species consistently used as a sensor species in lab environments. For these reasons, we believe *A. bahia* is a good choice as a test species for the environmental changes brought upon by climate change. The rise in ocean temperatures has led to increasing interactions between species because of a change in their distributional boundaries. Rhode Island salt ponds are ideal sites to determine the genetic variability of mysids because their location straddles the distribution limits of *A. bahia* and *A. bigelowi*. We plan to use various locations in Rhode Island to assess whether competition and/or hybridization occurs between these two species. Since it is ambiguous to distinguish between these species based solely upon traditional morphological characteristics, a more concrete molecular basis is necessary to properly distinguish between species. Our laboratory has created a Single Nucleotide Polymorphism (SNP) library and has so far validated 96 loci. The goals of this study are to use our 96 SNPs to assess the genetic variability within and between species of mysids, and to better define species. Another goal is to assess the robustness of *A. bahia* in changing environmental conditions. We will achieve this goal by subjecting mysids to variable environmental conditions (temperature, salinity, pH) and determine their limits for survival. As part of our study we will record mortality rates, changes in behavior, and environmental effects on fecundity. These results will help us extrapolate on how the increasing climate temperatures will ultimately affect marine life. We will continue to screen our genetic library and to discover additional SNPs that will be added to our dataset which will increase our power to assess the state of mysid populations.

this work was supported by the Southeastern New England Educational and Charitable Foundation

Detection of Nitrate Ions in Water Using SERS-Doped Algae

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Excessive levels of nitrates caused by fertilizer runoff can cause dangerous algae blooms which often shut down beaches and other coastal areas. Certain strains of algae such as *Ulva* SPP use these nitrates in their metabolic cycles. This gives a potential to observe very small nitrate levels in the water as the *Ulva* SPP leaves act as a bio concentrator. *Ulva* SPP were doped with SERS active particles made in our laboratory by dipping the leaves into a suspension of the particles. The doping levels were obtained using thermogravimetric analysis. Sodium nitrate solutions were added to the doped algae, and strong Raman signals arising from the N-O stretch of the nitrate ions were detected. When SERS particle doped paper was used as a control, the Raman signals from the N-O stretch were weak and inconsistent. The algae therefore have good potential for detection of nitrate ions in water.

this work was supported by RI C-AIM

Inferring Moments of the Floe Size Distribution Using a Neural Network Derived from Satellite Imagery

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Sea ice is a key part of the climate system, maintaining cool temperatures in polar regions by reflecting sunlight, and loss of sea ice will further drive global warming. In sea-ice covered areas, the floe size distribution (FSD), f(r), is defined with f(r)dr is the fraction of the area containing floes of horizontal size r. How the FSD changes over time is important for climate predictions, but current methods of calculating the FSD are expensive and time-consuming. Our goal was to create a neural network that predicts the FSD using meteorological data common to all large-scale climate models, such as ice concentration, ice thickness, and ocean temperature. We created a computer visualization program that efficiently picks out sea ice floes from the Sentinel-2 satellite and calculates the FSD. Running this program on many images, we acquire data that, when coupled with large-scale meteorological data, allows us to train a neural network for diagnosing the FSD. The final result of this project is a neural network that can be easily added to current climate models, that outputs the scale of sea ice floes in given ice-covered regions.

this work was supported by RI C-AIM

Westbrook Ice Disk

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In winter 2018, a large circular ice disk formed in the Presumpscot river in Westbrook, Maine. The disk had a diameter of approximately 102 meters and an area of 8283 m^2. The disk persisted in the river for at least 7 days before being frozen in. Ice disks such as this one are comparable to much larger sea ice floes that exist in polar regions. Developing methods to study and track such floes is difficult because of the challenge of obtaining repeated measurements of floes in such remote areas. Using three days of continuous time-series observations of the Westbrook disk and surrounding climate, we developed an algorithm that tracks the perimeter, radius, and motion of the disk and analyzes these descriptors with respect to differing climate conditions such as temperature, wind speed, and precipitation. We use this information, along with meteorological data from a nearby weather station, to constrain what causes changes in the disk over time.

this work was supported by RI C-AIM

Characterization of Microbial Communities in Biofilms Colonizing on Microplastics

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Microplastics (MPs) are plastics that are less than 5 mm or smaller in diameter. They are formed by degradation of plastic wastes through mechanical and/or photo-oxidative pathways. When an MP particle enters an aqueous environment a biofilm is formed on its surface. We hypothesize that depending on the physiochemical properties of the polymer, additives and the compounds adsorbed on the MP particle's surface, the microbial community in the biofilm will vary. The biofouled MPs could potentially be pathogenic/toxic to aqueous organisms. The objective of this research effort is to identify the microbial communities in the biofilm that are formed on the surfaces of MP particles. Ten different polymers were chosen – high density polyethylene, polypropylene, polystyrene, polyethylene terephthalate, nylon, polyvinyl chloride, polybutadiene, polycarbonate, polyacrylamide and polytetrafluoroethylene. The MP particles were made from the polymers and incubated in Narragansett Bay seawater for a period of one, two and three weeks in an *in vitro* biofouling assay. After their respective incubation period, the total genomic DNA was extracted from the surface of MP particles and a 16S rDNA library was prepared using primers for the V4 region of 16S rDNA. Library sequencing was performed on the Oxford Nanopore MinION and data processed and analyzed using Guppy and Epi2Me software platforms.

this work was supported by RI C-AIM

Do You Really Want Solar Panels on Your Roof? Economic and Psychological Factors Determine Interest in Residential vs. Community Solar Programs in Rhode Island

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Household adoption of solar energy is an important piece of the transition from our fossil-fuel dominant energy system toward a more sustainable energy future. Rhode Island has implemented a variety of statewide policies and incentives aimed at increasing the use of alternative energy at the household level. More recently, they have designed the Community Renewables Program to promote solar adoption among low-to-moderate income (LMI) households, renters, and others who cannot install solar on their properties. We designed a survey of Rhode Island residents to examine what economic, psychological, and demographic factors differ in leading residents to prefer installing solar panels at their home (residential solar) vs. subscribing to a community solar project.

The survey was mailed to 500 households in North Providence with a response rate of 20% (N = 99). The survey measured a variety of economic factors including (1) perceived costs, (2) perceived benefits, (3) consumer novelty seeking, and (4) present bias; psychological factors including (1) risk-taking orientation and (2) personal experience of climate change; and demographic factors including (1) dwelling type and (2) income. The survey also included a description of both the residential and community solar programs. After reading each description, respondents indicated their interest in each program, which program they prefer, and their likelihood of adopting solar through either program in the next 5 years.

Preliminary data analysis suggests that actual income-level and dwelling type were unrelated to people's preference or interest in either solar program. However, economic and environmental concerns as well as personality factors correlated with preference for the two programs. Specifically, those concerned with 1) perceived costs and those who are 2) risk takers, 3) present biased, and 4) novelty seekers prefer the community solar program. Those that have a 1) strong preference for conserving energy and 2) are concerned about climate change prefer the residential solar program. Those who believe switching to solar would save money (perceived benefit) have a higher preference for both programs.

Results suggest that there is a core group interested in community solar, potentially expanding solar adoption to a wider range of Rhode Islanders. However, more communication is needed to convey how community solar may benefit the renters and LMI households it is designed to attract.

this work was supported by RI C-AIM

Genetic Connectivity of *Fundulus heteroclitus* Populations from Polluted and Clean Habitats

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Fundulus heteroclitus, an estuarine fish found along the coast of the eastern United States, is notable in its hardiness and ability to adapt to highly polluted waters. This tolerance, combined with their relative abundance, makes them a useful organism for examining evolved tolerance to pollution. In collaboration with the EPA's Atlantic Ecology Laboratory in Narragansett, tissue samples were collected over the course of the last several weeks for the purpose of determining SNP (single nucleotide polymorphism) genotypes. Future use for the *Fundulus* data include fine-scale location-based analysis of fish adaptation to pollution, using genetic markers to identify the genetic population connectivity between various sites in southern New England, including especially polluted sites such as New Bedford harbor. A preliminary analysis of broad scale population structure using a set of SNP markers will also be presented.

this work was supported by RI C-AIM

Assessing Bimolecular and Spatial Distribution Changes of Marine Biofilm on PDMS

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The purpose of this study is to develop protocols to identify changes of biomolecules and spatial distribution during the growth of marine biofilm on microfluidic sensor to be deployed in Narragansett Bay. To examine the biofilm growth, polydimethylsiloxane (PDMS) samples were placed in two different conditions at the URI Ann Gall Durbin Aquarium building. Water entering the tanks was continuously pumped from the Narragansett Bay. One tank contained a sand filter and live animals, while the other was a “raw” tank containing only the unfiltered seawater. The water quality and content of the tanks were examined for effects on biofilm growth. After performing two test trial that ran for three week, samples were extracted and analyzed using two techniques Fourier Transform Infrared Spectroscopy (FTIR) and High Content Confocal Microscopy. In the case of FTIR, a two-step biofilm extraction method from the PDMS was developed and harvested biofilm was collected to form a pellet for FTIR analysis. Biomolecule identification with this pellet was used to detect the changes in molecular composition as the biofilm matured. The Opera Phenix High-Content Screening System was used for confocal microscopy assessment, quantification, and determination of spatial distribution. Various stains were obtained to evaluate different components of the biofilm such as proteins, polysaccharides, and eDNA. By using these fluorescent stains, the Opera Phenix can then provide the necessary insight into the structure of the biofilm, and the source of its adherence. These two complementary techniques have proved to be an aid in the future development of anti biofouling strategies.

this work was supported by RI C-AIM

Patient and Provider Communication about Cancer-Work Management among Recently Diagnosed Employed Breast Cancer Survivors

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Background

Cancer treatment can result in a range of physical and psychosocial symptoms that limit the ability to work during and after treatment (Feuerstein, *et al.*, 2010). Yet, many cancer survivors may need or decide to continue to work during treatment (Vanderpool, *et al.*, 2013; Swanberg, *et al.*, 2017). National estimates indicate that among cancer survivors employed at diagnosis about 70% had a conversation about with a health care provider about employment (de Moor, 2018). Yet, limited research has explored employed survivors' experiences discussing employment and cancer-work management issues with healthcare providers (Nichols, *et al.*, 2017). To address this gap, this study uses data from the EMPOWER Study to describe patient-provider communication (PPC) related to managing cancer and work.

Methods

The EMployment and Potential Outcomes of Working through cancER (EMPOWER) Study is a longitudinal, mixed methods pilot study designed to evaluate how employment influences treatment decisions among women diagnosed with breast cancer. A semi-structured qualitative interview was administered to assess cancer-work decisions. Interviews were digitally recorded and transcribed for coding and analysis. A constant comparative method was used to analyze data. Codes were analyzed for key themes and organized into six key domains, featuring themes and subthemes within each domain.

Results

Of the fifty women enrolled, 48 (96%) completed the three-month qualitative interview. The majority of women (83%) reported PPC about managing work and treatment. Thirty-two percent of these women had an in-depth conversation with their provider about managing work and treatment, consisting of open ended dialogue tailored to the individual's unique circumstances. For the remaining 68% PPC discussions about cancer-work management were more transactional in nature. The majority of the time (42%) the conversations were initiated by a member of the care team; only 23% of PPC were initiated by the women. The majority of provider-initiated conversations were brief and exchanged specific information. Patient-initiated conversations, in contrast, were more likely to be in-depth discussions tailored to the survivor's cancer treatment in relation to work responsibilities.

Conclusion/Implications

Further research is needed to better understand the content and quality of PPC about cancer-work management and the immediate and long-term effects on survivors' overall well-being.

this work was supported by RI INBRE

NOVA Food Processing Classification in the POUNDS Lost Study and Association with Weight Loss, BMI, Waist Circumference, and Percent Body Fat

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Background

The NOVA system is a novel guide for diet quality, categorizing foods by the nature, extent, and purpose of processing. The NOVA food groups are: (1) unprocessed or minimally processed foods; (2) processed culinary ingredients such as oils, butter, sugars, and salt; (3) processed foods, made by combining Group 1 and 2 foods; and (4) ultra-processed foods (UPF): formulations made mostly or entirely of substances derived from foods and additives, with little if any intact Group 1 foods. UPF are highly palatable due a generally high content of saturated fat, added sugar, sodium, and refined grains. UPF are often energy-dense, which when paired with excessive consumption, can lead to even greater energy intake. A positive trend between per capita UPF sales and population BMI has been found and numerous studies suggest UPF are positively associated with obesity. A recently published randomized-controlled-crossover trial reported a 0.9 kg weight gain when participants were on an UPF diet versus a 0.9 kg weight loss on an unprocessed diet.

Given that diets proportionally higher in UPF are associated with greater energy intake and higher BMI in healthy populations, it is plausible that an energy-restricted diet proportionally higher in UPF would be associated with decreased weight loss and BMI, waist circumference, and %body fat reduction. This study will explore if UPF consumption is associated with weight-loss trajectories in the weight-loss trial Preventing Overweight Using Novel Dietary Strategies (POUNDS Lost).

Objectives

To determine if there were changes in %kcal from UPF and unprocessed foods between baseline, 6, 12, and 24 months and if changes between baseline and 6 months are associated with %weight loss, %waist circumference reduction, BMI, and %body fat at 6, 12, and 24 months.

Methods

Over 3,625 unique food codes were identified from baseline, 6, 12, and 24 month POUNDS Lost diet recall data. To access nutrient information and match to NOVA groups, codes were merged to ~7,250 Food and Nutrient Database for Dietary Study (FNDDS) codes, which are composed of ~2,900 USDA National Nutrient Database for Standard Reference codes (SR codes). Handmade mixed dishes made at home or commercially were disaggregated into SR codes for accurate classification. Unique food codes not matched with FNDDS and SR database merges (5.7%) were manually coded by two research assistants. Coding and analysis were done in Stata 16.0.

this work was supported by RI INBRE

Design and Implementation of a Low Cost, Open Source Flight Computer for a High Altitude Balloon

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The purpose was to design and build a high altitude research balloon to take photographs and measure environmental variables across various altitudes using micro-controllers and electronic sensors. This was done by using Arduino IDE, an open source software to develop or modify codes for programming low cost micro-controllers and sensors. The electronics were programmed to store all of the data obtained from the sensors onto a MicroSD card, collect live GPS coordinates, and transmit GPS coordinates via radio (Automatic Packet Reporting System) to a website with live data accessible to the public. The final product included a flight computer, antenna, various sensors, a radar reflector, balloon, and parachute that could transmit signals via radio, be seen by airplane radar, float, and be used nearly anywhere. The high altitude balloon flight had a maximum altitude of 71,249 feet and traveled from Pittsfield, MA to Stonington, CT. A step by step website guide for the project was developed so the project could be easily replicated or modified. The project demonstrated that scientific data can be collected by nearly anyone at a low cost and can expand the future of citizen science.

this work was supported by

MARINE SYSTEMS

CENTER FOR BIOTECHNOLOGY & LIFE SCIENCES
1ST FLOOR HALLWAY

ODD -NUMBERED POSTERS ARE TO BE PRESENTED FROM 9:30 – 11:00 AM
EVEN-NUMBERED POSTERS ARE TO BE PRESENTED FROM 11:00 AM – 12:30 PM

Tracking Domoic Acid Levels in Phytoplankton and Mussels in Narragansett Bay, Rhode Island

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Narragansett Bay (NB) is home to various species of the diatom *Pseudo-nitzschia*. During *Pseudo-nitzschia* blooms, certain species produce a neurotoxin called domoic acid (DA). In other marine regions, such as the North American west coast, DA is detected in high amounts because of *Pseudo-nitzschia* blooms, bioaccumulating in shellfish such as mussels and subjecting humans to potential intoxication through ingestion. The first *Pseudo-nitzschia* bloom in NB that resulted in high levels of DA in shellfish meat resulting in a fisheries closure was in October of 2016, despite *Pseudo-nitzschia* presence in NB for over 50 years. Following the 2016 bloom, it has become necessary to understand levels of DA and seasonal fluxes of the toxin in NB. We filtered water collected at sampling stations in NB, extracted samples of phytoplankton for DA analysis and enumerated *Pseudo-nitzschia*. Additionally we tested mussels collected at various locations in NB at the height of the June 2019 *Pseudo-nitzschia* bloom and at the decline. We developed a methodology to extract DA from mussel tissue. This consisted of a 4 hour extraction with 70% Isopropanol and 30% acetic acid and running the sample through an SPE column. Using LC-MS/MS with MRM monitoring based on the protonated DA molecule, we analyzed the samples to detect the presence of DA in phytoplankton cells and mussels tissue. We detected a spike in DA levels in phytoplankton samples during the June 2019 bloom and did find DA in certain mussel samples, although the levels are below those that would trigger a shellfish closure. Understanding the seasonal patterns of DA concentrations in NB is important to management agencies and the public's consumption of shellfish, as consuming shellfish with DA can cause memory loss and even irreparable damage to neurons. It will be interesting to determine if the DA spikes we have recorded in June 2018 and June 2019 will reoccur in future years.

this work was supported by RI C-AIM

Narragansett Bay Fishes: Who is Eating Who?

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With rising seawater temperatures, Narragansett Bay, RI has become a landing spot – or at least a pitstop – for a number of species that previously preferred waters further south. A perfect example is the striped sea robin. While they've been known to utilize the bay for decades, recent catch data suggests they are arriving earlier in the season and departing later each year than ever observed before. A major concern of this growing pattern is their predation of larval winter flounder, which are still small and vulnerable by the time sea robins are pouring into the bay. Striped sea robins are remarkably well-adapted benthic feeders, using their unique pectoral fin rays to stir up bottom sediments and flush various prey. As of now, there is very little diet composition data for this species, which was identified by the RIDEM as a data gap. An important component in understanding the ecological significance of striped sea robins is learning what they eat. My summer has been spent going out on the weekly GSO fish trawl, collecting sea robin samples, and bringing them back for dissections. I have also been catching them by rod and reel to sample water that cannot be reached by a trawl net (i.e. shallow or rocky conditions) to observe any potential spatialized diet shifts. Ultimately, the goal is to fill the present data gap so we can continue to learn about this species and its ecological impacts in Narragansett Bay.

this work was supported by RI C-AIM

Investigating Perceptions of Coastal Conditions at Sabin Point Park

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Sabin Point Park in East Providence (RI), a coastal public access site in the Upper Narragansett Bay which has a public boat ramp, playground, basketball court, sandy beach, shaded picnic area, and fishing pier, has experienced recent infrastructure upgrades and water quality improvements. This study examined recreational activities and perceptions of conditions (e.g., water quality, access, artificial reef installation) at Sabin Point. Intercept surveys of Sabin Point users were conducted on weekends and weekdays in the morning, afternoon, and evening in order to gauge public perceptions of the site's conditions. In addition, attitudinal questions were asked about artificial reefs and their potential to enhance the quality of fishing. Site characteristics were also recorded at each visit, such as the number of people present and weather conditions. This poster will describe preliminary findings from these surveys. These findings will inform coastal management at Sabin Point and provide insight into the public's perceptions of the impact of artificial reefs on the area.

this work was supported by RI C-AIM

Understanding Experiences of Women in Marine Science Fields in Rhode Island

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A common theme among all STEM fields and careers is the underrepresentation of women. This study examines how women view the field of marine science, and some of the challenges they face within marine science fields. In a collaboration with researchers at MIT Sea Grant, semi-structured interviews were conducted with marine natural and social scientists in Rhode Island and Massachusetts who identify as women. Interview questions asked respondents how they got involved in their field of work, what they do in their current position, how they envision success in their position, and their views on some of the challenges and advantages to being a woman in marine science. The research team recruited scientists in both academia and governmental sectors. This poster will describe preliminary results from the first set of interviews in Rhode Island, highlighting common themes that emerged from the interviews. Direct quotations will be used where appropriate to provide richer understanding of the themes. These findings bring to light the unique challenges faced by women in marine science in the office, lab, research vessels or other environments. Findings from this study aim to bring awareness to these challenges, in order to create more equality and fairness in this particular field.

this work was supported by RI C-AIM

Using Machine Learning to Expedite Data Cleaning for Oceanographic Models of Narragansett Bay

Jonathan Benoit, Alice Foster & Baylor Fox-Kemper

Earth, Environmental, & Planetary Sciences, Brown University, Providence, RI

Computational models of Narragansett Bay are in the early stages of development. To improve these models, historical oceanographic data from the Rhode Island Department of Environmental Management are compared to hindcast runs of the models. Before being used for comparison, the observational data is cleaned manually because fouling and other unexpected effects produce erroneous data. There is currently a multi-year long gap between the data that has been taken and the data that has been cleaned. Historical data over the past few years is needed to expand the scope of hindcast model runs, and near real-time data is needed to develop a forecasting model. This project investigated the usefulness of machine learning in expediting data cleaning. By training a classification algorithm with samples of clean and raw data, a preliminary tool was developed that could accelerate the cleaning process by flagging data spikes and timeframes where fouling calculations are necessary. The machine learning algorithm was trained using two methods. In one method, all nine variables were input for one time, while in a second method, a single variable was input along with neighboring values in time. Though neither of these algorithms produces publication-ready cleaned data, this project is a proof of concept and creates a tool for speeding up data cleaning and providing preliminary data for predictive model runs.

this work was supported by RI C-AIM

Assessing the Skill of the Regional Ocean Modeling System in Narragansett Bay

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The Regional Ocean Modeling System (ROMS) is a framework for oceanic models that has been used to understand Rhode Island's coastal waters. The model is important in predicting hypoxia events, beach closures, biological activity, and the effects of climate change on economies and ecosystems. This project sought to quantitatively assess the skill of ROMS when applied to Narragansett Bay. To do so, we compared model predictions of temperature and salinity in the winter and summer of 2006 to observational data from that year. Time series were sourced from National Oceanic and Atmospheric Administration (NOAA) fixed-elevation sensors and Rhode Island Department of Environmental Management (RI DEM) buoys, and depth profiles were derived from Insomniacs and Daytrippers ship-based observations. We assessed the covariance and bias between the model and observations with respect to both time and depth. The model varied in accuracy depending on geography, depth, and whether temperature or salinity was evaluated.

this work was supported by RI C-AIM

Changes in the Abundance of Kelp and Rockweed in Narragansett Bay

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Narragansett Bay contains a variety of important seaweed ecosystems that are home to diverse fish and invertebrate communities. Rockweed, *Fucus* spp., in the intertidal, and kelp, *Saccharina latissima* and *Laminaria digitata*, in the subtidal have historically been prevalent in the bay. Surveys conducted in the 1970s and 1980s have provided historical data on the location and abundance of rockweed and kelp habitats. In order to monitor changes in these habitats, video surveys on SCUBA were conducted at 24 sites chosen for their previous abundance of rockweed or kelp from historical studies in Narragansett Bay. Video surveys were conducted in both the fall and summer using a metal ski mounted with GoPro cameras, underwater lasers, and lights along a 30m transect in order to document seaweeds, invertebrates, and fishes at all sites. Current percent cover of rockweed and kelp at all 24 sites was determined by analysis of the seaweed videos based on the total time that rockweed or kelp was seen along the transect. Additionally, subsamples were collected during surveys to determine rockweed and kelp density and biomass at each site. The results from these surveys will highlight the current abundance of kelp and rockweed habitats in the bay, as compared to the historical record, and determine which invertebrates and fishes are associated with these habitats. As the ocean climate continues to change, these seaweed habitats will also be subject to change, and subsequently their associated invertebrate and fish communities. Continuing to survey these vital habitats in the future will create a better understanding of the importance and abundance of diverse seaweed habitats in Narragansett Bay.

this work was supported by RI C-AIM

Boat on a Bay, Bay in a Bottle: Dynamics of the Toxic Diatom *Pseudo-nitzschia*

Meagan King[1], Alexa Sterling[2], Riley Kirk[3], Matthew Bertin[3], Patrick Wilson[2], Jacob Strock[4], Heather McNair[4], Susanne Menden-Deuer[4] & Bethany Jenkins[2,4]

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Diatoms are photosynthetic aquatic unicellular organisms that account for approximately 20% of the world's primary productivity. Diatoms in the *Pseudo-nitzschia* genus can produce a neurotoxin, domoic acid (DA), that causes amnesic shellfish poisoning in humans and economic harm from fisheries closures. An understanding of what causes these diatoms to produce the neurotoxin can aid in preventing shellfisheries' lost income in addition to protecting human health. Since 2016, *Pseudo-nitzschia* has become an increasing problem in Narragansett Bay, RI, and it is unclear as to what is driving its toxicity. In response, starting in September 2017, weekly samples have been collected at three sites: the Narragansett Bay Long-Term Plankton Time Series, West Passage mouth, and East Passage mouth. This year in 2019, there was a DA event in late May through early June, with similar timing to what was observed last summer. The highest cell-associated DA concentration detected during the 2019 event was 25.26 ng/L of seawater, which is under the threshold to closure fisheries. At the *Pseudo-nitzschia* bloom peak and decline that occurred one week apart, we collected water with the community of toxin-producing *Pseudo-nitzschia* to investigate how DA production was affected by grazing pressure from microzooplankton using 24-hour bottle incubations with a two-point dilution. Bulk phytoplankton growth rates were 0.01 per day during the bloom peak and 0.66 per day during the decline. Grazing rates on the entire phytoplankton community were 0.08 per day and 0.25 per day with the higher rate occurring during bloom decline. Specific growth of *Pseudo-nitzschia* will be determined from cell counts and DA production in each experimental treatment will be measured. The results from this experiment may add to the understanding of what conditions cause *Pseudo-nitzschia* to increase toxin production; this is important for Narragansett Bay because shellfish is harvested during times of the year when blooms occur which could negatively impact humans from shellfish consumption.

this work was supported by RI C-AIM

Red White and Big Blue: How Rising Ocean Temperatures Affect Red and White Muscle Recruitment in Fish Species

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Red and white muscle recruitment in fish allow them to swim at sustained speeds or accelerate to higher velocities. Red muscle occurs only in small bands in the fish's bodies and is aerobic, while white muscle makes up the majority of the muscle they possess and is anaerobic. The goal of this research is to evaluate the recruitment of red and white muscles of Narragansett Bay fish species at varying temperatures of 20 and 24° C to determine if the different temperatures affect muscle mechanics. Other studies have been conducted that show how red and white muscle contract, but few have delved into how temperature affects them *in vivo*. We collected Summer Flounder, Winter Flounder, Black Sea Bass and Scup from the Narragansett Bay and placed electromyography electrodes into their red and white muscle at 50 and 75% of the fish's total length under different temperatures. We analyzed muscle recruitment parameters such as duration of contraction, duty factor and magnitude to determine if higher temperature leads to suboptimal muscle mechanics. Using these data as well as cross sections from each species to measure the amount of red muscle available in their bodies we will be able to compare recruitment to total area of red muscle and species specific capacity to adapt to higher temperatures in the Bay. After analyzing the percentage of red and white muscle at medial and caudal end of the four species mentioned above, we found that Summer Flounder had the largest percent of medial white muscle with 93%, while Winter Flounder had the largest percent of caudal white muscle accounting for 94% of cross sectional area.. Black Sea Bass had the largest percent of medial red muscle with 27% and the largest percent of caudal red muscle with 38%. When comparing the intensities of the muscle recruitment burst intensity was higher at 24°C than at 20°C for Winter Flounder, for all the muscles tested except red caudal. This seems to indicate that at higher temperatures Winter Flounder spend more energy in stronger muscle recruitment which could leave less energy available for growth.

this work was supported by RI C-AIM

Taking the Heat: Climate Change Effects on Muscle Physiology of Fish in the Narragansett Bay

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An organism's ability to alter its physiology in response to external pressures reveals their adaptability and tolerance of future environmental conditions, especially in the case of climate change. We expect fish species including, black sea bass, scup, winter and summer flounder will recruit more red muscle when exposed to higher temperature waters in order to complete simple movements. Sedentary species like the flounder will likely demonstrate less of a physiological adjustment than the more active pelagic species. Red and white muscles at half the body length and at the caudal end were all monitored via electrodes hooked up to an electromyography data acquisition system in order to analyze their recruitment during different movements. Fish then swam at each of the experimental temperatures (18 and 22°C) to determine muscle mechanics. Respirometry experiments were also conducted within isolated tanks measuring the dissolved oxygen in the water, quantifying mass corrected oxygen consumption for each species at the two temperatures. Winter flounder exhibited a significant (p-value < 0.05) decrease in burst duration in both caudal muscle fibers and mid white muscles, revealing that muscle contractions occur quicker under higher temperature conditions. Furthermore, caudal red muscles saw stronger recruitment at 22°C conditions, as seen in greater intensity bursts. Mid red muscle had a significantly (p-value < 0.01) lower duty factor at higher temperatures, meaning that the muscles were more active for a shorter portion of the cycle than under 18°C conditions. These results confirm that red muscles were recruited more at 22°C than at 18°C. Aerobic red muscles operate more efficiently under warmer temperatures, explaining why the red muscle of the studied fish contracted faster and more often in the 22°C experiments. Further studies will be needed to understand the energetic costs associated with these physiological changes, as well as how other physical and chemical changes accompanying climate change will impact fish muscle recruitment. In Narragansett Bay, studying the muscle function and oxygen consumption of various fish species will indicate their fitness in the coming years and how local stakeholders must also adapt in order to achieve long-term fishery sustainability.

this work was supported by RI C-AIM

Characterization of Viral-Host Interactions Following Antagonistic Co-Evolution

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Cyanobacteria and the viruses that infect them are abundant in coastal marine environments. When cyanobacteria and viruses interact, co-evolution occurs in which the cyanobacteria evolve resistance to the viruses, and viruses then evolve to overcome that resistance. As the viruses evolve, their host range increases with the virus infecting both ancestral and co-evolved bacteria. However, few studies have explored the infection kinetics of pairwise interactions among co-evolved cyanobacterium and virus. In this project, we examined the level of viral resistance in four co-evolved hosts and the degree of infectivity of four co-evolved viral isolates. To examine the genetic mutations that are responsible for host resistance and virus host range expansion, we sequenced the whole genomes of all ancestral and co-evolved cyanobacterial hosts and viral isolates. In cyanobacterial isolates, viral resistance typically resulted from only a few single point mutations, and different combinations of mutations could lead to resistance to the ancestral virus. Likewise, in the co-evolved viral isolates, different sets of mutations enable the virus to overcome host resistance. One noted difference in the virus, however, was that multiple mutations were often observed in the same gene. Pairwise cross-infectivity assays among all viral isolates and cyanobacterial host isolates revealed that levels of resistance vary among the cyanobacterial isolates and similarly, the degree of infectivity differs among the viral isolates. For example, the co-evolved viral isolate RIM8 G-167-3 did not infect cyanobacterial isolate WH7803 G-84-sc3, however it evolved to overcome resistance and infect cyanobacterial isolates WH7803 G-112-sc3 and WH7803 G-167-sc2, but with varying degrees of infectivity. Even when co-evolved viral strains can infect bacterial host isolates, cross-infectivity assays suggest that the rate of replication may differ between cell types. The next step of this study is to analyze how well co-evolved viral isolates replicate on the ancestral and co-evolved cyanobacterial isolates. Understanding how co-evolution influences viral-host interactions will elucidate the effects viruses have on overall host mortality in natural environments.

this work was supported by NSF EPSCoR (Track 2)

Effects of Varying Environmental Conditions on Marine Viral Community Composition in Mount Hope Bay, Green Hill Salt Pond, and Quonochontaug Salt Pond

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Cyanobacteria are photosynthetic bacteria found in the oceans and they are one of the most important primary producers in the marine environment. Cyanophages are viruses that infect these cyanobacteria. There are many different types of cyanobacteria and cyanophage with different phages infecting different bacterial cell types. It is known that the cyanophage community composition changes seasonally, with an increase in viral abundance and diversity in the summer months. However, less is known about how environmental conditions during a given season affect the cyanophage community composition. The objective of this research was to examine how different environmental conditions such as temperature and salinity in Green Hill Pond, Quonochontaug Pond, and Mount Hope Bay influence the community composition of cyanophages. These different sites were sampled and the community compositions of *Synechococcus*-infecting cyanophages were analyzed. Viral abundance was estimated through most probable number assays and viruses were isolated using extinction dilution assays with *Synechococcus* strain WH7803. The DNA polymerase gene from the viral isolates was amplified via PCR and then sequenced. These sequenced viral isolates from Mount Hope Bay, Green Hill Pond and Quonochontaug pond were grouped into OTUs based on a 99% sequence similarity. It was estimated that there were 2.9x105 viruses per milliliter of water in Green Hill Pond, 210 viruses per milliliter in Mount Hope Bay, and 39 viruses per milliliter in Quonochontaug Pond. The sites had some variation in terms of environmental conditions which may have led to the differences in viral abundance and diversity of OTUs. A higher temperature correlated with a higher abundance of cyanophages as seen at the Green Hill Pond site. Preliminary results also show that among all three sites there are at least 26 OTUs with some overlap of OTUs between sites, however many of the OTUs in Green Hill Pond were unique to that location. Since viruses are the most abundant entities in the ocean, having a significant impact of nutrient cycling when they infect and kill their hosts, it is important that we understand them and how environmental changes due to global warming may affect microbial communities in the marine environment.

this work was supported by RI C-AIM

Multiplexed Optical Detection of Heavy Metal Contaminants in Plants

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In the Narragansett Bay ecosystem there are various types of plant life and soil that allow the sea life to remain healthy and prospering. Within these plants there are heavy metal contaminants that threaten to disrupt this ecosystem. Absorbance spectroscopy and inductively coupled plasma-mass spectroscopy are two methods that can be used to detect such contaminants, however they are time consuming and expensive to produce on site testing. Single-walled carbon nanotubes emit long-lasting near-infrared (n-IR) fluorescence that can be examined using N-IR fluorescence spectroscopy. These nanotubes are ideal biosensors in that they are photostable, and shift fluorescence in the presence of analytes including but not limited to heavy metal ions. Here, using the basis that plants can internalize single walled nanotubes we will use an n-IR fluorescence microscope to observe and quantify exactly how the nanotubes fluorescence varies with the addition of heavy metal ions. Upon binding of the heavy-metal ion to the nanotube complex a characteristic red shift is expected to occur in the n-IR emission spectrum as the wavelength that the nanotube fluoresces at increases. Once this is controlled these nanotubes will be optimal nanosensors to inject into the plants and detect the concentration of the heavy metal ion contaminants.

this work was supported by RI C-AIM

Investigating the Plastisphere: The Role of Plastic-Associated Microbes on Microbead Ingestion by the Coral *Astrangia poculata*

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There are currently between 6,350 to 245,000 million metric tons of plastic in the global oceans. Macroplastics that enter the ocean often get weathered down into microplastics (<5mm), now found in all oceans, including remote regions and depths. Microplastics are consumed by a variety of marine organisms and harbor distinct microbiomes. It is unknown whether bacterial biofilms on plastics influence the mobility of plastics throughout the food web. The local coral *Astrangia poculata* feeds on particles in the seawater. This study aims to test if plastics ingestion by *A. poculata* is influenced by surface-associated microbes. Colonies of *A. poculata* were presented polyethylene microbeads (200µm diameter) with various treatments in choice-feeding assays, in which *A. poculata* colonies were presented equal quantities of two treatments of microbeads suspended in seawater. Polyps were dissected to score intake of each treatment. Microbeads were biofilmed in aquarium tanks at Roger Williams University and *in situ* at Fort Wetherill State Park (RI) for three weeks. *A. poculata* consistently ingested non-biofilmed microbeads significantly more than biofilmed microbeads. *A. poculata* was provided pairwise choices of microbeads biofilmed in different bacterial isolates from the *Astrangia* Culture Collection. Ingestion appears to be significantly impacted by the presence of specific bacterial isolates, but it is not yet known whether this is due to avoidance of certain bacteria, or attraction to other bacteria. Ongoing studies include determining the fate of specific bacteria co-ingested with microplastics, via direct or indirect ingestion of microbeads. These studies also include investigating whether coral ingestion of microplastics is affected by an elevated temperature. Ultimately, these studies provide insight into the microbial aspect of the toxicological impact of microplastics in the marine environment, especially on the behavior of filter-feeding organisms.

this work was supported by RI INBRE

Localizing Key Bacterial Members of *Astrangia poculata*'s Microbiome and Monitoring the Changes in Taxa Diversity in Response to Holobiont Disturbance

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The facultatively symbiotic coral, *Astrangia poculata*, has a range from Buzzards Bay (RI/MA) to the Gulf of Mexico. In the northernmost population, *A. poculata* experiences seasonal temperature fluctuations from 2°C to 30°C, on average. Recent studies suggest that the *A. poculata* microbiome fluctuates according to season, but is still remarkably stable and predictable, compared to tropical corals. The highest levels of inter-colony variability in microbiome composition (beta diversity) occur in the winter months, and this is currently thought to be due to loss of regulation by the holobiont, which enters into quiescence in cold temperatures. In the spring months, as water temperatures warm, the microbiome recovers to a very predictable state that is dominated by a low number of prokaryotic taxa, including *Nitrosopumilus* sp., *Endozoicomonas* sp., *Amoebophilus* sp., and Roseobacterales. These taxa are also present in nearly all specimens that have been characterized for microbiome composition and are therefore candidate members of the *A. poculata* "core" microbiome. Based on our recent work, we hypothesize that the microbiome re-structuring in the spring is in part driven by antibacterial production by resident microbes in the *A. poculata* surface mucus layer (SML). The aim of the work proposed here was to design sequence-specific fluorescence *in situ* hybridization (FISH) probes to localize prokaryotic taxa of interest in wild symbiotic and aposymbiotic *A. poculata*, with a focus on previously identified candidate members of the *A. poculata* core microbiome and recently identified SML bacteria that inhibit growth of seawater microbes. Additionally, once localization protocols have been developed, they will be used to observe qualitative (and potentially quantitative) changes in taxa of interest in response to holobiont disturbance – for example, thermal stress, starvation, or changes in nutrient levels. FISH localization of different members of the *A. poculata* microbiome will provide new insight into microbe-microbe interactions in *A. poculata*, and their potential involvement in the response of *A. poculata* to disturbance.

this work was supported by RI INBRE

Determining How Echinoderms Eespond to a High CO_2 World

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Increased human activity has caused climate change to threaten the existence of a variety of species worldwide. For the ocean, future climate change conditions predict an increase in seawater temperature and acidity. The survival of organisms that rely on calcium carbonate ($CaCO_3$) is most at risk since this acidity will make it difficult for them to produce their calcareous body structures. *Psammechinus miliaris* (*P. miliaris*) is an Eastern Atlantic sea urchin which forms its test from $CaCO_3$. Although other studies have observed the effects of future climate change conditions on marine organisms, most have used short time frames (e.g. weeks) and focused on a single generation. Climate change is a process occurring across years and decades, and during this time animals will be developing and reproducing, therefore there is a need to look at how multiple generations respond. This internship examines this information gap by looking at how multi-generations of *P. miliaris* respond to present-day and year 2100 seawater CO_2 conditions. More specifically, this internship looks at gonad development across three generations of urchins derived from a 7 year study. It is crucial to understand how *P. miliaris* develops under climate change conditions as it is ecologically and commercially important.

this work was supported by RI C-AIM

Food Habits and Dietary Overlap Between Juvenile Cunner, Tautog, and Black Sea Bass in the Narragansett Bay Estuary (RI, USA)

Chloe Pearson[1], John Liberty[1], David Taylor[1], Lindsay Green-Gavrielidis[2], Niels Hobbs[2], Carol Thornber[2] & Giancarlo Cicchetti[3]

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The Narragansett Bay Estuary serves as a nursery habitat for cunner (*Tautogolabrus adspersus*), tautog (*Tautoga onitis*), and black sea bass (*Centropristis striata*). Due to their co-occurrence in the estuary and similar life history characteristics, there is the potential for dietary overlap and resource competition among the three species. In July-August 2018 and June 2019, juvenile cunner, tautog, and sea bass were collected using beach seines and traps at 24 sites in the Narragansett Bay. Fish were preserved in 70% ethanol (n = 51, 115, and 33 for cunner, tautog, and sea bass, respectively) and measured for total length (mm; TL) in the laboratory. Stomach content analysis was performed on each fish, and prey were identified to the lowest possible taxon. The overall contribution of prey to fish diet was determined by numeric percent, volumetric percent, and frequency of occurrence, from which an index of relative importance (%IRI) was calculated. The Schoener's index was also used to quantify dietary overlap among target species with respect to fish size: small (20-72 mm TL), medium (80-104 mm TL), and large (105-173 mm TL). Small cunner and tautog demonstrated significant dietary overlap ($\alpha = 0.84$), which was caused by their mutual reliance on copepods, amphipods, and crustaceans (cumulative %IRI = 93 and 90%, respectively). Medium cunner and tautog both consumed increasing amounts of algae (%IRI = 47 and 31%, respectively), although no significant dietary overlap was observed at this class size ($\alpha = 0.45$): medium cunner fed on copepods and crustaceans (cumulative %IRI = 37%), whereas equal-sized tautog continued to rely on amphipods (%IRI = 60%). Large cunner and tautog also demonstrated a significant overlap in diet ($\alpha = 0.74$) due to the high contributions of algae in stomach contents (%IRI = 91 and 65%, respectively). Finally, there were no differences in the diet between small sea bass and both cunner and tautog of the same size ($\alpha = 0.47$ and 0.33, respectively), though the sea bass similarly preyed on crustaceans and amphipods (cumulative %IRI = 87%). Overall, the results support dietary overlap between small cunner and tautog, after which dietary preferences diverged in medium-sized fish and re-converged in larger individuals. With respect to sea bass, there was no evidence of dietary overlap between this species and cunner or tautog.

this work was supported by RI STAC

Spatial and Temporal Variations in the Mercury Content of Narragansett Bay Sediments (RI, USA)

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Marine and estuarine sediments can provide a historical record of heavy metal contamination in the environment. In this study, stratigraphic profiles of sediment mercury concentrations were examined at four sites in the Narragansett Bay Estuary (Rhode Island, USA). Sediment cores were collected in May and June 2019 using a push piston corer, and in the laboratory, cores were sectioned at 2-cm increments. Sediments were then lyophilized and analyzed for total mercury content (Hg; ppm dry weight) and total organic carbon (TOC; % dry weight) using atomic absorption spectroscopy and loss-on-ignition, respectively. Overall sediment Hg concentrations and depth profiles varied across sites. The highest Hg concentrations were observed in the Providence River near Fields Point (maximum Hg = 2.02 ppm), followed by Greenwich Bay (0.85 ppm), Mt. Hope Bay (0.65 ppm), and mid-Bay, north of Conanicut Island (0.06 ppm). These patterns likely reflect each site's proximity to anthropogenic sources of Hg, as well as spatial variations in sediment TOC levels (range = 1.3 to 5.5%), i.e., Hg was observed to have a high affinity for organic material ($R2 = 0.574$; $p < 0.0001$). The depth of maximum Hg content also differed across sites (range = 1 to 51 cm), and this is attributed to site-specific sedimentation rates. Finally, sediment Hg concentrations were generally lowest in the deepest portions of the cores, coinciding with the pre-industrial period (< 1820s). In conclusion, the stratigraphic analysis of Hg and TOC in this study provided spatio-temporal insights into the history of Hg contamination in Rhode Island and surrounding waters.

The Design and Prototyping of the Multipurpose Autonomous Underwater Vehicle for Coastal Research

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As interests in the ocean and climate change have grown in recent years, the advancements in Autonomous Underwater Vehicles have made great progress and have made their way into many fields to adopt a wide range of military applications and oceanographic research in coastal and offshore waters. While any AUV's are very niche and expensive, we focused on designing the Multipurpose AUV (MAUVe) to be used in a vast range of applications. Beyond horizontal cruising, the MAUVe will be capable of hovering and vertical diving/climbing providing high-resolution water column measurements.

The MAUVe is designed to be less than 2 meters long with an outer diameter of 6 inches. The vehicle is designed to be one-man portable with a total weight less than 20 kilograms. In order to meet different mission requirements, the MAUVe is featured with two internal actuators, one linear mass slider and one mass rotator, to reposition the center of gravity relative to the center of buoyancy, and a pair of tilt-thrusters.

In this conference, we will focus on presenting the tilt-thruster design and prototyping. This unique tilt-thruster design features two through-housing magnetic couplings, folding propellers, and two adjustable NACA foils. This allows the thruster to reliably operate at a high RPM mitigating dynamic o-ring failure, propellers that are more resilient to obstacles and maintain a low profile when coasting. Currently, we are improving multiple aspects of the design, and thruster characterization is expected to be conducted in the coming Fall in the water tank at the department of Ocean Engineering. The MAUVe will primarily serve as a flexible and easy to reproduce research platform for future graduate students, post-doctoral studies, as well as commercial and military use.

MICROBIOLOGY

BEUPRE CENTER FOR CHEMICAL & FORENSIC SCIENCES
ROOM 130

ODD -NUMBERED POSTERS ARE TO BE PRESENTED FROM 9:30 – 11:00 AM
EVEN-NUMBERED POSTERS ARE TO BE PRESENTED FROM 11:00 AM – 12:30 PM

Overexpression of Yeast Bax Inhibitor (BXI1) Increases Cytosolic Calcium Levels

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The Bax Inhibitor (TMBIM) gene family has been linked to different cancers in human patients. Overexpression of the human Bax inhibitor gene, BI-1/TMBIM6 is known to drive the malignant phenotype of prostate cancer cells. Downregulation of BI-1 triggers apoptosis and tumor death suggesting that Bax inhibitor is anti-apoptotic in nature. The budding yeast, *Saccharomyces cerevisiae*, is known to have one TMBIM gene member that we have named BXI1. The protein is localized to the ER and vacuolar membranes. Studies in our lab suggest that it is a calcium channel when overexpressed in *E. coli* yet little is known about its endogenous function in yeast and how it inhibits the killing activity of Bax. To uncover its potential role in regulating calcium dynamics, we have measured the cytosolic calcium levels in yeast cells with loss-of-function and gain-of-function alleles of BXI1. Our preliminary data suggests that Bxi1p functions as a calcium channel in yeast that regulates cytosolic calcium levels.

this work was supported by RI INBRE

Measurement of Endoplasmic and Cytosolic pH in Bax Inhibitor Budding Yeast Mutants

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Bax Inhibitor proteins are pH-sensitive calcium channels that have been linked to a diverse range of human cancers. We have shown that yeast Bax Inhibitor (Bxi1p) is an ER localized calcium channel. By using a pH-sensitive GFP superfolder variant called pHluorin, we are monitoring the pH of both the endoplasmic reticulum and cytosol of living yeast cells, some of which carry Bax Inhibitor mutants including Δbxi1, Δcod1, and Δbxi1Δcod1. We are interrogating the change in pH of the endoplasmic reticulum and cytosol after the addition of calcium. Understanding the dynamic behavior of Bax Inhibitor in living cells will allow us to gain a better understanding for their importance.

this work was supported by RI INBRE

Detection of Bacterial Colonization and Endotoxin Content on Surgical Material and Tissue Allografts via a Rapid Visualization Assay

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Bacterial infection poses a serious problem in healthcare and the field of orthopedics. This challenge is especially problematic in implants and the insertion of fracture fixation devices, where infection rates can be as high as 28%.This high incidence rate of infections is magnified by the two million fracture-fixation devices that are inserted annually in the United States alone. In addition, these infections may lead to: extended hospital stays, increased cost, decreased quality of life, and the possibility of subsequent revision surgeries. Another potential complication is presented by endotoxin from gram-negative bacteria. Upon lysis of gram-negative bacteria, the lipopolysaccharide components of the cell wall are released as endotoxins, which are toxic substances that can cause harm to patients. Despite the significant problem with infections, current diagnostics like gram-staining, culturing, and PCR suffer from reliability, cost, length of time to diagnose, and efficiency.

This study evaluates a rapid visualization assay which employs fluorescent-conjugated antibodies and Confocal Laser Scanning Microscopy to detect the presence of bacteria and endotoxin on synovial fluid, tissues, surgical explants, and allografts in 30 minutes.

In an IRB-approved study, samples were collected by six orthopedic surgeons at Rhode Island Hospital, and were stored in 10% neutral buffered formalin. Synovial fluid samples were fixed on slides, while tissue samples and explants were analyzed in falcon tubes. All samples were stained with anti-LTA (lipoteichoic acid) antibodies conjugated to FITC488, labelling gram-positive bacterial cells green, and anti-LPS (lipopolysaccharide) antibodies conjugated to Dylight594, labelling gram-negative bacterial cells red. Images were obtained via Confocal Laser Scanning Microscopy, analyzed using ImageJ software, and compared to gram-staining images and the surgeon's clinical impression of the infection. This rapid visualization assay has the potential to be an effective diagnostic tool in the clinical setting due to its accuracy, low-cost, and ability to quickly identify the presence of bacteria.

this work was supported by RI INBRE

Hydrogen Peroxide Production During *Shewanella oneidensis* Aerobic Respiration Contributes to Death Phase Severity

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We are studying stress adaptation in the dissimilatory metal-reducing bacterium *Shewanella oneidensis*. We have observed that the fraction of surviving cells following death phase in batch cultures of *S. oneidensis* is inversely proportional to the starting nutrient concentration of the medium. Cell-free conditioned medium from post-death phase *S. oneidensis* cultures kills healthy exponentially growing S. oneidnesis cells. However, the ability of cell-free conditioned medium to kill cells rapidly disappears following harvest of the conditioned medium. Because oxidative stress responses are significantly induced during stationary phase in *S. oneidensis*, we hypothesized that the killing molecule in conditioned medium is hydrogen peroxide. We have found that hydrogen peroxide levels in *S. oneidensis* batch cultures are low during exponential phase and high during stationary phase and death phase. We are currently determining whether reducing the hydrogen peroxide levels in cell-free conditioned medium by enzymatic means mitigates the lethality of the conditioned medium. We are also testing whether boosting expression of katB, the *S. oneidensis* catalase gene, protects cells against the lethality of cell-free conditioned medium. Finally, we are determining whether the hydrogen peroxide concentration in cell-free conditioned medium is directly proportional to the starting nutrient concentration. Taken together, these experiments will reveal the extent to which hydrogen peroxide contributes to the severity of death phase in batch cultures of *S. oneidensis*.

Screening Environmental Samples for the Presence of Metal-Reducing Bacteria in the Genus *Shewanella*

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Dissimilatory metal reducing bacteria are capable of using extracellular metals as terminal electron acceptors during anaerobic growth. For example, *Shewanella oneidensis* strain MR-1 was isolated from Oneida Lake in New York based on its ability to reduce insoluble manganese dioxide (Mn(IV)O2) to soluble Mn(II). To determine whether environmental samples contain *Shewanella* species, we developed a polymerase chain reaction (PCR) assay that uses primers developed to amplify only 16S rRNA genes from the genus *Shewanella*. Our PCR assay is specific for *Shewanella*, as it amplifies 16S rRNA genes from *S. oneidensis*, but not from *Escherichia coli*. In addition we have found that this assay is sensitive enough to consistently produce a PCR product from ~10 *S. oneidensis* cells. We are currently using this PCR assay to screen a variety of sediment samples from Oneida Lake and aquatic sediments collected in Rhode Island. We are also developing and testing an anaerobic manganese dioxide reduction assay. We will use this assay to screen environmental samples that are positive for *Shewanella* 16S rRNA genes to determine whether bacteria in these samples are capable of reducing manganese dioxide. These experiments will allow us to begin cataloging the presence of metal reducing bacteria in Rhode Island.

Developing a Markerless Deletion System in *Haemophilus parainfluenzae*

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Haemophilus parainfluenzae is a facultatively anaerobic Gram negative bacteria found in abundance in human supragingival plaque. Microbiome sequence data indicates a positive correlation between the presence of *H. parainfluenzae* and *Streptococcus* spp. including hydrogen peroxide-producing *S. mitis*. However, our coculture experiments have shown that when in high abundance hydrogen peroxide produced by *S. mitis* can kill *H. parainfluenzae*. In most aerobic bacteria, catalase is the primary enzyme that is responsible for protection from hydrogen peroxide. Whilst *H. parainfluenzae* encodes and expresses catalase, its contribution to peroxide detoxification is minimal. We have demonstrated that single gene deletions for catalase and other known peroxide-detoxification genes do not render *H. parainfluenzae* sensitive to hydrogen peroxide. Thus, the likelihood of a single gene product being responsible for the breakdown of peroxide in *H. parainfluenzae* is unlikely. Our hypothesis is that *H. parainfluenzae* hydrogen peroxide resistance is a redundant system utilizing multiple gene products. To test this hypothesis a strain lacking multiple genes would need to be constructed. Our current methods have relied on allelic replacement with antibiotic resistant cassettes. However, due to the limited number of cassettes that function in *H. parainfluenzae*, the use of allelic exchange for this purpose is implausible. This work focuses on development and testing of a sucrose (SacB) counter-selection system that will enable the construction of markerless or “clean” deletions which circumvents the issues that arise from antibiotic cassette usage and facilitates rapid construction of multiple gene knockouts within a single strain.

this work was supported by RI INBRE

Recovery and Identification of Marine Microbes from Narragansett Bay and Assessment of Their Potential for Biofilm Formation

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The recovery of marine microbes in pure culture remains a challenge due to microorganisms being found in low abundance, not growing readily on standard growth media, and/or relying on interactions with other microbes for growth and viability. Studying the microbes found in Narragansett Bay will help us understand how microbial population vary in response to climate change and may lead to the identification of biomarkers for monitoring the health of our local marine ecosystem. The purpose of this study was to recover microbes in Narragansett Bay at various locations, to identify them at the genus level and to assess their ability to form biofilms. Water samples were collected from various locations around Narragansett Bay, microbes were recovered by filtration and centrifugation, and cultured on low-nutrient growth media. Recovered microbes were further subcultured to obtain pure isolates and subsequently identified through PCR and sequencing of the 16S rRNA region. The 60 isolates recovered belonged to 4 phyla and 17 genera. Pellicle formation was assessed for the 60 isolates representing 17 genera. Moderate pellicle formation was seen in 85% of isolates, while 3% of isolates showed strong pellicle formation. To date, biofilm formation has been tested for 20 isolates. Strong biofilm formation was seen in 45% of isolates, while 50% of isolates showed moderate biofilm formation. This project will extend to using the strongest biofilm producers to discover which types of materials and/or coatings are more resistant to biofouling and as such optimal for use in underwater sensors.

this work was supported by RI C-AIM

The Role of Flagella in Mediating Interactions Between *Salmonella enterica* and Red Leaf Lettuce

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Salmonella enterica is a gram-negative bacterium that is the causative agent of Salmonellosis, the leading cause of hospitalizations due to foodborne illnesses in the United States. The objective of this research was to determine the role of flagellar components in attachment, colonization, and persistence of *S. enterica* on red leaf lettuce. Flagellar genes fliC (flagellin, phase 1), fljB (flagellin, phase 2), flgK (flagellar hook gene), and fliB (flagellin methyltransferase) were targeted for deletion in several *S. enterica* serovars using Lambda Red homologous recombineering and the expression levels of these genes were determined using qPCR. The deletion of these genes is expected to lead to loss of flagellin expression (one or both phases) or methylation, which we hypothesize will impair attachment, colonization, and/or persistence on red leaf lettuce. Antibiotic resistance cassettes were successfully amplified for all genes and transformed into *S. enterica* serovars expressing lambda red proteins by way of electroporation and recombinants were selected on media containing antibiotics. To date, putative deletion mutants in fliB, fliC, and flgK have been obtained for *S.* Typhimurium. While we have obtained antibiotic resistant clones, suggesting recombination of the cassette onto the chromosome, PCR amplification of the target region has not yielded expected products. As a result, these mutations have not yet been confirmed. Analysis of expression levels of the target genes under a range of growth conditions is currently underway. Lettuce adherence assays show significantly higher levels of attachment when serovars are grown in media with lower salinity. By understanding the role of the flagellar components in plant-bacterium interactions, strategies can be developed to interfere with these interactions and subsequently decrease the frequency of Salmonellosis cases worldwide.

this work was supported by RI INBRE

Recovery and Identification of Marine Microbes from Narragansett Bay and Assessment of their Potential for Antibiotic Synthesis and Resistance

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Antibiotic resistance is a public health crisis. The discovery of new antibiotics is urgently needed, as multi-drug resistant pathogens continue to emerge. Bacterial antibiotic resistance occurs when bacteria themselves, not people or animals, become immune to the medicinal effects of the antibiotics. The ocean is an untapped reservoir of marine bacteria that have the potential to produce novel antibiotics effective against harmful pathogens. The goal of this study is to explore the antimicrobial-producing and antibiotic resistance properties of bacteria found in the Narragansett Bay, a semidiurnal estuary located in the north Atlantic waters of Rhode Island. Bacteria from 7 water samples collected from around the bay were isolated by filtration and cultured on low nutrient media, identified by 16S rRNA sequencing, and screened for their resistance to common antibiotics as well as cross-streaked to examine their antibiotic-producing capabilities. To date, 17 genera of bacteria belonging to 4 different phyla were isolated in pure culture. Isolates belonging to 7 of these genera were tested for antibiotic susceptibility on R2A and AIA plates using a disk diffusion assay with a total of 71.4% of the genera showing resistance to at least one antibiotic. Three isolates inhibited the growth of gram-negative bacteria suggesting possible antibiotic production. Future studies will continue to assess antibiotic production and further characterize strains showing either high levels of antibiotic resistance or production.

this work was supported by RI C-AIM

Role of Flagellin Methylation in *Salmonella enterica*

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Salmonella enterica is the leading cause of hospitalizations due to foodborne illnesses in the United States. It is an important environmental health issue that can lead to costly multistate outbreaks. Lettuce is part of a healthy diet however these leafy greens have increasingly been associated with a higher risk of food poisoning. The bacterial flagellum has been implicated in motility, adhesion to surfaces and invasion of host cells. Flagellin methylation has been detected in *S.* Typhimurium, but the biological role of this post translational modification is unknown.

The goal of this study is to determine the role flagellin methylation plays in a range of serovars. It is expected that methylation levels will correlate with surface hydrophobicity, flagellin methyl-transferase (fliB) expression levels and enhanced interactions with plant and host cells. Flagellins were purified and subjected to Western blotting with an anti-methyl-lysine antibody, while Microbial Adhesion to Hydrocarbon (MATH) assays were performed to measure surface hydrophobicity of the cells. Western blotting revealed that flagellin methylation occurs in serovars Agona, Enteriditis, Montevido and Senftenberg and is not confined to *S.* Typhimurium. Serovar Agona expressed the highest methylation levels per mg of protein while serovar Montevido expressed the lowest methylation levels. This correlates with low surface hydrophobicity in *S.* Montevido, but does not correlate with surface hydrophobicity in *S.* Agona. Future research will focus on comparing methylation levels under a range of growth conditions such as Lennox and Low Salt plates, and using qPCR to determine levels of fliB expression under these conditions.

this work was supported by RI INBRE

Role of Flagellin Methylation in Attachment of *S. enterica* to Plant Cell Walls

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Salmonella is a gram-negative, rod-shaped bacterium that causes a form of food poisoning called salmonellosis, which occurs in 1.2 million people each year in the United States. Outbreaks are increasingly being linked to fruits and vegetables, which are usually consumed raw and as such pose a higher risk of infection. It is known that *S.* Typhimurium methylate its flagellum, however, the exact role of this post-translational modification is unknown. The purpose of this research is to determine the role of flagellin methylation in attachment to plant cell walls. It is hypothesized that if flagellin methylation of *S. enterica* favors hydrophobic interactions with plant cell wall components, then these interactions will be impaired by deleting the methyltransferase gene (fliB). The fliB gene was targeted for deletion using Lambda Red recombineering, and the ability of wild-type and mutant strains to interact with red leaf lettuce was assessed. Analysis of pooled data from all serovars revealed that attachment to red leaf lettuce was better when cells were grown in Lennox agar plates compared to low-salt plates, but broth-grown cells did not attach differently in response to medium salinity. Conversely, colonization and persistence levels were not influenced by medium salinity. However, these trends mask the influence that medium salinity has on the ability of individual serovars to interact with the lettuce. In Lennox media at 37°C serovars Enteritidis and Javiana showed the highest levels of hydrophobicity in broth, while serovar Agona was the most hydrophobic on agar plates. In low-salt media at 37°C, all serovars except Agona showed equal levels of hydrophobicity in broth, while serovar Enteriditis was the most hydrophobic on agar plates. Future work will seek to correlate surface hydrophobicity to strength of interactions to red leaf lettuce and to confirm deletion of the fliB gene to further our understanding of methylation in plant-bacterium interactions.

this work was supported by RI INBRE

Sugar, Spice and a Glycan Slice: Investigating Changes to *Oxyrrhis marina* Surface N-Glycans During Prolonged Starvation.

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Oxyrrhis marina is a globally distributed heterotrophic dinoflagellate commonly found in marine and saltwater coastlines. Their cell surfaces are covered in N-linked polysaccharides (N- and O-linked). These carbohydrates are used by cells for communication, adhesion, and regulation. In a previous study with *O. marina*, changes to their neutral lipid content during starvation was observed, which ultimately led to curiosity of their surface glycan profiles. In this study, *O. marina* was used as a model to investigate the changes to the cell's surface N-glycans under saturation and starvation conditions. The *O. marina* cells in the saturated condition were actively fed with the prey Isochrysis Gambana, a common alga found in saltwater. The prey's surface glycan profiles were also analyzed. In order to label the surface carbohydrates, they first needed to be taken off the cell itself. The N-glycans were liberated via treatment with PNGaseF, purified by cold ethanol precipitation, and labeled with 2-aminobenzamide (2AB) followed by analysis by MALDI-TOF mass spectrometry. PNGaseF effectively liberated N-linked oligosaccharides from glycoproteins from intact cells. The cold ethanol precipitation allowed contaminating protein to be removed from the aqueous solution. Carbohydrates ionize poorly in mass spectrometry, labeling with 2AB provided a fluorescent label and ionizable group on the glycans through reductive amination. During active feeding on the cyanobacteria Isochrysis, the *O. marina* surface should present a specific set of N-linked glycans. No detectable Isochrysis N-glycans were observed in the satiated *O. marina*. Over a 3-week starvation period, a shift in the *O. marina* surface N-glycan profile occurred. Starvation conditions result in a unique array of carbohydrates. Our characterization of the *O. marina* surface provides opportunities for a greater understanding of cell signaling and communication in saturation and starved conditions.

this work was supported by RI C-AIM

Dechlorination of Organic Pollutants in Microcosms Amended with Cellulose and Chitin.

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Medium-Chain Chlorinated Paraffins (MCCPs) are industrial chemicals of increasing environmental concern. They came into wide use about 30 years ago as substitutes for the very persistent, toxic, and eventually banned polychlorinated biphenyls (PCBs). There is some evidence that MCCPs accumulate in animal tissues and aquatic sediments, but their distribution and environmental fate is not well described or understood.

It is known that aquatic sediments contain bacteria capable of breaking down chlorinated organics by a process called reductive dechlorination. These microbes, especially members of the Dehalococcoides genus, have been shown to break down other persistent chlorinated pollutants like PCBs, but as far as we know, nothing has been published on their action on MCCPs. If MCCPs were found to be effectuvely dechlorinated by natural bacteria, it would possibly open up new treatment technologies in the field to reduce the bioaccumulation and human exposure to MCCPs.

The experiment involves laboratory incubations of polluted or clean sediment with added MCCPs to understand whether and how bacteria break them down over a period of up to 6 months. Once the incubations are terminated, samples will be extracted and analyzed for MCCPs by gas chromatography coupled to mass spectrometry. In addition to MCCPs, some cultures receive nutrient chemicals (cellulose or chitin) to see whether the rate of breakdown can be accelerated. The abundance of Dehalococcoides and of a key dechlorination gene in the cultures at various time points will be estimated using real-time PCR, to understand how the microbial population respond to the various treatments, and whether the microbial changes explain the changes observed in the concentration of MCCPs. This work has important implications for the environmental chemistry and bioremediation of MCCPs.

this work was supported by RI INBRE

Spatial and Temporal Patterns of Free-Living Microbial Community Diversity in Narragansett Bay, RI

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Microbes play pivotal roles in marine ecosystems such as in biogeochemical cycling (i.e carbon and nitrogen cycles) along with animal and human health. Narragansett Bay is a well-mixed estuary that supports diverse habitats from salt marshes to shellfish reefs and is both ecologically and economically important. While many of the important processes occurring in the bay are mediated by microbes, little is known about the taxonomic composition or function of the microbial communities. Here, we apply 16S rRNA community profiling to examine the spatial and temporal patterns of microbial diversity in the surface waters throughout Narragansett Bay. The free-living fraction of microbes (0.22-5.0 micron) in the water column was sampled from seven sites across the mid and lower bay from September 2017 to August 2018. The V4 region of the 16S rRNA gene was targeted using Illumina MiSeq sequencing for the identification of community profiles. From this study, several major taxa were identified across all samples, including the Alphaproteobacteria, Gammaproteobacteria, Bacteroidetes, and Verrucomicrobia. Initial results indicate potential correlations of the alpha and beta community diversity with sampling months rather than sites. Although only one year of sampling has been completed, a potentially reproducible community structure (by season) through beta diversity analyses has been identified that calls for additional analysis. Ongoing analyses include the identification of abundant and variable taxa by month. For example, there is a significantly higher relative abundance of the genus *Vibrio* in the month of August. Understanding the identity of the microbes in Narragansett Bay is the first step in elucidating their functions in ecologically and economically relevant processes.

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Metabolic Modeling of a Novel *Mycoplasma* Identified from Eastern Oyster Microbiome

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Bacteria of the genus *Mycoplasma* are commonly known for having pathogenic or symbiotic relationships with their hosts. They generally have a relatively small genome that lacks pathways for the synthesis of essential biomass components. As a result, survival of the *Mycoplasma* species is dependent on the accessibility of rich nutrient resources from their environment. In our prior study, a specific *Mycoplasma* strain was identified from oyster gut samples, and its draft genome was assembled from metagenomic sequencing. Here, we construct a genome-scale metabolic model of this strain and use the model to provide insights into its potential roles in the gut microbiome of oyster. A draft model was first generated using ortholog mapping to the KEGG database. Manual curations were then performed using references from other *Mycoplasma* species and using sequence comparisons with annotated transporters from the Transporter Classification Database. The model was then used to identify a list of essential nutrients that would be required for growing the oyster-associated *Mycoplasma* strain. Overall, this project represents a first metabolic model of a bacterial species in the oyster gut microbiome. In the next step, we will attempt to isolate this bacterial strain from live oysters and continue to investigate the roles of this bacteria in oyster health and ecology.

this work was supported by RI C-AIM

MOLECULAR BIOLOGY

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EVEN-NUMBERED POSTERS ARE TO BE PRESENTED FROM 11:00 AM – 12:30 PM

39 and Me: Evolution of Dogs Through the Study of Genomics

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Darwin referred to artificial selection, such as that in dog breeds, to introduce the controversial concept of natural selection. Over the last few hundred years, humans have created breeds by selecting different traits, which has led to the great diversity in breeds today. Every time a new dog breed is created, a reduction in genetic variability results from only using a select group of individuals with the desirable traits, which frequently leads to the practice of inbreeding. In order to assess this reduction, we use powerful molecular tools to establish connections between physical traits and the genes that code for them. We focus on one specific breed, the hovawart, to better understand any mutations and genetic predispositions they may have developed in their evolution. In German folklore, the hovawart is claimed to be one of the oldest established breeds in European history. To test this claim, we want to determine the phylogenetic place of hovawarts among all other breeds. In addition, we aim to understand the evolutionary history of dogs through the use of 200 single nucleotide polymorphisms (SNPs) by contrasting the genetics traits of hovawarts against other breeds. We have close to 400 samples of canine DNA, including 350 hovawarts across Europe and North America. We also use DNA samples from other canid species, such as coyotes and foxes, as outgroups. To create a more robust collection of breeds, we have accessed large datasets from the literature representing the seven groups of dog breeds defined by the American Kennel Club. At the end of our study, we will be better at working with hovawart clubs from around the world and advising them on healthy breeding practices. Our data indicates which breeding pairs will help maintain overall genetic variability and avoid inbreeding. Ensuring genetic variability will help breeders maintain the traits that they desire, and more importantly, prevent passing on diseases. Many diseases are genetically inherited, and through our knowledge of the dogs' medical histories, we expect to prevent the passing of diseases over generations.

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Evaluating Divergence in the Polymorphic Haitian Freshwater Fish *Limia nigrofasciata* Using Mitochondrial and Nuclear Gene Sequences.

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Evaluating patterns of biodiversity is important for understanding variation within and among species, as well as underlying evolutionary mechanisms producing it. Biodiversity within the Caribbean is exceptionally high; estimates suggest that 75% to 95% of species across the Caribbean are endemic, unique to a single location. The complicated biogeographic history of the region is likely responsible for the creation of these biodiversity hotspots. Over the last several million years the Caribbean has been altered, shaped profoundly by sea level rise and fall caused by glaciation events and flooding, resulting in island fragments merging from the mainland to the Caribbean. Additionally, the region has a long history of tectonic movement, rearranging landmasses above and below sea level. A representative model of colonization, divergence, and speciation in the Caribbean are the small, freshwater fish in the genus *Limia*. *Limia* are distributed across the Caribbean, are highly speciose, with high incidence of endemism. On the island of Haiti, one putative species, *Limia nigrofasciata* from Lake Miragoane shows patterns of behavioral, ecological, and morphological variation that suggest it may represent a complex of species instead of a single polymorphic one. To evaluate if *L. nigrofasciata* represents a complex of species, we generated sequence data from eight captive bred fish representing two varieties of *L. nigrofasciata* (golden morph, and tiger morph). Sequences were compared among these eight samples other to evaluate levels of genetic similarity and divergence, as well as to previously generated sequences obtained from GeneBank, to reconstruct historical patterns of movement into Haiti.

this work was supported by the NIH

Assessing Physiological Plasticity in the Face of Climate Change in Natural and Lab Reared *Drosophila* from Thermally Stable Low Elevation Sites

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Climate change models predict increases in variability and shifts in temperatures likely resulting in thermal stress for organisms. Thermal strategies that have evolved under previous conditions will likely become unfit under future conditions. In order to elucidate mechanisms of adaptation to thermal stress, we investigated physiological plasticity and gene expression of pathways associated with heat shock proteins (hsps) in a variety of wild-caught *Drosophila* species and laboratory-reared populations of *Drosophila pseudoobscura*. All flies originated from low elevation thermally stable habitats (wild caught: San Diego, CA; lab-reared: Catalina Island, CA). Comparing the responses of wild caught and lab-reared flies will allow us to assess the effects produced by long-term captivity and reduced population size. Prior to experimentation, all flies were cultured in three developmental temperatures (18, 25, and 30°C) for at least two generations. We tested the minimum and maximum thermal limits in adult flies by ramping external temperatures until flies became unconscious. Following thermal testing, we evaluated the expression of genes associated with heat shock pathways using qPCR. We hypothesize that if species evolved under thermally stable conditions have lost plasticity associated with thermal stress; they will show a decrease in ability to activate thermally protective physiological and molecular mechanisms. If this is supported, we expect variability around the average minimum and maximum thermal limits to be low across developmental treatments, with little change in gene expression of hsp genes across all developmental temperatures. If small population sizes of cultures maintained in the laboratory and stock center affect genetic and epigenetic mechanisms of thermal tolerance, we expect to see a reduced range between thermal minimum and maximum, and very little change in gene expression across developmental temperatures. This research was conducted as part of a collaborative project (www.thermofly.org) and was supported by funding from NSF EPSCoR RII Track 2 FEC (1826689): From Genome to Phenome in a Stressful World: Epigenetic regulatory mechanisms mediating thermal plasticity in *Drosophila*.

Investigating Mechanisms of Physiological Plasticity in the Face of Climate Change: Assessing Natural and Lab Reared *Drosophila* from Thermally Variable High Elevation Sites

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Thermal stress has a substantial impact on organisms, affecting metabolic activity, fecundity, and life cycle thermal cues. Climate change models predict an increase in temperature variability, and understanding how organisms can respond will become important for predicting future biodiversity. In response to climate change organisms have three potential options; range shift, failure to adapt leading to death, or coping with and/or adapting to new conditions. Coping can occur through behavioral modifications, physiological plasticity, or evolutionary change. Organisms that are currently adapted to variable thermal regimes, such as those in habitats that experience seasonality and strong shifts in minimum and maximum annual temperature may have an evolutionary advantage in the face of increasing variability due to climate change. We investigated physiological plasticity in fruit flies (*Drosophila*) in order to determine the connection between genetic and epigenetic mechanisms to cope with thermal stress in relation to evolutionary background. We tested wild caught *Drosophila* species as well as lab-reared *D. pseudoobscura* to evaluate effects of long-term captivity and reduced population size. Flies were cultured at low, ideal, and warm temperatures (18, 25, and 30°C) for at least two generations, and then tested for minimum and maximum thermal limits. Following thermal testing expression of genes associated with heat shock pathways were assessed. We predict that, if an evolutionary background adapted to a thermally variable climate causes an increase in plasticity mechanisms then we expect variability around the minimum and maximum thermal limits to be high and a sizable increase in gene expression of heat shock proteins (hsp) across all developmental temperatures. This research was conducted as part of a collaborative project (www.thermofly.org) and was supported by funding from NSF EPSCOR RII Track 2 FEC (1826689): From Genome to Phenome in a Stressful World: Epigenetic regulatory mechanisms mediating thermal plasticity in *Drosophila*.

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Generation of a CRISPR-Activating System to Evaluate the Effect of Gene Overexpression on Yeast Life Span

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CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats) is a powerful tool that is used for gene editing. The CRISPR system is comprised of a protein component and a short guide RNA that can be generated to specifically target a genomic region of interest and create a double-strand break (DSB). CRISPR proteins can be modified to perform other functions, such as activating gene transcription, by deleting the nuclease domain of the CRISPR protein and fusing the protein to a strong transcriptional activator. Here, we create a CRISPR-activating system to overexpress genes in baker's yeast *Saccharomyces cerevisiae* using the CRISPR protein Cpf1 from lachnospiraceae bacteria. We cloned a codon-optimized nuclease-dead Cpf1 fused to the transcriptional activator VPR into a yeast expression vector. We also designed a guide RNA to bind 134 bp upstream of the SOD2 (superoxide dismutase) transcription start site, and expressed this from plasmid harboring a yeast RNA polIII promoter. This system will be used to investigate the role of SOD2 over-expression on the life span of yeast, and can be adapted to explore the effect of over-expression on other genes that may be important regulators of longevity.

this work was supported by RI INBRE

Conformational Heterogeneity of 4-Aminobiphenyl Bulky Adducts

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4-Aminobiphenyl (ABP) is a known bladder carcinogen which can form adducts with guanine nucleotides resulting in a bulky DNA lesion. These bulky lesions may interfere with DNA replication and damage repair depending on the conformation of the adduct and the flanking sequence of the modified nucleotide. ABP displays a conformational heterogeneity in which it can exist in the major groove (B conformation) or in a stacked (S conformation) motif. It is hypothesized that conformational heterogeneity will influence relative mutagenicity of the B and S conformers for the ABP lesion. The present study aims to measure the B/S conformational heterogeneity of the ABP adduct in one known mutagenic sequence in the p53 gene, 5'-CTTCCG1*G2ATTC-3'. Fluorine NMR (19 F-NMR) and circular dichroism (CD) were used to elucidate the conformation of the adducts. Isothermal titration calorimetry (ITC) was performed to explore thermodynamics of the adducted DNA strands and UV spectroscopy was used to measure the melting point of the modified duplex DNA.

this work was supported by RI INBRE

Conformational Heterogeneity may Affect Mutations with 4-ABP

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4-Aminobiphenyl (4-ABP) is a human bladder carcinogen to which humans are mainly exposed through cigarette smoke and direct contact with certain dyes. 4-ABP is known to modify DNA by forming adducts with guanine residues, resulting in a bulky lesion that may interfere with DNA replication and repair. These lesions can adopt two distinct conformations, where the adduct either populates the major groove (B) or is stacked within the DNA helix (S). This conformational heterogeneity, combined with differences in the flanking sequence around the modified base, may lead to differences in mutagenicity of the lesion. Herein, the proportion of B to S conformation and effects of the flanking sequences were determined in the non-mutagenic, '5-CCTTAG1*G2CCTC-3' 11 base-pair sequences of the p53 gene. This was done using circular dichroism (CD), Fluorine NMR spectroscopy (19F-NMR) with FABP, the fluorinated analog of 4-ABP, and isothermal titration calorimetry (ITC) and UV spectroscopy to determine the thermal stability of the modified DNA.

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Characterizing the Effect of N-Terminal Acetylation on Structure and Phase Separation of the RNA-Binding Protein FUS

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N-terminal acetylation, the most common post-translational modification (PTM) in mammals, alters the charge and interactions of the N-terminus of proteins. This PTM has been shown to promote helicity and protect from degradation, yet its effect is unknown for most proteins. FUS (Fused in Sarcoma) is a ubiquitous protein with roles in RNA metabolism and processing. FUS is physiologically acetylated at the N-terminus and is known to assemble via liquid-liquid phase separation (LLPS) into functional RNA granules and to aggregate into ALS-associated neuronal inclusions. Importantly, N-terminal tags are known to alter the behavior of FUS, yet N-terminal acetylation has been absent from previous *in vitro* studies using recombinant protein. Here we tested the effect of N-terminal acetylation on the structure, LLPS, and aggregation of the disordered, prion-like domain comprising the first 163 residues (FUS LC). Using NMR spectroscopy, we find that N-terminal acetylation has only a slight enhancement of helical structure and slowing of local motion of the first 16 residues of FUS LC. Conversely, we found that acetylated FUS LC phase separates more avidly than unacetylated FUS LC. This difference in LLPS may arise due to N-terminal acetylation removing the positively charged NH3 of the nearly uncharged (only 2 negatively charged residues, no positively charged residues) FUS LC. We also report our preliminary findings regarding the effect of N-terminal acetylation on FUS LC aggregation and the phase separation and aggregation of full-length FUS.

this work was supported by RI INBRE

Effects on Structure and Function of Spontaneous Peptidyltransferase Center Mutations in *Rhodothermus marinus*

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Rhodothermus marinus is a thermohalophilic bacterium isolated from submarine hot springs off the Reykanes peninsula in the Isafjardardjup Bay, Iceland. This bacterium has the ability to undergo cellular processes in conditions of extreme heat and high sodium concentrations, such as those present in its native habitat. Studying a bacterium's adaptations to extreme environments can be done by exposing a wild type strain to a variety of selective pressures, and isolating individual colonies that were able to undergo spontaneous mutant phenotypes. By comparing specific areas of interest, like the peptidyltransferase center (PTC), located in the large subunit of the ribosome, we can identify mutations that allow survival despite potential effects on growth. The PTC has one of the most highly conserved sequences across the board, in addition to remaining highly resilient to mutations, contributing to preserving ribosome function which is critical for cell survival. Because the PTC is an ideal target for antibiotics, isolating spontaneously arising *Rhodothermus marinus* antibiotic resistant mutants and analyzing mutations located in the genome where protein synthesis is controlled, we can understand more about the importance of the PTC, protein synthesis, and the benefit of adapting. Mutations were identified in the peptidyl transferase center of the ribosome in the rRNA of *R. marinus*, which provides more evidence that *R. marinus* may be an ideal model for the study of ribosomal mutations and their effect on structure and function.

this work was supported by RI INBRE

The Expression and Purification of N-Terminally Acetylated Ssa1 Chaperone from *Saccharomyces cerevisiae*

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Nα-terminal acetylation is an irreversible post-translational modification that adds complexity to the cellular proteome by constituting an acetylome wherein modified proteins exhibit altered dynamics and functions from their fundamental counterparts. Nα-terminal acetylation is catalyzed by a collection of N-terminal acetyltransferases (NATs), which transfer the acetyl group from acetyl-coenzyme A to the -amino group at N-terminal residues of the client protein. One target for Nα-terminal acetylation is Ssa1, a Hsp70 class molecular chaperone endogenous to yeast (*Saccharomyces cerevisiae*). Ssa1 works in tandem with a Hsp40 co-chaperone, either Ydj1 or Sis1, and a variety of nucleotide exchange factors to cycle through a transient, ATP-dependent mechanism to restore the native conformation of client proteins. The transient mechanism of Ssa1 prove limiting to *in vivo* studies, while *in vitro* and structural studies are limited as they require large starting volumes of highly pure chaperone, which often proves to be both a time-consuming and resource-intensive endeavor. We previously developed a one-step, native purification model for Ssa1 by adapting previous purification methods for our use. Here, we present our results which indicate that our method isolates high yields of pure, enzymatically-active Ssa1, allowing for further investigation regarding the effects of N-terminal acetylation on Ssa1 function. Additionally, we confirm that our purification method is capable of purifying both modified and unmodified Ssa1 and present preliminary evidence indicating that the acetylation status of Ssa1 may affect potential binding partners.

this work was supported by RI INBRE

The Effect of N-Terminal Acetylation on the Protein Tau

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The microtubule-associated protein tau (MAPT) plays a critical role in many neurodegenerative diseases such as Alzheimer's Disease. Tau functions to stabilize microtubule structures that are essential for transport within the neuron, and disruption of transport leads to a loss of neuronal function. Tau binding is regulated by phosphorylation, a post-translational modification (PTM) where a phosphate group is added by a specific kinase. Phosphorylated Tau blocks the microtubule binding sites, then phosphatases remove these phosphate groups which expose the microtubule-binding regions. Tau is also found in a hyper-phosphorylated state, which causes Tau to self-assemble into oligomers and higher order aggregates. It is clear that post-translational modification of Tau plays a key role in the dysregulation of neuronal function due to abnormal conformational changes, however not all PTMs are as thoroughly studied as phosphorylation. N-terminal acetylation is the most common PTM of all proteins and Tau is predicted to be a target of N-terminal acetylation. N-terminal acetylation is a co-translational process that is catalyzed by N-terminal Acetyltransferases (NATs), which adds an acetyl group to the N-terminus of a Tau polypeptide, thus neutralizing the positive charge on the N-terminus. Commonly, studies endogenously express Tau in a prokaryotic system that lack PTMs. Here we utilize a co-expression system in *E. coli* that allows us to purify Tau with a modified N-terminus. Purifying modified Tau with Fast Protein Liquid Chromatography (FPLC) will allow us to probe the structural and functional effects of N-terminal acetylation by assessing changes in Tau's aggregation and microtubule binding affinity. Furthermore, our studies of the N-terminus of Tau will provide insight into the role of the N-terminal projection domain and potentially provide new targets for therapeutical intervention in the treatment of Tau related diseases.

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Determining the Effects of N-Terminal Acetylation on Tau Phase Seperation Dynamics

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Neurodegenerative diseases such as Alzheimer's Disease and Parkinson's Disease have a distinct characteristic such that abnormal neurofibril tangles form within the brain. This is a result of the microtubule associated protein Tau, that is abundantly found within neurons, misfolding and aggregating with itself. Tau's primary purpose is to stabilize neuron microtubules. Many studies have focused on the post translational modification (PTM) of the C-terminus of Tau, in respects to its microtubule binding domain and its propensity to form aggregates within neurodegenerative diseases. Hyperphosphorylation of the microtubule binding region is a highly studied PTM of tau and is correlated to cause cell toxicity and aggregation. However, the structure and function of the N-terminus is still unknown, as it is often removed when investigating the effects of PTMs on Tau. The N-terminal domain lacks any structural elements and it is irreversible post translationally modified via N-terminal acetylation. We developed a purification scheme that will allow us to express and purify full length Tau and its first 100 amino acids, to see how N-terminal acetylation changes the secondary structure of the N-terminus. Future experiments will include liquid-liquid phase separations (LLPS) as it is driven largely by interactions between its negatively charged N-terminus and the positively charged middle and C-terminus regions. Once the purification and study of the effects on the N-terminus are refined, we can begin to look at ways to treat and slow Tau aggregation within patients suffering from neurodegenerative diseases.

this work was supported by RI INBRE

The Role of the FANCD2 Protein in Mitotic DNA Synthesis

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Fanconi anemia (FA) is a rare genetic disease characterized by birth defects, an increased risk for bone marrow failure, and cancer. Cells from FA patients are extremely sensitive to DNA damaging agents because of an inability to efficiently repair DNA damage. This disease is usually diagnosed in early childhood and is caused by mutations in one of the 22 genes that code for FA proteins. In previous studies, an important FA protein, FANCD2, was shown to be essential for the efficient replication of chromosomal fragile sites. Chromosomal fragile sites are regions of the genome that are particularly difficult to replicate and are hot spots for breakage and rearrangement in cancer. Replication of these sites can often proceed into early M-phase of the cell cycle - a phenomenon referred to as mitotic DNA synthesis or MiDAS. In this study, we have examined the importance of the FANCD2 protein in MiDAS. We have used short interfering RNA (siRNA) to deplete the FANCD2 protein from the U2OS osteosarcoma cell line. Western blotting was used to confirm the depletion of FANCD2 and examine levels of the mitotic markers H3 pS10 and CDC2 pY15. Cell cycle stage was also analyzed using flow cytometry and FlowJo software to determine the effects of FANCD2 depletion on M-phase progression.

this work was supported by RI INBRE

Analyzing the Impacts of Loss of the FANCA Protein on Chromatin State

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Fanconi Anemia (FA) is a rare human genetic disease, which occurs 1 in 160,000 individuals. Patients with FA have a high risk for organ malformations, bone marrow failure, and cancer. This disease is usually diagnosed during childhood and the treatment options for these patients are extremely limited. FA is caused by mutations in any one of 22 different genes. The FA genes encode for proteins that function in the repair of damaged DNA. Mutations in the FANCA gene are responsible for about 60 percent of all cases of FA. FANCA is an essential protein in the FA core complex which activates two proteins, FANCD2 and FANCI. This is done through the attachment of a small protein, ubiquitin. In this project, we analyzed the role of FANCA in chromatin plasticity by determining if the absence of FANCA impacts chromatin state. Previous studies have shown that FANCA associates with BRG1, a subunit of the SWI/SNF chromatin remodeling complex. The SWI/SNF complex restructures nucleosomes to make DNA accessible for transcription, translation, and DNA repair. It is possible that FANCA recruits this complex to facilitate chromatin remodeling during DNA repair. To study the role of FANCA in this process, we analyzed the levels of specific chromatin modifying enzymes and histones in cells from an FA-A patient and the same cells complemented with FANCA. We also performed micrococcal nuclease (MNase) assays in these cells to analyze the differences in nucleosome packing in the absence of FANCA. These studies will help further elucidate the physiological function of the FANCA protein.

this work was supported by RI INBRE

Phosphorylation of FANCD2 and ID2 by Cyclin Dependent Kinases

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Fanconi anemia (FA) is a rare genetic disease characterized by developmental abnormalities, bone marrow failure, and increased rates of leukemia. FA patient cells are extremely sensitive to DNA crosslinking agents because of a defect in DNA repair. There are 22 known FA genes and the proteins encoded by these genes function in the FA pathway to repair damaged DNA. The FA repair pathway is comprised of the main complex that recognizes the ICL, a ubiquitin ligase, a heterodimer, and additional proteins that help repair the DNA downstream. One of the core parts of this pathway is the ubiquitination of the heterodimer, which consists of two proteins, FANCI and FANCD2 (ID2). The ubiquitination of ID2 signals for additional proteins like nucleases and recombination enzymes, to finish the repair. We have identified several putative cyclin-dependent kinase (CDK) phosphorylation sites in the FANCD2 protein. The major goal of this research project was to determine if the FANCD2 protein is a CDK substrate. To test this, a CDK kinase assay was developed to detect the phosphorylation of purified protein. Two methods of detection were used, Pro-Q Diamond Phosphoprotein gel stain which stains any phosphorylated protein, and a pan phospho-CDK antibody which recognizes proteins phosphorylated by CDKs. The two methods were used in conjunction to test purified full length human ID2 and human FANCD2, as well as, Xenopus ID2. Our results suggest that both FANCD2 and FANCI are phosphorylated by CDK. In addition, our results indicate that FANCI may be preferentially phosphorylated over FANCD2 when the proteins are assembled in the ID2 heterodimer. This work provides important new molecular insight into the regulation of the FA DNA repair pathway.

this work was supported by RI INBRE

Molecular Phylogeny of Gregarine in the Narragansett Bay Area

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Gregarines are a group of unicellular eukaryotic parasites that inhabit the intestines of both aquatic and terrestrial invertebrates. They fall within the phylum Apicomplexa, which contains many highly pathogenic organisms including the causative agents of malaria and toxoplasmosis. Gregarines are known to infect many species of invertebrates, especially arthropods, annelids, and mollusks. They infect their hosts via an orofecal cycle; gregarines enter the host through the oral cavity as an oocyst, making filter feeders particularly susceptible to infection. Soon after, the oocyst bursts, releasing sporozoites. As obligate parasites, the sporozoites require the host to develop into their adult stage, the trophozoite, where they anchor themselves to intestinal host tissue and feed. These adults produce new oocysts which are then expelled by the host, perpetuating the cycle. The purpose of our study was to gain a better understanding of the diversity of the gregarine populations in Rhode Island. Here, we characterize the molecular phylogeny of gregarines by sequencing the 18S region in order to determine where Rhode Island gregarines fall within the clade.

this work was supported by RI INBRE

Biological Evaluation of 4-Aminobiphenyl DNA Adduct

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Environmental influence is culpable for the overwhelming majority of cancer developments. One of the most commonly understood sources of carcinogenic agents regularly exposed to the public are conceived from tobacco consumption. 4-aminobiphenyl (ABP) is a by-product of cigarette smoking linked to DNA damage and certain cancers. ABP has been determined to produce bulky lesions responsible for interference with tumor suppressor genes, DNA replication, and repair.

In this study, the mutational effects of ABP were further examined first through utilization of chemical synthesis and identification of modified oligonucleotides containing an APB adduct. Then analysis was accomplished by employing Competitive Replication and Adduct Bypass (CRAB) and Restriction Endonuclease and Post-labeling (REAP) assays which assessed resulting lesion bypass, mutational types and frequency.

this work was supported by RI INBRE

Using CRISPR/Cas9 to Identify Genes Required for Zebrafish Pigment Cell Migration and Stripe Formation

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Melanoma, the deadliest form of skin cancer, results from the neoplastic transformation of pigment cells called melanocytes. Melanocytes originate in a transient embryonic tissue called the neural crest. Neural crest cells are multipotent and differentiate into various cell types including neurons, pigment cells, glia and other derivatives. During embryonic development, pigment cells in zebrafish migrate along distinct migration pathways to arrive at their final destination, in dorsal, lateral, or ventral stripes. Metastatic melanoma cells share many characteristics with embryonic neural crest cells including invasive and migratory behaviors.

Using a CRISPR/Cas9 screen, the objective of our current study is to identify genes that are associated with pigment cell migration and pigment pattern formation during zebrafish embryonic development. In conjunction with the screen, we produced 28 gRNAs to knock out 14 genes. We injected the gRNAs along with Cas9 protein into 1-cell zebrafish embryos and used PCR to verify mutagenesis. To document possible mutations, we analyzed and imaged the phenotypes both 3 and 5 days post-fertilization. Our goal is to identify genes that are necessary to stop migration and promote differentiation of pigment cells in zebrafish embryos. By exploring and discovering such genes, we simultaneously build on the body of knowledge related to human pigment cells and their association with metastatic melanoma.

this work was supported by RI INBRE

Identification of CRISPR/Cas9 Induced Mutations in Zebrafish ALX Homeobox 4a

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Animal pigment patterns are recognizable traits that serve important ecological roles. The pigment cells that produce these patterns originate in the neural crest, an embryonic population of undifferentiated cells that gives rise to an array of different cell types such as neurons, pigment cells, and craniofacial cartilage. Embryonic neural crest development is a classic system for studying the processes of specification, morphogenesis, differentiation and patterning. Zebrafish, *Danio rerio*, have three different neural-crest derived pigment cells: Black melanophores, yellow xanthophores, and iridescent iridophores. Previous work suggested that at least some melanophores and iridophores are derived from bipotent precursors but the mechanisms of lineage specification are not well understood. Last summer, as part of an ongoing CRISPR/Cas9 genetic screen, we found that knockout of the gene ALX homeobox 4a (alx4a) caused a severe reduction in iridophores. We hypothesized that alx4a is required to repress melanophore fate and promote iridophore fate by repressing expression of the master regulator of melanocyte fate, mitfa. To further investigate the role of alx4a in iridophore development, we sought to isolate specific CRISPR/Cas9 induced mutations in alx4a. This summer, I identified two distinct mutations in exon 1 of the alx4a gene: an eight base pair insertion and a four base pair deletion. Using PCR and restriction digest analysis, I genotyped F1 progeny to identify carriers of either alx4aex1+8 or alx4aex1-4. Carriers of alx4aex1+8 were then intercrossed and pigmentation phenotypes were examined. The isolation and characterization of loss of function alx4a mutants will allow us to further explore the role of this gene in pigment cell fate specification and differentiation.

this work was supported by RI INBRE

Investigating the Control of Ribosomal Protein Expression in *Francisella tularensis*

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Francisella tularensis is the Gram-negative intracellular bacterial pathogen that causes the disease tularemia and is considered a potential bioweapon. The *F. tularensis* gene encoding the small ribosomal protein bS21-1, rpsU1, has been identified as critical for virulence, although it is not known why. Furthermore, the conditions that induce rpsU1 expression are unknown. In this study, our goal is to investigate how expression of the rpsu1 gene is controlled in *F. tularensis*. To monitor expression of rpsU1, we cloned and utilized an allelic exchange vector to synthesize a reporter strain incorporating the lacZ gene downstream of rpsU1 as a transcriptional fusion. The rpsU1-lacZ reporter strain allows us to use production of the protein encoded by lacZ, beta-galactosidase, as a proxy for rpsU1 transcription. Using our rpsU1-lacZ reporter strain, we can monitor the transcription of the rpsU1 gene by measuring the amount of beta-galactosidase in cells grown under different conditions to identify conditions that increase or decrease rpsU1 transcription. Specifically, we are investigating how temperature and genetic background influence transcription of rpsU1. These studies will help us understand the conditions under which the *F. tularensis* virulence factor encoded by rpsU1 is expressed.

this work was supported by RI INBRE

Developing an *in situ* Hybridization Method for the Identification of *Merocysits kathae* in the Atlantic Sea Scallop *Placopecten magellanicus*

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The Atlantic sea scallop (*Placopecten magellanicus*) is the basis of one of the most economically important fisheries in the US. Starting in 2004, adductor muscles from many of the fished scallops from Georges Bank in the North Atlantic appeared gray to brown with variable meat shrinkage, resulting in a an unmarketable produce and a tremendous loss of income. This "grey meat disease" has since been attributed to infection by an apicomplexan parasite (*Merocysits kathae*) previously identified as the cause for mortality in populations of the Icelandic scallops (*Chlamys islandica*). Using PCR methods, combined with histological evaluation of the adductor muscle, a proposed positive correlation between abundance of the parasite and muscle color and condition was not supported by the ADL. This project builds on the ADL data to further determine if a positive relationship exists between the quality of the adductor muscle and the number of *M. kathae* parasites by using PCR, histology, *in situ* hybridization and sequencing data. This work is important since other factors may be the proximal cause of poor meat quality, and not the parasite infection, thus resulting in a change in management methods of the scallop fishing grounds.

this work was supported by RI C-AIM

Effects of Maternal Opioid Usage on the Insilin and Leptin Signaling Pathways in F1 Offspring

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The opioid epidemic is a pressing issue in the United States, with approximately 1000 being treated daily for opioid use at emergency rooms. Another health concern in our country is also the high numbers of individuals with diabetes and or obesity. Both pathologies share common pathways that are deregulated contributing to their pathophsiology and therefore the effects of one on the other require investigation for potential treatment strategies applicable to both diseases. As a result of maternal opioid history and specific offspring obesogenic diets, the animals of a previous study (referred to as MORF1) developed metabolic dyshomeostasis. The current study aims to identify underlying mechanisms mediating the observed glucose dysregulation. We had not observed in these previous studies changes associated with body weight, but consistently noted increased levels of glucose in both the free fed state as well as the fasting state in TFFA1 females. We also noted alterations in insulin, corticosterone, and glucagon in these animals which would suggest deregulated endocrine receptor related pathways. Both the insulin and leptin receptor signaling pathway are important for glucoregulation and can be disrupted in the diabetic state; thus, we focused on examining Fox01, Insulin receptor substrate (IRS), JAK, STAT as these proteins comprise these pathways. Studies are utilizing hepatic tissue of MORF1 and control (SALF1; maternal history of saline exposure) to test the hypothesis that MORF1's fed a high fat diet demonstrate defects in the insulin and leptin pathways. The homogenized samples of liver were calibrated into standard curve in concentration with BCA assay to then be run through western blots to compare the concentrations of these proteins of interest between female F1 subjects maintained on a control, high sucrose, or high fat diet. Future studies will examine these pathways in male F1 offspring of the same treatment conditions. Collectively, findings will highlight endocrine mechanisms that are multigenerationally altered in response to opioids and its putative next-generation metabolic effects.

this work was supported by RI INBRE

Diet-Induced Changes in the Gluconeogenesis and Glycogenolysis Metabolic Pathways in F1 Offspring as a Result of Maternal Adolescent Opioid Exposure

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Metabolic Syndrome is an ongoing public health issue in the United States. Glucose dyshomeostasis can lead to features of metabolic syndrome such as obesity, insulin resistance, and type II Diabetes. These glucose impairments can increase the risk of other health issues such as cardiovascular disease. Previous studies have shown that maternal exposure to opioids can inflict adverse metabolic phenotypes in offspring unexposed to opioids in tandem with high fat diets. Such findings suggest that maternal opioid exposure may cause impaired fasting glucose levels across multiple generations. This study aims to identify the mechanisms by which glucose dysregulation is mediated in F1 offspring with a maternal adolescent opioid history. The gluconeogenic and glycogenolytic metabolic pathways are important for maintaining glucose homeostasis. Gluconeogenesis is the process by which glucose is generated by non-carbohydrate substances, and glycogenolysis is the process by which stored glycogen is broken down to glucose. Both processes contribute to maintaining glucose homeostasis in times of fasting via a series of protein interactions. In this study, we are focused on proteins that contribute to these metabolic pathways including PCK1, Glut4, PFKP, PFKB3, and Glycogen Synthase. Liver, pancreas, and brown adipose tissue samples from male and female F1 rats of a maternal morphine exposed background or maternal saline exposed background have been obtained. Rats of each background were fed either a standard control diet, high sucrose (70%) diet, or high fat (45%) diet. All tissue samples have been homogenized and protein lysates obtained; however, the liver is the focus of the current study. The BCA assay was utilized to determine total protein concentration in each sample. We are utilizing Western immunoblotting to probe for specific proteins within the gluconeogenesis and glycogenolysis metabolic pathways. Current studies have probed for several of these proteins in F1 female liver samples, while planned experiments will examine male liver samples to begin to understand potential sex-differences. Overall, results will add to our knowledge of the multigenerational effects of drug history by diet interaction in drug-naïve offspring.

this work was supported by RI INBRE

The Effects of Maternal Opioid Usage on the Glycolytic Pathway in F1 Offspring

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About 1⁄4 of the world population has a metabolic syndrome, with increasing diagnoses of type II diabetes mellitus (T2DM) and obesity in both developed and developing nations, making this a global health epidemic. However, the contributing factors to this metabolic dyshomeostasis are currently being investigated, including the inherited effects that may be induced from maternal environmental exposures. Over the past few years the US has seen an increase in drug over-dose deaths due to opioids, thus research is being conducted to examine the effect of opioids on not only the health of the user, but also how the usage of opioids may lead to a cascade of inherited metabolic dysregulation.

Proceeding previous findings, this study was designed to examine the metabolic modifications of F1 progeny as a result of maternal adolescent exposure to morphine or saline. The metabolic responses of these F1 progeny were comparatively analyzed based on their dietary conditions as well. Each F1 rat was fed either a control diet, a high sucrose diet (HSD) or a high fat diet (HFD). Hepatic transcriptome analysis of HFD-fed F1 males previously revealed significant dysregulations in transcriptional networks involved in cellular bioenergetics concurrent with weight gain and glucose dyshomeostasis. F1 females, also display impairments in glucose homeostasis; thus the current study sought to investigate whether defects in bioenergetics pathways contributed to the observed phenotype. Hepatic samples of these mice were homogenized, and protein concentration was measured via BCA analysis. SDS PAGE and Western Blots were made for female liver samples and then probed for specific proteins. The proteins of interest in this study were: the glucocorticoid receptor (GR), phosphoglucocorticoid receptor (pGR), phosphofructokinase platelet (PFKP), and Sirtuin-1 (Sirt1) were focused on in this study.

The results of this research will contribute to further studies examining the extent to which inherited metabolic dysregulation may occur from adolescent maternal opioid usage, and whether these effects are sex-linked.

this work was supported by RI INBRE

Implementing a Baculovirus Expression System to Optimize the Solubility of DNA Polymerase Theta

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DNA polymerase Theta is a low fidelity enzyme involved in DNA repair. Even though it is error-prone, little is known about the selection process it uses during nucleotide incorporation. Understanding the mechanism is critical for understanding how to prevent cancer which is thought to occur at the DNA level. To elucidate this mechanism, we have sought to develop a FRET labeling system similar to others developed for DNA polymerases that monitors the conformation dynamics between the enzyme and the DNA substrate. Understanding these dynamics is important as it is suggested that nucleotide selection and genome fidelity is influenced by these movements. The challenge is to label the enzyme in such a way to maximize FRET and ensure these movements are captured during a rapid chemical event. Previous labeling studies using enzymatic means were not site specific enough to monitor this mechanism. To ensure direct labeling we changed our approach to implement thiol labeling, a more site specific labeling technique which utilizes conjugation to a cysteine. Due to the complexity of Pol θ, 16 out of 17 endogenous cysteines needed to be mutated to serines to allow for only one solvent exposed reactive group. Preliminary solubility studies in *E. coli* suggest these mutations are not viable. In order to improve solubility, we have altered our strategy for expression in insect cells. This process includes various molecular biology techniques to create appropriate expression vectors for full length POLQ, the serine mutated truncated POLQ (SQM1), and its control, truncated POLQ (QM1) as well as optimization of expression conditions. Future studies will include DNA polymerase assays of serine mutated Pol θ compared to WT to ensure proper structure and function.

this work was supported by RI INBRE

Investigating the Role of Human MRE11 in Cancer

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UV radiation and toxic chemicals induce DNA breaks which have detrimental effects on genomic stability, potentially leading to cancer and other genetic diseases. To combat this, cells utilize several DNA repair enzymes. This project focuses on MRE11, which is a nuclease that detects DNA alterations and recruits other repair proteins. A recent screening from breast and lymphoid cancer patients identified S104C mutation in the conserved region in MRE11. Overexpression of S104C in MCF7 cells suggests hypersensitivity to carcinogens. We hypothesize that this mutation alters Mre11 activity. Thus, we will express and purify S104C Mre11 in insect cell to investigate a potential biomarker for cancer.

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Cancer-Associated Variants of DNA Polymerase Theta Display Altered Bypass Activity on Damaged DNA

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DNA Polymerase theta (POLQ) belongs to the proofreading-deficient A-family of DNA polymerases that are involved in DNA replication and repair. Pol theta is primarily involved in double stranded DNA repair but has also been shown to have translesion synthesis capabilities to bypass damaged DNA. Recent evidence suggests, while error prone, bypass promotes replication and protects against unchecked cell growth, especially against UV damage and skin cancer. We have identified, through a collaboration with the Tissue Research Core of the Yale SPORE in Skin Cancer, several variants of POLQ from patients with significant sun-exposed related tumors. We hypothesize that these variants are unable to bypass damaged DNA and thus lack the cancer protection that might occur from a normal functioning Pol theta. In this project, we have expressed and purified the patient-derived variants from *E. coli* and assessed the bypass activity compared to wild-type Pol theta. Our preliminary results suggest that the variants have altered bypass activity and that UV-damaged repair may be hindered in patients expressing these variants. From these experiments we aim to identify potential biomarkers for melanoma and improve early detection of disease.

this work was supported by RI INBRE & the RI Project SEED program

Heat Shock Physiology: Measuring the Metabolic Impacts of Thermal Stress in *Drosophila melanogaster*

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Organisms live in thermally dynamic environments and have evolved mechanisms to survive acute thermal stress. These include behavioral responses such as movements toward preferred temperatures and postural modification and molecular mechanisms such as biochemical adaptation and expression of heat shock proteins which act as chaperones to protect the three dimensional structure and function of critical cellular machinery. These responses have consequences for organismal fitness and evolutionary adaptation, but what are the costs of the stress response? We hypothesized that the energetic costs of resilience to acute thermal stress are detectable as elevations above resting metabolic rate and are likely to persist long after the initial exposure and recovery. Using one-hour long hot and cold temperature shocks, we measured the metabolic rates of adult male and female *Drosophila melanogaster* in a controlled and repeated measures experimental design. This research was conducted as part of a collaborative project (www.thermofly.org) and was supported by funding from NSF EPSCOR RII Track 2 FEC (1826689): From Genome to Phenome in a Stressful World: Epigenetic regulatory mechanisms mediating thermal plasticity in *Drosophila*.

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Designing a Graphical User Interface for the Modeling of Metabolic Networks

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Science is continuously producing new tools and technologies that can help improve the future of society. In order for these tools to have an effective impact, it is important to make sure they are accessible and easy to understand for all potential users. Graphical user interfaces (GUIs) work as a bridge between a scientists highly detailed application and a user with little knowledge of the app's inner workings. The goal of this project was to create a graphical user interface for the PSAMM application programming interface (API). PSAMM is an open-source software package that performs analyses on metabolic models. It provides a trackable platform that supports collaborative model curations and integrates mathematical simulations with network topology analysis. Previously PSAMM was released as a command-line program, which limits its accessibility to a broader user community. Our development of the new GUI system allows the user to access all functions in PSAMM by pressing a few buttons and inputting a few words. The system also allows the user to easily upload their own models from anywhere in their local computer and download the results of the PSAMM analyses. While previously a user needed to run extra help commands to find what functions and arguments were available, all options are clearly laid out in the GUI for the user to see and use. The PSAMM GUI was built using the Shiny package in R, and it supports certain JavaScripts and popup messages using the shinyjs and shinyalert packages. The GUI has a clean design and contains detailed instructions on how to use each PSAMM function to limit confusion for users. To prevent errors, default values are provided for text inputs that were problematic when left null and buttons that require prerequisite inputs are deactivated until inputs are set by the user. The PSAMM GUI is accessible to anyone on the internet, and hence it provides a generic platform for the analysis of metabolic models. By making PSAMM easy to obtain and use, scientists can perform metabolic model analyses more quickly and therefore reach new findings sooner.

Link to the PSAMM app:
https://keshiny.itrcs.uri.edu/psamm-sierra/

this work was supported by RI INBRE

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PMSF Reduces α-Synuclein Aggregation in Yeast Modeling Parkinson's Disease

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Parkinson's Disease, PD, is the second most common neurodegenerative disease in humans. PD is marked by Lewy body formation in the brain, which disturbs the dopamine transfer system across neurons. Previous studies have shown that the protein, α-Synuclein, is a major contributor in the formation of Lewy bodies. In this study, we modeled α-Synuclein aggregation in the Budding Yeast, *Saccharomyces cerevisiae* and treated the cells with Phenylmethylsulfonyl fluoride (PMSF) to see how this chemical would affect aggregation, while also monitoring the health of the yeast. Our Preliminary data has suggested that a 4mM concentration of PMSF significantly reduces protein aggregation. Our lab will continue to investigate the role of PMSF in the prevention and breakdown of α-Synuclein aggregates.

this work was supported by RI INBRE

Tracking Neuropeptide Release In the Cold Stress Circuit of *Drosophila melanogaster*

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All organisms are exposed to environmental stressors throughout their life, making mechanisms to combat stress essential for survival. The nervous system plays a critical role in stress response by integrating multiple sensory cues and altering physiology to maintain homeostasis, however, the exact mechanisms by which these circuits function remain poorly described. In this study, I used a novel neural circuit underlying acute cold stress in *Drosophila melanogaster* to elucidate mechanisms of neural stress response circuits. Preliminary work implicated two neuropeptides, corazonin and tachykinin, as signaling molecules required to mediate physiological response to cold stress, but the critical downstream target tissues remain unknown. I surveyed candidate downstream target tissues of the cold tolerance circuit using cell-specific knockdown of corazonin and tachykinin receptors and evaluated the cold tolerance abilities of the resulting animals. Identification of the downstream tissue types that mediate physiological responses to stress is the first step in identifying universal signaling mechanisms used by the nervous system to maintain homeostasis in an ever changing environment.

this work was supported by RI INBRE

The Mushroom Body's Role in Metabolic Stress

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All organisms are confronted with stress throughout their lives, as stress can be defined as anything that disrupts homeostatic conditions. Studies have shown that neurons respond in many ways to stress, including changes in expression of molecules such as microRNAs and transcription factors. I examined neural circuit mechanisms that govern systematic physiological response to stress using a novel acute cold stress response circuit in *Drosophila melanogaster* as a model system. The Mushroom Body (MB) of the *Drosophila* brain, specifically the α'β' lobes, has previously been found to regulate acute cold stress. I hypothesize that the prime lobes of the mushroom body function more broadly than originally thought and act as a general stress regulator. This hypothesis was tested by inactivating neurons from the previously described cold tolerance circuit then assessing response to stressors. Inactivation of the α'β' lobes resulted in a significant decrease in survival time under starvation conditions, suggesting that this brain region also plays a role in mediating metabolic stress. Inactivation of either the αβ lobes or MBON showed no significant difference in metabolic stress response. Based on the data collected, the MB, specifically the α'β' lobes, may have a role in metabolic stress, suggesting that the mushroom body prime lobes act as a central stress regulator. These findings will provide insight into the modulation of neural circuits during stress response through a better understanding of the mediation of metabolic stress in *Drosophila*.

this work was supported by RI INBRE

The Role of Corazonin and Tachykinin Neuropeptides in the Physiological Response to Cold Stress in *Drosophila melanogaster*

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All organisms are exposed to environmental stressors including temperature, chemical exposure and starvation. Stress exposure has acute effects on physiology, which disrupt homeostasis and can lead to death. Studies show the importance of neural circuits in the modulation of physiology in response to environmental stressors, but the mechanisms underlying the function of these circuits remain poorly understood. I used a novel neural circuit underlying acute cold stress response in *Drosophila melanogaster* to probe signaling mechanisms underlying thermal stress response. Circuit activation leads to systemic neuropeptide signaling through the release of two neuropeptides, tachykinin (tk) and corazonin (crz). Though neuropeptides have been shown to modulate physiology in response to acute cold exposure, the downstream target tissues remain unknown. In this study, I used cell-specific RNAi to knockdown expression of corazonin and tachykinin receptors in target tissues and used behavioral assays to determine the role of each tissue in thermal stress response. By learning more about the specific tissues involved in the cold tolerance response, we can better understand how neural circuits induce physiological responses to cold stress.

this work was supported by RI INBRE

Effects of PFOS on Neuronal Development of *C. elegans*

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Perfluorooctane Sulfnate (PFOS) is a man-made toxin that was used to manufacture many consumer products, resulting in widespread human exposure. Current studies have demonstrated that PFOS is able to cross the blood-brain barrier, increasing concerns about potential side effects from neurotoxicity. Studies have shown detrimental effects of PFOS exposure on the nervous system, including altered brain development and neurobehavioral defects in mice, though the underlying mechanisms of these neurological defects have yet to be elucidated. In this study, we aim to develop a model system to study molecular mechanisms of PFOS exposure in the nervous system using the well-defined *C. elegans* motor system. *C. elegans* is an ideal model system for these studies due to a well-defined nervous system, ease of genetic manipulation and high level of gene homology with humans. We characterized changes in nervous system development following preconception exposure by tracking neuronal morphology and outgrowth throughout the lifespan of the worm. This morphological approach was complemented by a functional assessment of motor system function at each developmental stage using worm tracker software. This initial characterization is the first step in establishing a genetically tractable model in which we can determine the molecular mechanisms by which PFOS is able to alter the nervous system.

this work was supported by the NIH

Daily Negative Experiences and Behavioral and Physical Health Among College Students

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Past research conducted predominately in laboratory settings or using self-report has suggested that how individuals handle stress emotionally and physically has implications for behavioral and physical health during adolescence and emerging adulthood (Powers *et al.*, 2016, Allwood *et al.*, 2011). Yet we know little about how these daily stressors affect youth outside of the laboratory context in their daily lives limiting the ecological validity of findings. A particularly effective way to increase ecological validity and capture associations among daily stressors and behavioral health outcomes is the use of daily diary approaches for collecting data. Daily diary approaches are a class of methodologies for examining everyday experience and typically involve participants self-collecting data across several days in their daily lives (Laurenceau & Bolger, 2005). To date few studies have examined the associations among stressful experiences and health outcomes in college students using a daily diary approach. Given the dearth of research on this important topic, this study will consider the effect of daily stress on health outcomes. Participants consisted of 30 friendship dyads (60 youth total; 18-20 years old) who were predominately female and Caucasian. A daily diary approach was used across three days and involved participants self-collecting morning and evening surveys sent via text messaging, as well as cortisol samples taken 4 times across the day to capture cortisol upon awakening and diurnal variation in cortisol. To assess stressful events participants reported on the Daily Stress Events Checklist, which consisted of a list of 19 items that late adolescents might experience throughout the day. Participants also self-reported on 7 different risk behaviors (e.g., I broke rules) and physical health outcomes (e.g., headache). Saliva Samples were collected to assess diurnal variation in cortisol response. Preliminary analyses using hierarchical linear modeling was used to examine the effect of daily stressful events on health outcomes. Results indicated that on days that participants reported more stressful events they were also more likely to report negative health outcomes. Cortisol is still being analyzed and will be examined as an additional outcome once those results are available. Taken together findings highlight the importance of considering how stressful events in late adolescence may be an important contributor to health outcomes.

this work was supported by RI INBRE

TAG-1 Controls Motor Neuron Wiring During Embryonic Development

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Spinal motor neurons signal effector muscles to contract, initiating movement. During embryonic development, motor neuron cell bodies must remain confined within the central nervous system, while motor axons extend into the peripheral nervous system. Motor neurons are divided into unique motor column populations. The medial motor column sends axons to the epaxial muscles, whereas the lateral motor column innervates the limbs. Neurons belonging to these distinct populations can be distinguished based on their molecular profile and position within the spinal cord ventral horn. One of the molecules that is essential for the establishment of motor neuron cell body localization and axonal trajectory is transient axonal glycoprotein type 1 (TAG-1), a cell surface protein expressed by motor neurons. In the absence of TAG-1, motor neuron cell bodies aberrantly exit the spinal cord, and motor axons are defasciculated and misguided. These defects are observed in early embryonic TAG-1 knockout mice, but whether these defects persist into later developmental stages, including postnatal life, is unknown. To start to test this, we conducted immunohistochemistry for various motor neuron markers at early and late embryonic stages of development. We found that subsets of these molecules are expressed throughout development, while others are lost towards the end of embryogenesis. We thereby identified markers that can be used to indicate motor neuron defects in postnatal TAG-1-/- mice. We are currently carrying out additional analyses to determine which, if any, of the TAG-1 knockout mice phenotypes persist past birth.

this work was supported by RI INBRE

Erector Muscles of the Spiny Dorsal Fin Show Higher Activity Under Turbulence Even When a Neuromuscular Blocking Agent is Injected in Bluegill Sunfish

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Fish are an extremely diverse taxonomic group and thus have multiple key innovations that can be explored to find bio-inspired designs to improve human health. In this experiment, turbulent and non-turbulent experimental treatments were applied to detect muscle activity in bluegill sunfish *Lepomis macrochirus* when gallamine triethiodide (Flaxedil), a non-depolarizing neuromuscular blocking agent or muscle relaxer, is injected directly into the dorsal fin muscles. We expect that when motor control is compromised, fish exposed to an agitation such as turbulence will show no activity in the spiny dorsal fin erector muscles and the fin will not erect. However, activity from other fins will be present and the fish will recover the initial heading with a higher latency. We quantified fin erector muscle activity using electromyographic recordings and high-speed video of dorsal and lateral views. Despite the application of a muscle relaxant, lateral video kinematic data from our experiments shows that the spiny dorsal fin erector muscles are responsible for moving the fin up, as onset and offset of muscles coincided with changes in fin height. Our electromyography results show higher magnitude of muscle contraction during turbulence closer to the cranial portion of the spiny dorsal fin. Other muscle mechanic properties (e.g. frequency and duration of contraction) were kept constant under turbulence and non turbulence conditions. This can be due to the possibility that the muscle contraction starts more cranial than our flaxedil injections or that flaxedil concentration and volume are not sufficient. Studying how fish maintain their stability under turbulent forces will advance the understanding of neuromuscular control in the spiny dorsal fin of these fish and can be used as a proxy for human prosthetics.

this work was supported by RI INBRE

Getting a Sense: Effect of a Sensory Nerve Inhibitor on Dorsal Fin Spines of Bluegill Sunfish, *Lepomis macrochirus*, Under Turbulence

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In derived bony fishes the dorsal fin is in part composed of spiny fin rays that depress and erect in response to perturbation and are essential for fish to recover stability. These collapsible spiny fin rays can be used as a proxy for other hinge joints in humans, like elbows and knees. To assess motor control of the spiny dorsal fin under induced perturbations, muscles at the base of the spiny fins of bluegill sunfish, *Lepomis macrochirus*, were injected with Lidocaine, a sensory nerve inhibitor. Muscle activity was then recorded and analyzed via electromyography. Data obtained from the electromyographic recordings were analyzed to determine duration, intensity, magnitude, cycle duration, and frequency. These were then compared across four different locations associated with the spiny dorsal fin on both left and right sides — epaxial under the dorsal fin and spiny erectors from 3rd, 5th and 7th dorsal fin spines — to look for statistically significant differences in function in the presence or absence of turbulence. At the same time videos were collected and analyzed to measure body posture, fin movements, center of mass displacement, and the time it took the fish to recover stability when faced with turbulence. Erratic behavior shown by the fish when faced with turbulence suggests inability to effectively control the dorsal fin as a result of blocking the nerves from receiving sensory information from the water, thus making it harder to recover stability. Removing afferent information, using Lidocaine, appears to prevent the fish from sensing turbulence in its surroundings. Data obtained from electromyographic recordings shows similar muscle contraction parameters with and without turbulence. Thus, we can conclude that when sensory information is removed the fish is unable or unwilling to erect the spiny dorsal fin to recover stability, leading to erratic behavior.

this work was supported by RI INBRE

The Hidden Path to Understanding: a Nerve Roadmap of the Bluegill Sunfish Fins (*Lepomis macrochirus*)

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Little is known about the function of spiny fins in fish under unsteady conditions. We aim to understand how muscles that drive stability receive motor information, efferent innervation, and how the fins detect perturbations, afferent innervation. This study utilized histological staining methods to identify the location of nerves in the bluegill sunfish (*Lepomis macrochirus*). The pectoral fin and spiny dorsal fin were stained with either Sudan Black B, Luxol Fast Blue, or Cresyl Violet to visualize nerve pathways. Our study also investigated muscle activation of the dorsal fin muscles under turbulence and no turbulence. As a control to treatments that inhibit afferent and efferent function, fish were injected with a saline solution. Muscle activity was recorded using electromyography and the duration and magnitude of bursts were analyzed. We hypothesized that muscle recruitment and burst duration would be higher during turbulence. The best staining protocol was Cresyl Violet, which stained the nissl bodies of neurons purple. Sudan Black B, a myelin stain, was also effective at staining nerves black. Although over and understaining were common, slight protocol modifications improved staining visibility of nerves. Histology of the pectoral fins showed branching innervation that derived from the spinal cord and continued to the base of each pectoral fin, before branching out to individual fin rays. Muscle innervation of the spiny dorsal fins was not as clear, but it appears similar with branching from the epaxial to all the fin rays. In terms of muscle function, duration of muscle activity near the right 5th spiny erector muscle was higher during turbulence than in no turbulence, although other muscles did not show significant differences (p-value > 0.05) between the two treatments. Further research is needed to assess how dorsal fin muscles contribute to stability under different conditions and stimuli, as well as how innervation controls muscle behavior. A better understanding of connectomes, i.e. a complete map of neural connections, will help scientists understand the integration of appendages as a part of electrophysiological control processes and has greater implications for human applications.

this work was supported by RI INBRE

Changes in Glycinergic Interneuron Morphology from Spinal Cords of SOD1 Mouse Model of ALS

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Many studies have examined vulnerable motoneurons in amyotrophic lateral sclerosis (ALS), but few studies focus on the premotor interneurons that drive (or inhibit) motoneuron activity. This study assessed inhibitory interneurons from SOD1G93A x GlyT2 eGFP mice well before symptom onset (postnatal day 5-11) using cell counting and anatomical reconstructions. Cells were counted by a blind researcher using Stereo Investigator software, and were then anatomically reconstructed using Neurolucida 360. All data was analyzed using SPSS statistics software running MANOVA, with significance at $p \leq 0.05$. Hundreds of interneurons from 16 animals (n=16) were reconstructed, and the results show no significant decrease in area and cell body length in the spinal cord of SOD1 animals (n=6) when compared to control animals (n=10). This indicates that SOD1 ALS mice do not yet exhibit significant changes in interneuron morphology in ages p5-11.

this work was supported by RI INBRE

Temporal and Spectral Analysis of EEG Signals for BCI Applications

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Introduction

Brain-computer Interfaces (BCIs) are communication devices that allow a user to control computer applications through their brain activity. Non-invasive BCIs use various types of brain signals, such as the P300-based event-related potentials (ERP) or motor imagery (MI)-based event related desynchronizations (ERD) and synchronization (ERS), from scalp electroencephalograms (EEG). These unique and "large" signals are often buried due to several factors including volume conduction effect of the skull and background noises such as eye blinks, environmental noise, etc. Using signal analysis techniques, we are able to clean the signal and highlight the activity of interest for an optimal BCI use. In this SURF project, EEG data related to two main tasks that elicit ERPs and ERD/ERS were analyzed.

Methods and results:

In the first dataset, to acquire the P300 based ERP, a 6x6 Speller matrix with a standard 64 electrode montage was used. The temporal analysis was then applied to extract the ERP segments. We observed a positive increase in the amplitude of brain signals around 200 and 300 ms after the stimulus was presented. In the topographical maps of the brain, this increase was mainly particular to the parietal lobes.

In the second dataset, for the ERD a One-dimensional Sensorimotor Rhythm (SMR) cursor control task was given, with a 13 electrode montage. To extract the frequency bands of Mu (8-12 Hz) and Beta (18-25 Hz), an 80 point fast Fourier transform (FFT) was applied.Three different spatial filters were used: Common Average Reference (CAR), Small Laplacian (SL), and Large Laplacian (LL) to localize the signal over the correct physiological location from which it originates. For the CAR filter, there was a distinct concentration of the ERD surrounding the channels located over the sensorimotor cortex in comparison to the unfiltered signals that were referenced to the right ear. The LL showed to be better at localizing the signal than the SL because it had averages coming from over a larger area of the cortex and could "see" the ERD better.

Conclusion

Selecting optimal activities through various types of signal processing techniques such as spatial filters is essential for providing practical BCI systems.

this work was supported by RI INBRE

Creating Permanent and Temporary Lesions to the Rat Posterior Parietal Cortex

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Previous studies have examined the posterior parietal cortex (PPC) as a multimodal hub using permanent lesioning techniques. However, when attempting to lesion the PPC as a whole, researchers have generally only managed to lesion the dorsal portion of the PPC (dPPC) without lesioning the caudal portion (cPPC). This study aimed to refine and improve the methods for successful targeting and lesioning the entire PPC. In two pilot lesions, we successfully created permanent excitotoxic lesions to the entire PPC using NMDA. Quantification of lesion extent in 19 subsequent surgeries (10 lesion; 9 sham), however, showed that like previous researchers, we were not able to target the cPPC. We attribute this to the increased size of the rats at the time of surgery compared to that of our initial lesions and calculated coordinates. The coordinates were thus modified to ensure that the caudal as well as dorsal limb were targeted. With refined coordinates and injection amounts, we have begun to administer temporary lesions to both the dPPC and the cPPC through the use of Designer Receptors Exclusively Activated by Designer Drugs (DREADDs). We piloted DREADDs virus injections in two male Long-Evans rats with slightly varying coordinates and injection amounts. Results from the first subject show that our coordinates accurately target both the dPPC and the cPPC, with improved localized spread. With these enhanced PPC coordinates and sharpened DREADDS methods, we will be well positioned to answer questions about the function of both the dPPC and cPPC, which may be functionally different.

this work was supported by RI INBRE

Adverse Neonatal Outcomes of Infants with Prenatal Opioid Exposure

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Background
Opioid use has greatly increased in the United States over the past decades. Evidence from several studies indicates that the prevalence of opioid use during pregnancy has also increased. However, the potential effect of prenatal exposure to prescription opioids on neonatal health outcomes, particularly in women without opiate dependency remains unknown.

Objective
To assess the association between gestational prescription opioid exposure and adverse neonatal outcomes in infants.

Methods
Women aged 12-55 years with one or multiple liveborn infants were identified from Rhode Island Medicaid and infants were linked to the mothers based on Birth Certificates between 2008 and 2015. Women exposed to confirmed teratogenic medications or diagnosed with cancer, opioid use disorder, or chromosomal abnormalities were excluded. The adverse neonatal outcomes, identified by diagnosis codes or birth certificates, were defined as any abnormal newborn conditions, including preterm birth, low birth weight (<2500 g), neonatal Intensive care unit admission, apgar score at 5 minutes, cesarean delivery and assisted vaginal delivery. Unadjusted and adjusted odds ratios with 95% confidence intervals (CI) were estimated using univariate and multivariate regression analyses.

Results
Among 9,429 mother-infant pairs included in this study, 690 (7.3%) infants were exposed to one or multiple opioid prescriptions anytime during pregnancy. Prescription opioid use in pregnancy were more common in women who had depression, anxiety, tobacco abuse, alcohol/drug abuse or were complicated by pain-related conditions and comedication use. Infants with prenatal exposure to prescription opioids were at an increased risk for a composite of adverse neonatal outcomes (unadjusted OR = 1.35; 95% CI: 1.12,1.54) and cesarean delivery (unadjusted OR = 1.53; 95% CI: 1.28,1.82). After adjusting for maternal demographic characteristics, comorbidities, comedications use and pain-related indications, no significant association was found for the overall adverse neonatal outcomes.

this work was supported by RI INBRE

PHARMACEUTICAL SCIENCES

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EVEN-NUMBERED POSTERS ARE TO BE PRESENTED FROM 11:00 AM – 12:30 PM

Evaluation of the Placental Transcriptomic and Microbiome Response to Perfluorooctanesulfonic acid (PFOS) Exposure in Mice

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Classified as a member of the per- and polyfluoroalkyl substances (PFAS) family, perfluorooctanesulfonic acid (PFOS) is a bioaccumulative environmental toxicant consisting of a synthetic perfluorinated eight-carbon backbone with a sulfonic acid head group. Evidence suggests that PFOS can induce developmental toxicity in humans and in rodents via maternal exposure and is associated with low infant birth weight in humans. Furthermore, PFOS is detected in human and rodent umbilical cord blood and breast-milk, and transporters are expressed in placenta that can facilitate PFOS deposition to the fetus. The aim of this summer bridge project was to elucidate the effects of developmental exposure to PFOS at 0.3 mg/kg/day and 3 mg/kg/day on the placenta *in vivo* using C57BL/6 mice. Based on the overarching theory that the microbiome plays a vital role in human health, the effects of PFOS on the placental microbiome and transcriptome was assessed. Previous characterization of the human placental microbiome revealed associations of the placental microbiome with a remote history of antenatal infection (Aagaard *et al.*, 2014). However, the effects of PFOS on the placental microbiome remain unknown to our knowledge. Genomic bacterial DNA was isolated from fetal placentas.The relative abundance of key bacterial strains found in the placenta was quantified by real-time quantitative PCR to screen samples before progressing to 16S rRNA sequencing. RNA integrity was confirmed, libraries prepared, and then next-generation sequencing (RNA-Seq) was performed to correspondingly investigate the impact of PFOS exposure on the placental transcriptome. The impact of PFOS on the placental transcriptome and placental bacterial content will be presented.

this work was supported by RI INBRE

Sun Sensitivity in PPIX and Copper-Cysteamine Nanoparticles

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Photoporphyrin IX (PPIX) and Copper-Cysteamine (Cu-Cy) are both photosensitizing agents. They have the ability to absorb light and produce free radicals. Those free radicals damage DNA in cancer cells at a high rate, helping to eliminate tumors. These capabilities are beneficial to photodynamic therapy treatment. Unfortunately, the use of the agent, PPIX, has a concerning side effect which damages the skin when exposed to sunlight. Side effects such as this may not be as detrimental when using Copper-Cysteamine nanoparticles. Unlike PPIX, these particles were only recently discovered as potential photosensitizing agents. Therefore, it is necessary to test whether Cu-Cy is less damaging to the skin than PPIX when exposed to light. This experiment could provide justification that Cu-Cy nanoparticles are less sun sensitive, increasing its potential as an agent for future cancer treatment. If so, it could be a better treatment strategy for photodynamic therapy than PPIX. To study this, mice tissue containing PPIX and Cu-Cy nanoparticles were exposed to UV light and tested for DNA damage. Seven solutions were made, each varying in the concentration of the two particles. These solutions were deposited onto shaven spots of mice, which were then placed under a sun simulating light. After ten minutes of exposure, the skin tissue samples containing the seven solutions were collected for DNA extraction. DNA damage can be detected and measured using a qPCR-based assay. The concentration of PPIX and Cu-Cy that produced the most DNA damage will be used for further testing. A second experiment will be done that requires the same methods as the first, but it focuses more on the effect of different times that the particles are exposed to light and its level of skin damage. Future experiments would eventually be conducted to test other properties and possible side effects of Cu-Cy nanoparticles. These studies can increase our understanding of Copper-Cysteamine nanoparticles and its possible use in enhancing the effects of cancer treatment in humans.

this work was supported by RI INBRE

Ubiquitin Specific Peptidase 2

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Since 1980, liver cancer incidence has nearly tripled, with approximately 42,000 new cases in the United States for 2019. Death rates caused by liver cancer has also increased by over 2% per year since 2017. The most common type of primary liver cancer in an adult is Hepatocellular carcinoma (HCC). Studies have shown that Farnesoid X Receptor (FXR) acts as a master regulator of the bile acid homeostasis in the liver and plays a protective role against HCC. Additionally, Ubiquitin Specific Peptidase 2 (USP2) was recently identified as a bona fide oncogene to promote prostate and bladder cancer and is involved in the pathogenesis of breast, ovarian, lung cancer and gliomas. Currently, it still remains unknown as to whether USP2 plays any roles in the pathogenesis HCC. However, preliminary studies have shown that USP2 is a novel target regulated by FXR and ERα signaling and the dysregulation of the FXR and ERα signaling can lead to HCC. Also, USP2 expression was dysregulated in HCC. Thus, the purpose of the study is to investigate the effects of modulating FXR and ERα signaling pathway with FXR and ERα agonist and antagonist on USP2 (USP2a and USP2b) expression through the use of immortalized liver cancer cell line HepG2. HepG2 cells were transfected with FXRα2, ERα and pcDNA vector as a control, followed by treatment of the transfected cells with either FXR or ERα agonists or antagonists for 30h. The expression levels of the USP2 including isoform USP2a and USP2b were detected and quantified with Real-Time PCR. In the HepG2 cells, after transfecting FXRα2 and ERα, treatments of transfected cells with FXR agonist GW4064 increased while FXR antagonist DY268 decreased USP2b expression. On the other hand, treatment of transfected cells with ER agonist and antagonist both increased USP2b expression. However, no significant changes were detected for USP2a expression following FXR or ER agonist or antagonist treatment. In conclusion, USP2b was transcriptionally regulated by the FXR and ERα signaling pathway while limited effects on USP2a were detected.

this work was supported by RI INBRE

Aspirin VR App

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The Aspirin App is an interactive virtual reality experience designed to teach students about the history and pharmacology of aspirin. The goal is for students with little knowledge of aspirin to learn basic concepts in natural products, biochemistry and pharmacology using aspirin as the example. The app starts with a brief tutorial on virtual reality then ventures into a natural products area where the users learns about historical use of willow bark as a medicinal remedy. Following that is a museum that delves into the history and development of aspirin, giving students insight into the global relevance of aspirin and its role in creating the modern pharmaceutical industry. Up next is the clinical area delving into pharmaceutical uses of aspirin, alternative drugs to aspirin, warnings and side effects of aspirin, and modern long-term studies involving aspirin. The final section covers the biosynthesis and pharmacology of aspirin using interactive biochemistry tools. Users are tested on their knowledge through interactive quizzes and displays such as an interactive biosynthesis module that allows the user to create aspirin from its precursors. The app concludes with a display of the cyclooxygenase receptor showing the binding of salicylic acid (aspirin prodrug). Overall the students should leave the app with an experience reinforcing their knowledge that they could not have achieved any other way.

this work was supported by RI INBRE

Development of Lipid-Polymer Nanoparticles for Inhalational Drug Delivery

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Purpose

Poly lactic-co-glycolic acid (PLGA) is a well known biocompatible and biodegradable polymer used to deliver anti-cancer drugs. The immune system often reacts to drugs that are administered, decreasing the ability of the drugs to remain in the body or carry out their intended function. Coating the PLGA nanoparticles (NPs) with a biomimetic material (1,2-Dipalmitoyl-sn-glycero-3-phosphocholine (DPPC) or lung surfactant (LS)) helps improve the ability of the drug to stay in circulation and reach the intended target site. Hence, the present investigation was intended to develop biomimetic lipid-coated PLGANPs and evaluate for its efficiency in lung delivery by 2 dimensional (2D) and 3 dimensional (3D) tissue models.

Methods

Coumarin-6-dye-loaded PLGA nanoparticles (PLGANPs) were prepared using water-in-oil emulsion-solvent evaporation method. Lipid-coated PLGANPs were prepared using thin film hydration technique. Particles were characterized using dynamic light scattering to determine size (d.nm), polydispersity index, zeta potential (mV), and using transmission electron microscopy (TEM) to evaluate morphology. A cell uptake study was performed against A549 lung cancer and NR8383 lung macrophage cell lines. A cytocompatibility study will be performed. 3D lung tumor models were developed and imaged, so that they can be used to test the NPs in the future.

Results

PLGANPs, Coumarin-6 LS_PLGANPs and Coumarin-6 DPPC_PLGANPs were characterized to have sizes of 167.9 d.nm, 295.6 d.nm and 291.8 d.nm respectively. The NPs had zeta potential of -37.4, -24.8 and -17.0 mV respectively, indicating that the NPs are stable. Cell uptake study showed that LS coated NPs decreased uptake by the NR8383 cell lines. This indicated that the coating may help the NPs to remain in the lungs for a longer time and be better taken up by cancer cells. The TEM images show that the lipid-coated NPs were relatively uniform in size and spherical in shape. It was determined that 3D spheroids increase in size over time.

Conclusion

Significantly lower uptake of LS_PLGANPs than PLGANPs was observed by NR8383 cells, which indicated that the LS coating can potentially allow the NPs to evade macrophage uptake. The 3D lung cancer models are in the process of being optimized to allow for the NPs to be tested on a model that is more similar to *in vivo* conditions than 2D monolayer cultures.

this work was supported by RI INBRE

Chemical Characterization of a Maple Syrup Extract (MSX) Produced by Fermentation Method

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Maple syrup is a natural sweetener produced by concentrate sap from the certain maple species including sugar maple (*Acer saccharum* L.). A novel maple syrup derived extract, namely, maple syrup extract (MSX), was developed by our laboratory for nutraceutical applications. We have conducted extensive compositional studies of MSX for its macronutrients (carbohydrates, primarily sucrose), and micronutrients (minerals and vitamins), and phytochemicals (primarily phenolics). MSX was reported to show several biological activities including anti-cancer, anti-diabetic, anti-glycation, and neuroprotective effects. While MSX has proven to be an interesting nutraceutical ingredient, the current method of obtaining pure maple syrup extract by chromatographic method is cost-prohibitive, time-consuming, and only suitable for small scale production. Herein, we propose to explore a fermentation method to produce MSX by reducing sugar content and yielding a polyphenol-enriched MSX product. The chemical composition of MSX products from the fermentation methods were characterized by evaluations of their sugar content, total phenolic content, and antioxidant potential. Additionally, all MSX samples were analyzed by high-performance liquid chromatography (HPLC) method to identify phytochemicals in MSX using standards from our previously reported studies. Data from this study will provide a more cost and time-effective means of creating maple syrup extract.

this work was supported by RI INBRE

Development of a Biocompatible Nanoparticle/Hydrogel Composite for the Treatment of Glioblastoma

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Glioblastoma (GB) is the most aggressive and highly metastasizing form of brain cancer with bleak prognosis. The three-year survival rate of GB patients is about 2% and long-term survivors are rare. Unfortunately, there has been little change in the prognosis and treatment of GB. The present study aims to develop a nanoparticle/*in-situ* gelling drug delivery system using bioinspired materials for the treatment of GB. Polymeric nanoparticles were incorporated into the optimized *in situ* gelling system. An *in vitro* biocompatibility study demonstrated that the developed nanoparticle/hydrogel composite was not toxic to human GB cells (U87). Rheological studies demonstrated that the in-situ gelling system gelled within 4 minutes at 37°C, which will be suitable for future animal studies. Furthermore, a florescent dye (DiI) was encapsulated into nanoparticles, and the *in vitro* release and cellular uptake of nanoparticles in GB cells (U87) were investigated.

this work was supported by RI INBRE

Assessing the Effect from an Earlier Plating Time on Gene Expression of PFAS Exposed Human Hepatocytes

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Per- and polyfluoroalkyl substances are man-made compounds that are ubiquitous in the environment. These compounds originate from the use of aqueous film forming foams (AFFF), non-stick, water-resistant household and personal items. Perfluorooctane sulfonate (PFOS), perfluorooctanoic acid (PFOA), and perfluorohexane sulfonate (PFHxS) have all been linked to liver damage, as well as an increased liver weight. Human primary hepatocytes express uptake transporters (i.e. OATP1B1, OATP1B3, and NTCP) that facilitate the movement of the PFASs into hepatocytes. It was described that time in culture down-regulates membrane transporter expression, which can confound PFAS potency interpretation in hepatocyte models. Thus, this study was conducted to observe transporter- and PFAS-regulated gene expression when treatment occurred at an early time (e.g. 4 hours) or traditional time (e.g. 24 hour) after plating. It was hypothesized that because transporters are more abundantly expressed 4 hours after plating, then PFAS uptake into the hepatocyte will be greater and a greater/more potent toxicodynamic effect will be observed. Cryostax 5-donor pool of cryopreserved human hepatocytes were cultured following the manufacturer's protocols. Hepatocytes were treated with PFOS, PFOA, and PFHxS (10 nM, 0.025 µM, 0.25 µM, 2.5 µM, 25 µM) at either 4 or 24-hours after plating. 48 hours after PFAS treatment, the cells were lysed and relative expression for 15 transcripts were quantified using a Thermo QuantiGene 2.0 Bioplex assay and analyzed on a Bio-Rad Bio-Plex 200 platform. Gene expression results will be analyzed and used to compare the expression transporters in the human hepatocytes with PFAS induction of target genes. This information will indicate whether PFAS potency in hepatocytes may be influenced by uptake transporter expression and suggests that transporter expression should be considered when evaluating PFASs *in vitro*.

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