2016 Rhode Island Summer Undergraduate Research Fellowship Conference

Friday, July 29, 2016
8:00 AM

Richard E. Beaupre Center for Chemical & Forensic Sciences
University of Rhode Island

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Rhode Island NSF EPSCoR
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9TH ANNUAL RHODE ISLAND SUMMER UNDERGRADUATE RESEARCH FELLOWS CONFERENCE

FRIDAY, July 29, 2016
RICHARD E. BEAUPRE CENTER FOR CHEMICAL & FORENSIC SCIENCES
UNIVERSITY OF RHODE ISLAND
KINGSTON, RI

8:00 – 9:00 AM	**CONTINENTAL BREAKFAST AND POSTER SET-UP**
9:00 – 9:30 AM	**WELCOMING REMARKS**
	DR. DAVID DOOLEY, PRESIDENT, UNIVERSITY OF RHODE ISLAND
	SCOTT JENSON, DIRECTOR, RI DEPARTMENT OF LABOR & TRAINING
	DR. ZAHIR SHAIKH, RI- INBRE PRINCIPAL INVESTIGATOR & PROGRAM DIRECTOR, UNIVERSITY OF RHODE ISLAND
	DR. CAROL THORNBER, INTERIM ASSOCIATE DEAN OF RESEARCH, UNIVERSITY OF RHODE ISLAND
	DR. GEOFFREY BOTHUN, RI NSF EPSCOR PRINCIPAL INVESTIGATOR, UNIVERSITY OF RHODE ISLAND
9:30 – 11:00 AM	**POSTER SESSION A**
11 AM – 12:30 PM	**POSTER SESSION B**
12:30 PM	**Lunch**

EXHIBITORS

Located in the Lobby on the 1st 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 manned for presentations according to the schedule below.*

Poster Session	**Posters to be Manned**
Session A 9:30 AM – 11:00 AM	All themes Odd-numbered posters
Session B 11:00 AM – 12:30 PM	All themes Even-numbered posters

POSTER LOCATIONS

All posters will be located in the Beaupre Center, grouped by research theme as follows

Research Theme	**Location**
Cell & Molecular Biology (CMB) • #1-18 • #19-36	 Room 145 Room 150
Chemistry (Chem)	2nd floor lobby
Environmental Science (ES)	Room 140
Genetics (Gen)	Room 130
Marine Science (MS) • #1-16 • #17-24	 Room 120 Room 130
Microbiology (Micro)	Room 135
Neuroscience (Neuro)	Room 155

CELL & MOLECULAR BIOLOGY

LOCATED IN ROOMS 145 AND 150 ON THE 1st FLOOR OF THE BEAUPRE CENTER

The Parkinson's Disease Protein α-synuclein Alters the Microenvironment of the Endoplasmic Reticulum in *Saccharmomyces cerevisiae*

Trevor McBride & Nicanor Austriaco

Department of Biology, Providence College, Providence, RI

The protein α-synuclein forms aggregates in human dopaminergic neurons triggering apoptosis and Parkinson's Disease (PD). To better understand the link between protein aggregate formation and cell death, we have overexpressed human α-synuclein in the budding yeast, *Saccharomyces cerevisiae*. Our experiments suggest that these aggregates trigger the unfolded protein response (UPR) in yeast and that different clinically relevant variants of α-synuclein up-regulate the UPR to different degrees. Moreover, we show that the overexpression of α-synuclein alters the redox state and the calcium dynamics of the yeast ER. Finally, our preliminary data suggests that sub-lethal doses of tunicamycin and β-mercaptoethanol, drugs that trigger the UPR, may alleviate the aggregation of α-synuclein. Our results point to possible pharmacological interventions that may lower protein aggregation in PD.

[Our laboratory is supported by grant NIGMS R15 GM110578, awarded to N. Austriaco.]

Expression of Tau in *Saccharomyces cerevisiae* to Determine the Effects of N-Terminal Acetylation

Jess Anderson[1], William Holmes[1], Heather J. Axen[2] & John-David Swanson[2]

[1]Department of Biology, Rhode Island College, Providence, RI
[2]Department of Biology & Biomedical Sciences, Salve Regina University, Newport, RI

Protein aggregates are found in the brains of people who have been afflicted with neurodegenerative diseases, which are disorders such as Alzheimer's disease that affect the functioning of the brain. One of these proteins is Tau, a protein that works to stabilize microtubules in neuronal cells. Tau does not normally aggregate, but post-translational modifications, which are changes to a protein's structure after the protein has been made, can lead to aggregation. Therefore, it is important to understand the post-translational modifications of Tau in order to understand why it aggregates. One type of post-translational modification is N-terminal acetylation (the addition of an acetyl group). Tau is N-terminally acetylated. Based off of the amino acid sequence of Tau, it is predicted to be acetylated by the N-terminal acetylation complex NatA, which is responsible for acetylating a majority of proteins. Without the presence of NatA – and therefore without acetylation - Tau may be more prone to aggregation and toxicity. Here we utilized *Saccharomyces cerevisiae* as a model system to express Tau and examine its effects with and without the presence of NatA. To facilitate this, Tau was integrated into the genome of *S. cerevisiae* strains – both wild-type strains and strains each lacking an N-terminal acetyl transferase complex (NatA, NatB, and NatC) - therefore generating strains that produce consistent amounts of Tau protein. The next step is to visualize changes to the aggregation of Tau in these cells.

Characterization of Oxygen Radical Formation in *Saccharomyces cerevisiae*

Victoria Hallisey[1], Ann Kleinschmidt[1] & Linmary Darosa[2]

[1]Department of Biology, Providence College, Providence, RI
[2]Central Falls High School, Central Falls, RI

The growth of many eukaryotic cells is regulated by a mechanism commonly known as apoptosis or programed cell death. Cancerous cells lack this process and proliferate in an unrestricted manner. We are working to better understand the mechanisms of programmed cell death by investigating how the budding yeast, *Saccharomyces cerevisiae*, dies. One common precursor of programed cell death in many cell types is the formation of highly reactive oxygen radicals (ROS). We have begun to interrogate the role of ROS in yeast cell death by looking at how and when yeast cells normally produce ROS. The presence of these radicals can be visualized using the red fluorescence stain dihydrorhodamine-123 (DHR) or an H_2O_2-sensitive RFP reporter called HyPer. Our experiements suggest that when grown in a fermentable carbon source such as glucose, yeast does not produce many oxygen radicals. However, with a nonfermentable carbon source like ethanol, the organism must respire and thus produce more oxygen radicals. Using these visualization methods we hope to link oxygen-radical formation to programed cell death.

[Our laboratory is supported by grant NIGMS R15 GM110578, awarded to N. Austriaco.]

Expressing and Purifying the Mammalian Protein Tau in *Escherichia coli* to Assess How N-Terminal Acetylation Alters Structure and Function.

Anna Lally & William Holmes

Department of Biology, Rhode Island College, Providence, RI

Alzheimer's disease is a neurodegenerative disease resulting from the death of neurons. One cause of this cellular degeneration is the aggregation of the protein Tau. Tau's endogenous function stabilizes polymerized microtubules in neurons, an integral part of nervous system. Since a protein's function is completely dependent upon their structure, it is essential to understand any modifications. Bacteria, specifically *E. coli*, have been widely used to express and purify proteins efficiently and cost effectively, by utilizing recombinant plasmid DNA. However, prokaryotic cells do not make the same modifications that eukaryotic cells have evolved with. These variations of the final protein, also called post-translational modifications (PTMs), can drastically change aspects of the protein. N-terminal acetylation is a PTM in which an acetyl group is added at the N-terminus, changing the charge and structure of the protein, potentially affecting its function. There are six different N-terminal acetyltransferase complexes, Nat A – Nat F, and over 80% of mammalian proteins are N-terminally acetylated by one of these Nat complexes at the ribosome. N-terminal acetylation is determined by the amino acid sequence of the newly synthesized protein; specifically, the first two amino acids. When this complex is expressed in, *E. coli*, it binds to the ribosome and are able to N-terminally acetylate a protein in the same manner as eukaryotic cells. Tau is predicted to be N-terminally acetylated based on its amino acid sequence, and additionally, the N-terminus is implicated in altering aggregation rates. To determine the effect of N-terminal acetylation on Tau aggregation, we co-expressed Tau with the Nat A protein complex in *E. coli* to determine if Tau is N-terminally acetylated, which will be used to subsequently purify Tau and determine any effect on the structure and function.

Characterization of Cell Death in the Filamentous Fungus, *Pseudogymnoascus destructans*, the Causative Agent for White Nose Syndrome in North American Bats.

Ann Kleinschmidt & Victoria Hallisey

Department of Biology, Providence College, Providence, RI

Over the past ten years, White Nose Syndrome has killed over six million North American bats including bats in Rhode Island. It is a disease caused by the filamentous fungus, *Pseudogymnoascus destructans*, a pest that kills bats by disrupting their hibernation patterns. We are investigating programmed cell death (PCD) in *P. destructans* in order to better understand its drug susceptibilities. Our preliminary data suggests that agents known to trigger PCD in yeast, including hydrogen peroxide and farnesol, also leads to cell death in *P. destructans* with markers indicative of apoptosis.

[Our laboratory is supported by grant NIGMS R15 GM110578, awarded to N. Austriaco.]

Protein A tagging of Hsp70 Ssa1 in *Saccharomyces cerevisiae* [PSI+] Cells

Jeremy Boutin & William Holmes

Department of Biology, Rhode Island College, Providence, RI

In order for proteins to carry out necessary biological reactions their primary amino acid chain must first fold into a functional conformation. Numerous neurodegenerative disorders such as Alzheimer's disease, Parkinson's disease, and Huntington's disease are shown to be a result of protein misfolding. Another example of toxic protein aggregates are prion proteins, however prions are self-propagating in that they induce conformational changes in native proteins that results in toxic aggregates. Molecular chaperone proteins are essential in influencing prion aggregates by properly folding proteins into their native conformation. *Saccharomyces cerevisiae* has multiple classes of molecular chaperones called heat shock proteins (Hsp) that work together to properly fold proteins. Ssa1 is a Hsp70 class chaperone that works with the Hsp40 Sis1 co-chaperone to bind to non-native polypeptide chains and refold them into a functional conformation. Many proteins have been shown to fold spontaneous *in vitro*, however *in vivo* they require chaperone proteins to maintain a catalytic amount of functional protein. *In vitro* protein assays can help study chaperone activity to understand why they are necessary for protein folding under cellular conditions *in vivo*. However, studying chaperones *in vitro* becomes difficult because active Hsp70 and co-chaperone complexes are extremely difficulty to isolate in mammalian cells. The gene encoding for Ssa1 in Yeast [PSI+] cells were transformed by a PCR amplified cassette encoding for a Protein A (Pra) tag to create a one step purification model for Ssa1 in active chaperone complexes. Yeast [PSI+] cells were used to verify that the mutant cells are still active with the Ssa1-Pra tag construct because they encode for the prion isomer of Sup35, which expresses a loss of phenotype once prion aggregates form. The purified Ssa1 protein will be used in further assays to better understand its interaction with Hsp40 Sis1 and the effects of post-translational modifications such as N-terminal acetylation on chaperone function.

Yeast Bax Inhibitor (BXI1) Is Involved in Redox Homeostasis of the ER in *Saccharomyces cerevisiae*

Joseph Alisch[1], David Eagan[2] & Nicanor Austriaco[2]

[1]Department of Engineering-Physics-Systems, Providence College, Providence, RI
[2]Department of Biology, Providence College, Providence, RI

Yeast Bax inhibitor-1 (BXI1/YBH3) encodes a protein that belongs to the Bax Inhibitor (TMBIM) family of proteins that all contain a transmembrane BAX inhibitor motif. The crystal structure of a prokaryotic member of the family, BsYetJ, has revealed that the Bax inhibitor proteins are pH sensitive calcium leaks. In mammals, the Bax inhibitor family of proteins has cytoprotective properties that are most evident in paradigms of endoplasmic reticulum (ER) stress. Our published studies have shown that yeast Bxi1p is localized to the endoplasmic reticulum and is involved in the unfolded protein response (UPR) that is triggered by endoplasmic reticulum (ER) stress. BXI1 is thought to act via a mechanism involving altered calcium dynamics. We now show that cells lacking BXI1 have an altered redox microenvironment in their ERs: Δ*bxi1* cells exposed DTT and tunicamycin, two drugs known to induce the unfolded protein response (UPR), undergo more reduction in the ERs than their wildtype counterparts. This suggests that Bxi1p is involved in regulating the yeast ER microenvironment.

[Our laboratory is supported by grant NIGMS R15 GM110578, awarded to N. Austriaco.]

Cardiac Na^+ Channels are Co-expressed with Neural Na^+ Channels in Cultured Human Melanoma Cells

An Xie[1], Benjamin Gallant[2], Alfredo Gonzalez[2], Matthew Clark[2], Audrey Madigan[2], Feng Feng[1], Samuel Dudley Jr.[1] & Yinsheng Wan[2]

[1]Lifespan Cardiovascular Institute & the Warren Alpert School of Medicine, Providence, RI
[2]Department of Biology, Providence College, Providence, RI

Resting membrane potential (RMP) and intracellular Ca^{2+} concentration ($[Ca^{2+}]i$) have been suggested to be associated with tumorigenesis and metastasis. In this study, we discovered that functional cardiac Na^+ channels are expressed in human melanoma cells (WM 266-4) and participate in RMP maintaining and Ca^{2+} homeostasis. Confocal microscopy was used to detect Na^+ channels. Patch-clamp technique was employed to record Na^+ currents and action potentials. Cytoplasmic Ca^{2+} was measured by loading Fluo-4. Our results showed that only cardiac (Nav1.5) and neural (Nav1.6) Na^+ channels are expressed in WM 266-4. Tetrodotoxin (TTX) could dose-dependently block Na^+ currents. Nav1.5 plays a predominant role in Na^+ currents. UV light could induce similar action potentials in both human skin melanocytes (HMC) and WM 266-4, which could be abolished by transient receptor potential A1 channels specific blocker, 100 μM HC-030031. Compared with HMC, RMP is substantially depolarized in WM 266-4. TTX could prominently hyperpolarize RMP in WM 266-4 at a concentration of 30 μM, which facilitates Ca^{2+} influx. Compared with HMC, $[Ca^{2+}]i$ is significantly higher in WM 266-4. $[Ca^{2+}]i$ could be elevated by TTX. Taken together, our data have shown that cardiac Na^+ channels are co-expressed with neural Na^+ channels, depolarize RMP and inhibit Ca^{2+} uptake in WM 266-4. Our data suggest that those channels agonists may be developed to treat melanoma.

Yeast Bax Inhibitor (BXI1) Is Involved in Calcium Homeostasis of the ER in *Saccharomyces cerevisiae*

Liam McDonough & Nicanor Austriaco

Department of Biology, Providence College, Providence, RI

Yeast Bax inhibitor-1 (BXI1/YBH3) encodes a protein that belongs to the Bax Inhibitor (TMBIM) family of proteins that all contain a transmembrane BAX inhibitor motif. The crystal structure of a prokaryotic member of the family, BsYetJ, has revealed that the Bax inhibitor proteins are pH sensitive calcium leaks. In mammals, the Bax inhibitor family of proteins has cytoprotective properties that are most evident in paradigms of endoplasmic reticulum (ER) stress. Our published studies have shown that yeast Bxi1p is localized to the endoplasmic reticulum and is involved in the unfolded protein response (UPR) that is triggered by endoplasmic reticulum (ER) stress. BXI1 is thought to act via a mechanism involving altered calcium dynamics. We now show that cells lacking BXI1 accumulate higher levels of calcium in their ER as compared to their wildtype counterparts. Our preliminary data with Δ*bxi1* Δ*pmr1* double mutants suggest that Bxi1p and Pmr1p, the Golgi-ER calcium pump, function in an antagonistic manner.

[Our laboratory is supported by grant NIGMS R15 GM110578, awarded to N. Austriaco.]

Nanoparticles Mimic Exosomes and Attenuate Growth Factor-induced Cell Migration in Melanoma Cells

Benjamin Gallant[1], Matthew Clark[1], Audrey Madigan[1], Andrew Sano[2], Yali Cui[3] & Yinsheng Wan[1]

[1]Department of Biology, Providence College, Providence, RI
[2]Johnston Senior High School, Johnston, RI
[3]Department of Biology, Northwest University, Xi'an, Shaanxi, China

Melanoma is notoriously aggressive and still considered untreatable, in spite of some of the most recent breakthroughs related to immunotherapy. Our previous studies have shown that melanoma cells, compared to untransformed normal human melanocytes, exhibit constitutive mTOR activity and resist oxidative stress. Exosomes, with various sizes between 30-100 nm, are membranous vesicles that are recently being intensively studied. These vesicles are involved in cell-cell communications and aggression of cancer cells. We hypothesized that constitutive mTOR activity in melanoma cells is attributable to biogenesis and the release of exosomes, resulting in the aggression and metastatic potential of melanoma. Externally applied nanoparticles, with similar sizes to exosomes, may confuse melanoma cells and thus have biological effects or therapeutic benefits. Using phagokinetic motility assay, we observed that gold and iron nanoparticles (50 nm) attenuated serum-induced cell migration in cultured melanoma cells (WM266-4 cells). Similarly, inhibitors of PI3K/AKT, MEK/ERK, and mTOR showed inhibitory effects. Confocal microscopy results showed that while above inhibitors decreased mTOR activity in melanoma cells, gold and iron nanoparticles also inhibit serum-induced mTOR activity (expressed in phosphorylation of ribosomal protein S6), and MEK/ERK activity, which are critical for cell migration. Western blot analysis demonstrated that serum induced biogenesis and release of exosomes, as expressed by two of the markers, CD63 and Mart1, were inhibited by inhibitors of PI3K/AKT, MEK/ERK, and mTOR. Together, our data indicates that nanoparticles, mimicking exosomes, inhibit mTOR, resulting in the attenuation of cell migration of melanoma cells. Our results suggest that nanoparticles with similar sizes to exosomes may provide therapeutic benefits to melanoma treatment.

Optimizing Chemotherapeutic Temporal Delivery Profiles Using Remotely Activated Biomaterials

Anne Reisch[1], Tani Emi[1], Tanner Barnes[2] & Stephen Kennedy[1,2]

[1]Department of Chemical Engineering, University of Rhode Island, Kingston, RI
[2]Department of Electrical, Computer & Biomedical Engineering, University of Rhode Island, Kingston, RI

Alone in 2016, the American Cancer Society estimates that more than 1.5 million new cases of cancer will be diagnosed. Modern treatments for cancer can involve intravenous cytotoxic drug delivery (i.e., chemotherapy). However, while the cytotoxins kill cancer cells, healthy cells in the body are also exposed to these toxins, often resulting in undesirable side effects. To reduce complications of chemotherapies, we propose a more direct-to-tumor-site approach using drug-laden biomaterials. These biomaterials allow for a more direct drug delivery at the tumor site. However, traditional biomaterials release drug by diffusion and produce unalterable, sustained tumoral drug concentrations. We hypothesize that these sustained deliveries could be improved upon by using stimuli-responsive biomaterials to produce pulsatile deliveries. Thus, we tested to see if pulsatile chemotherapeutic deliveries killed cancer cells more effectively than sustained concentrations. Indeed, we found that pulsatile mitoxantrone deliveries destroyed B16-F10 melanoma cells significantly more effectively than sustained deliveries.

Exosomes-mediated mTOR Activation is Involved in Ovarian Cancer Cell Aggression and Migration

Audrey Madigan[1], Matthew Clark[1], Benjamin Gallant[1], Andrew Sano[2] &Yinsheng Wan[1]

[1]Department of Biology, Providence College, Providence, RI
[1]Johnston Senior High School, Johnston, RI

Ovarian cancer remains difficult to treat, with the remission rate still high and its drug resistance obvious. Our previous studies have demonstrated that ovarian cancer cells resist chemotherapeutic drugs via activation of EGFR-PI3K-AKT mTOR cell survival signaling pathway. Recent studies have suggested that exosome biogenesis and release is associated with cancer cell aggression, migration and cell-cell communication. We hypothesize that exosomes-mediated mTOR activity is involved in ovarian cancer cell metastatic potential and migration. Using phagokinetic motility assay, we observed that inhibitors of PI3K/AKT, MEK/ERK, and mTOR reduced cell migration in ovarian cancer cells (CaOV3 cells). We also found that inhibitors of exosome biogenesis and release, GW4869 and Brefeldin A, attenuated EGF- and serum-induced cell proliferation and migration. Western blot analysis showed that EGF induced expression of CD63, one of the markers of exosomes, in a dose dependent manner. Serum treatment also enhanced CD63 releases into culture medium. Interestingly, while Brefeldin A blocked serum-induced mTOR activation, GW4869 appeared to enhance mTOR activity in CaOV3 cells. Collectively, our data suggest that exosome biogenesis and release feedback-regulate mTOR activity in ovarian cancer cells, with a result of aggression, migration, and drug resistance. Modulation of exosome activities may provide therapeutic options of clinical ovarian cancer.

NS1 Protein ELISA on Paper-based Platform

Adannia Nwangwu[1], Alan Rothman[2], Mohammad Faghri[3] & Constantine Anagnostopoulos[3]

[1]Biotechnology Center, University of Rhode Island, Providence, RI
[2]Institute for Immunology & Informatics, University of Rhode Island, Providence, RI
[3]Mechanical Engineering & Applied Mechanics, University of Rhode Island, Kingston, RI

Dengue fever, the mosquito-borne tropical disease caused by dengue virus, has negatively affected millions of people throughout the world each year. To confirm the infection at early stages, there are tests to detect viral RNA and NS1 antigen. Both are viewed as a diagnostic marker to confirm the dengue illness. The issue at hand is that not everyone around the world has the resources to test it or otherwise even afford the materials to do so, especially for second and third infections in which the concentration of the NS1 protein is substantially reduced. The “Lab on a Chip” project being conducted at the University of Rhode Island, seeks to develop high sensitivity lateral flow tests to detect various proteins in biological fluids of animals, humans, and in the environment. Using a product like this to detect Dengue virus would be less expensive and require fewer resources than the tests currently available. This paper based device makes it possible to have multiple fluids in one chip triggering one another at certain times. The objective of this project is to transfer a bench-top ELISA (enzyme-linked immunosorbent assay) method to the paper-based platform for Dengue fever detection. Pilot assays were run testing different dilutions of the antigen, capture and detection antibodies, and enzyme conjugate to determine the optimal experimental conditions for the bench-top assay. Dengue 2 Virus NS1 Protein was detected on 96 well plates using a sandwich ELISA with a 1:50 dilution of the Capture Antibody (rabbit polyclonal anti-NS1 antibody), a 1:100 dilution of the Detection Antibody (mouse monoclonal anti-NS1 antibody), and a 1:500 dilution of the enzyme conjugate (anti-mouse Ig-horseradish peroxidase). The same procedure will be tested on the paper-based platform. Based on this study and further research, hopefully this type of device with its higher sensitivity capability can be used in the future to help detect Dengue Fever for patients who cannot afford nor have available the more expensive tests.

Evaluation of Hepatic AlkB DNA Repair Enzyme Expression in Mouse Models of Metabolic Disease and Nutrient Deprivation

Marisa Pfohl, Emily Martell & Angela Slitt

Department of Biomedical & Pharmaceutical Sciences, University of Rhode Island, Kingston, RI

The Center for Disease Control and Prevention states that more than one third of the United States adult population is considered to be obese. Obesity is a major risk factor for non-alcoholic fatty liver disease (NAFLD), which ranges from simple steatosis to steatohepatitis. In addition, obesity and NAFLD are associated with an increased risk of developing certain cancers, such as hepatocarcinoma. Cancer is due to the replication of damaged DNA. DNA damage repair is a process that corrects physical abnormalities within the DNA from replication. If the DNA remains damaged, it can cause gene transcription to be disabled and an increase risk of cancer. The DNA repair enzyme family that is responsible for repairing alkylation damage to single stranded DNA is AlkB homologs (Alkbh). These alkylation repair enzymes reverse alkylated DNA or RNA through an oxidative demethylation mechanism, where the removal of a methyl group from single stranded DNA. The AlkB family consists of 8 enzymes: Alkbh1-8. We hypothesized that obesity and NAFLD decrease Alkbh enzyme expression in liver, which may predispose the liver for increased risk for hepatocarcinoma in conditions such as obesity and NAFLD. Specifically, the goal of this summer work was to profile Alkbh1-8 mRNA expression in livers of various diet and genetically-induced mouse models of obesity and NAFLD using pre-existing samples from published studies performed in Dr. Slitt's laboratory. Total RNA isolated from liver was checked for integrity and concentration using gel electrophoresis and absorbance 260/280. Once RNA integrity was confirmed, cDNA synthesis was performed followed by qPCR using SYBR green to quantify the relative abundance of Akbh1-8 transcripts. The results from the qPCR for this study will be presented in the poster.

Getting Things Where They Need to Go: FANCD2 Localization to Chromatin

Nicholas Mamrak, Karissa Paquin & Niall Howlett

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

Fanconi anemia (FA) is a rare autosomal and X-linked genetic disease characterized by congenital abnormalities, bone marrow failure, and increased cancer susceptibility. The proteins encoded by FA genes function together in the Fanconi anemia - Breast Cancer (FA-BRCA) pathway, one major function of which is to repair a specific type of DNA damage; interstrand crosslinks (ICLs). The central step in this pathway is the addition of a single ubiquitin molecule to the FA protein FANCD2. Monoubiquitinated FANCD2 localizes to chromatin where it facilitates the recruitment of DNA repair proteins. Chromatin, an aggregation of nucleosomes containing DNA wrapped around core histone proteins, plays a key role in the cellular response to DNA damage. Specifically, histone tail modifications control the recruitment of DNA repair proteins: reader domains in these proteins recognize a specific histone tail modification state. For example, chromodomains (CD) recognize methylated histone tails. Previously, our lab identified a putative CD in FANCD2 through homology comparison to known chromatin-binding proteins. This FANCD2 CD exhibits specificity for methylated Histone 4 on lysine 20 (H4K20). Using site-directed mutagenesis, single amino acid changes in FANCD2 were made to characterize this potential chromatin binding motif; FANCD2 H1056A and FANCD2 W1075A. Using lentiviral expression vectors, these mutant proteins were stably expressed in FA-D2 patient cells. Unlike wild type FANCD2, these missense mutants fail to correct the ICL sensitivity of FA-D2 patient cells. The monoubiquitination of FANCD2 is inducible through exposure to DNA damaging agents. To investigate the consequences of these mutations, treatment with various damaging agents will be used to determine the ubiquitination, localization, and repair consequences of these FANCD2 mutations. To test the recruitment of FANCD2 to different methylation states of H4K20, transient gene knockdowns were made in HeLa and MCF10A cells targeting KMT5A transcripts using RNA interference. KMT5A is the methyl transferase responsible for the monomethylation of H4K20 which all subsequent di- and tri- methylations are based upon. Without the ability to epigenetically modify H4K20, it is suspected to result in an altered FANCD2 localization pattern. Characterization of FANCD2 localization is essential in fully elucidating the FA-BRCA pathway, in turn providing insight into potential therapeutics and genetic screening.

Dengue Virus Impairs Drp1-Triggered Mitochondrial Fission

Siraj Janoudi, Vincent Barbier, Darshika Udawatte & Carey Medin

Institute for Immunology & Informatics, University of Rhode Island, Providence, RI

Dengue virus (DENV) is a mosquito-borne human pathogen responsible for major diseases in the tropics, ranging from acute febrile illness to more severe forms such as dengue hemorrhagic fever. To study viral-host interactions at the cellular level, our laboratory analyzed organelle changes in DENV infected cells by immunofluorescence. We observed that DENV-infection induced elongation of mitochondria and that this phenotype correlated with a reduction in the fission factor, Dynamin-related protein 1 (Drp1) in mitochondrial fractions of cells. DENV protease, NS2b3, was previously reported to cleave mitochondrial fusion proteins. We hypothesized that NS2b3 may also be involved in the reduction of Drp1 levels during DENV infection. To test this hypothesis, cells were co-transfected with plasmids expressing wild-type Drp1-YFP and NS2b3 and levels of Drp1 were analyzed by western blot analysis. Drp1-YFP levels were reduced in DENV infected cells. Mitochondria form an intimate association with the endoplasmic reticulum through mitochondrial associated membranes (MAMs). We further hypothesize that DENV proteins at the MAM interface are involved in inhibition of mitochondrial division. Interestingly, we found DENV proteins in the mitochondrial fraction of infected cells and a reduction in the MAMs marker, FACL4, indicating that DENV infection decreases MAMs association with mitochondria. Further analysis will be required to determine the interaction of DENV proteins with mitochondria and whether this is necessary for defects in mitochondrial fission.

Analyzing the Function and Regulation of PTEN SUMOylation

Morganne Adroved, Elizabeth A. Vuono & Niall Howlett

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

Fanconi Anemia (FA) is a rare genetic disease caused by mutations in any of the 20 known genes. These genes encode for proteins that function in the FA-BRCA DNA repair pathway to remove interstrand crosslinks (ICLs). Many FA proteins are regulated by phosphorylation but no known phosphatase had been linked to the pathway. Our lab has recently connected the Phosphatase and tensin homolog (PTEN) gene to the pathway, specifically establishing an epistatic relationship between PTEN and FANCD2. PTEN is one of the most highly mutated genes in human cancer encoding for a phosphatase that removes phosphates from both lipids and proteins. PTEN is best known for its lipid phosphatase activity by antagonizing the PI3K/AKT pathway. Recent studies have established a PI3K/AKT-independent nuclear function. However the mechanism in which nuclear PTEN is regulated is unknown and under investigation. Recent studies have determined that PTEN is posttranslationally modified by a small ubiquitin like modifier (SUMO) to on lysine 254, however, the function of PTEN SUMOylation is poorly understood. We have recently determined that PTEN is robustly SUMOylated following inhibition of the proteasome. This leads us to speculate that PTEN SUMOylation may regulate its stability. Specifically, we hypothesize that the RNF4 STUbL (SUMO-targeted ubiquitin ligase) may recognize SUMOylated PTEN, catalyze its polyubiquitination, and target it for degradation by the proteasome. To examine the role of PTEN in ICL repair, we complemented PTEN-/- cells with a PTEN missense mutant K254R (SUMOylation defective) and examined the sensitivity to mitomycin C (MMC), a DNA damaging agent. We have examined PTEN-SUMO localization upon DNA damage by performing a cellular fractionation comparing wild type and mutant cells. In HeLa cells we have silenced RNF4 using siRNA and performed a cellular fractionation observing increased PTEN expression in the nuclear fraction. To further examine PTEN SUMOylation, we will be using a U2OS-HA-SUMO2 cell line to determine the regulation of PTEN and the correlation of PTEN-SUMO to genome stability. We have observed a reduction of PTEN-SUMO in the absence of FANCD2 and speculate that FANCD2 may transcriptionally regulate the SUMO E3 ligase, PIAS1. Using patient lines lacking FANCD2 and corrected with FANCD2 we can observe the expression level of PIAS1 via western blot. This will help determine the role FANCD2 in the regulation of sumoylation and PTEN in DNA repair.

Cloning of DENV-2 Proteins to Investigate their Effect on Mitochondrial Fission

Alexandra Chasse[1], Vincent Barbier[2], Sierra Valois[2] & Carey Medin[2]

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Dengue virus (DENV) is a mosquito-borne pathogen that is the leading cause of illness and death in tropical and subtropical areas. DENV infection induces significant organelle changes in infected cells, including mitochondria. Mitochondria undergo continuous cycles of fission and fusion, which are essential for cellular functions. Previous work in the laboratory reported an inhibition of mitochondrial fission due to reduced levels of the mitochondrial fission factor, Dynamin-related protein 1 (Drp1), in DENV-infected cells. Interestingly, nonstructural protein (NS)4b was found in mitochondrial fractions of infected cells suggesting a potential role of the viral protein in mitochondrial changes. We hypothesized that NS4b interacts with mitochondria at sites of Drp1 localization and inhibits mitochondrial division. To test this hypothesis, NS4a, NS4b and 2K-NS4b protein-coding regions from DENV-2 16681 genome were cloned into expression vectors using the Gateway system, as these proteins are successive in the genome. Drp1 and NS protein expression were analyzed by western blot. To assess the effect of NS4b on Drp1-triggered fission of mitochondria, we analyzed mitochondrial morphology in cells expressing NS proteins and localization of NS proteins with Drp1 using fluorescence microscopy.

The Effects of Dioxin-like Chemicals on Cardiovascular development of Little Skate *Leucoraja erinacea*

Kenneth Hughes, Christopher Rei-Mohammed, Mikayla Lopes, Casie Pendexter, Timothy Bock & Rebeka Merson

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Dioxin-like chemicals are legacy chemicals present in soil and sediment. They are bioaccumulative, toxic chemicals linked to cardiovascular problems, compromised immune system response, and developmental disorders. Aquatic organisms, especially apex predators, are often exposed through biomagnification. Our goal was to determine the effects of exposure to a dioxin-like chemical, 3,3',4,4',5-pentachlorobiphenyl (PCB 126) on the cardiovascular system of the Little Skate, *Leucoraja erinacea*, as a model of vertebrate development. Excased embryos (removed from their egg cases) were exposed to PCB 126 by waterborne exposure. Embryos were then observed and imaged for subsequent weeks to determine presence of blood clotting, hemorrhaging, and blood flow. Blood smears were taken to determine effects of PCB 126 on whole blood composition and erythrocyte morphology. Differences were observed between controls and exposed embryos, supporting a dose-response in changes in vasculature and blood flow. Abnormal cell morphology was observed in erythrocytes of exposed embryos. Our results support a positive correlation between the exposure to Dioxin-like chemicals and toxicity in the cardiovascular system. The continued persistence of these toxic chemicals in the environment is a potential threat to the stability of ecosystems and breeding populations of aquatic organisms. These effects may be magnified in apex predators, which universally have long embryonic periods.

Advanced Curation and Comparative Analysis of Genome-scale Metabolic Models

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Genome-scale models of biological networks simulate genotype-phenotype associations using combined biological information from literature, experimental data, and statistical analyses. The aim of the model is to construct an *in silico* system to facilitate the understanding of fundamental behaviors of a biological system. The information contained in the model can include molecular properties of biological components and their interactions in metabolic, signaling, or gene regulatory networks, as well as information from omics data like genomics, proteomics, transcriptomic, and others. These models integrate a very large amount of data, and computational optimizations are useful tools for exploring the biologically significant solutions that mathematically represent the underlying mechanisms of living cells. The Zhang group has developed an open source software package, Portable System for the Analysis of Metabolic Models (PSAMM), for connecting existing biological annotations with mathematical simulations. There are 57 annotated models in a repository within the software for the user's selection for analysis, including models to human, yeast, and bacterial cells. In this project, we expand on this existing infrastructure by implementing a Metabolic Adjustment by Differential Expression (MADE) approach for integrating differential gene expression data into metabolic models. The MADE approach matches the computationally simulated gene presence/absence with statistically determined up-/down-regulation of genes under different environmental conditions. Thus, it effectively integrates gene expression data into modeling the variable functional pathways under different conditions. The new implementation was applied to a case study of a deep-sea bacterium in the Shewanella genus using transcriptomic data of the species under high temperature anaerobic, high temperature aerobic and low temperature aerobic conditions. The goal of this work was to understand if this implementation creates a better representation of the organism's gene regulation under extreme conditions of low temperature and high pressure.

Effects of Waterborne Exposure to PCB 126 in Little Skate (*Leucoraja erinacea*) Excasing Model, a Vertebrate with Extended Embryonic Development

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Dioxin like chemicals (DLC) in the environment induce CYP1A expression via activation of the aryl hydrocarbon receptor signaling pathway. CYP1A protein activity is associated with embryotoxicity and developmental deformities in vertebrates. The little skate (*Leucoraja erinacea*) excasing model was used to determine the impacts of DLC to the activation of CYP1A in a vertebrate with an extended embryonic period. Embryo's were exposed to waterborne 3,3',4,4',5-pentachlorobiphenyl (PCB 126) in concentrations ranging from 2 parts per trillion (PPTr) to 20 parts per billion (PPB). Following exposures for up to 7 days, embryos were allowed to develop in clean filtered seawater for up to 33 days with periodic assessment of morphological responses. Polymerase chain reaction with RNA extracted from embryo livers demonstrate CYP1A expression when exposed to concentrations as low as 2 PPTr (nominal) of PCB 126. Little skate embryos are extremely sensitive to low concentrations of DLC. Thus, this model proves to be effective for investigating DLC effects on vertebrates with longer embryonic developmental periods.

Motif and Structure Guided Screening for the Protein Phosphatase 1 Interactome

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Protein Phosphatase 1 (PP1), a serine/threonine phosphatase, is a potential drug target due to its ability to interact with a diverse set of regulatory proteins and its function in the progression of a wide range of human diseases including Alzheimer's, the regulation of transcription of HIV-1, and cancer. Only a few experimentally solved structures exist for the PP1:regulator holoenzymes of the approximately two hundred PP1-binding proteins. However, the structures have provided significant insights into the diversity of PP1-binding motifs. Of a variety of PP1-binding sites, the RVxF motif has been shown to be present in a majority of known PP1-binding proteins where it serves as an anchor for interaction. Through consensus of several existing representations of the RVxF motif, we seek to identify novel PP1-binding proteins in the human proteome in silico. A motif guided search of the human proteome has been performed that identified proteins that contain a putative RVxF binding site. Additional PP1-binding motifs, although not as ubiquitous as the RVxF motif, have also been characterized in the human proteome. These included the SILK, MyPhoNE, ΦΦ, and Arginine motifs along with structural features, such as the alpha helix used by some PP1-binding proteins (e.g. NIPP1) to interact with PP1. Predictions of disordered regions and signal peptides within these proteins further restricted the candidate PP1 interactors. Additional analyses are underway to further evaluate the top candidates before they will be tested in vitro. Expanding the PP1 interactome through the identification of novel PP1-binding proteins will assist in identifying the mechanisms proteins use to interact with PP1.

Enzymatic Studies of Nicotinamide Phosphoribosyl Transferase (NAMPT) with Molecular Activators

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Nicotinamide adenine dinucleotide (NAD^+) is a critical component to cellular life. Cancer cells have a higher NAD^+ metabolic requirement than healthy cells. Additionally, cancer cells have an up-regulation of the NAD^+-consuming enzyme poly (ADP-ribose) polymerase (PARP), the activity of which depletes NAD^+ pools and yields nicotinamide (NAM) as a byproduct. Thus, cancerous cells demand constant replenishment of NAD^+ which is accomplished by the NAD^+ salvage pathway. This is a two-step process in which NAMPT is the rate limiting step. Thus, NAMPT is responsible for NAD^+ homeostasis and a therapeutic target of interest. NAMPT catalyzes the condensation of NAM and phosphoribosyl pyrophosphate (PRPP) to nicotinamide mononucleotide (NMN), which in turn is adenylated to give the product NAD^+. Previous studies in our lab have identified small molecular activators of NAMPT. To elucidate the effect of these activators on NAMPT function, six single point mutations, selected based on known post translational modification sites of NAMPT (K389A, K229A, S472A, S200D, Y188D, and Y188F) were generated. Purified protein from each mutation was used in a fluorescence activity assay, allowing for the monitoring and quantification of the NMN product. Comparisons of these mutations with respect to wild type NAMPT will be presented, as well as the effect of small molecule activators. These studies could help to clarify the regulation of NAMPT within the NAD^+ salvage pathway.

Genetic Variability of the Hovawart Breed

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Scientists interested in dog evolution have recently been able to improve their understanding of dog domestication, levels of variation among breeds, and inherited diseases with recent advances in molecular methods. We are particularly interested in learning about the genetic differences within and between breeds. Breeders have artificially selected unique traits resulting in varying allelic frequencies of different genes per breed. We are taking advantage of these breeding practices to learn about the inheritance of traits with the use of molecular markers (SNPs and microsatellites). We utilized an ABI Genetic Analyzer to screen a dozen microsatellite loci and a Fluidigm EP1 Genotyping Analyzer to screen 152 SNPs. We have obtained 95 samples of dogs from either blood or cheek swabs, corresponding to 12 pure breeds and 5 mixed breeds. We are currently analyzing the results obtained from this large dataset, assessing allele frequencies within and among populations, levels of heterozygosity, and assessing gene expression of some of these loci previously associated with dog diseases like cancer and neurodegenerative disorders.

Assessing the Effects of Gallic Acid on Gastrointestinal Cancers Using Real Time Cell Analyses

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Gastric cancer is a particularly deadly form of cancer with a 5-year survival rate of less than 30%. It is most commonly treated with chemotherapy or surgical removal. Chemotherapy is known to have many aggressive side effects that can be life changing and is less than 100% effective, thus necessitating the discovery of alternative treatments and preventatives for gastric cancer. Nutraceuticals, naturally occurring plant compounds, are known to cause cellular apoptosis, or regulated cell death, as well as provide the human body with beneficial antioxidants, representing an appealing area to investigate for gastric cancer prevention and treatment. Gallic acid is one such nutraceutical; found in high concentrations in berries such as blackberries and raspberries and other fruits. This compound has been shown to have anti-proliferative effects selectively on cancerous cells. Cells were grown in gold plated wells, which allow us to measure impedance of electricity as cells attach and grow. We investigated the effect of different concentrations of gallic acid on the cell response to gallic acid in two immortal gastric cancer cell lines (MKN28 and AGS) using the Roche xCelliegence Real Time Cell Analysis system at the URI core facility. Cells were allowed to establish for 60 hours, and were then serum starved to sync all cells into G1 of the cell cycle. Cells were then treated with gallic acid in varied concentrations (0, 10, 20, 40, and 100 μM) for 48 hours. The cell lines were compared through three separate phases: initial cell growth (0-60 hours), serum starving (70-118 hrs.), and gallic acid treatment (118-150 hrs) with impedance measurements taken once every minute. We will normalize the sets of the data measured by impedance at the time point immediately prior to treatment to assess the cells rate of survival by observing how the slopes of the different treatments compare to the no gallic acid control. Overall, the cells are growing logistically during the first 60 hours, then they all level out when they are serum starved, then during the treatment phase the impedance increases, indicating cells detaching from the well as the time goes on. This supports the hypothesis that gallic acid is effective in halting cancer cell growth.

Melanoma Derived DNA Polymerase Theta Variants Exhibit Altered Polymerase Activity

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Cancer is one of the leading causes of death worldwide. In the United States, skin cancer is the most commonly diagnosed. According to the American Cancer Society (ACS), melanoma accounts for only about 1% of skin cancers, however, amongst them all, it has the lowest survival rate. ACS estimates that about 76,380 new cases of melanoma will be diagnosed in 2016 alone. From 2001 to 2005, Rhode Island had the 8th highest melanoma death rate in the U.S. – nearly 14.8% higher than the national average. Although an exact biological cause of cancer is unknown, DNA damage may play a significant role. Cells can maintain genomic stability through DNA repair pathways using enzymes that remove and replace damaged DNA regions. One such enzyme is DNA Polymerase Theta (POLQ, Pol θ), which is involved in double strand break repairs. Studies have correlated POLQ upregulation in breast cancer patients to poor survival rates, as well as polq deficiency in cells can lead to cellular stress and DNA damage. This suggests a potential role for POLQ as a driver of cancer. Furthermore, several somatic mutations in POLQ have been identified in a melanoma patient screen. To date, there have been no studies highlighting the effect on DNA fidelity from a cancer-associated Pol θ. This study aims to elucidate how aberrant forms of Pol θ can affect genomic stability. Pol θ variants L2538R and E2406K located in the palm domain and fingers domain of Pol θ were generated via site-directed mutagenesis. Variants were expressed in E.coli, purified via affinity chromatography and assayed for polymerase activity. Preliminary studies suggest that the variants experience altered polymerase activity compared to wild-type Pol θ, suggesting these melanoma derived mutations repair DNA differently compared to wild-type, and may contribute to overall genomic instability.

Fenofibrate Reduces Pro-inflammatory Cytokine and Chemokine Secretion in Human Macrophages: Implications for the Treatment of Chronic Cholestatic Liver Diseases

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Chronic cholestatic liver diseases result from an impairment or disruption of bile production and cause intracellular retention of toxic bile constituents. The pathophysiology of cholestasis includes the critical activation of inflammation, mainly by liver kupffer cells (macrophages), which release pro-inflammatory cytokines and chemokines, i.e. TNFα, IL-1β and -8, and determine the subsequent degree of organ damage. In particular, IL-8 is elevated in chronic liver diseases, evidenced by elevated IL-8 biliary and serum concentrations, mRNA expression, and bile duct staining in patients with cholestasis. Complications of unresolved cholestasis include prolonged hepatic inflammation which progresses to fibrosis and cirrhosis and eventually end-stage liver disease or the development of malignancies, including liver, bile duct, gallbladder, or colon cancer. Currently, the only therapy available is ursodeoxycholic acid (UDCA), yet, many patients have a sub-therapeutic response and UDCA does not improve survival for some types of cholestasis. Thus, novel therapeutic strategies to target and reduce hepatic inflammation are urgently needed. Fenofibrate is FDA-approved for the treatment of hypercholesterolemia and it is a peroxisome proliferator-activated receptor-alpha (PPARα) agonist. The nuclear receptor PPARα is highly expressed in the liver and it has been shown to inhibit inflammation. Interestingly, fenofibrate reduced symptoms and liver function abnormalities in patients with cholestasis who do not respond to UDCA, yet the mechanism(s) remains unknown. A human macrophage cell culture system was successfully established with THP-1 cells, a human leukemia monocyte cell line that differentiates into a macrophage-like phenotype when treated with phorbol-12-myristate-13-acetate. Pro-inflammatory cytokines (TNFα, IL-1β and IL-8) were measured in cells treated with DMSO (vehicle control) or LPS ± pretreatment with fenofibrate by ELISA. LPS treatment stimulated peak concentrations of TNFα, IL-1β, and IL-8, which were reduced by pretreatment with fenofibrate, in a dose-dependent manner. Preliminarily, we have found that fenofibrate reduced human pro-inflammatory cytokine secretion in vitro. In addition to other PPARα-mediated actions, fenofibrate likely exerts human anti-cholestatic actions through its anti-inflammatory mechanisms.

Temperature Effects on Marine Invertebrate Physiology

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Due to the expected impact of global climate change, Rhode Island waters are predicted to rise by as much as 4°C. Studies on the native Rhode Island marine invertebrate *Ciona intestinalis*, a species of sea squirts, indicate a potential negative effect on the species' reproductive success. In this project, we looked at the impact of environmental stressors which might hinder the animal's embryonic development. This research was conducted by rearing local *C. intestinalis* animals in both a projected stressed temperature of 22°C and the high temperature limit for normal development of 18°C. We then carried out a cross fertilization, further analyzing the impact on embryonic development. We focused on the physical development of embryos by fixing them in formaldehyde and scoring them based on five categories: 1) unhatched and uncleaved eggs, 2) unhatched and cleaved eggs, 3) hatched eggs with normal embryonic tails, 4) hatched eggs with kinked embryonic tails, and 5) hatched masses with no developed structure. Our preliminary studies suggest that *C. intestinalis* reared at the elevated temperature produced far fewer viable embryos, often with little/no development or altered development. However, specimens reared at the 18°C oceanic temperature more often produced viable embryos with normal development. In addition, we conducted further "stress tests" based on modified pH and salinity levels consistent with the expected effects on the oceanic environment due to global warming. Our results illustrate that sea squirt embryos reared from animals at the stressed temperature of 22°C are less likely to survive exposure to other stressors (changes in pH and salinity, for example). Both of these stressors are predicted effects of global warming. These results suggest that the reproductive success of the *Ciona intestinalis* species will be hindered by increased water temperatures and other effects of global warming.

Crystallizing the Enzyme Nicotinamide Phosphoribosyltransferase: Understanding the Potential for Cancer Drug Development

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Nicotinamide Adenine Dinucleotide (NAD) is a coenzyme found in all living things. Made up of two subunits, its structure is easily altered by additional enzymes making it a model molecule for reactions in a biological system. NAD mainly serves as an electron carrier in metabolic oxidation and reduction reactions. The concentration of NAD is continuously changing due to cleavage of the molecule into its two subunits, one of which is Nicotinamide (NAM). NAM is transformed back into NAD in a two-step process. The first step involves Nicotinamide phosphoribosyltransferase (NAMPT), an enzyme that when inhibited is fatal for the cell. NAD is not only involved in metabolic pathways but many others including post-translational modifications. Given NADs importance in numerous cellular processes, the inhibition and activation of the regenerating enzyme NAMPT makes it an excellent target for drug discovery. In crystallizing the NAMPT protein, a crystal structure can be analyzed using x-ray diffraction which generates a detailed structure of the protein. This structure aids in the understanding of the protein and provides insight on how a drug could alter its typical functionality to potentially treat and/or prevent human diseases.

Structural Basis for Micro-Compartmentalization of D2-Dopamine Receptors

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The D2-Dopamine Receptor (D2R) is a clinically important receptor as it is the major target of all available antipsychotic drugs. D2R is also targeted in the pharmacotherapy of Parkinson's disease and depression. Previously, the Kovoor lab utilized a novel in-cell biotin transfer protocol to demonstrate that the majority of plasma membrane-expressed D2R is micro-compartmentalized within detergent-resistant structures. Studying how receptors are compartmentalized in the plasma membrane is important because the presence of such micro-compartments could explain how cells prevent cross-talk between receptor signal transduction pathways. The goal of my project was to identify the structural components of D2R that were important for the cellular micro-compartmentalization. D2R is a member of the seven-pass transmembrane G-protein coupled receptor superfamily and previously the lab showed that a D2R construct that extended from the N-terminus to the end of the 4th transmembrane domain (4TMD) was micro-compartmentalized but a similar construct that extended to the end of the 3rd transmembrane domain was fully accessible to other plasma membrane proteins. Thus the goal of my project was to determine if the 4TMD of D2R was both necessary and sufficient for micro-compartmentalization of D2R. Towards this end I designed and made DNA constructs for expressing the 4TMD of D2R in both bacterial and mammalian cells. These constructs were generated by PCR of the DNA segment encoding for the D2R 4TMD and ligation of the fragment into the mammalian and bacterial expression vectors, pCDNA3.1 and pET28a, respectively. Expression of D2R 4TMD in mammalian cells and evaluation of the accessibility of this construct will allow us to determine if the 4TMD is the structural feature of D2R that is both necessary and sufficient for D2R micro-compartmentalization. Expression of 4TMD in bacteria allows for the expression and purification of large quantities of the D2R 4TMD for biochemical studies. For example, evaluating the detergent-solubility of the purified D2R 4TMD will allow us to ask if detergent-insolubility is an intrinsic property of the 4TMD or requires interactions with other portions of D2R or with other cellular proteins. Similarly, reconstitution of the pure 4TMD in liposomes will allow us to ask if the D2R 4TMD can self-assemble into compartments.

Toxic Metals Inhibit the AlkB Family DNA Repair Enzymes by Replacing Fe(II) Ion in the Catalytic Center

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DNA and RNA lesions are constantly generated from exogenous and endogenous chemical attacks, and if left unrepaired the damages may result in cancer and other genetic diseases. To prevent accumulation of toxic adducts in the genome, all living organisms have evolved multiple DNA repair mechanisms. One such pathway involves the α-ketoglutarate(Fe(II)-dependent AlkB family enzymes that catalyze direct removal of alkyl adducts, ultimately recovering the undamaged DNA bases. Recent studies have shown divalent toxic metals can inhibit the AlkB human homolog ABH2 by replacing the Fe(II) ion in the catalytic center. This project aims to study the inhibition capacity of toxic metals, such as Ni, Cd, Hg and others, on AlkB and its human homologs ABH2 and ABH3 by evaluating the IC_{50} and K_i values.

Stablilzation of Dengue Peptides by HLA-A*11

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Dengue viruses (DENV) are a group of four serologically closely related viruses which belong to the family Flaviviridae. The viruses are transmitted to humans by the mosquito vector *Aedes aegypti*. Natural Killer (NK) cells are part of the immune system and express inhibitory and activating receptors on their cell surface. Killer-cell immunoglobulin-like receptors (KIR) are inhibitory receptors that interact with HLA molecules and regulate the function of NK cells. Recently, we identified an interaction between a DENV peptide presented on HLA B57 and an inhibitory KIR receptor, KIR3DL1. To expand our studies to HLA molecules commonly seen in dengue endemic areas, our project focuses on HLA-A*11 restricted DENV peptides. We received an NK sensitive cell line 221.721.ICP47-A*11 and tested different conditions to optimize HLA-A*1101 expression on the surface of 221 A*11 cells. We evaluated whether known HLA-A*11 DENV peptides can stabilize HLA-A*11 on the cell surface by using an antibody against HLA-ABC. The data was then analyzed by using FlowJo Software. Three variant peptides (D1.1, D2.2 and D3.1) stabilized HLA on the surface with the highest expression detected with the D3.1 epitope. In order to determine which amino acids are important for HLA A11 stability, we tested different alanine mutant peptides of the D1.1 epitope. Our results indicated that amino acids at position 8 and 10 were important to stabilize HLA-A*11. In the future, we plan to utilize this cell line stabilized with HLA*A11 DENV peptides to determine functional NK cell activity.

The Ascidian Myogenic Regulatory Factor N-Terminal Domain, an Evolutionary Novelty

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This summer, our lab is investigating the role in muscle development of the N-terminal domain of CiMRF, the *Ciona intestinalis* Myogenic Regulatory Factor (MRF). MRFs are basic helix-loop-helix transcription factors that regulate metazoan muscle development and feature the unique ability to stimulate muscle gene expression when they are expressed in non-muscle cell types. We exploited this ability to study the properties of MRFs by expressing them in the endoderm of embryos of the ascidian *Ciona intestinalis*. Previously, we found that only MRFs of *C. intestinalis* and the closely related ascidian *C. savignyi* were able to stimulate muscle gene expression in our assay. This result was surprising in view of published studies showing that MRFs exhibit a high degree of functional conservation. Subsequently, we found that the N-terminus of CiMRF was essential for promoting muscle development in our assay, a result that was further supported by studies carried out this summer. Bioinformatics analysis of the MRFs of three additional ascidian species revealed that they also possess a large N-terminal domain that is not present in the non-ascidian MRFs that we examined. This summer we are investigating whether the N-termini of four different ascidian MRFs are functionally conserved. In addition, we are using two complementary approaches to test the possibility that the CiMRF N-terminus acts, at least in part, as a transactivation domain. In the first we replaced the N-terminus with the transactivation domain of the Herpes simplex viral protein 16; in the second we created a fusion protein consisting of the N-terminus of CiMRF and the DNA binding domain of *Ciona* Brachyury, a notochord-specific gene regulatory protein. If the CiMRF N-terminus functions as a transactivation domain, then in the first approach the fusion protein is predicted to drive muscle gene expression in the endoderm and in the second approach the fusion protein is predicted to drive notochord gene expression in the endoderm. Collectively our research indicates that the N-terminus of ascidian MRFs is an evolutionary novelty essential for the activity of these transcription factors.

Cloning and Modification of a Full-length T Cell Receptor Beta-chain Gene for the Creation of Stably Transfected Cell Lines

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Single-cell analysis technologies are of growing interest in many biological fields, especially immunology, where well established technologies like ELISpot or flow cytometry are reaching their limits of resolution. Our laboratory will establish a single-cell analysis platform to investigate the capability of T cells to become activated and produce cytokines when stimulated with antigens. This PCR-based method will use an approach where T cell receptor (TCR) genes will be linked with several effector function genes (like *IL-13* or *IL-21*) if expressed in response to a stimulation with antigen. To validate our method and for the use as internal controls, cells with known TCR and effector gene expression are needed. This poster shows the cloning of a TCR gene (TCR beta-chain gene, TRB) which will be used, together with plasmids containing effector function genes, to create these cell lines. The cloning strategy includes the modification of a TRB gene amplified from human adult PBMC. This modification, necessary for the identification of an otherwise 'naturally expressed' TRB gene during spike-in experiments (internal validation controls), was done using fusion PCR. The complete experimental workflow consisted of the following steps: (1) isolation of nucleic acid from human PBMC, (2) cloning of TRB genes into vectors with subsequent sequencing, (3) primer design based on a functional TRB gene, (4) fusion PCR, and (5) final sequencing to confirm a functional and modified TRB gene. The successfully cloned TRB gene was later used for the creation of TRB expressing cell lines.

Antibody-Dependent Enhancement in Dengue Fever and its Effect on Disease Severity and Mitochondrial Elongation

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It has been observed that during secondary dengue virus (DENV) infection, an individual is at a higher risk for hemorrhagic fever. One factor thought to contribute to this risk is antibody-dependent enhancement (ADE), where antibodies produced during primary dengue virus infection actually help the virus enter the cell instead of neutralizing it. To permit further studies of ADE using different dengue virus serotypes, this project tested different experimental conditions for ADE using dengue virus serotypes 2 and 3 (DENV-2 & DENV-3). Different monoclonal antibodies and antibody concentrations were compared for their effects on DENV infection of K562 cells, a human myeloid cell line. The percentage of infected cells was determined using flow cytometry after samples were stained with a fluorescent-conjugated monoclonal antibody. Samples were also stained to determine if the virus had any effect on the mitochondria, which are known to elongate during dengue virus infection. Stocks of DENV-2 and DENV-3 differed in the percentage of infected K562 cells. Using a multiplicity of infection (MOI) of 2 and dilutions of monoclonal antibodies of 1:1000 to 1:4000, enhanced infection of K562 cells could be demonstrated for both DENV-2 and DENV-3. The experimental protocol developed should allow the measurement of ADE using samples of serum or antibodies from patients with dengue to test whether ADE correlates with disease severity.

Oxidative Stress Resistance and Neuroprotective Effects of *Mucuna pruriens* in *Caenorhabditis elegans*

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Mucuna pruriens (mucuna; velvet bean) is widely cultivated in India where it has been used as a food source and traditional medicine for centuries. Mucuna has been used traditionally, and in recent years, to treat the symptoms of Parkinson's disease (PD). Mucuna contains significant levels of L-3,4-dihydroxyphenylalanine (L-DOPA) which is used for the treatment of PD, and there are several commercial dietary supplements based on this natural product. In PD, a stressful cellular environment could contribute to the death of dopaminergic neurons, leading to the pathology of the disease. Herein we investigated the oxidative stress resistance properties and neuroprotective effects of a chemically characterized and standardized commercial Mucuna extract on wild-type and transgenic *Caenorhabditis elegans*

CHEMISTRY

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Optimizing the Purification of KmtR

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Mycobacterium tuberculosis (Mtb), infects close to one-third of the world's population. An estimated 9 million new active cases were found in 2013, of which 1.5 million perished from the disease. The WHO has estimated that an approximate 480,000 new cases of multidrug-resistant TB (MDR-TB) will appear in the coming years, of which 9% will be extensively drug-resistant (XDR-TB), so the development of new therapeutic strategies that target other essential pathways in Mtb is critical. Mtb encodes many metal transport systems including KmtR, the second Ni(II) and Co(II) metalloregulator in the bacteria, which is essential for its survival in phagosomes because the metal concentrations are constantly changing.The goal for this summer was optimize the purification conditions of the target protein, KmtR. The future work of our research is to use spectroscopic techniques to understand why KmtR is responsive to only Ni(II) and Co(II) binding.

G4 DNA-Binding Properties of Tolyl Terpyridine Tethered with Various Phosphonium Groups

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G-quadruplex DNA are guanine rich sequences that occur in the telomeric region of DNA. Past studies have shown that it is possible to intercalate planar molecules into the G4 structure and in turn cause apoptosis of the cell due to cellular stabilization. Because of this, many potential anti-cancer strategies involving planar molecules have revolved around G-quadruplex DNA. Phosphonium salts are known to selectively target cancer cells because of their positively charged and lipophilic nature. Phosphonium moieties should increase the cell permeability of large planar molecules and subsequently increase their cellular uptake. In this study, we focus on augmenting the planar 4'-(4-Methylphenyl)-2,2':6',2''-terpyridine (TTPy) with triphenylphosphine (PPh_3) in hopes to enhance their selectivity in cancer cells. A small library of complexes was made by synthesizing TTPy with various PPh_3 groups attached to the tolyl group to form TTPy-PR^{3+} molecules. Herein, we report the synthesis, characterization, and G-quadruplex DNA-binding studies of several of these novel substances.

Expression and Purification of a Metalloregulator from *Mycobacterium tuberculosis*, KmtR

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Approximately one-third of the world's population is infected by *Mycobacterium tuberculosis* (Mtb). There has been an increase in the number of drug-resistant strains of this bacteria and the development of new therapeutic strategies that target other essential pathways in Mtb is critical. *M. tuberculosis* requires two Ni(II)- and Co(II)-responsive metalloregulators, one of which is KmtR. The objective of this project was to explore the structure function relationships in KmtR from Mtb. Specifically, the short-term goals include determining the coordination number and geometry of the metal sites and to measure the metal binding affinities of both cognate and noncognate metal ions to KmtR. The process began by cloning KmtR from the genomic DNA. A plasmid was constructed for expression of the protein and the identity of the gene was confirmed by DNA sequencing. Optimal protein expression conditions have been determined and future work includes optimization of protein purification and obtaining X-ray Absorption Spectroscopy data (XAS).

Synthesis, Characterization, and Binding Properties of Metal-Based G-4 Intercalators Containing a Phosphonium Tether

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G4-quadrupli appear in the telomeric regions of DNA and are responsible for maintaining the structural integrity of the genome. Past studies indicate that it is possible for large planar molecules to intercalate G4-DNA and ultimately trigger cellular apoptosis. Because of this, G4-DNA has been the center of many novel anti-cancer strategies revolving around large planar molecules. However, the selectivity of these molecules is poor and as a result healthy cells are also damaged in vivo. Phosphonium salts have been known to selectively target cancerous cells due to their lipophilicity and delocalized positive charge. By incorporating the phosphonium salt moiety into the previously studied 4’-(4-Methylphenyl)-2,2’:6’,2’’-terpyridine (ttpy), we aim to enhance the selectivity of this molecule. Additionally, a series of noble metals (Pd, Rh, and Pt) will be incorporated into ttpy in order to promote stability and the likelihood of binding to the quadruplex. Herein, we report the synthesis, characterization, and DNA binding properties of a variety of compounds exhibiting the [M]-ttpy-PR3 motif.

Synthesis of BODIPY Appended β-Cyclodextrin Architectures for Improved Sensing and Understanding of Molecular Interactions

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Non-covalent energy transfer involving fluorophores and cyclodextrins provides a highly effective investigatory tool for the study of molecular interactions due to being both highly sensitive and dependent on multiple factors. In particular, our group has explored the use of the high quantum yield fluorophore BODIPY and the cyclodextrin isomer of γ-cyclodextrin extensively in these studies. (1) BODIPY is often chosen due to its high quantum yield and low stokes shift, while γ-cyclodextrin is a common choice as a supramolecular scaffold due to its well-documented ability to accommodate two small guest molecules in the interior of the cavity simultaneously. However, the use of γ-cyclodextrin carries a number of known limitations, predominantly stemming from its relatively large cavity size; although that size enables the cyclodextrin to bind two small molecule guests, it often endows the complexes with limited specificity and poorly-defined host-guest geometries. (2) β-cyclodextrin circumvents many of these issues due to its smaller size, and can provide a higher degree of sensitivity to and selectivity for a given analyte. Herein, we report the synthesis of a variety of BODIPY-attached β-cyclodextrins, where the key adjustable parameter is the length of the covalent tether between the BODIPY fluorophore and the cyclodextrin host. These compounds will be used to study and understand the basic criteria for efficient complex formation that results in successful fluorescence energy transfer.

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Interactions Between G4-DNA and Copper Complexes With a Tolyl Terpyridine Phosphonium Motif.

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Derivatives of the 4'-(4-Methylphenyl)-2,2':6',2"-terpyridine (ttpy) ligand have been shown to bind to G-quadruplex DNA. In our study, several different copper complexes were synthesized with a tolyl-terpyridine phosphonium motif. Copper complexes in particular have been reported to have their own mechanism of DNA interaction and cleavage. The addition of the phosphonium to the ttpy ligand is expected to increase selectivity toward inherently negative cancer cells. In order to increase solubility, glycine was ligated to our copper complexes. Copper-ttpy complexes have been synthesized with a diverse collection of different phosphonium groups to compliment the ttpy ligand. These complexes are currently being characterized and tested for their DNA-binding properties.

Detection and *In Situ* Fluorescence-Based Monitoring of Hydrocabon Food Sources in Complex Marine Environments

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Since the 1940s, organochlorine pesticides have proven effective in eliminating pests and securing a profitable harvest. However, they are also extremely environmentally persistent and remain in the soil, air and water long after their initial application (1). These analytes can be detrimental to both human health and marine food systems (2), and are therefore very important to monitor as climate change continues, ultimately magnifying the amount of pesticides present in the environment. We utilized our well-developed cyclodextrin-promoted fluorescence modulation technique to measure the presence (and amounts) of chlordane, heptachlor and lindane in the Providence River, Narragansett Bay, Narragansett Beach and Arcadia Lake, which are selected bodies of water throughout the state of Rhode Island. We were able to successfully identify all three pollutants with 100% differentiation in each body of water. Identification of these toxicants in various water bodies throughout Rhode Island is beneficial for researchers who study marine life as well as those who study pesticides in the environment, and it is additionally significantly beneficial to the residents of Rhode Island who can be potentially exposed to such pesticides through inhalation, ingestion, and skin contact. As long as global warming remains a concern around the world, it is important to continue monitoring the presence of these analytes in Rhode Island water and study their effects on both marine and human life.

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Inhibition of LuxR Mediated Quorum Sensing by β-Keto Esters

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Bacteria have become resistant to antibiotics, causing infections that are more difficult to cure. Quorum Sensing (QS), which allows bacteria to communicate with one another, is a chemical signaling system that is regulated by small complex molecules called autoinducers. Autoinducers released by the bacteria promote gene expression when bound to the receptor protein; genes expressed through QS include virulence factor production, swarming motility, and biofilm production which harm the body. The focus of this project is to inhibit the autoinducer from binding to the receptor by finding a compound with a similar structure so it will bind to the receptor and block the autoinducer, preventing the infection from spreading. Specifically, various β-keto esters were tested in a wild type strain of the bacteria *V. harveyi* for their ability to inhibit luminescence, a quorum sensing controlled phenotype. Each compound was observed to have no growth inhibition, with the exception of ethyl-3-oxohexanoate, ethyl (4-trifluoromethylbenzoyl) acetate, and ethyl (4-nitrobenzoyl) acetate that delayed the growth. Structure Activity Relationship studies showed the most active compounds to be ethyl (4-methoxybenzoyl) acetate, ethyl (3-methoxybenzoyl) acetate, ethyl (4-fluorobenzoyl) acetate, and ethyl (4-iodobenzoyl) acetate. These compounds also inhibited green fluorescent protein production in *E. coli* JB525, thus confirming that their activity is due inhibition of the QS pathway.

The Fluorescent Detection of BPA Derivatives and Learning Organic Synthetic Techniques

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Bisphenol-A (BPA) is an organic compound, which is well known for its use in the production of plastic. BPA is a known endocrine disruptor and studies have shown that this can have harmful effects to both humans and the environment as a result of its prevalence in production (1). Prolonged BPA exposure could result in detrimental effects such as impaired cognitive development in babies and young children, breast cancer, and heart disease. Efforts to replace BPA have focused on BPA derivatives, which behave in similar harmful manners. In the Levine laboratory, work is focused on the detection of BPA and its derivatives by using a fluorescence modulation technique using polymer nanoparticles. The fluorescent output by the nanoparticles in response to the addition of analytes demonstrates distinctive modulations. In addition, novel macrocycles are synthesized using Nitro-reduction, Suzuki-coupling, and Schiff-base reactions. These macro cycles are utilized as potential fluorescent detectors for a variety of analytes such as PAHs. Our objective is to study the interaction between the fluorescent polymers, macrocycles, and the analytes in order to better understand the reasoning behind its shift in fluorescent levels.

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Quantification of Pyocyanin in *Pseudomonas aeurginosa* Treated with Potential Quorum Sensing Inhibitors

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Pseudomonas aeruginosa is an opportunistic human pathogen that continues to grow resistant to numerous antibiotics essential for the treatment of various life-threatening infectious diseases. In order to combat this antibiotic resistance, a new mechanism causing bacteria to become dormant is being identified through the inhibition of quorum sensing. Quorum sensing is a population density-dependent mechanism of communication essential for bacteria to coordinate different actions. By targeting the quorum sensing pathways, the bacteria can be rendered dormant without killing the bacteria. In the wild-type strain of *Pseudomonas aeruginosa* (PA01), quorum sensing is the means of virulence factor production regulated by N-acyl homoserine lactone (AHL) signaling molecules. Of the virulence factors produced by PA01, pyocyanin is a blue-green toxin capable of oxidizing and reducing native molecules in the human biome. Pyocyanin production when treated with various quorum sensing inhibitory compounds was quantified through extractions with chloroform and re-extractions with hydrochloric acid. Absorbance was measured at 520 nm (OD520) using a UV-visible spectrophotometer following re-extraction with hydrochloric acid. The extraction process was used to isolate the pyocyanin in the acid layer, where it will exhibit a reddish-pink color. Through this quantification method, the quorum sensing inhibition of *Pseudomonas aeruginosa* virulence factors can be observed. Overall this assay protocol has been particularly difficult due to very poor consistency and reproducibility. A variety of liquid media, growth conditions, and incubation periods were tested. Consistency has finally been achieved, and a variety of potential quorum sensing inhibitors will be tested against this quantitative assay.

Development of a Hand-portable Chemosensor Device to Aid in Combatting the Trade of Illegally Caught Fish through the Cyanide Fishing Method

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Roughly 70% of all tropical aquarium fish are available solely through live-caught fishing methods [Mak, et. al.]. One very inexpensive and highly effective method of catching these fish is through cyanide fishing. Contrary to its effectiveness, this method has been deemed illegal due to the severe mortality rates it has on the fish and environment where this type of fishing takes place [Cervino et. al.]. One proposed method to deal with this practice is by developing a portable chemosensor to electrochemically detect thiocyanate, a metabolite secreted by the fish after being poisoned with cyanide. The handheld device will be created by using active and inactive electronics, along with the computing power of a Raspberry Pi®. Together these will be used to create three circuits that work cooperatively to mimic cyclic voltammetry measurements to selectively detect thiocyanate with a high degree of sensitivity using electrodes prepared with bound metalloporphyrins on transmissive conducting substrates.

Synthesis Of A Phevalin Derivatives Library For Quorum Sensing Activity

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Antibiotic resistance is becoming a large concern in today's society due to the fact that infectious diseases are being treated with compounds that kill or inhibit bacterial growth. One process used by bacteria to communicate with one another is called quorum sensing (QS). QS allows the bacteria to function as a whole rather than an individual cell; the ability to communicate is through a chemical known as an autoinducer. Autoinducers are small molecules that are released by bacteria, initiating the expression of quorum sensing genes. Inhibition of the QS pathway has the ability to control infectious bacteria without interfering with growth, making it less likely for bacteria to develop resistantance. Our goal is the synthesis of molecules that have the ability to inhibit QS. Phevalin has been successfully synthesized, and we have demonstrated its ability to inhibit QS using the reporter strain, *Vibrio harveyi*. We have also created a small library of derivatives using various amino acid starting materials and have investigated each compounds ability to inhibit quorum sensing. The most active derivative thus far is the phenylalanine-leucine derivative, with an IC_{50} of 39.11 μg/ml.

Developing a Metalloporphyrin-based Photo and Electrochemical Detector of Thiocyanate Ions in Marine Environments

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Coral reef ecosystems around the world are threatened by many destructive factors, including the common practice of cyanide fishing. The salt, sodium cyanide is toxic, yet it is used to immobilize exotic fish species for sale in Asian and North American aquarium markets. Fish caught in this manner secrete thiocyanate as a metabolite, which can be used as a marker for analysis. We are exploring an analytical method through the use of an array of metalloporphyrin chemosensors that can bind and detect thiocyanate with a high degree of sensitivity. These metal-centered macrocycles have distinctive absorption and electrochemical features, which should change quantitatively once bound to the anion thiocyanate. Metal centered meso-tetratolylporphyrins will be synthesized and then chemically functionalized onto silanated, semiconductive surfaces through Sonogashira coupling, including: iron (III), ruthenium (II), cobalt (II), zinc (II) and manganese (III). Once functionalized onto the solid-state substrate, cyclic voltammetry and UV-Vis absorption spectroscopy are used to quantify the response of these complexes to thiocyanate samples.

Selective Toxicity of Synthesized Molecules in Cancer vs. Normal Cells in Culture

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Arylphosphonium salts (APS) and some small polypeptides have selective affinity for cancer cells. We have observed selective toxicity of APS for mouse breast cancer cells by cytometry. The effect is amplified when RGD tripeptides are co-administered. The goal of this project is to optimize these results in cell cultures of normal and malignant mouse breast cancer cells and identify small molecule leads for toxicity studies in human cancer cells. RGD and APS are synthesized utilizing microwave assisted synthesis. The RGD's are prepared by solid state polypeptide synthesis assisted by microwave. Cells will be cultured from frozen stock cells, taken through three or four passages, stained with the appropriate dyes, co-cultured, then dosed with the APS co-administered with a synthesized polypeptide for observation by flow cytometry. We will determine EC_{50}'s and SAR's for a library of APS and modify the most promising leads to increase toxicity and selectivity.

siRNA Release from Lipid-coated Magnetic Nanoparticles (LMNPs)

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Glioblastomas (GBMs) are rapidly growing tumors that are found in the brain and spinal cord. Because of their rapid rate of growth, most GBMs are malignant and will lead to brain cancer. GBMs are very difficult to treat for a few reasons: the tumors contain many different types of cells which respond differently to certain therapies, and the blood-brain barrier poses a challenge for drug-delivery. This project focuses on Lipid-coated magnetic nanoparticles (LMNPs) as a means for targeting, diagnosing, and treating GBMs. The nanoparticles used in this study are iron oxide (Fe_3O_4) nanoparticles which were coated with varying compositions of 1, 2-Dioleoyl-3-trimethylammonium-propane (DOTAP) and 1, 2-Dimyristoyl-sn-Glycero-3-Phosphoethanolamin-N-[Methoxy(polyethylene glycol)-2000] (PEG2000) lipids. The iron oxide nanoparticles possess magnetic properties. This allows the nanoparticles to be directed to the GBM, as well as provide contrast that allows the GBM to be identified on an MRI scan. The small size of the nanoparticles, approximately 30-40 nm in diameter, allows them to easily pass through the blood-brain barrier. These LMNPs also spin and heat up when exposed to an electromagnetic field, such as one produced from a radiofrequency (RF) inductive heater. The final characteristic of these nanoparticles that makes them advantageous for GBM treatment, is that they can be coated with lipids. The lipids provide protection from the immune system, as well as allow the loading of cancer drugs such as siRNA onto the nanoparticles. The purpose of this study is to record and characterize the size and zeta potential of nanoparticles containing different compositions of DOTAP and PEG2000 in their lipid coating using dynamic light scattering, as well as measure the release of siRNA when the nanoparticles are exposed to an electromagnetic RF field using fluorescently tagged siRNA and a photospectrometer. The sizes and zeta potentials of the nanoparticles remained relatively constant over time, which demonstrates the ability to consistently prepare stable LMNPs. and it was shown that the heating of the nanoparticles caused the release of siRNA from the LMNPs, however whether the spinning of LMNPs also caused release is inconclusive. We were also able to show the release of siRNA from the LMNPs through the use of the electromagnetic RF field. This shows that there is potential to initiate the intracellular release of siRNA through the use of an electromagnetic trigger.

Synthesis of Triarylcinnamylphosphonium Salts for DNA Melting Studies and Toxicity Screening in Cell Culture.

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Synthesis of the title compounds was done by benchtop and microwave techniques. Variations in the cinnamyl scaffold were chosen since the "parent" compound proved the most active/toxic in prior studies of DNA melting. Much is known about the cytotoxicity of these salts and their ability to cross the cell membrane and penetrate DNA. The protocols involved in the synthesis are limited to a three to five step solvent-free synthesis. These compounds were analyzed using a MolInspiration informatics software to look for “hits”. Once a molecule was established that met our criteria, we synthesized our compounds and ran infrared spectroscopy to indicate the formation of esters. After establishing that ester peaks were formed through IR we synthesized our compounds in macroscale quantities to yield a white crystallized end product. These compounds will then be tested for selective cell toxicity in normal and malignant cancer cells.

Binding of the Emerging Contaminants Perfluorinated Compounds (PFCs) to Bovine Serum Albumin

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Perfluorooctanesulfonate (PFOS) and Perfluorooctanoic acid (PFOA) are two emerging environmental contaminants known collectively as perfluorinated chemicals (PFCs) that have been used in the production of such common products as Teflon, pizza boxes, and firefighting foams. Due to their unique chemical structures, these fluorine-heavy compounds have been under scrutiny by the National Institutes of Health and the Environmental Protection Agency for their possible negative effects on human health. This project identifies and examines the interactions of these PFCs with bovine serum albumin (BSA), a standard protein in blood. Methods including differential scanning calorimetry (DSC) and circular dichroism (CD) spectroscopy are employed to identify the thermodynamic and structural nature of PFC-BSA interactions. DSC and CD measurements of BSA solutions are taken with varying concentrations of PFOS or PFOA in the solutions, and a dose-dependent change in the thermodynamic properties of BSA is identified. This work serves as a starting point for future studies of the binding and structure of PFCs.

The Effect of Monovalent and Divalent Metal Cations and Arylphosphonium Monovalent Cations on Melting of dsDNA

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As calf and salmon DNA is heated in solution, it begins to denature. The native double helix form of DNA dissociates into single strands, indicating melting. Many factors affect the temperature at which DNA transforms from a double stranded form to a single stranded form. The presence of cations is one such factor that can have a stabilizing affect on the double helix form of DNA. The size and charge of the cation in solution are key factors that effect the stabilization of DNA. This experiment tested each of the variables with respect to divalent and monovalent ions of different size and charge (K^+, Mg^{2+}, and Ca^{2+}) in order to determine which cation had the most effective combination of charge and size that would increase the melting temperature. This was accomplished by altering one variable while the other remained constant. It was determined that potassium had little or no effect on the melting temperature, while magnesium and calcium increased the melting temperature. Furthermore, magnesium outperformed calcium and was found to be the most effective ion for stabilizing DNA.

Synthesis of Molecular Probes for Xenon-129 MRI

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Magnetic Resonance Imaging (MRI) can be improved by biosensors that selectively bind to biochemical targets. Cucurbit[6]uril (CB6) is a molecular cage that can reversibly bind the noble gas xenon, a process that can be detected by xenon-129 NMR spectroscopy. This unique binding ability makes CB6 a potential scaffold for constructing biosensors that could be detected by xenon-129 MRI. The key to the creation of a biosensor for xenon-129 MRI is the construction of a covalent bond that could tether target-specific ligand to the CB6 scaffold. Unfortunately this key step has not been accomplished to date. We have developed a method by which CB6 can first be hydroxylated by photochemical means to produce a mixture of polyhydroxylated CB6, and the monohydroxylated product can be chromatographically separated using CHP-80 resin or silica gel. Subsequently, the hydroxylated CB6 can be propargylated to produce a scaffold that can be readily conjugated to target-specific ligands via a routine click reaction.

Evaluation and Improvements to the Purification Strategy for the *Entamoeba histolytica* Bifunctional Alcohol Dehydrogenase (EhADH2) Enzyme

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The parasitic protist *Entamoeba histolytica* is responsible for the development of amoebiasis in humans, which results in over 500,000 infections and 100,000 deaths worldwide annually. *E. histolytica* lacks mitochondria and obtains energy from the anaerobic conversion of glucose into ethanol. The bifunctional alcohol and acetaldehyde dehydrogenase EhADH2 enzyme turns acetyl coenzyme A to acetaldehyde and acetaldehyde to ethanol in the last two steps of the pathway. We have previously shown that EhADH2 is essential for trophozoite growth and survival. A thorough understanding of the role of this enzyme and its function will aid in the development of more effective inhibitors for better management of amebiasis. The ehadh2 gene was cloned in pet23A and inserted into an adhe-deficient *E. coli* for expression. Initial purification through emulsification, centrifugation, and ammonium sulfate precipitation prepares samples for desalting through size exclusion chromatography using a Superdex 200 column. Desalted samples are run through a HiTrapQ XL anion exchange column and individual fractions are tested for their enzyme activity. Spectrophotometric assays are performed to monitor the conversion of the cofactor NADH to NAD^+ in order to determine the kinetic activity of the enzyme. Although significant progress has been made on this purification progress, a stable active protein is obtained reliably. Effective inhibitor molecules that were previously identified through growth inhibition studies will be tested for their specificity to EhADH2 and optimal inhibitor concentrations and delivery methods will be determined.

Using the “Molinspiration”® Docking Program to Find Structures that Bind Cellular Targets.

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Triphenylphosphonium cations are of particular interest because of their ability to passively transfer across cell membranes and permeate cell mitochondria. Both processes are driven by the negative membrane potential. The purpose of this research is to observe how variations in the structure of these molecules can change predicted bioactivity. Four different cationic scaffolds were studied, using Molinspiration to draw the structures, calculate physical properties and predict docking to molecular targets. This computational analysis reveals potential bioactivity and drug-likeness. It is observed that changes in the molecular structure cause changes in calculated results (sometimes substantially) for the drug targets: GPCR ligands, kinase inhibitors, ion channel modulators and nuclear receptors. It is important that many of the active compounds are accessible by laboratory synthesis and the actual compounds then screened for actual biological activity.

Unprecedented Rate Acceleration of Organocatalytic Ring-opening Polymerization through the Application of Bis- and Tris-(thio)urea H-bond Donors

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The organocatalytic ring-opening polymerization (ROP) of cyclic ester monomers, namely δ-valerolactone and ε-caprolactone, has been performed using various H-bond donating mono-, bis-, and tris- (thio)urea cocatalysts with H-bond accepting bases. Polymerizations catalyzed by the tris-urea cocatalyst exhibited reaction rates up to 100 times those of ROPs employing the previously disclosed mono-thiourea. Despite this significant rate enhancement, these polymerizations retain the characteristics of 'living' polymerizations: low polydispersity, predictable molecular weight, and linear evolution of molecular weight with conversion. In addition, the concept of increasing the number of (thio)urea moieties per molecule has been applied to a slate of chiral catalysts, which are hypothesized to be able to effect stereoselective ROP at enhanced rates. A mechanism for the rate acceleration with both bis- and tris- catalysts is proposed.

Screening for Inhibition of Sulfotransferase 2A1 in Mouse Liver Cytosol

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Sulfotransferases are enzymes that catalyze the transfer of a sulfonate group from a donor molecule to a particular substrate. Dehydroepiandrosterone (DHEA) is an endogenous steroid which is sulfonated by the specific sulfotransferase called SULT2A1. SULT2A1 is important physiologically to control steroid hormone homeostasis in both males and females. It also controls activity of cholesterol and cholesterol derivatives important for normal liver function. Thus, chemicals which may alter the rate of SULT2A1 action on endogenous substrates may disrupt the normal physiological roles of these substrates. Our lab is screening a set of compounds to test their ability and potency to inhibit the SULT2A1. This study specifically investigates the potential for inhibition of DHEA-sulfonation by mouse liver cytosolic sulfotransferase 2A1. The method includes mixing small volumes of the reaction components in the presence of increasing concentrations of the test compounds, incubating at 37°C for a specified time, boiling to stop the reaction, and centrifugation to remove denatured protein. The resulting components of the reaction mixtures were analyzed for DHEA-sulfate concentration using HPLC separation and UV detection. The test compound inhibition potency was calculated after plotting the DHEA-sulfate concentration versus test compound concentration. Results will be shown in detail for selected test compounds.

New Synthetic Methods towards Complex Nitrogen-containing Heterocycles

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Our research is focused on new methods in organic synthesis and chemical biology. The first of four projects involved is using palladium to catalyze the formation of complex β-carboline heterocycles in a minimum number of steps. This reaction forms multiple bonds and multiple rings simultaneously in the same flask. We have been screening various factors, such as solvent, ligand, temperature, stoichiometry, time, and substrate structure to optimize the reaction yield. Similarly, a second project is dedicated to developing and optimizing a palladium-catalyzed synthesis of α-carbolines via a one-pot Sonogashira coupling and [2+2+2] cyclization. Our third project involves the synthesis of eudistomin U analogs. We have prepared more than 25 of these β-carboline-containing molecules via a Suzuki reaction and will test their binding the 5-hydroxytryptamine receptor. Finally, we have initiated a project involving the fluorination of alkynamides as an easy method to synthesize α-fluoroamides. We have been exploring various reaction conditions, including: source of electrophilic fluorine, base, solvent, temperature, etc. We hope that these conditions will enable the formation of fluorine stereocenters in the future.

N-Heterocyclic Carbene-catalyzed Epoxidation of Alpha-amino Alcohols

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N-Heterocyclic carbene (NHC) catalysts serve great utility in catalyzing a wide range of nucleophilic additions. In this application, various imidazolium-derived NHC catalysts are employed to catalyze the conversion of an alpha-amino alcohols to the corresponding epoxied. This reaction would be the first reported case of an NHC acting as an electrophile rather than a nucleophilic. The alpha-amino alcohols used as the substrate for this epoxidation can be derived from amino acids via a two-step conversion: reduction with sodium borohydride and iodine followed by alkylation with formaldehyde to afford an N,N-dimethylamino alcohol. Using procedures found in the literature, this conversion is time-consuming and requires extensive purification. By using a microwave reactor, the conversion can be accomplished in less than 3 hours and with no get her purification needed.

Lock and Key: The Virtual Screening of Glycosyl Triazoles and Diamides against the N-acetylglucosaminidase FlgJ from *Salmonella enterica*.

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Salmonellosis is one of the most common causes of food poisoning in the United States, causing an estimated 1.4 million cases of foodborne illness and more than 400 deaths annually. Raw poultry and eggs are the usual culprits for *Salmonella* contamination. However, food recalls of fruits and vegetables can occur from cross-contamination. FlgJ is a flagellar-associated protein in *Salmonella* that is essential for rod protein assembly and passage of the assembly through the peptidoglycan (PG) layer. PG is a heteropolymer composed of alternating N-acetylglucosamine (GlcNAc) and N-acetylmuramic acid (MurNAc) linked via a beta 1-4-glycosidic linkage. Attached to the lactyl group of MurNAc is a stem peptide composed of D and L amino acids. Adjacent glycan strands are cross-linked via the stem peptides to form a 3-dimensional structure that confers shape and resistance to turgor pressure. FlgJ is an N-acetylglucosaminidase (GlcNAcase) that cleaves the glycan strands of PG between GlcNAc and MurNAc. With the inhibition of FlgJ, the flagellar filament will not form, resulting in a loss of motility. Previous work by our collaborators at the University of Guelph identified several glycosyl triazoles and diamides that inhibit FlgJ in vitro. Computational experiments using Autodock Vina were employed in an effort to understand how the compounds bind and interact with the FlgJ protein [5dn4], compare compounds, and identify more potential active compounds. The glycol triazole BI.fgba (IC_{50} 829 μM) and the diamide fgka (IC_{50} 142 μM) were identified by our collaborators mentioned above as the most likely candidates for inhibition of FlgJ. Computational analysis revealed key structural features that are important to binding. Validation of compounds identified via this virtual screen is provided via cell-based assay to prevent flagellar assembly. This work can eventually identify potent inhibitors that can be used as potential anti-virulence antibiotics.

Investigation of Nitric Oxide as an Inducing Agent for Bacterial Secondary Metabolism

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Marine bacteria have frequently been a source of novel bioactive secondary metabolites, however whole genome sequencing has shown that there are large amounts of microbial compounds that are inaccessible to the scientific world due to the inadequacy of laboratory growth techniques. New strategies are needed to crack into the world of "Microbial Dark Matter," especially to discover new antibiotics for fighting drug-resistant bacteria. Previous studies have shown that nitric oxide (NO) can influence phenotypes by modulating intracellular concentrations of the signaling molecule cyclic-di-GMP. For example, NO decreases cyclic-di-GMP in the human pathogen *Pseudomonas aeruginosa* leading to dispersal of biofilms. In this study, we investigated if NO could induce the production of secondary metabolites when provided to a panel of marine bacteria. Sodium nitroprusside (SNP) was used as an NO source in bacterial cultures. Two strains of *Loktanella*, two strains of *Pseudoalteromonas*, and one strain of *Bacillus* were cultured in the presence or absence of SNP at two concentrations (1µM and 10µM) after reaching stationary phase. An hour after the administration of SNP, XAD16 resin was added and left for 72 hrs. The resin was then collected, washed, and extracted. These extracts will be tested for new antibiotic activity and analyzed by HPLC for the presence of NO-induced compounds.

ENVIRONMENTAL SCIENCES

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The Relationship between Land Use, Pool Substrate Characterization, Pool Variability, and Temperature on Stream Macroinvertebrate Communities along Bailey Brook, Rhode Island.

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The objective of this study was to determine the influence of land use and land cover on the physical, chemical and biological features of small urban watersheds, focusing on Bailey Brook in Middletown, RI. Bailey Brook is especially important because it is situated in a heavily urbanized area and it is the primary drinking water source for southern Aquidneck Island. A number of freshwater macroinvertebrate species are good indicators of stream quality because they are sensitive to chemical and thermal pollution. We used biological indicies based on tolerance scores associated with Ephemeroptera, Plecoptera, Trichoptera (EPT) relative abundance, and an EPT/Diptera ratio. During the summers of 2014-2016 Macroinvertebrates were sampled at each site twice monthly with concurrent measurements of stream temperature, pH, and conductivity. The data collected for Bailey Brook were compared to Cork Brook, located in heavily forested Scituate, RI, serving as a reference site. EPT scores and EPT/Diptera ratios increased moving downstream, indicating higher water quality in lower reaches of the watershed. This unexpected result may be due to lower temperature in deeper pools and riffles, an increase in pool substrate characterization and an increase in pool variability in the lower reaches. An increase in firmer sediment types and rooted aquatic plants support a wider variety of organisms than a pool substrate dominated by mud or bedrock with few plants, despite higher levels of nutrient (P, N) pollution in these areas. However, the highest EPT scores in Bailey Brook were lower than scores at Cork Brook, the control. Data from RIGIS, associated with land use on Aquidneck Island, will be used to uncover key physical parameters and land use patterns such as, buffer zone width that would significantly alter the water quality at key sites along Bailey Brook.

Public Perception of Dams: How Their Removal and Construction Affects the People and Environment around Them

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With over 14,000 dams in the New England, many of them aging and under increasing pressure from climate change related weather fluctuations, and over 50 New England dams scheduled for relicensing in the next ten years, scientists and public officials need to take a closer look at how dams may be impacting the environment and local communities. This poster describes my work collecting and analyzing media discourse about New England dam decisions in local, regional, and national news media, as well as compiling a case study about dam decisions on the Pawcatuck River.

The Effect of Channel Flow Status, Pool Variability, and Phosphate Levels on Macroinvertebrate Communities on Maidford River.

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Agricultural and urban watersheds provide vital ecological services, such as filtering water as it moves downstream, erosion control, and flow regulation. Monitoring the integrity of the watershed is important for people who may use this water for drinking or other recreational uses. Watersheds also give macroinvertebrates a habitat to live in and but only if a certain range of conditions are met such as the correct amount of phosphate or dissolved oxygen. Due to their different pollution tolerances freshwater macro invertebrates can be used to determine the quality of water. This is measured by Ephemeroptera, Plecoptera, Trichoptera (EPT) scores as well as EPT/Diptera. This study tries to connect the downstream change of EPT scores and EPT/Diptera ratio to the changes in pool variability, channel flow status, and also Phosphate levels on Maidford river which is in an agricultural watershed. From summer 2014 to 2016 eleven sites in Rhode Island were monitored daily for temperature, pH, conductivity and biweekly for dissolved oxygen, nitrate, and phosphate. Macroinvertebrates were sampled at each site monthly. Ten sites were located on Aquidneck Island five sites on Bailey's Brook and five on Maidford River. The last site was located on Cork Brook in Scituate, RI as a reference. The results of this study show EPT/Diptera and EPT scores increase moving downstream on Maidford River. This may be due to the increase of pool variability and channel flow status in the same direction. Pools increase in variety from few small shallow to a mix of large and small pools moving downstream resulting in a higher EPT score and EPT/Diptera ratio. Channel Flow Status also increases moving downstream due to a larger area contributing to the flow of the river. This could explain why Phosphate levels also decrease downstream. The larger area leads to more water in the river downstream which dilutes the phosphate. The highest EPT scores were found in waters that had low phosphate levels suggesting a negative relationship between the two.

Public Opinions on Dam Removal

Francesca Soluri

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Through the Student Undergraduate Research Fellow (SURF) Program, Hannah Dallas and Francesca Soluri worked with mentor Dr. Caroline Druschke on a project titled “Public perceptions of dam removal and migratory fish passage in an era of climate change.” This project is part of a larger research group, funded by the National Science Foundation (NSF), that aims to study the tradeoffs that are made when deciding whether or not a dam should be removed. Starting with the research question “How can scientific findings and forecasts in the area of climate variability and marine life be made more accessible to the public through a variety of media,” Soluri and Dallas studied and collected data on public reactions and opinions concerning these tradeoffs, in order to establish a baseline overview of public knowledge and perceptions concerning dams and their removal. Soluri, based in Providence, was primarily focused on examining and collecting data concerning dam sites of Rhode Island, and the public opinions about these dams, through a textual and discourse analysis of news media throughout the state. This project involved compiling and examining state newspaper archives for articles on dam removal, contributing to a data bank of related articles in New England. Other tasks included completing an annotated bibliography of academic journals that examine the social factors of dam removal projects, and conducting case studies of dam sites in Rhode Island, focusing mainly on the mill complex and dam located at Potter Hill in Westerly. With the hundreds of dams in Rhode Island, there is still much ground to cover, decisions to be made, and discussions to be had, but with better understanding of public knowledge and perspectives about dam sites, we can better communicate the scientific findings about the environmental impacts of dams, and bring a broader perspective to these continued discussions.

Niche Partitioning among Two Dominant Detritivorous Snails in Narragansett Bay, Mud Snail and Dog Whelk.

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Narragansett Bay is an expansive marine community with estuaries that harbor a multitude of fish and macroinvertebrates. Detritivores such as crabs and snails play a key role in the cycling of energy and nutrients that link primary producers with consumers that fuels the rest of the food chain. Therefore detritivore populations are directly related to the productivity and biodiversity of an ecosystem. Specifically, in Narragansett Bay, the relationship between eastern mud snails (*Ilyanassa obsoleta*) and dog whelk (*Nucella lapillus*) was observed throughout 33 locations of western Narragansett Bay during the summers of 2015 and 2016. It was predicted that habitat partitioning would reduce competitive interactions between these generalist detritivore snails. It was also predicted that snail abundance would be highest in areas of high primary productivity and community biomass. Over the course of 10 weeks, a total of 33 minnow traps were set at along the bay and checked every few days for species count and identification. Substrate size was measured at all locations in the intertidal zone as a measure of habitat selection. Water quality (temperature, pH, DO, and SPM) was measured and chlorophyll density was measured as an index of productivity. The results showed that although competition between the two species exists over resources, whelk are drawn to more sandy habitats while mud snails prefer brackish and muddy waters and therefore there is rare spatial overlap. Although no significant pattern was observed between whelk abundance and biodiversity, areas with high numbers of mud snails did reflect low biodiversity but high total abundance of mud snails, an apparent allee effect. Greater whelk abundance was correlated with low productivity, an indication of areas with higher wave energy and larger substrates. But greater mud snail abundance was correlated with high productivity particularly in polluted areas where nitrate concentrations are high. While neither detritivore reflect bottom up control, they appear to respond to substrate and wave energy. Projections of climate mediated sea level and consequent habitat change may affect the abundance and distribution of these important detritivores. Further sea temperature increase and ocean acidification may additionally impact these important nutrient cyclers in Narragansett Bay, having cascading effects in the ecosystem.

The Effect of Nutrient Concentrations on the Growth of *Ulva rigida* in Narragansett Bay, RI

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Over the past decade there has been a noticeable increase of green macroalgae in Narragansett Bay, RI. The two main species found include *Ulva compressa* and *Ulva rigida*. The extensive proliferation of these species have resulted in the formation of Harmful Algal Blooms (HABs) that tend to outcompete other species in the environment for sunlight and nutrients, causing critical economical and ecological damage. In the search to determine the underlying causes of these macroalgae blooms, we seek to identify the influence of increasing nutrients including: nitrogen, phosphorus, and potassium on growth by culturing *U. rigida* in the laboratory with higher doses of these three nutrients. These particular nutrients are being investigated due to the fact that increased levels are often found in runoff waste that frequently enters Narragansett Bay due to fertilizer use from agriculture. With this, we hypothesize that the increase in concentration of nutrients in the water, has a direct correlation on the growth of the green macroalgae. *Ulva rigida*, was collected from Oakland Beach in Warwick, RI and weighed before being grown in culture. Samples were then grown under conditions mimicking typical summer photoperiods, with 16 hours of light and 8 hours of dark under different nutrient concentrations (0, 5, 10, 25, and 50 µM). These concentrations represent the range of nutrient levels found in runoffs. Each week culture media was changed and each sample was weighed to observe the growth associated with nutrient levels. In addition we extracted samples for RNA extraction for qPCR. We expect to observe a significant difference in the growth of the samples reflecting increasing nutrient concentrations. Further work will observe the mechanistic effect of nutrient concentration on growth of *Ulva* species in Narragansett Bay by assessing gene expression shifts using qPCR.

Distribution and Abundance of Asian Shore, Spider, and Green Crabs in Narragansett Bay Based on Substrates and Energy Levels.

Courtney Conklin, Katarzyna Kos & Jameson Chace

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Narragansett Bay is a prominent estuary in southern New England and provides a diversity of habitats for a rich community of marine invertebrates and fish. During the summers of 2015 and 2016 this study focused on nine sites spanning western bay from Dutch Island north to Conimicut point, with 33 subsites that comprise a range of near shore sheltered bays and coves to deeper sites with greater wave energy and daily tidal flow. At each subsite minnow traps were baited with mackerel in relatively shallow water (< 3 m) and checked at least once every four days and moved to new subsites each time to avoid oversampling the same location. Three species dominated the near shore environments *Hemigrapsus sanguineus* (Asian shore crab), *Carcinus maenas* (green crab), and *Libinia emarginata* (spider crab). The hypothesis that substrate, or habitat, structure principally determines the abundance and distribution of these three detritivore crabs was found to be important in site selection by these crabs. Asian shore crabs generally prefer cobble dominated substrates and tolerate high but prefer low energy levels, green crabs showed a preference for finer grained substrates such as sand, and gravel with lower energy levels, while, spider crabs prefer mud and sand substrates and eelgrass beds with high or low energy levels. The hypothesis that net primary productivity is the primary driver of crab abundance and distribution was determined by the features of the sites and resources available to each crab species. Finally, green crabs, first, and Asian shore crabs, more recently, are invasive species and have outcompeted native species in the near shore environment. We tested the hypothesis that crabs competitively exclude each other through correlation analysis and found that while there was no significant negative correlation in abundance of each species there was numerical dominance of Asian shore crabs. This work has been able to determine characteristics of sites were best suited for each type of crab, and has elucidated some key factors leading to the numerical dominance of Asian shore crabs. Species-specific ecological niche models such as these are important for mapping future climate-driven distributions of species that comprise the Narragansett Bay community.

Determining Bloom Identity of *Ulva compressa* and *Ulva rigida* in Narragansett Bay, RI Using Microsatallite Analysis

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Green macroalgae especially *Ulva* spp., have been causing harmful algal blooms (HABs) in coastal ecosystems around the world, including Narragansett Bay, RI. The blooms in the Narragansett Bay are mainly composed of two closely related species of green macroalgae, *Ulva compressa* and *Ulva rigida*. In this study we evaluate population dynamics of blooms in Narragansett Bay, Rhode Island, to determine if blooms are the product of a single individual rapidly multiplying, or of many individuals. By collecting and examining extracted DNA from samples of *U. compressa* and *U. rigida*, we can determine if one individual or multiple individuals are causing the proliferation of HAB in the Narragansett Bay. Samples of these two species were collected at three different sites: Chepiwonoxet Point, Sandy Point, and Oakland beach. Determination of the bloom identities required the development and identification of polymorphic primers for *Ulva* spp. Microsatellite primers were designed using Microsatellite Commander V2 to identify short, repeated sequences of non-coding DNA. First, these primers were tested on two samples from each species and amplified using PCR. Gel electrophoresis was then used to visualize PCR products; amplicons, and compared to a standard 100bp ladder to evaluate banding patterns. Next, we tested five samples of each species from the three collection sites using the above methods. This allowed us to examine the different banding patterns within the same species, therefore allowing us to identify different individuals. Using this information, we were able to determine the identity of each bloom and elucidate if the blooms are a single rapidly growing individual or multiple individuals.

Opossum Shrimp Genomics, Adaptive Capacity and Cimate Change in Rhode Island

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Due to human interference, the balance of oceanic ecosystems is changing. Without further study these altered environments can drastically effect the balance of the food web, directly affecting RI fish populations. Our research studies a lower tier food web species, the opossum shrimp, and plans to use them as a model through which we can predict the effects on other economically important, and difficult to test species. We have been assessing the phylogenetic relationships among six species of mysids of the genus *Americamysis*. These species were described in the 1990's based on morphological traits. Due to their overlapping distributions, highly similar physical appearance, and ability to hybridize we have been using DNA sequencing to characterize their taxonomic boundaries. The addition of a molecular approach will be a much less subjective way of classifying the species. For our study we plan to use a combination of mitochondrial, nuclear, and morphological traits to better characterize these species. We will use a fourfold method of characterization. Our first step was to use microsatellites to assess the genetic variability within and between species of mysids. In our second step, we created a series of inbred lines. The inbred lines were created by isolating pregnant females from our stock tanks into separate tanks until offspring were seen. The offspring were then bred among each other, until a generation of shrimp was born that had lost all reproductive capabilities (third generation). High quality DNA was obtained from the inbred lines, and was then extracted and sent to the Genomics Diversity Facility at Cornell University, where they used next generation sequencing to construct a DNA library for mysids. We will use the DNA sequences obtained by Cornell for SNP discovery. We will design primers for these sequences and use them to screen mysid populations. By using these SNPs, we will be able to contrast the genetic variability among populations. In our third step, we will use these SNPs across other species and localities to assess within and between species variation. The final step will be to create new inbred lines with minimal within-line genetic variability to assess what mutations, if any, allow these organisms to survive in non-ideal environments.

Contrasting Results from XRD and FTIR Data Sets of Hydrothermally Altered Sediment Collected from the Axial Seamount (Pacific Ocean offshore WA/OR, USA)

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A series of earthquakes occurring in 1999 along the Endeavor portion of the Juan de Fuca plate released an abundance of dissolved gases into the Pacific Ocean consisting of large amounts of CO_2, H_2, and H_2S (Seyfried et al., 2003), indicating that relatively smaller seismic events could have lead to similar abrupt and lasting changes in the vent fluid chemistry in the Juan de Fuca ridge. While the effects of CO_2 dissolution on shallow ocean chemistry is generally understood, not much research is available regarding the changes CO_2 dissolution has on deep ocean chemistry or on the sediment-hosted communities that inhabit hydrothermal vents in the Juan de Fuca Ridge. In this experiment, vertically profiled samples of cores obtained during the Northern 2014 Expedition research dives directed at the Monterey Bay Aquarium Research Institute (MBARI) were characterized by Fourier Transform Infrared Spectroscopy (FTIR) and X-Ray Diffraction (XRD) for mineralogical constituents, which serve as solid substrate for geomicrobiological activity. Cores for this study were collected near the Axial Seamount submarine volcano (46.0600° N, 130.0000° W) off the Pacific coast of North America near the common border of Washington and Oregon. The Axial Seamount lies in the center of a geologic hotspot in between the spreading Juan de Fuca ridge and the Pacific plate known for its rectangular shaped caldera that is lined with sulfide emitting hydrothermal vents that fuel biological communities. Push cores collected during the cruise were subsampled in order to resolve fine differences in mineralogy (via XRD) and screened for differences in organic loads (via FTIR). FTIR and XRD data provide different spectra which are analyzed accordingly. FTIR spectra were analyzed by creating a binary histogram, where the wavenumber (cm^{-1}) that the atoms in the sample respond to by vibrating and then absorbing is recorded in a spreadsheet. XRD analysis was done through XPower, a peak matching software that takes the spectra that the XRD produces and matches each significant peak to a known mineral until all peaks are accounted for. Collectively, the data provide insight towards understanding the mineralogy of the samples and how consistent their composition is with each respective dive they were taken from

Haplotyping Populations of the Invasive Species *Aethina tumida* in Rhode Island Beehives

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The small hive beetle (SHB, *Aethina tumida*) is an invasive species in North America and parasite of honey bees (*Apis mellifera*) and native bee species. SHB cause severe damage to hives by consuming honey, comb and bee larvae, compromising healthy hives and entirely destroying others; additionally, the very presence of SHB introduces yeast which ferment and ruin produced honey, preventing its use by the hive and beekeeper alike. Originating in sub-Saharan Africa, these beetles have undergone a rapid and widespread diaspora throughout a number of countries, including the United States. We have undertaken a detailed survey of SHB populations in Rhode Island by monitoring 35 apiaries throughout the state. In 2015, SHBs were collected from mineral oil trays over ten weeks in a previous study assessing population counts in 35 beehives in Rhode Island. To determine the extent of genetic variation within the Rhode Island SHB population, we are using single-nucleotide polymorphisms in the mitochondrial Cytochrome C Oxidase I (COI) gene, to determine SHB haplotypes. By comparing haplotypes of Rhode Island SHBs to subpopulations within Africa, we will trace SHB origin and determine whether single or multiple introductions occurred.

Do Nitrous Oxide Fluxes Reflect Ammonium Concentrations in Different Zones at Mary's Creek Salt Marsh?

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Coastal salt marshes serve as highly productive habitat that is important ecologically and economically. Not only do they provide a nursery for important fishery species, but they also protect the shores against flooding and erosion. Recently, anthropogenic factors such as increased nutrient loading have been damaging and degrading coastal marshes on a global scale. Coastal marshes here in Southern New England are experiencing changes to their floral communities due to factors such as invasion, increased nitrogen (N) inputs and sea level rise. Mary's Creek, a back-barrier marsh located in Warwick, RI, has historically received high N influxes. Nitrous oxide (N_2O) is a greenhouse gas that can be formed as a byproduct of microbial activities such as denitrification in marsh soils. This is a natural process that occurs in the soils of salt marshes. The purpose of this study is to test and compare N_2O fluxes from different vegetated and dieback areas at Mary's Creek. Dieback areas occur when a disturbance causes the marsh vegetation to die. For this study, flux measurements took place in three different vegetated zones and three vegetation dieback zones. To measure the N_2O fluxes, the Picarro G2508 and Los Gatos Research N_2O/CO greenhouse gas analyzers were both utilized. A transparent chamber connected to the analyzer via tubing in a closed loop was placed over an installed collar to measure net N_2O fluxes from the marsh. There were three replicates per zone. In addition, before gas measurements began, salinity levels were collected from each collar using a portable refractometer and porewater samples were collected to test for ammonium concentrations. Nitrous oxide fluxes were calculated in R to plot concentration over time. Ammonium analysis took place using the Orion Aquamate 7000 VIS spectrophotometer. Statistical analyses will be preformed in JMP to determine relationships between N_2O and ammonium, and also test for flux differences between the zones. A positive correlation between N_2O fluxes and ammonium concentrations are hypothesized to show that they reflect each other in a marsh environment. It is important for future studies to include a variety of salt marsh "zones" because each plant species contributes to the overall diversity and high productivity of the marsh. By testing multiple areas in marshes, the long-term health of coastal marshes may be better understood. With this, conservation efforts may be better tailored towards specific issues.

Metabolic Dynamics: From Individuals to Whole Colonies

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Ants (Hymenoptera: Formicidae), live and behave in functionally integrated social groups that promote the well being of their colony as whole rather than their existence as individuals. Their collective behavior is responsible for the performance of colonies as super-organisms, but relatively little is known about the physiological causes and consequences of these behaviors. By studying the respiratory characteristics of individual ants and whole colonies, we aimed to identify distinct metabolic signatures of eusociality. To measure the ventilation and metabolic dynamics of ants, we conducted flow-through respirometry to detect real-time changes in the concentrations of oxygen and carbon dioxide induced by the metabolism of organisms within airtight chambers using the Sable Systems FoxBox and Li-Cor 7000 gas analyzers. Baseline air was supplied by an ultra zero compressed air tank and regulated at 50-1000 mL per minute depending on the size of the organism using an Omega MFC. Motion was detected using an infrared activity detector and also by tracking individual positions from recorded video. The wet masses of ants were measured to the nearest 0.01 mg using a Mettler Toledo XS analytical balance. Ants were collected from locations on campus at Providence College and Lincoln Woods State Park in Rhode Island and maintained in the lab in artificial nest enclosures and with food and water provided ad libitum. We measured the ventilatory characteristics of individual ants from *Camponotus floridanus*, *Formica subsericea*, *Formica fossaceps*, *Tetramorium caespitum*, and *C. pennsylvanicus* and whole colonies of *Temnothorax curvispinosus*. Preliminary results indicate that individual ants exhibit a metabolic allometry with larger individuals (e.g. super majors) exhibiting significantly lower mass-specific metabolic rates than smaller individuals (e.g. minors). In addition to this effect of morphological caste, the scaling was also shown on an interspecific basis. Three fundamentally different ventilation patterns were exhibited by individual ants including continuous, cyclic, and discontinuous gas exchange. We tested the hypothesis that whole colonies would exhibit cyclic ventilation due to synchronized breathing among individual ants within the same colony. Future research on this project will use Fourier transform to examine the periodicity of ventilation and the correlation between locomotory and respiratory dynamics.

GENETICS

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The Effects of Gallic Acid on Changes in Gene Expression Profiles in the Immortal Gastric Adenocarcinoma Cell Line AGS as found through Microarray Analysis

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Gastric cancer is the fifth most common cancer with a fatality rate of over 70% in the Western world. Standard treatments such as chemotherapy incur unfavorable side effects, necessitating the development of less invasive treatments. One promising area of study focuses on nutraceuticals, naturally occurring plant secondary metabolites. One such compound of particular interest, gallic acid, is found in high concentration in blackberries and raspberries. In previous studies, gallic acid has been shown to cause cancer cell death while leaving noncancerous cells unaffected in immortalized cell lines. In this study, we identified changes in gene expression profiles in the immortal gastric adenocarcinoma cell line AGS when treated with 100µM gallic acid over a period of 24 hours using Affymetrix human transcriptome arrays (2.0). AGS cells were grown under standard culture conditions at Salve Regina University. Cells were serum starved for 48 hours and treated with 100µM gallic acid; untreated AGS cells were given DMSO as a negative control. RNA extractions were performed at 0, 6, 12, and 24-hour time points. RNA was hybridized at Brown University's genomics center and microarray data was analyzed using the Affymetrix Expression Console and Affymetrix Transcriptome Analysis Console. A total of 763 genes were differentially expressed among all treatments. Cells treated with 100µM gallic acid for six hours displayed the greatest number of differentially expressed genes compared to untreated cells at the zero-hour time point. 70 genes were upregulated and 147 genes were downregulated between these two treatments. This suggests that a 100µM concentration of gallic acid (equivalent to 1.7% of the gallic acid in a single berry) has the greatest effect on AGS six hours into its 24-hour doubling time. Thus, gallic acid serves as an intriguing potential alternative treatment for gastric cancer.

Functional and Mechanistic Responses of the Adenocarcinoma Immortal Cell Line AGS to Low Dose Treatments with the Nutraceutical Gallic Acid at 24 and 48 Hours

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Gastric cancer is the third leading cause of cancer-related deaths globally and the second most common cancer death in United States. The most common treatment options of treatment for gastrointestinal cancer includes chemotherapy and resection of primary tumors, both of which are particularly invasive, non-specific, and can have many deleterious side effects to the patient, which highlights the need for improved treatment techniques. Gallic Acid (GA) is a secondary plant metabolite found naturally in plants such as, raspberries and blackberries. In the plants cells, GA promotes rapid cell proliferation; however in cancer cells, GA has been shown to contribute to cell cycle arrest, without affecting normal, healthy cells. We evaluated the effects of GA on the cell cycle, and treated cells from the immortal gastric adenocarcinoma cancer line AGS, an epithelial cancer of the stomach lining with GA. To evaluate minimal effect dose, we investigated the effect of low concentrations of GA on cell cycle and gene expression in the cell line of AGS. This study investigated the cell line of AGS was serum starved for 48 hours and treated with fine doses of GA (0, 5, 10, 15, 20, 40, 60, 80, 100µM) for 24 and 48 hours. Cell cycle analysis was performed by using flow cytometry; used to analyze the phase in which the cells has stopped proliferating. The mechanistic response to GA was analyzed using qPCR, to analyze the expression of several genes of interest. These included genes involved in the expression of genes important to the cell cycle (P21, Cdk4, Cdk6, Cyclin D1), angiogenesis (MMP9) and apoptosis (Bax, Bcl2, RhoB). Gene expression at 24 hours was highly variable and currently under further investigation. However, expression of Cyclin D1 and MMP9, at 48 hours, at low doses of 5-10µM were slightly up-regulated. This suggests that Gallic Acid is effective in very low concentrations as low as 5-10µM may be useful for preventing gastric cancer. Low concentrations of gallic acid can easily be obtained in a glass of wine or a handful of blackberries.

Investigating the Effect of High and Low Doses of Gallic Acid on Cell Cycle, Apoptosis, and Angiogenesis Gene Transcripts in Gastric Cancer Cell Line MKN-28

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Gastric cancer is a significant global health concern; it is the fifth most common cancer in Western countries and the five-year survival rate is less than 30%. Current treatment options include chemotherapy, radiation, and resection of the primary tumor. These routes of treatment are non-specific to cancerous cells, expensive, and have adverse side effects on the patient. Nutraceuticals, which are naturally occurring, biologically active compounds in food that have medicinal benefits in humans, provide potential treatment alternatives. The nutraceutical gallic acid, a phenolic compound commonly found in blackberries and raspberries, has been shown to selectively target mammalian cancer cells and impede their division. In this study, we investigated the effects of high and low doses of gallic acid on gene expression in cancer cells from the immortal gastric cancer cell line MKN-28 over 48 hours compared to untreated cells. Prior to treatment, cells were cultured to approximately 80% confluence, serum starved for 48 hours, and then treated with gallic acid at concentrations of 0 (no treatment control), 20 (low effective dose), and 100 µM (high dose). Cancer cells were sampled at various time points (0, 3, 6, 12, 18, 24, 36, and 48 hours). Using qPCR, we investigated expression changes of gene transcripts associated with cell cycle regulation (P21, CyclinD1, Cdk4, Cdk6), apoptosis (Bax, RhoB, Bcl2), and tumor angiogenesis (MMP9). Fold changes were calculated using the constitutively expressed gene RPL29 as a baseline. While the 20µM dose of gallic acid induced changes in gene expression, we found that the 100µM dose was more effective. The genes RhoB and Bcl2 were upregulated while MMP9 was downregulated. Determining which of these gene transcripts are upregulated or downregulated is crucial to understanding the effects of gallic acid on the immortal gastric cancer cell line MKN-28. An average blackberry contains gallic acid at a concentration of 6,000µM which is far higher than the demonstrated effective dose of 20µM. Therefore, ingesting blackberries or other foods with high concentrations of gallic acid, has the potential to prevent and treat gastric cancer while avoiding the problems associated with traditional cancer treatments.

Investigating the Antiproliferative Effect of Gallic Acid on the Gastric Cell Line MKN28 Through Flow Cytometry and Quantitative PCR

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Cancer is the second leading cause of death in the United States, accounting for 22.92% of all fatalities. Gastrointestinal cancers are especially lethal with a 5-year survival rate less than 30%. The standard course of treatment for gastric cancer includes chemotherapy and resection of primary tumors, both of which are non-specific and present many negative side effects. Alternative treatments are being investigated in the form of nutraceuticals, nutrients that can be utilized to prevent and treat diseases. Gallic acid is a plant phenolic found in high concentrations in fruits such as raspberries. In plants gallic acid stimulates cellular proliferation, but studies have shown that in mammalian systems, gallic acid specifically targets cancer cells by halting cellular proliferation. This study investigated the minimum effective dose of gallic acid needed to cause cell cycle arrest in the immortal gastric cancer cell line MKN28. Furthermore the effect of gallic acid on key genes found in several molecular pathways important for cancer including: progression through the cell cycle (P21, Cdk4, Cdk6, CyclinD1), tumor angiogenesis (MMP9), and apoptosis (Bax, Bcl2, RhoB) was studied. Cells were treated with gallic acid in varied concentrations of 0, 5, 10, 15, 20, 40, 60, and 100 uM over both 24 and 48 hour time periods. Cell cycle arrest was observed with flow cytometry and gene expression was assessed using qPCR by comparing genes of interest against a constitutively expressed housekeeping gene (RPL29). Using flow cytometry, we found that MKN-28 cells, which normally have a doubling time of approximately 36 hours, are held in the G1/S phase of the cell cycle when treated with gallic acid resulting in a longer doubling time. We found that when the cells were treated with the lower doses of gallic acid (10, 15, and 20 μM), expression of our genes of interest were generally up regulated. Whereas doses higher than 20 μM resulted in a down regulation in all of our genes of interest. This suggests that gallic acid has a minimal effective dose on the pathways between the concentrations of 10 and 20 μM. Differences between gene up-regulation were also seen between the 24 and 48 hour samples, further indicating that gallic acid alters the duration of the cell cycle of gastric cancer cells. When used as a nutraceutical gallic acid can halt proliferation of cancer cells and therefore potentially reduce the negative effects associated with common cancer treatments.

The Diversity of *Nephromyces* Species Found within *Molgula* tunicates

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Apicomplexa is a large, diverse phylum of metazoan parasites that includes *Plasmodium* and *Toxoplasma*, the causative agents of malaria and toxoplasmosis, respectively. Apicomplexa was previously considered a purely parasitic clade, however, species in the genus *Nephromyces* have been recently described as mutualistic partners with their host, *Molgula* tunicates. *Nephromyces* are located in a specialized organ called a renal sac, and have been found in nearly every mature tunicate surveyed. Previous studies have determined that every molgulid tunicate has multiple species of *Nephromyces* within its renal sac. Further complicating the relationship between tunicates and *Nephromyces* are the endosymbiotic bacteria found within species of *Nephromyces*. To better understand the diversity of *Nephromyces* and its bacterial endosymbionts within *Molgula* tunicates, the contents of ninety-four renal sacs were sequenced using ribosomal RNA primers for eukaryotes (18S), bacteria (16S), and a mitochondrial primer set for the cytochrome oxidase I gene (CO1). Here we describe the diversity of species within this complex symbiosis and the implications for the biology of these organisms.

Changes in Transcriptome Expression in the Immortal Gastric Cancer Cell Line Mkn-28 after Gallic Acid Treatment Revealed through Microarray Analysis

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Cancer is the second leading cause of death in the United States, accounting for 22.92% of all fatalities. Gastrointestinal cancers are especially lethal, with a 5-year survival rate less than 30%. Current courses of treatment for gastrointestinal cancer includes chemotherapy and resection of the primary tumor, both of which are particularly aggressive, non-specific, and have many deleterious side effects. Gallic acid is a plant phenolic found in high concentrations in raspberries and blackberries that has been shown to specifically target cancer cells, halting cellular proliferation. We investigated the effects of gallic acid on gene expression in the immortal gastric cancer cell line MKN-28. Transcriptomes from the gastric adenocarcinoma immortal cell line, MKN-28 were after with 100uM GA and compared to untreated. Prior to treatment, cells were serum starved for 48 hours, and biological replicates were treated with media containing gallic acid dissolved in dimethyl sulfoxide (DMSO) to a final concentration of 100µM. Samples were collected at 6, 18, 24, and 36 hours post exposure. Controls included a media-only, with cells grown in complete media, and a DMSO control, with cells grown in media with DMSO. RNA was extracted using Trizol, was quantified and hybridized to Affymetrix's GeneChip® Human Transcriptome Arrays 2.0 at the genomics center at Brown University. Transcriptome expression was analyzed using Affymetrix® Expression Console™ (EC) and Transcriptome Analysis Console (TAC) Software. Initially, all time points were compared against each other, which showed that the 6 and 36 hour time points had the greatest number of differentially expressed transcripts in comparison to all other time points. The 6 and 36 hour time points were then compared against the media only and DMSO controls. When compared against the DMSO control, there were 438 genes up-regulated and 332 genes down-regulated in the 100µM/6hr sample, and 237 genes up-regulated and 241 genes down-regulated in the 100µM/36hr sample. The functional role of differentially expressed transcripts was then determined to evaluate pathways important in gallic acid's ability to halt cellular proliferation. The roles potentially involved in antiproliferation included genes known to be cell cycle regulators. This suggests that gallic acid treatment affecting genes key to cell cycle regulation that is allowing for the anti-proliferation of MKN-28 gastric cancer cells.

Characterizing Motor Neuron Degeneration in a *Drosophila* Model of ALS

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Amyotrophic Lateral Sclerosis (ALS) is a devastating neuromuscular disease characterized by motor neuron degeneration leading to paralysis, and afflicted individuals typically surviving only 3-5 years after diagnosis. Mutations in the superoxide dismutase 1 (SOD1) gene produces a dominant form of familial ALS in humans. To better understand the pathogenesis of ALS at a cellular and molecular level, we have introduced ALS-causing *SOD1* point mutations within the endogenous *Drosophila Sod1* locus by homologous recombination. Homozygous *Drosophila sodH71Y* mutants show a decreased life-span and exhibit neurodegeneration and associated locomotor deficits. We have focused on characterizing progressive motor neuron degeneration in the metathoracic leg, as well as at the neuromuscular junction. *sodH71Y* flies were aged and legs dissected. Immunocytochemistry revealed a decreased axon bundle width through the leg, as well as decreased motor neuron arborizations between aged and non-aged flies. Additionally, disruption in proteostasis due to both the ubiquitin-proteasome system (UPS) and autophagy has been associated with many neurodegenerative diseases including ALS. Immunocytochemistry revealed that ubiquitin, a marker for proteins targeted to the proteasome, and LC3, an autophagy marker, showed a dramatic increase in *sodH71Y* aged flies. These studies will allow us to further compare motor neuron changes in various ALS models across species.

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Caribbean Reef Fish Response to Coral Restoration

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The decline of Caribbean coral reefs since the 1970s has led to widespread coral reef restoration during the past ten years. *Acropora* corals are fast growing and create complex 3D structures which generate habitat for fish and other marine organisms. The *Acropora* genus has faced a 97% decline from their 1970s population levels. These reasons have lead *Acropora* to be used for virtually all coral restoration projects all around the Caribbean. Previous research has been focused on perfecting methods to regrow and repopulate *Acropora* populations but little attention has been given to the recovery of the rest of the reef community. In addition to *Acropora*, many other coral species have declined, as have many fish and invertebrates. The hypothesis is that restoring *Acropora* populations on the reef will induce a population increase in fish, lobsters and other reef organisms. In this study we are looking to test the hypothesis that Acropora restoration benefits reef fish communities. Specifically, we are testing if there is some threshold of amount of coral that must be restored to increase fish densities. To test this hypothesis, we visited five locations around the Caribbean (Guana, BVI; St. Croix, USVI; Punta Cana, Dominican Republic; Nassau, Bahamas; and Kingston, Jamaica) where active *Acropora* restoration has been occurring. At each location we surveyed several restoration sites that differed in how long ago restoration occurred (1-9 years) and how many corals were initially outplanted. We conducted fish counts within defined areas (30m by 1.5m belt transects) and surveyed current coral abundance using point intercept methods along on a 30m transect. To test the restoration effect, we compared fish densities at restoration sites to pre-restoration densities (temporal controls) or to nearby non-restored sites (spatial controls). We are thus evaluating the broader ecological effectiveness of coral reef restoration and encourage restoration studies to include the reef community response as an important factor in determining the success of a study.

Chemical Competition of Native and Nonnative Algae

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Presence of the nonnative red alga *Grateloupia turuturu* has increased in Narragansett Bay since its arrival in 1994. This species is known to be problematic for native red alga, such as *Chondrus crispus*, because it has extended its biological range. There have been many studies documented the impacts of *Grateloupia turuturu* in its invasive range, but few studies have looked at the production and release of chemical compounds by *G. turuturu* that inhibit the growth of other macroalgae (i.e. allelochemicals). In order to determine whether *G. turuturu* produces allelochemicals that impact *Chondrus crispus*, we set up two laboratory trials. In each trial there were experimental mesocosms (n=7) consisting of native *Chondrus crispus* and invasive *Grateloupia turuturu* separated by mesh to allow water exchange, but not physical contact of the two species. There were also control mesocosms that consisted only of *Chondrus crispus* (n=7) in order to determine its growth rate without the influence of *Grateloupia turuturu*. In each eight-day trial, we determined the wet mass of *Chondrus crispus* every other day and determined growth rate over time. Nitrate was measured daily and essential nutrients were replenished to verify that the only major factor impacting the growth rate of *Chondrus crispus* was based on the allelochemicals from *Grateloupia turuturu*. Our results suggest that there was no significant effect of *Grateloupia turuturu* on the growth rate of *Chondrus crispus*. In other words, *G. turuturu* does not produce and release allelochemicals. Therefore, any negative effects of *G. turuturu* on *C. crispus* are not a result of chemical competition.

Do Food Web Changes and Habitat Loss Explain Population Declines of Coral Reef Fishes?

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Many populations are in decline, and correlational analyses imply that habitat loss is often responsible. For most coral reef fishes, a critical habitat feature are crevices in the reef that provide structural refuges to which they flee when threatened by a predator. Experiments show that a fish's chance of escape increases as the ratio of fish to refuges increases. Despite the fact that these interactions between fish, predators and refuges control the abundance of many reef fishes, we know little about how predator-prey interactions have changed as reef habitats degrade. In this study, we tested for changes in the behaviors of the bridled goby (*Coryphopterus glaucofraenum*), the behaviors of their predators, and the availability of refuges on the reef, to explain their population decline. We repeated experiments that manipulated prey population density and refuge availability in 2000 (pre-decline) and 2015 (after substantial decline). In 2016, we performed an identical manipulation within the same eighteen 4 x 4 m plots of reef used in the two previous studies. We are testing whether declines in goby abundance are due to (1) increased predator density or attack rates over time or (2) declines in refuge availability or quality over time that have led to intensified competition for hiding places. We thus monitored predator density, visitation rates, and attack rates in each plot using procedures identical to those in 2000 and 2015. To assess competition for refuges, we monitored goby feeding rates, aggression, refuge use and mortality, also using methods identical to those employed in the past. Understanding the basis for habitat-related population declines should help predict the consequences for fish, when influenced by management actions designed to protect reef habitats.

Macroalgae Cause Mortality in Oyster Larvae: The Effect of Nutrients

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The numbers of harmful macroalgal blooms have been increasing, and are expected to continue to increase, as a result of a warming climate. Macroalgae have been known to produce and release chemical compounds that negatively impact shellfish (i.e. allelopathic compounds). Shellfish farming is an important and profitable industry that could be greatly impacted by allelopathic chemicals from macroalgal blooms. While we know macroalgae produce allelopathic chemicals, we do not know the nutrient conditions that promote the production of these chemicals. In this study we exposed two species of macroalgae (*Ulva rigida* and *Ulva compressa*) to nutrient replete and nutrient deplete conditions. *U. rigida* and *U. compressa* were cultured for at least two days in both nutrient replete and nutrient deplete conditions. After the nutrients had been used by the replete samples, two to ten day old oyster larvae were then exposed to the deplete and replete culture water from each species for two weeks. Larval mortality was recorded every other day over the course of the two-week period. Data suggests that the two species of algae have a different response to nutrients. There was no significant difference in the survivorship of oyster larvae exposed to replete and deplete *U. rigida* water. Conversely, oysters had significantly lower survivorship in nutrient replete *U. compressa* water than in nutrient deplete *U. compressa* water.

Can Probiotic Treatments Slow the Progression of Epizootic Shell Disease in Lobsters?

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The lobster industry is a cornerstone of the seafood market in the United States. In the coastal waters of the northeastern U. S., that cornerstone is being chipped away at by Epizootic Shell Disease (ESD): an illness that causes deep lesions that spread over the shell. The frequency of harvesting lobsters with ESD is causing harmful repercussions to coastal shellfish economies. In this study, we are exploring probiotic treatments as a means to slow the progression of ESD. Lobsters with ESD will be treated with a blend of potential probiotic bacteria isolated from the outer surfaces of apparently healthy *Homarus americanus*, the species native to Rhode Island. Percent coverage of ESD on shells will be measured over time for both treated and untreated animals. This presentation will describe the image analysis methods utilized to measure ESD progression.

Seasonal Variation of the Macroalgal Community in Napatree Lagoon

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Macroalgae are important components of coastal ecosystems that provide habitat for invertebrates and serve as the base of many food webs. In May of 2015, we began surveying the macroalgal community in Napatree Lagoon, Watch Hill, Rhode Island. Monthly surveys were conducted from May through September 2015 and were repeated in 2016. In 2015, there were differences in macroalgal abundance within the lagoon and between months, with the highest abundance of macroalgae collected in July. In July 2015 the maximum intertidal biomass was 1100 g/m^2 and the maximum subtidal biomass was 2200 g/m^3. Ongoing analysis is being conducted to determine whether yearly and seasonal fluctuations in the biomass and species composition of the macroalgal population in Napatree Lagoon is correlated with temperature, storms, and/or nutrients in the water column. It is important to understand the drivers of changes in macroalgal communities in order to predict future changes in these important ecosystems.

Effect of Extraction Solvent on Analysis of Chlorophyll *a* from Phytoplankton

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Chlorophyll *a* (chl *a*) is a phytoplankton pigment commonly used to estimate phytoplankton biomass. The standard procedure for analysis of chl *a* utilizes acetone as an extraction solvent. A number of studies suggest similar, if not enhanced, efficacy of ethanol compared to acetone as an extraction solvent. Ethanol has benefits in that it is less toxic, less volatile, and can be used at room temperature to extract chl *a*, making it preferable over acetone for shipboard analysis. Samples from Narragansett Bay and cultures of four separate phytoplankton species (*T. weissflogii*, *H. triquetra*, *S. marinoi*, and *P. micans*) were used to compare the extraction of chl *a* in acetone, ethanol, and isopropanol, with particular attention to the time necessary for extraction, temperature during extraction, and volume of sample necessary for filtration. In general, ethanol extracted chl *a* concentrations were 7.6-11.7% higher than acetone extracted concentration in whole water samples from Narragansett Bay. In contrast, the range in concentration was more variable between the two solvents in mono-specific phytoplankton culture samples, ranging from 0.3-11% with no particular solvent yielding higher or lower values than the other, even within the same phytoplankton species. Time needed for ethanol to extract chl *a* was found to reinforce previous studies, as 6-24 hrs produced comparable results. Variation in volume of sample filtered resulted in a linear correlation with mass of chl *a* on the filter over a relatively large mass range, suggesting that both solvents are effective over a wide range in concentration. In addition, the coefficient of variation between replicate filters decreased as the volume filtered increased. Approximately 0.2 µg chl *a* on the filter resulted in consistently comparable results between solvents, with a coefficient of variability below 10%. Preliminary studies using Isopropanol as an extraction solvent also demonstrated a strong linear correlation between volume of filtration and the mass of chl *a* on the filter, suggesting solvents not commonly reported in the literature may be worth further consideration. Ethanol and acetone yielded similar chl *a* concentrations, generally within 10% of each other. Using our data, we will provide suggestions for a standard method for analysis of chl *a* utilizing ethanol as the solvent for extraction.

The Effects of Allelochemicals from *Ulva* spp. on the Mortality and Behavior of Oyster Larvae

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Macroalgae can produce and release chemical compounds (i.e. allelopathic compounds) that inhibit the growth and/or behavior of other organisms, including commercially important shellfish. Determining the allelopathic effects of macroalgae on shellfish larvae can be used to help develop macroalgal bloom management strategies and help select sites for future oyster reef restoration. Oysters help with water quality and the decline of oysters can degrade water quality. We exposed shellfish larvae to seawater conditioned with macroalgae (*Ulva rigida* and *Ulva compressa*). Oyster larvae were cultured for 14 days and counts of dead larvae were conducted on Day 2, 5, 7, 10, 12, and 14 to determine the mortality over time. Our preliminary results indicate that both species of *Ulva* produce allelochemicals that cause mortality in oyster larvae. Current experiments are under way to determine the effect of these allelochemicals on the swimming behavior of the larvae and on the ability of the larvae to settle.

Spatial Variations in Mercury and Selenium Concentrations in Marine Fishes of Rhode Island: Risks and Benefits to Human Health

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Mercury (Hg) is a prevalent environmental contaminant that poses risk to human health and exposure occurs mainly by consuming fish. The U.S. Environmental Protection Agency (EPA) introduced a Hg action level of 0.3 ppm (wet weight) in fish tissue, above which consumption may become a health risk. Selenium (Se), a trace element that mitigates Hg toxicity, is also present in fish, increasing their health benefits. Total Hg and Se concentrations were measured in the muscle tissue of five fish species collected from the Narragansett Bay (inshore) and Rhode Island/Block Island Sound (offshore), including summer flounder (*Paralichthys dentatus*), scup (*Stenotomus chrysops*), bluefish (*Pomatomus saltatrix*), striped bass (*Morone saxatilis*) and black sea bass (*Centropristis striata*) (inshore: n = 19-20 per species and offshore: n = 19-20 per species) . Data were analyzed relative to spatial variations (inshore and offshore) and fish body size to assess bioaccumulation patterns. Health Benefit Values (HBV) were calculated to estimate the relative health risk vs. benefit of each species for human consumers. There is evidence supporting that offshore bluefish, black sea bass, and summer flounder have less total Hg than inshore conspecifics. Total Se concentrations did not vary spatially. Total Hg concentrations were positively related to total length for all fish, and values routinely exceeded the U.S. EPA action level at larger body sizes for inshore and offshore fishes (except summer flounder). Se concentrations were relatively constant across fish size. HBVs were inversely related to length, suggesting that larger fish pose greater health risks. Among all species, summer flounder had the lowest Hg concentration, yet the highest Se content; therefore this species provides the most health benefits according to the matrices of this study. Future work includes increasing the sample size of the offshore species for analysis of total Hg and Se concentrations.

Hemocytic Neoplasia in Hard Clams, *Mercenaria mercenaria*: Transmissibility of Neoplastic Cells

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In the summer of 2009, numerous 2-3 year old adult hard clams (*Mercenaria mercenaria*) in Wellfleet, Massachusetts began to surface and die. Pathological evaluation identified a new neoplastic disease, termed hemocytic neoplasia. Examination of a hemolymph (blood) sample from the pericardial sac of affected animals showed few to abundant large, unusual cells. These tumorous cells caused significant obstruction of the vascular system as well as a loss of normal hemocytes. Histological evidence did not suggest that the causative agent was bacterial, fungal, or toxic in origin leading to the hypothesis that it is caused by a viral agent. The neoplastic disease has continued to occur in the intervening years and is most prevalent in May and June, almost disappearing from the population in the summer and fall. Preceding studies in soft-shell clams and cockles have successfully transmitted a similar neoplastic disease present in each species into naïve animals of the same species by injecting them with abnormal circulating cells from the affected animals. This study looked at disease transmission in *M. mercenaria* by injecting naïve animals with neoplastic cells as well as cell free filtrates harvested from hard clams with the disease. The information from this study will be vital to the containment of this disease which could significantly impact the fishery and aquaculture industries.

Foraging Ecology of Blue Crabs (*Callinectes sapidus*) and Their Potential Impact on Winter Flounder (*Pseudopleuronectes americanus*)

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The blue crab, *Callinectes sapidus*, is a temperate species that is expanding its geographic range northward, thus possibly altering benthic community structure in Southern New England waters. This study examined the potential impact of blue crabs on local fauna by analyzing their abundance, size-structure, and diet. Potential crab predation on winter flounder, *Pseudopleuronectes americanus*, was of particular interest due to locally declining populations of this flatfish species. Crabs were collected from the Seekonk River (RI) and Taunton River (MA) from May to August 2012-2016, and subsequently preserved in 95% ethanol. In the laboratory, crabs were measured for carapace width, and prey contents were extracted from stomachs and identified to the lowest practical taxon. Crab abundance exhibited both spatial and temporal variations in the rivers, but overall estimates were consistent with southern Mid-Atlantic populations. Moreover, decomposition of crab length-frequency distributions revealed three distinct cohorts, suggesting that multiple life history stages utilize the riverine habitat. Direct visual analysis of stomach contents indicated that crabs undergo ontogenetic dietary shifts. The main prey of small crabs were crustaceans (e.g., amphipods/isopods, shrimp, and crabs), whereas larger conspecifics preferentially consumed bivalves. There was also evidence of crabs consuming fish, including winter flounder, with rates of predation positively related to predator-prey size ratios. The incidence of crab predation on flounder was minimal, however, and thus crabs may not be an important source of mortality for juvenile flounder. Future research will continue to examine the feeding habits of blue crabs via visual/genetic analysis of stomach contents and measurements of stable nitrogen and carbon isotope signatures in chelae muscle tissue.

Hemocytic Neoplasia in Hard Clams (*Mercenaria mercenaria*): Assessment of Neoplasia through Flow Cytometry

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In 2009 a disease known as hemocytic neoplasia was found to be the cause of death for hard clams (*Mercenaria mercenaria*) in Wellfleet Massachusetts. This poses a problem as the mortality of the clams could lead to a notable impact on the fisheries and aquaculture farms in the area. Hemocytic neoplasia is responsible for the formation of large, abnormal cells characteristic of a large nucleus and a high nucleus to cytoplasm ratio. These abnormal cells clog the vascular system of the animal leading to mortality. Neoplasia can be typically diagnosed by hemocytology and histology. Both are efficient methods to determine if the disease is within adult clams and histology is believed to be the most efficient and costly method. Clams with neoplasia typically have tetraploid DNA while healthy clams display diploid DNA. Ploidy level is believed to increase with the severity of the disease. Ploidy was observed using Flow Cytometry to aid in identifying Neoplasia in two different transmission treatments. The transmission experiments consisted of injecting naive animals with neoplastic cells as well as a cell free filtrate taken from clams diagnosed with neoplasia. Previous literature states that experiments have been performed studying the cells caused by neoplasia using processes like Ploidy, hemocytology, and histology. In this particular study we focused on the transmission of the disease by exposing naïve non-neoplastic clams to the neoplastic cells in order to better understand the disease. The information gained from this study of transmission could possibly lead to containment.

Blue Crab Predation on Juvenile Winter Flounder in New England Waters Assessed through PCR-based Methods

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Increasing water temperatures in the Northwestern Atlantic have resulted in blue crabs (*Callinectes sapidus*) extending their geographic range northward to Southern New England waters, including the Narragansett Bay Estuary and associated tidal rivers and coastal ponds. The increased abundance of blue crabs in these areas may have important consequences to resident biota. For example, blue crabs may adversely affect juvenile winter flounder (*Pseudopleuronectes americanus*) populations via trophic interactions. In this study, Polymerase Chain Reaction (PCR)-based methods were used to detect crab predation on juvenile flounder. DNA extractions of crab stomach contents were done using a Qiagen DNeasy Blood and Tissue Kit and then amplified using a winter flounder-specific 208 base-pair primer set, specifically attaching to the U12068 (D-loop) position. A total of 122 crab stomachs were analyzed, of which 26 tested positive for winter flounder DNA. This 21.3% positive detection exceeds predation rates estimated from traditional visual analysis of stomach contents, and further suggests that crabs may be an important source of predator-induced mortality for juvenile flounder. Dynamics in this predator-prey interaction were unrelated to crab/flounder body sizes or flounder densities. Conversely, crab predation on flounder significantly decreased at low dissolved oxygen concentrations, possibly due to reduced crab foraging during hypoxic conditions (<4 mg DO/L). Future work will include the analysis of crabs collected in 2015 and 2016, as well as the comparison of PCR results with visual analysis of the stomach contents.

Variation in Parasite Tolerance among Selectively-bred Oyster Families

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Disease presents a major obstacle for most agricultural species. Species cope with disease in different ways. One way that is of interest to the agriculture industry, is tolerance, which is defined as a host's ability to minimize a pathogen's effect on fitness (e.g. survival and reproduction). The eastern oyster production in the US is limited by Dermo disease, a fatal condition caused by the protozoan parasite *Perkinsus marinus*. This has led to widespread interest in developing tolerant oyster stocks for industry. In my summer research, I worked as part of a team to investigate variation for Dermo tolerance among selectively bred oyster families through controlled laboratory experiments. Four families, each differing in pedigree, were exposed to five doses of the parasite and disease response was evaluated by quantifying survival and the time course of infection. This line of research should help us determine whether Dermo tolerance is an appropriate target of selection for shellfish breeding programs and will ultimately provide the eastern oyster aquaculture industry with stocks that perform well in the presence of Dermo disease, thereby reducing loss and increasing productivity.

Fatty Acid Profiles of Marine Fishes from Rhode Island Coastal Waters

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Marine fish are an excellent source of omega-3 fatty acids, including eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), which provide numerous health benefits to human consumers. Further, the majority of consumed fish are of marine origin, thus underscoring the importance of research focused on this topic. In this study, fatty acids were analyzed in Rhode Island coastal fishes, including summer flounder, *Paralichthys dentatus*; black sea bass, *Centropristis striata*; striped bass, *Morone saxatilis*; scup, *Stenotomus chrysops* ; winter flounder, *Pseudopleuronectes americanus* ; and bluefish, *Pomatomus saltatrix* . Fatty acid profiles of fish muscle tissue were determined by esterification and gas chromatography. Data were categorized as mono-saturated, saturated, omega-3 and omega-6 fatty acids, and results were expressed as concentrations (mg/100 g wet weight; [FA]) and percent of total fatty acid content (%FA). Future research will examine total mercury and selenium concentrations of each fish species to further evaluate their respective health risks and benefits to human health.

Understanding Propulsor Placement to Find a Fluid Mechanical Basis of Universal Natural Bending

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There are few morphological examples of natural solutions for propulsion in fluids that are as ubiquitous among animals as stereotypic bending kinematic patterns. Bending location and extent are remarkably similar across disparate animal lineages moving in different fluid media and conform to a variety of propulsor sizes and materials. Current approaches to understanding propulsor placement and bending, which focus on the dynamics of straight, steady-swimming motion, offer little insight into why such diverse animal species bend with such predictable regularity. We suggest that swimming and flying animals have converged on constrained bending kinematics due to fluid dynamic forces that affect maneuvering in fluids. We propose to use animal models to document organizing principles for application of these forces. By studying multiple species of birds, fish, cetaceans, bats, reptiles, and pteropods, and showing that the relative wing or pectoral fin placement has converged on a small range of values, we will begin to test the general hypothesis that position of propulsor bending predictably determines propulsive forces.

Better Quality Oysters Grown via Flip Bag Method

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Aquaculture of oysters is no new thing to New England, however there are always new methods and gear being developed to produce a better product. The purpose of this study, conducted in cooperation with the Northeast Aquaculture Research Farm Network (NARF-Net), was to investigate the efficacy of flip bag farming in comparison to standard oyster farming techniques. The purpose of flip bags is to have the oysters in constant motion which hopefully causes the shells to grow deeper instead of longer. This makes for a more uniform and better shaped oyster which is considered a better product and commands a higher price from buyers. This experiment included four conditions with two experimental flip bag set ups and two controls or standard growing methods. Each condition had three bags each stocked at 150 oysters and located at the Roger Williams University Learning Platform. The experimental conditions included a standard ADPI oyster grow out bag converted to be used as a flip bag as well as an Australian made SEAPA basket. The control conditions used were floating ADPI bags attached to an anchored long line and ADPI bags attached to racks that sit on the bottom. Every week after the experiment started thirty oysters were taken from each bag, measured for length width and depth and then placed back into the same bag to continue growing. After five weeks, samples from each bag were taken and condition indexes performed to determine significant differences in shell size, weight and meat weight.

Impacts of Increased CO_2 on Deep Ocean Microbial Communities

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Surface waters of the global oceans absorb approximately one third of current atmospheric CO_2, which continues to increase due to anthropogenic emissions, and the dissolved CO_2 provides an important carbon resource to life in Earth's oceans and seabed (Doney, 2008). Oceanic uptake of CO_2 acidifies the surface waters by lowering the pH, and is likely to cause an associated shift in bottom water pH levels (Woods Hole Oceanographic Institution, 2007). Studies have shown important, harmful effects of changing surface water chemistry on marine organisms (Doney, 2008), however, very little is known on the potential effects of ocean acidification on the microorganisms thriving in the deep ocean and seabed. To begin to answer this question, cores of deep ocean sediments, impacted by black smoker hydrothermal activity at the Pescadero Basin and Alarcon Rise in the Gulf of California, (Monterey Bay Aquarium Research Institute cruise, R/V Western Flyer, April 3-13, 2015) were characterized to determine the mineralogy of the seabed sedimentary substrate hosting deep ocean microbes. This will spur further study on how changes in the chemistry of ocean bottom waters could impact the habitability of the seabed in sites of this type. This mineralogy is important for understanding inorganic resources available to microbes and to predict how microbes may react if that environment changes. Results so far show that the lithology of these environments is a combination of common marine sedimentary minerals, such as quartz, halite, and albite, mixed with some hydrothermally altered minerals such as berlinite, illite and pyrochroite. Conclusions indicate that if ocean bottom water becomes even subtly more acidic, the stability of these minerals may be impacted and thus change the habitable niche in the seabed sediments.

Bull-raking Effects on Infaunal Benthic Communities

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Bull-raking is the predominant method to harvest the quahog (*Mercenaria mercenaria*) in Narragansett Bay. Although quahog harvesting is a profitable industry, little is known about its potential impacts on the benthic communities of the Bay. To investigate impacts on quahog harvesting, a study was undertaken to monitor changes in benthic infauna associated with a substrate disturbance similar to one generated by a bull rake. Divers collected benthic sediment samples from two substrates, hard and soft, that had been disturbed. In each substrate area, core samples of sediment were taken from a bull raked area and a non-bullraked control area. Sampling was performed daily for a week after the bull raking occurred and then once a week during the following three weeks. Benthic organisms were identified and enumerated from replicated samples collected at each time point and location. Changes in the benthic community were compared between disturbed and control plots in the two substrate types. This process helps identify potential community disruption from the harvesting technique, bull raking, and how long it takes for infaunal species to return to an area once it has been disturbed by bull raking. Results pending.

Growth Response of *Crassostrea virginica* to Increased Nutrient Loading in Two Sites across an Estuarine Gradient in Pt. Judith Pond

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Eutrophication in coastal waters and wetlands has become an issue of growing concern in recent years, especially in Narragansett Bay. It has been well documented that oysters play an important role in nitrogen cycling in coastal waters by their ability to help regulate nitrogen levels through the process of denitrification. This in turn can help mitigate the effects of eutrophication. There have been many studies that test rates of denitrification in oysters, but few that focus on oyster growth under anthropogenic stress. Since oysters are suspension feeders, when more nutrients are available (i.e. from anthropogenic activities) in the water they are able to utilize it and allocate more energy towards growth. We investigated the impact that increased nitrogen loading had on the growth of oysters at two sites of Pt. Judith pond when compared to those under ambient conditions. At each site across the estuary, there are a total of six oyster cages at each site, three control and three experimental. The experimental group has fertilizer attached to the cage so it can dissolve around the oysters and imitate the effect of nutrient loading in the water column. Growth measurements were taken once every two weeks along with water temperature and salinity. Oysters directly exposed to increased nutrient levels should be expected to grow more than their control counter parts. This study will help expand our understanding of the potential effects anthropogenic activities can have on commercial shell fisheries.

Does Full Measure CAL (FMC) Supplement Increase Shell Mass in Oysters?

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Oyster farming, as with any farming, presents numerous challenges. In many cases, farmed oysters are provided with a surplus of food, which causes them to grow so quickly that they cannot produce enough shell material to protect themselves from their environment. The farmed oysters may be brittle, resulting in their shells breaking upon being handled, which damages or kills the oysters and results in serious losses to the farm. Oysters and other shellfish make their shells largely out of calcium carbonate, which they form using carbonate extracted from the surrounding seawater and calcium extracted from their food. To counter shell fragility, it may be possible for oysters to ingest calcium carbonate artificially introduced to the environment in the form of an additive, such as Full Measure CAL (FMC) to supplement and strengthen their shells. Full Measure CAL is a 30% calcium carbonate solution developed by Full Measure Industries for use in agriculture, due to its ability to encourage healthy cell growth and help plants more effectively absorb nutrients from the soil. Replicate samples of early post-set oysters were allowed access to a supply of FMC mixed into a microalgal food mixture for several weeks in a bottle upweller system. Oyster growth rate and shell mass were compared to that of oysters which grew under similar conditions for the same amount of time but without access to FMC.

Exploring Microscopic Life at the Edge: Analysis of an Estuarine Microbial Community

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An estuary is a moderately enclosed coastal body of water with one or more rivers flowing into it with a free connection to the open sea. The productivity and variety of estuarine habitats support abundance and diversity of species. The microbial community is responsible for degradation of compounds that are required for nutrient cycling. Our research is targeted on samples from the India point park and from the Oxford Street. India Point Park, situated at the confluence of Providence River and Seekonk River has a history of contamination. On the other hand, the Oxford street sampling site is also highly contaminated with a history of it containing large oil storage units and is now surrounded by the metal recycling facility. We are trying to explore the Providence/Seekonk river estuarine microbiome by three methods:

a) Analyzing the extracellular enzymatic activities that are involved in nutrient cycling (C,N,P)
b) Identifying the microorganisms (bacteria and archaea) by 16srDNA followed by second generation gene sequencing
c) Conducting the nutrient analysis of water focusing on nitrogen, phosphorous and sulfur.

The relationship of these three things will help us understand the microbiome as a whole. This would help us understand how industrial effluents impact the overall microbial colony. We hypothesized that since both the sites share a common history of excessive contamination by industrial effluents, we would observe an augmented microbiome thriving in it as compared to more pristine estuarine environments. The samples collected were exposed to artificial substrates that helped in tracking the rate of substrate hydrolysis. This helped us in determining the extracellular enzymatic activities by recording the concentrations of cellulose, chitin, phosphate, cellobiose and peroxidase using colorimetric and fluorometric analysis. This analysis would help us determine the presence of certain things like peroxidase activity can suggest the presence of polyaromatic hydrocarbons in the sediment as well as degrading plant matter such as lignin, or heavy metal stress. Our expected outcome would be that the heavy industrial history and current industrial activity along the estuary had an impact on nutrient cycling. We found out that the sites have high phosphate concentration (Avg. 0.09149 micromoles/hr. g) and a very high salinity (Avg. 25.7ppt). Further, the DNA extraction was carried to detect the presence of zeta and sulfate reducing bacteria.

Impacts of Macroalgal Accumulation on Salt Marsh Environments

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Our study examines the effects of different densities of two macroalgae (*Ulva* spp. and *Fucus* spp.) on the local halophyte, *Spartina alterniflora* (*S. alterniflora*). As a result of climate change and anthropogenic influences, macroalgal growth is expected to increase with rising temperatures and nutrient input; it is predicted that interactions among algal species and *S. alterniflora* will also intensify. Through physiological processes and soil microbial interactions, *S. alterniflora* has been known to emit or absorb different greenhouse gases at various rates, specifically nitrous oxide (N_2O), methane (CH_4), and carbon dioxide (CO_2). The purpose of this study is to determine how algal coverage affects the survival and greenhouse gas fluxes from *S. alterniflora*-vegetated soil in a 20-cm diameter core. A laboratory experiment was conducted at the University of Rhode Island's Graduate School of Oceanography during the summer of 2016. Twenty-five cores of *S. alterniflora* were collected at Fox Hill Salt Marsh in Jamestown, Rhode Island and placed under five varying treatments (n=5). The treatments included: (1) *S. alterniflora* cores covered with 210g/m^2 of *Ulva* spp., (2)-cores with 210g/m^2 of *Fucus* spp., (3)-cores with 105g/m^2 of *Ulva* spp. and 105g/m^2 of *Fucus* spp., (4)-cores with 210g/m^2 of *Ulva* spp. and 210g/m^2 of *Fucus* spp., and (5)-cores with no algal treatment as the control. The stem density, stem growth, stem health (measured via PAM fluorometry), and the N_2O, CH_4, and CO_2 gas fluxes of the *S. alterniflora* cores were measured in June (after one month). It is expected that cores consisting of *Ulva* spp. will show a greater decline in stem survival due to smothering effects than observed in the *Fucus* spp. treatment. Results thus far suggest that no significant N_2O fluxes were observed, there was a trend of increased CH_4 emissions in all treatments that consisted of *Fucus* spp. Relative to controls, and all algal treatments showed a trend of higher CO_2 emissions. As our work was part of a continuous experiment scheduled to end in September, our results are solely based upon the effects of each treatment after a one month period. However, we expect to observe similar and more significant gas flux patterns as the experiment persists.

Approaching a Holistic Understanding of Coral Bleaching: Using the Coral *Astrangia poculata* to Understand How the Coral Microbiome is Influenced by *Symbiodinium*

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Corals exhibit a multi-partner symbiosis with multiple microbes, including zooxanthellae from the genus *Symbiodinium* and complex prokaryotic communities. However, microbe-microbe interactions that regulate composition of the microbial communities are not fully understood. Unlike many tropical corals, the temperate coral species *Astrangia poculata* exhibits a facultative relationship with its zooxanthella *Symbiodinium psygmophilum*. Because the symbiosis is facultative, *A. poculata* can survive with varying densities of zooxanthellae, which makes it a model organism for developing a better understanding of the potential influence of *Symbiodinium* on prokaryotes. Colonies can vary from all brown polyps, to all white polyps, or even to a mixture of white and brown polyps. *Symbiodinium* densities are heavily influenced by abiotic factors such as sunlight and temperature, which play an important role for colonies in Rhode Island, considering the large seasonal fluctuations in these factors. Our lab is currently using 16S rRNA amplicon sequencing to explore whether prokaryotic diversity varies with symbiotic state in wild *A. poculata* colonies from Narragansett Bay, RI. As a part of that work, the primary objective of this study is to advance our ability to characterize microbial metabolic processes that are differentially abundant in each symbiotic state via metagenomics and metatranscriptomics. However, host DNA contamination is often an obstacle to obtaining high enough quantities of microbial DNA for 'omics approaches, and therefore it is nearly impossible to obtain genomic sequence from the microbiome without an enrichment process. In this project, we are developing and evaluating an enrichment process, involving cell separation, for microbial meta-omics applications of *A. poculata* tissue. Quantitative PCR (qPCR) is being used to evaluate whether the cell separation method has successfully enhanced microbial DNA content and significantly reduced host DNA content. Our method can be used for future DNA and RNA preparation for 'omics procedures that will build a conceptual model of microbe-microbe and microbe-host interactions within the coral *A. poculata*.

MICROBIOLOGY

LOCATED IN ROOM 135 ON THE 1st FLOOR OF THE BEAUPRE CENTER

Entamoeba spp. as Models of Aquatic Environmental Changes in Pathogenic Marine Protists

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Entamoeba spp. are a lineage of free living, opportunistic and pathogenic protists. The pathogenic *E. histolytica* is responsible for more than 100,000 human fatalities globally per year. *E. invadens* VK-1:NS and *E. invadens* IP-1 are parasitic in salamanders or snakes and commensals in turtles. As climate change persists, environmental conditions are shifting and influencing the ecological conditions for protists at large. *Entamoeba* spp. can be used as models to study adaptability of pathogenic and non pathogenic species to global climate change conditions. *In vitro* assays were performed to identify the effects of pH and salinity on *Entamoeba* spp. survival and cell health. Increasing temperatures, ocean acidification and aquatic salinity are all modifications commonly associated with climate change. Growth media was adjusted to simulate changing pH and salt concentrations. Cell health was assessed by measuring cell surface area and monitoring cell shape, confluency, cell density, bacterial growth, and aggregation. Protein gel electrophoresis was preformed to identify potential acid or salt shock proteins released into the extracellular environment by *Entamoeba* spp trophozoites. This study suggests that climate change could result in the increased presence of pathogenic protists such as *E. histolytica*, *E. invadens* VK-1:NS and *E. invadens* IP-1.

Analysis of Hfq and Catalase Functions in Oxidative Stress Adaptation in the Metal-reducing Bacterium *Shewanella oneidensis*

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Hfq is a bacterial RNA chaperone protein that has been widely implicated in the function of small, regulatory noncoding RNA molecules that contribute to adaptive gene expression. We have previously found that loss of the RNA chaperone Hfq in the dissimilatory metal reducing bacterium *Shewanella oneidensis* results in slow exponential phase growth as a result of reduced heme production, a reduced terminal cell density in stationary phase, a striking loss of colony forming units in extended stationary phase, and an exquisite sensitivity to both hydrogen peroxide and superoxide stress. We are investigating the molecular basis for the oxidative stress hypersensitivity of the *hfq* mutant. Increasing available heme, which is the catalytic portion of the hydrogen peroxide-degrading enzyme catalase (KatB), does not rescue oxidative stress survival in the *hfq* mutant. However, pretreatment with sub-lethal doses of hydrogen peroxide boosts catalase activity and increases survival when treated with lethal doses of hydrogen peroxide suggesting that the *hfq* mutant adapts slowly to oxidative stress. Consistent with this hypothesis, increasing KatB levels by expression from an inducible plasmid fully protects the *hfq* mutant against lethal dose challenge with hydrogen peroxide. To elucidate the nature of the *hfq* mutant's oxidative stress adaptation defect, we are utilizing *katB* reporter fusions and catalase activity assays to characterize differences in *katB* expression kinetics and magnitude between the *hfq* mutant and the wild type *S. oneidensis* strain.

Proteomic Analysis of Signaling Molecules in *Entamoeba* spp.

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Entamoeba spp. are protists that belong to Archamoebae and comprise parasitic, free-living and commensal species. Clone-recognition experiments were performed with seven *Entamoeba* lineages (*E. invadens* IP-1, *E. invadens* VK-1:NS, *E. terrapinae*, *E. moshkovskii* Laredo, *E. moshkovskii* Snake, *E. histolytica* HM-1:IMSS and *E. dispar*). This study examined whether aggregation and kin-discrimination among *Entamoeba* varieties is based on intra- and inter- strain chemical signaling. Trophozoites secreted extracellular signaling proteins into the growth media, which encouraged aggregation between cells of the same lineage. Secreted proteins from *E. histolytica*, *E. dispar*, and *E. invadens* were analyzed through protein gel electrophoresis and LC-LC tandem mass spectometry. A number of proteins were identified that are likely involved in motilty and aggregative mechanisms in *Entamoeba* spp and may play a key role in kin discrimination and pathogenesis. Mass spectrometry analysis of in-gel and liquid media samples identified ubiquitin, RasGap/ankirin, coronin, actin, heat shock, and kinase proteins. These protiens are required for cell proliferation, motility, actin polymerization, cytokinesis, G-protein modulation, encystation, adhesion and other cellular mechanisms. Because the parasitic protist *Entamoeba histolytica* is responsible for the development of amoebiasis in humans, which results in approximately over 500,000 infections and nearly 100,000 deaths worldwide annually, these findings may be used to inhibit chemical signaling and reduce aggregation for alternative strategies for managing amebiasis.

Analysis of Hfq And RpoS Functions in Stationary Phase Survival in the Metal-reducing Bacterium *Shewanella oneidensis*

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The bacterial RNA chaperone protein Hfq has been broadly implicated in the function of small, regulatory noncoding RNAs that contribute to adaptive gene expression. In previous work we have found that loss of Hfq in the dissimilatory metal reducing bacterium *Shewanella oneidensis* results in slow exponential phase growth, reduced terminal cell density in stationary phase, a high level of sensitivity to oxidative stress, and, in striking contrast with the wild type strain, a complete loss of colony forming units in post death phase cultures. We are investigating the molecular basis for the stationary phase survival defect of the *hfq* mutant. Because the stationary phase sigma factor RpoS promotes cell survival in other bacteria, our working hypothesis is that deficient RpoS function underlies the stationary phase survival defect of the *hfq* mutant. Consistent with this hypothesis, we have found that strains carrying a mutation in *hfq*, relative to the wild type strain, have diminished levels of *rpoS* expression during exponential phase, the transition into stationary phase, and stationary phase. We have also shown that increasing *rpoS* expression using an inducible expression plasmid rescues the stationary phase survival defect of the *hfq* mutant. Intriguingly, our preliminary data suggests that exponential phase expression of *rpoS* is responsible for promoting stationary phase survival of *S. oneidensis*. Initial detection of RpoS protein levels in *Shewanella* via western blot analysis suggests that Hfq influences RpoS protein production in the hours leading to and surrounding stationary phase.

Inhibitory Effects of Purified Plant Extracts on the Growth of *Entamoeba histolytica*

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The protist *Entamoeba histolytica* is the causative agent of human amebiasis. This intestinal disease results in approximately 50 million cases and over 100,000 deaths worldwide annually. Amebiasis is primarily treated with metronidazole, an antimicrobial drug that has adverse side effects including neurological complications. Discovering alternatives to metronidazole is of great interest to improve the management of this infectious disease. Purified extracts from plants and subsequent analogs have shown significant antimicrobial character in previous studies. Both crude and purified plant extracts have been analyzed for their efficacy in inhibiting the growth of *E. histolytica*. This study identified pomegranate, rhubarb (Rhein), and maple leaf (maplifa) extracts as potential inhibitors of *E. histolytica* growth. Interestingly, purified Rhein and its analogs showed successful inhibition *E. histolytica* trophozoite growth. Future studies will determine the molecular and biochemical mechanism of action for these inhibitors and test their relative toxicity in humans compared to that of metronidazole. Besides showing promising preliminary antiamebic properties, the anti-inflammatory effect of maple syrup extract could neutralize or defuse the severe inflammatory colitis caused by intestinal amebiasis.

Environmental Conditions Affecting Polysaccharide Intercellular Adhesin (PIA) Expression in *Salmonella enterica* Serovars

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Salmonella enterica is a Gram negative bacterium that is responsible for a variety of diseases, ranging from basic food poisoning to typhoid fever. Adhesion to host and environmental surfaces contributes to the virulence of this bacterium by promoting colonization and increasing survival. Polysaccharide intercellular adhesin (PIA), the main exopolysaccharide component in biofilms belonging to more than 40 species of bacteria, fungi, and parasites, has been found in *Escherichia coli* which is very closely related to *S. enterica*. Due to the high genomic similarity between these two bacterial species (>95%), we predicted that PIA would also play a role in biofilm formation in *S. enterica*. In order to test this prediction, six *S. enterica* serotypes (Agona, Enteriditis, Javania, Newport, Montevideo and Typhimurium) were cultured in 96-well plates in two types of media at two different temperatures and treated with either periodate, which interferes with PIA expression or proteinase K, which digests proteins. Biofilms were then visualized by staining with crystal violet. Results show that there is an inherent difference between the serovars' ability to form a biofilm, Javania having the most biofilm production and Typhimurium having the least. Proteinase K had no adverse effect on growth of *S. enterica* serovars, but did interfere with biofilm formation of all serovars tested. Periodate interfered with growth of all serovars, and led to decreased biofilm formation. Future studies will focus on determining the effect of periodate on growth of planktonic cells in broth, and expanding the assay to include multiple strains of each serovar.

Dead on Target: Cloning and Expression of the GlcNAcase LytB from *Streptococcus pneumoniae*, the Putative Target of Diamide Inhibitors

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Streptococcus pneumoniae is a Gram-positive, alpha-hemolytic, facultative anaerobic member of the genus *Streptococcus*. *S. pneumoniae* can cause many illnesses. This consists of pneumococcal pneumonia, bacteremia, ear infections, and sinus infections. Additionally, it is the leading cause of meningitis. These bacteria have the ability to cross the blood-brain barrier, inflaming the meninges, and resulting in pressure on the brain and spinal cord. Recently, according to a study done by Johns Hopkins University School of Medicine, strains of *S. pneumoniae* are increasingly developing antibiotic resistance. One vital structure of the *S. pneumoniae* strain is its peptidoglycan layer, which acts as a backbone, and is responsible for conferring shape and the osmotic stability of the cell. It is comprised of two general structures: a peptide moiety and glycan strands. The peptide moiety is made up of alternating L and D amino acids. Uniform glycan strands of alternating β-1, 4-linked N-acetylglucosamine (GlcNAc), and N-acetylmuramic acid (MurNAc) residues are also spread throughout the layer. Adjacent glycan strands are cross-linked via the stem peptides to form a 3-dimensional mesh structure. β-N-acetylglucosaminidases (GlcNAcase) are one class of autolysin, and cleave the glycan backbone between GlcNAc and MurNAc. Our focus is on the metabolism of peptidoglycan, and the enzymes that are responsible for breaking it down, particularly GlcNAcases. Previous results have identified a number of compounds that can successfully inhibit the growth of *S. pneumoniae*. Work with the model organism *Bacillus subtilis* has identified the molecular target of these inhibitors to be the GlcNAcase LytG. Our intent is to clone and express the LytG homolog LytB from *S. pneumoniae* (SpLytB). In previous studies, the endo-β-N-acetylglucosaminidase LytB has demonstrated to be highly important for cell division and nasal colonization in *Streptococcus pneumoniae*. By successfully cloning LytB, we will be able to confirm this is the molecular target of our inhibitors through *in vitro* experiments. We are constructing two forms of the LytB gene. One form is a full-length form with an N-terminal His-tag. The other form is an N-terminal truncation that lacks the first 40 a.a lipidation sequence, and contains an N-terminal his-tag. LytB will be cloned into the pET28 vector for subsequent expression in *Escherichia coli*. Protein expression and purification results will be discussed.

Expression of LPS and CPS O-antigens under Varying Growth Conditions in a Range of *Salmonella enterica* Serovars

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Salmonella enterica is a common bacterium that causes gastrointestinal disease in humans. More recently *S. enterica* outbreaks have been linked to contaminated fresh produce. There are multiple structures on the outer surface of *S. enterica* that could aid in its interaction with fresh produce such as the O-antigen, which is serovar-specific. The O-antigen is present on the surface of *S. enterica* in two forms: attached to lipid A-core as lipopolysaccharide (LPS), and independent of lipid A-core as an O-antigen capsule (CPS). The overall goal of this research is to determine the effect of growth conditions on the expression of O-antigen (LPS and CPS) in various serovars. Seven serovars of *Salmonella enterica* [Agona, Enteritidis, Javiana, Montevideo, Newport, Poona, and Typhimurium] were chosen to study based on the food commodities they are typically associated with. The cells were grown on media of varying salt concentration (LB Lennox and LB low salt), at two temperatures (2°C versus 37°C), and in varying media states (plates, biphasics, and broths). Cell-free lysates were analyzed by SDS-PAGE gel and visualized under a silver stain (LPS), and Alcian blue and silver stain (CPS). The data for LPS show that varying serovars express O-antigen of different chain lengths, while there is little difference in chain lengths between varying states of media for a given serovar. All serovars tested appeared to produce CPS, with increased CPS expression in biphasic media. Future studies will focus on antibody-based detection of O-antigen molecules in Western blots in an effort to enhance visualization of high molecular weight LPS.

Rearranging the Cell Wall: Ugi-derived Diamides Alter Autolysin Activity

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Antibiotic resistance in bacteria has been a leading concern in healthcare, causing deaths from previously non-fatal pathogens. The cell wall of Gram-positive bacteria is composed of a thick peptidoglycan (PG) layer and teichoic acid. The PG layer is composed of a polysaccharide backbone comprised of N-acetylglucosamine (GlcNAc) and N-acetyl muramic acid (MurNAc). Adjacent polysaccharide strands are cross-linked via pentapeptide side chains attached to the MurNAc providing a dynamic 3-dimensional structure that confers shape and strength to resist turgor pressure (1). Enzymes produced by bacteria known as autolysins have the capability to break down the PG of cell walls. These enzymes are involved in processes such as cell growth, division, and motility (2), which influence severity of bacterial infections. It has been proposed that PG synthesis in Gram-positive bacteria is turnover-mediated via hydrolysis of the cell wall (3). Previous work with a library of Ugi-derived diamides has identified inhibitors specific to individual Gram-positive organisms (1). A chemical genomics approach is being utilized to determine if these compounds have the ability to target more than one autolysin. *Bacillus subtilis* is an easily grown non-pathogenic Gram-positive bacteria widely studied in relation to PG synthesis and metabolism, making it ideal to use as a model organism. This project investigates the potential of targeting PG metabolism through the inhibition of autolysins as an antibiotic strategy. In addition, *B. subtilis* will help validate the compounds as tools for studying cell wall metabolism. The compound fgkc was the top inhibitor of *B. subtilis* 11774, with an MIC between 1.7 – 2.1 μg/mL. Previous results revealed treatment with fgkc at 0.8xMIC resulted in incomplete cell division. Autolysins LytC (an L-alanine amidase), LytD (a glucosaminidase), and LytF (an endopeptidase) are important for regulating cell motility and division (4). At least one target of fgkc has been identified in *B. subtilis* LytG, and other *B. subtilis* strains lacking autolysins were screened against fgkc to investigate additional autolysin targets of fgkc. Results indicate increased sensitivity in single mutants. Microscopy revealed changes in cell motility and morphology in mutants. We have initiated phenotypic studies using phenotypic arrays to explore the use of these compounds for chemical biology. Preliminary data has been collected with the LytD mutant in an osmolyte array.

The Effect of Salt Concentration and Temperature on FliC and FljB Expression in *Salmonella enterica* Serovars

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Salmonella enterica is a species of bacteria that causes a type of food poisoning called salmonellosis, which is typically transmitted to humans through contaminated poultry and eggs but has been recently been linked to outbreaks involving produce. This study investigates how *S. enterica*'s flagella affect its ability to persist on leafy greens, as literature has shown that the flagellum aids in host attachment. *S. enterica* can express two distinct flagellin proteins, FliC or FljB, where the expression of one flagellin represses the other. Seven serovars of *S. enterica* with varying frequencies of produce-derived outbreaks were examined: Agona, Enteritidis, Javiana, Montevideo, Newport, Poona, and Typhimurium. Flagellin expression was assayed in Lennox formula LB versus low salt LB media, in cells grown at room temperature versus body temperature, and in media of varying physical states (solid plates, biphasic flasks, shaking and static broths). Cells were harvested after a 24 hour growth period, flagella were sheared from the cell bodies by vortexing and the flagellin proteins were precipitated with acetone. The flagellins were analyzed on Coomassie-stained SDS-PAGE gels and the identity of the flagellins was confirmed by Western blotting. Flagellins represented the bulk of the proteins isolated from Lennox media, while it represented a minor fraction of the total protein isolated in low-salt media. Additionally, in high salt conditions flagellin expression was highest in biphasic media for Agona, Montevideo, Newport, and Typhimurium. There was little to no difference in flagellin expression between biphasic and plates in low salt conditions. Expression of FliC or FljB was serovar-dependent, however serovars grown in low salt conditions had a higher instance of expressing both phases of flagellin. The results of this study will be used to optimize flagellin production in further research to determine whether one phase over the other enables the bacterium to better colonize and survive on produce.

Prey Range of Predatory *Bdellovibrio* Strain Isolated from Bioswale Soil

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Bdellovibrio are members of the delta-proteobacteria with an obligate predatory lifestyle. *B. bacteriovorus* invades the periplasm of Gram-negative prey bacteria, whereas *B. exovorus* attaches to the outside of prey cells. Both species digest prey bacteria in order to propagate. *Bdellovibrio* are found in a wide range of environments and may attack multiple different prey species, including animal and plant pathogens. This trait makes *Bdellovibrio* an attractive alternative to antibiotics, which are losing effectiveness with the rise in antibiotic resistance. To understand variation in prey range and predation efficiency within *Bdellovibrio*, we aimed to isolate naturally occurring strains of predatory bacteria from soil. Initially, we isolated potential prey bacteria from soil and classified them by 16S rRNA gene sequencing as strains of *Pseudomonas* and *Serratia*. We then used these strains as prey in enrichments of soil samples from a bioswale, which is an artificial landscape feature used to filter rainwater runoff from buildings on the Providence College campus. After purification by plaque formation on double agar overlay plates, we obtained an isolate showing predation on both *Pseudomonas* and *Serratia*. 16S rRNA gene sequencing classified this isolate as a member of *Bdellovibrio*, with ~96% identity to *B. bacteriovorus* 16S rRNA gene sequences and ~92% identity to *B. exovorus* 16S rRNA gene sequences. Based on 1000X phase-contrast microscopy, this soil *Bdellovibrio* isolate appears to invade prey cells, similar to the predatory strategy used by *B. bacteriovorus*. We will perform additional microscopy to determine whether this isolate invades the prey cell periplasm. In addition to *Pseudomonas* and *Serratia*, we are challenging this isolate with other Gram-negative bacteria, including isolates from freshwater environments, to assess prey range and investigate whether this isolate specializes on particular types of prey. We are also working to sequence and annotate the genome of this isolate for comparison with other strains of *Bdellovibrio*. This work will contribute to our understanding of predation in bacteria and explore the potential for therapeutic applications of predatory bacteria in the control of pathogens.

The Effect of Growth Conditions on Biofilm Formation and Cellulose Expression in *Salmonella enterica* Serovars

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Biofilms occur when a group of bacteria growing on a substrate excrete a slime-like material composed of exopolysaccharides, proteins and nucleic acids. This multicellular lifestyle improves bacterial resistance to harsh environmental conditions. Evidence suggests that cellulose, a glucose polymer found in biofilms of *Salmonella enterica*, plays a role in colonization of fresh produce. The present study was conducted to determine the effect of various growth conditions on biofilm formation and expression of cellulose in *S. enterica* serovars Agona, Enteritidis, Javiana, Montevideo, Newport, and Typhimurium. Excluding Agona, these serovars have been linked to colonization of fresh produce. Biofilm formation and cellulose expression were tested under conditions of varying temperature (37^{o}C and 25^{o}C) and media type (Lennox LB and low salt LB). Biofilm assays were conducted by growing serovars in triplicate in a 96-well plate. After rinsing the wells and staining with crystal violet, the OD600nm was determined. Cellulose expression was determined by growing cells on solid media containing Calcofluor White or Congo Red, reagents known to bind cellulose. Biofilm assays showed Javiana and Enteriditis were the strongest biofilm formers, while Typhimurium was the weakest. The majority of serovars tested showed increased biofilm expression in low salt media. When grown on media containing Congo Red, serovars Javiana and Montevideo were the only ones to display the typical wrinkled colony morphology indicative of cellulose expression. Fluorescent imaging of colonies grown on media containing Calcofluor showed that Javiana and Montevideo had the brightest fluorescence, suggesting they had the highest expression of cellulose. The next phase of the study is to look at cellulose expression on a cellular level using Calcofluor White and fluorescence microscopy.

Isolating and Characterizing Predatory Bacteria from Natural and Built Freshwater Environments

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Bdellovibrio are predatory bacteria that prey upon gram-negative bacteria. *Bdellovibrio*, the most widely studied of predators, attach to host cells and penetrate the periplasm. The host cell then bursts via predator propagation. A variety of predatory bacteria species have been observed across environments ranging from terrestrial to aqueous. Understanding variation in prey range and predation efficiency could inform development of predatory bacteria as alternatives to antibiotics. To further explore variation among predatory bacteria, we aimed to isolate and characterize predatory bacteria from natural and built aqueous environments via culture-independent and dependent approaches. In culture-independent approaches, we validated genus-specific PCR to assay the presence of *Bdellovibrio* DNA. Using this PCR, we tested metagenomic DNA extracted from our samples. In culture-dependent approaches, we used gram-negative bacteria previously isolated from a freshwater stream as prey to obtain a pure predatory bacteria isolate via enrichments and double agar overlay. As a first step, we sampled from two ponds and one urban stream. From the two ponds, we took water samples directly from the water column. From the urban stream, we took swab samples from the biofilm on abiotic surfaces, such as plastic, glass, and rocks, as well as biotic surfaces, specifically snail shells. From culture-independent analysis, we found evidence of *Bdellovibrio* on surfaces in freshwater environments, but not the water column. Swab samples were also collected from a janitorial closet drain located at Providence College. Using the *Bdellovibrio* 16S rRNA gene as a positive control, we ran PCR with the drain samples that provided positive evidence of predatory bacteria. Enrichments using *Aeromonas* and *Raoultella* were tracked via spectrophotometric analysis and 1000x phase contrast microscopy. Enrichments were plated using double agar overlay technique. Plaque formation varied in size and appearance, suggesting the possibility of multiple morphologies. Moving forward, we will classify the drain isolate using 16S rRNA gene sequencing. We will isolate predatory bacteria found on the biotic surfaces of snail shells. Prey range and predation efficiency will also be tested across a wide assortment of gram-negative bacteria. This will contribute to our understanding of predation in bacteria and explore the potential for therapeutic applications of predatory bacteria in the control of pathogens.

Identifying Helpful Probiotic Bacteria for Lobsters using Matrix-Assisted Laser Desorption Ionization Mass Spectrometry

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The American lobster *Homarus americanus* is a commercially important Rhode Island fishery. In recent years, lobster shell disease has dramatically impacted this fishery. Understanding this complex disease is important to their continued economic success. This complex disease is thought to be caused by certain types of pathogenic bacteria, which can infect the lobster shell, creating lesions or pits. Identifying potential probiotic bacteria capable of neutralizing this deleterious effect are being sought to provide a natural sustainable treatment for this disease. The process of identifying probiotic bacteria is lengthy and requires the screening of many bacterial isolates. We aim to use matrix-assisted laser desorption ionization (MALDI) mass spectrometry to screen bacteria to the genus level. MALDI mass spectrometry can be used for a quick and easy way to help identify different substances, such as biopolymers like DNA, proteins, peptides and sugars. This research will be used to create a database to do a quick, in-house screening of the bacteria to check for duplicate strains before further testing. A strain of *E. coli*, #1100 was used to model the conserved proteins. MADLI mass spectrometry data has been previously published on this strain and our goal was to be able to obtain consistent results for this strain. We were able to accurately observe all conserved proteins, and are currently working to replicate the results.

Laboratory and Probiotic Strains of *Escherichia coli* Induce Toxicity in *Candida albicans*

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Fungal infections are among the leading causes of death in immunocompromised patients. Particularly, systemic *Candida albicans* infections have a mortality rate of 35-40% even with the administration of anti-fungal therapy. A key reason why fungal infections are so deadly is the lack of effective and non-toxic antifungal treatments. The focus of our research is to identify novel antifungals through microbial competition. Evidence shows that competition between *C. albicans* and the probiotic strain of *Escherichia coli* Nissle (1917) reduces *C. albicans* growth. We compared *C. albicans* growing by itself to *C. albicans* grown in competition with both a laboratory strain of *E. coli* (MG1655) and Nissle (1917). By doing so, we found that the presence of both *E. coli* strains induces toxicity in the *C. albicans*. We also demonstrate that this toxicity relies on a small, soluble molecule produced by *E. coli*.

Informatic Discovery of *H. pylori* and Gut Microbiota Common Determinants that Drive Extragastric Beneficial Effects of *H. pylori* Colonization.

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Heliobacter pylori is a bacterium found in the stomach, infecting millions of people around the world. It normally colonizes individuals in childhood and has been linked to causing stomach cancer in later life. *H. pylori* is more prevalent in the developing world than in Western countries where antibiotic therapy is available. It is known to persist in the body through T regulatory-mediated suppression of productive effector T cells that could eradicate infection. Previous studies have shown that this bacterium confers protection against allergic and extragastric inflammatory conditions such as asthma. It is hypothesized that T regulatory cells educated through commensal antigen presentation in the gut may expand in the periphery due to cross-reactivity with *H. pylori* antigens. These studies may demonstrate a novel mechanism for the capacity of *H. pylori* to persist in the stomach for years while conferring this extragastric protection. To test this hypothesis, a comparison of *H. pylori* must be made to the gut microbiota. The human microbiome consists of thousands of bacterial species, and appear in the gut at different time points throughout an individual's life. The earliest bacteria to enter the body were selected for preliminary screening due to the impact they have on the developing immune system. Recent studies have debunked the assumption that the womb is sterile, and bacteria have been found in meconium of newborns as well as the umbilical cord. We compiled two separate databases, one for bacteria identified to colonize the gut before and after birth. We began to screen and analyze T cell epitopes from *H. pylori* against these cohorts using JanusMatrix. JanusMatrix is an algorithm that predicts potential T cell cross-reactivity between input peptides and any sequence database by matching the specific amino acid sidechains that interact with the T cell receptor when bound to HLA-DR molecules on the surface of antigen-presenting cells. Input *H. pylori* sequences with many matches to microbiota may be indicative of cross-reactive T cell epitopes that contribute to persistence of *H. pylori* and its extragastric effects. Once these sequences have been identified, the aim will be to examine the phenotypes and functions of extragastric CD4 + Treg cells that cross-react with human microbiome homologs of *H. pylori* HLA Class II-binding sequences using peripheral Dendritic cell/T cell co-cultures derived from *H. pylori*-infected and –uninfected subjects.

NEUROSCIENCE

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Genetic Association of GABRG2 and Body Dysmorphic Disorder in a Mouse Model

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Body dysmorphic disorder (BDD), characterized by preoccupation with perceived slight or imagined flaws (Phillips, 2001; APA, 2013), is associated with high rates of functional impairment, suicidal ideation and suicide attempts (Phillips, Mernard et al. 2002). Estimated to affect 1-2% of the population, individuals suffering from BDD often report higher levels of stress and poor quality of life (Phillips, 2004). In a preliminary candidate gene study, Phillips, Zai et al. identified a potential link between a mutation in the gamma aminobutyric acid A receptor gamma subunit (GABRG2) and body dysmorphic disorder (2015). Investigating the genetic impact of this mutation, GABRG2 mutant mice underwent home cage monitoring and various behavioral assays to assess anxiety, depression, fear conditioning and behaviors including grooming. Furthermore, interested in the role of adolescence, the period when symptoms of BDD commonly manifest (Phillips, Didie et al. 2005), a sample of p30 mice were exposed to TMT to investigate the impact of an adolescent stressor on this genotype. The potential for this KO mouse as a model of BDD is explored.

Examining Associations of Neurobiological Responses to Stress across Adolescents in a Dyad

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Relationships are key contexts in which individuals emotionally react to and learn how to regulate stress. During adolescence, peer relationships are perhaps the most salient context in which emotional and physiological responses to stress are regulated and shaped. The reciprocal process by which stress responses may be shared and shaped within relationships has been referred to as co-regulation and can be measured by examining the degree of emotional concordance (e.g., matching arousal level) within a friendship dyad. The present study will examine if adolescents' friendships provide a context in which emotional responses to stress are shared and shaped in a sample of 60 friendship dyads. By using physiological indicators, as well as self-report data obtained through surveys, this study can collect accurate representations of stress within the dyad and interpret prevalence of co-regulation. Proposed measures of stress include heart rate, galvanic skin response, alpha amylase, cortisol, and self-reports of emotion. Participants will engage in a conflict task with a close, same-sex friend, which is used to invoke stress similar to typical daily interactions. Additionally, one individual from the dyad will participate in the Yale Interpersonal Stressor (YIPS), while the other completes a control task. The YIPS is a scenario designed to produce a stress response caused by feelings of peer rejection created through the use of two confederates. Co-regulation will then be observed through a support task in which the two participants discuss what was experienced during the YIPS. We have hypothesized that the stress response between the individuals will show a high level of concordance and that gender differences will be observed in the co-regulation of physiological stress responses such that female adolescents will be more likely to share stress response during interactions. Examination of this topic could shed light on the concordance of emotions at a neurobiological level and if shared emotions serve as a reinforcing dynamic that maintains or amplifies how individuals' regulate stress. This research is paramount given the importance of friendships in shaping behaviors and the known correlation between dysregulation of stress response and health problems.

The Effect of Stress Controllability on Anxiety and Nociception from Juvenility to Adulthood

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Previous anxiety models have taken into account the various effects of adolescent stress on anxiety, but have overlooked the importance of stressor control as a facilitator of anxiety reduction in late adolescence and adulthood. Within this experiment, the interaction of a juvenility stressor with stressors experienced during adolescence was explored in order to determine whether anxiety-like behaviors in adulthood manifested. Forty Sprague -Dawley rats were randomly selected, and divided, into four conditions all of which centered around stress exposure in juvenility and adolescence. During juvenility (PND23) rats were exposed to either predator odor (2, 4, 5 trimethyl--3--thiazoline) or saline controls. In adolescence (PND33), rats were assigned to yoked pairs consisting of one animal trained under signaled avoidance, which allowed for the escape and avoidance of shock. The corresponding animal was exposed to uncontrollable shocks of the same number and intensity as their yoked counterpart. Animals were tested for anxiety-like behaviors and changes in nociception following the stressor exposure in juvenility and adolescence. Subsequent testing also occurred in adulthood. Both behavioral assays were analyzed to assess the role of early and adolescent stressors in the development of anxiety-like behaviors in adulthood. Evidence may shed light on the role of stressors across the lifespan in the development and maintenance of human disorders of anxiety.

The Effect of Interpersonal Stress on Stress Reactivity and Risk Behavior

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Adolescence represents a critical juncture to examine stress as an etiological explanation for risk behavior due to increases in normative stressful experiences brought on by developmental transitions, as well as changes in neurobiological systems that are associated with how the body handles stress (Masten, 2004; Silk et al., 2012). Associating interpersonal stress with neurobiological mechanisms that explain increases in adolescent risk behavior and developing innovative ecologically valid ways in which to assess these associations has been recognized as imperative both for advances in basic science and to improve prevention efforts to reduce the incidence of risk behaviors. Thus to contribute to basic and applied research, this study examined the neurobiological mechanisms by which interpersonal stress within peer and family relationships impacts risk behaviors using ecologically valid stressors. Data was collected from fifty-one adolescents (M = 14.83, SD = .88) using a within-subjects design across two laboratory visits. During Visit 1, participants watched a parent-adolescent visual stressor to induce a stress reaction that might typically occur when adolescents experience conflict in their relationships with their parents. During Visit 2, adolescents participated in a peer rejection task that consisted of a computer-based interaction that involved acceptance and rejection by same age peers. Risk behavior using the Balloon Analogue Risk Task (BART) and physiological indicators of stress were measured before and after the stressors. Results indicated that the parent-adolescent stressor and peer rejection stressor were associated with increased stress reactivity as measured by alpha amylase and negative affect. In turn, increased stress reactivity to the parent-adolescent stressor was associated with increased risk behavior as measured by the BART. These results suggest that neurobiological responses to stress may be one mechanism that explains the effect of interpersonal stress on risk behavior during adolescence.

A Longitudinal Assessment of Dominance Hierarchies in Rats

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The study of dominance is essential to the understanding of animal social behavior, but the literature ignores the most widely used animal in research: the rat. Social hierarchies in ten group-housed Long-Evans rats were assessed throughout five stages of development: juvenility, early adolescence, late adolescence, early adulthood, and late adulthood. Late adulthood was further examined over an additional ten-week period to assess the long-term stability of possible hierarchies formed during development. To assess rank order, hierarchies were analyzed through two measures. First, rats were focal sampled in their homecage to record the outcome of naturally occurring dominant-submissive interactions. Second, a dominance based skill task, known as the tunnel task was implemented on every combination of animal pairs throughout the week of data collection. The tunnel task requires the more dominant rat to push his opponent out of the tunnel, in order to receive a food reward. For both experiments, dominance hierarchy orders were determined using Elo-ratings, a system that produces a representative number which dynamically decreases or increases based upon the predicted outcome of interactions. Results support the conclusion that rats have moderately stable dominance hierarchies. Hierarchies showed transient indications of stability, becoming correlated for a few weeks during each phase of testing, but hierarchies were not robustly stable over time. Between task correlations revealed that hierarchies were not the same across tasks, indicating that there is not just one hierarchy.

Assessing the Lower Bounds of Verb Comprehension

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During SURF 2015 we began a project to reliably assess verb comprehension across the second year. We test verb comprehension using the Preferential Looking Task (PLT). Participants view two videos (target and distracter) of actors performing actions (e.g., walk / dance) before (baseline trial) and after (test trial) the target action is labelled. Visual attention to target and distracter videos during baseline and test trials is monitored using a Tobii T60 XL eye-tracker system. Comprehension is defined as an increase in visual attention to the target video during test compared to baseline. We tested 16, 18 and 20-month olds on a set of 12 verbs. During SURF 2016 we completed data collection and analysis and found that 18 and 20-month-olds, but not 16-month olds, comprehend verbs. We have further extended data collection to 17-month-olds to identify the beginnings of verb comprehension. A follow-up study uses the PLT to examine comprehension in younger children (13-15 months) of words that represent early actions (e.g. bye, up, down) and routines (e.g., allgone, peekaboo) that are not encoded as verbs.

Memory Monitoring in Rats

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Metamemory is the ability to monitor the strength of one's memories and is a cognitive function that people utilize each day in making adaptive decisions. For example, in the game show "Who Wants to be a Millionaire," it is advantageous for a participant to phone a friend if she knows that she does not remember which actress played Phoebe on the T.V. show, "Friends." While metamemory has long been recognized in humans, debate still exist as to whether other animals possess this cognitive ability. We investigated the potential for memory monitoring in nine Long-Evans rats (*Rattus norvegicus*) in a series of three experiments using a delayed match to sample (MTS) scent task. Experiment 1 was a concurrent metamemory MTS test with two types of trials: choice trials in which subjects had the option to take or decline the memory test, and forced trials in which there was no such option. Declining the memory test resulted in a guaranteed, but less preferred reward. If rats possess metamemory, they should selectively decline tests in which they forget, which would result in higher performance on chosen trials as compared to forced trials in which there is no option to decline tests when the sample happens to be forgotten. Average performance on choice trials was significantly higher than forced trials, indicating adaptive use of decline test response, and thus, metacognitive responding. To examine generalization of appropriate use of the decline test response and control for possible external, rather than internal, sources of stimulus control, rats were tested on "no sample" trials in Experiment 2 in which no to-be remembered scent was presented. If rats possess metamemory, they should decline "no sample" trials frequently as there is nothing to remember. Results showed that the average decline rate was significantly higher on no sample trials than on sample trials, suggesting that rats use their memory state to cue in determining whether to take or decline memory tests. Together, these results indicate that rats utilize metamemory to gauge the strength of their memories.

Visual Attention to Faces When Making Rapid Judgments of Traits

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Perception of traits is known to occur very rapidly; often with surprising accuracy. We extended this work to consider visual attention to faces during the first 3 seconds of exposure. Asian, Black and White males and females (N = 60) were presented with faces from each of these racial groups and visual attention was measured, and faces were rated on personality traits. Target faces varied in centricity; the degree to which facial features depart from (high or low) average, prototypic features that define racial category membership. Faces from each racial groups with more Euro-centric features received more visual attention than faces with weaker Euro-centric features. For Asian and Black faces, the greater the visual attention to faces with augmented racial features (high centricity) that define category membership, the lower the judgments of verbal ability (Blacks and Asians). For Black and White faces with low centricity, the greater the visual attention the lower the rating of verbal ability (Whites) and the higher the rating of artistic ability (Blacks). An important new finding is that for low status groups (Asians and Blacks), the more visual attention to faces with high centricity; there was more negative stereotyping of ability. In contrast, for a higher status group (Whites) more visual attention to faces with high centricity was associated with positive stereotypes related to mathematical skill and a negative stereotypes related to physical skill.

Social Housing, Stress, and Grit in rats

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“Grit” is a personality trait that causes individuals to persevere when faced with stressors, and stay interested in long-term goals. Studies with rats have shown that different housing conditions can produce different levels of stress on the inhabitants. The current study investigated whether housing conditions, social and non-social, can be used to induce different levels of stress and, in turn, different levels of grit in Sprague-Dawley rats. Grit was measured using an impossible toy task, in which the more time spent attempting to retrieve a food reward from inside, the grittier the rat was. It was hypothesized that there would be a significant difference in corticosterone levels between socially and non-socially housed rats, and as a result there would be a significant difference in grit. The current study serves as a continuation of an earlier study of the same format, in which the socially housed rats were found to have significantly higher corticosterone levels, indicating a higher stress level, but no significant difference in grit. The present investigation aims to determine if the lack of a significant difference in grit was due to the rats being tested too early for the conditions to have made an effect or if the relationship between housing and grit does simply not exist. Results indicate that the socially housed rats were significantly more anxious, as measured by increased grooming behavior, but that the individually housed rats showed a trend in grittier behavior.

Wearable Diagnostics

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Parkinson's disease is a neurodegenerative disorder characterized by loss of motor control and muscle stiffness. When evaluating patients with PD, it is important for neurologists to have an accurate method of measuring motor functions and responses to medication. It's even more critical that the measurement method be designed with the comfort of the patient in mind. Electronic textiles [e-textiles] were designed as a wearable diagnostic tool. Flex sensors using the Arduino program were tested to collect data on degree and speed of finger motion, in conjunction with electrical components embedded with conductive thread. The conductive thread was an essential portion of the design to add comfort and flexibility to the glove. Motion data was then transmitted to a BLE controller Android app. Future development would involve patient testing and construction of different types of textiles. Another user friendly app could also be designed along with the e-textile so that both doctors and patients can utilize the tool.

Social Housing Improves Working Memory Performance in Middle Aged Male Rats

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Working memory is a core executive function that allows for the holding, processing, and manipulation of stored information. The prefrontal cortex, which supports working memory processes, deteriorates more and at a faster rate than other brain regions, and as a result, working memory functioning is among the first cognitive functions to decline with old age. In humans, studies have suggested that strong social relationships can be protective against memory-related dementias, but animal models of sociality and aging are lacking. The aim of this study was to determine whether social housing conditions provide protection against age-related cognitive decline in rats. Beginning in juvenility, 19 male Long-Evans rats were housed in identical enriched conditions with the exception that half of the rats lived together (socially housed) while the other half lived with no physical contact to other rats (non-socially housed). At month 13, when rats were middle aged, they were tested in a Delayed Alternation Task in a T-maze in which subjects had to alternate arm choices to receive reward after a delay interval and previous arm choice. At a 10 second delay, socially housed rats explored arm choices faster than non-socially housed rats and only subtle differences in performance. At longer delays of 1 and 5 minutes, the difference between socially and non-socially housed rats amplified as socially housed rats made fewer errors to previously visited arms, suggesting superior working memory performance in socially housed rats. These results indicate that, independent of general enrichment and exposure to exercise, social housing serves a protective benefit to age-related cognitive decline in working memory functioning.

The Effects of Alternate Feeding Patterns on Circadian Rhythms and the Health of Rats

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Circadian rhythms are the main determinants in feeding and sleeping patterns in animals. They are intrinsic in nature but can be entrained by outside stimuli such as sunlight and access to food. Night shift workers who have irregular activity patterns are much more likely to be overweight and develop diabetes. Irregular activity patterns have a direct correlation to glucose homeostasis and weight gain or loss. The mutation or deletion of circadian genes in mice has led to their inability to maintain glucose homeostasis. In this study, twelve male Fischer rats, housed in individual cages with running wheels, were entrained to a normal feeding cycle for 14 days where they had access to food *ad libitum*. In a 31-day study following the entrainment period, three groups of rats were set up. Group one, the control, had access to food ad libitum as consistent with the circadian rhythm entrainment in the prior fourteen days. Group two only had access to food during the day and not at night, and group three had access to food only at night. Each of the three groups had characteristic shifts in the activity data showing different circadian entrainments of the rats based on food availability. Blood glucose levels were measured at four time points per day on both a fasting and non-fasting day for three separate weeks during the study. Each group demonstrated characteristic changes in the ability to maintain blood glucose homeostasis. Rats in their respective groups either gained weight or maintained their weight like groups 1 and 3 or lost weight like rats in group 2 depending on their circadian entrainment. The goal of this research project was to elucidate the mechanisms of circadian entrainment to food. This correlates with many adverse health effects like the inability to maintain blood glucose homeostasis, weight gain or loss, and fluctuations in activity.

Measuring Sociability in Socially Housed and Non-Socially Housed Long-Evans Rats

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Sociability is the quality of interactions with conspecifics and can be quantified by determining the frequency and duration of social interactions. The purpose of this study was to create a measure of sociability and social novelty in order to examine the extent to which sustained social contact, as achieved by social living conditions, increased or decreased social behavior as compared to individually housed controls. Beginning in juvenility 19 male Long-Evans rats were housed in enriched environments, with half living socially in a large social group (9) and half living individually (10). After several months in these housing conditions, rats we tested on sociability (phase 1) and social novelty preference (phase 2) at month 14. In Phase 1, we found that non-socially housed rats spent more time with an unfamiliar rat as compared to an empty cage than the socially housed rats did, indicating that the non-social rats showed greater tendencies towards sociability in the presence of an unfamiliar rat. In Phase 2, we tested for social novelty preference by placing the now familiar rat from Phase 1 with a new stranger rat, and found that the non-socially housed rats visited the novel stranger more than the familiar rat as compared to the socially-housed rats. These results again show that non-socially housed rats display a greater preference towards sociability and social novelty than the socially housed rats.

Acceptance and Feasibility of a Biobehavioral Intervention that Simultaneously Targets Smoking Cessation and Weight Loss: Pilot Results

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Smoking and obesity are the first and second leading causes of preventable deaths in the United States (U.S.). Standard intervention for both behavior risks is either behavioral, pharmacologic or a combination of both. Acceptance and Commitment Therapy (ACT) is an empirically supported, behavioral intervention with proven efficacy for both smoking cessation and weight loss as separate behaviors. ACT conceptualizes both smoking and excess eating as forms of experiential avoidance, which is when a person is unwilling to remain in contact with a particular private experience (e.g., bodily sensations, emotions, thoughts, predispositions) and takes steps to alter the form, frequency, or situational sensitivity of these experiences even though it is not immediately necessary. Despite ACT's proven efficacy, to date, there are no ACT interventions that simultaneously target smoking cessation and weight loss. Phase 1 of this project piloted a novel ACT intervention that targeted smoking cessation and weight loss simultaneously. The 8-week group-level protocol consisted of 6 group sessions each covering a different component of ACT intervention along with psychoeducation on healthy eating and smoking cessation. Additionally, participants are given the Transdermal Nicotine Patch (TNP). Five eligible participants (2 women, 3 men, mean: 40.8, age range: 23-78) were successfully recruited and four out of five participants completed the intervention. All participants who completed the intervention quit smoking and remained quit and either lost or maintained weight from baseline to the 1-month follow-up assessment. This provides initial efficacy for the novel intervention and will guide phase 2 of this project which is to conduct a preliminary randomized controlled trial (RCT) comparing this novel ACT-intervention to a general health control.

Altered Stress Responses in a *Drosophila* model of ALS

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Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disease characterized by progressive motor neuron loss resulting in paralysis and death in afflicted individuals. A *Drosophila* ALS-model was created by knocking in point mutations within *SOD1*, a gene which when mutated causes familial ALS in humans. Previous phenotype analysis showed *SOD1* mutations caused recessive degeneration of motor neurons, progressive paralysis and lethality in flies. Using these ALS *Drosophila* mutants, we sought to characterize other phenotypes based on knowledge of disease mechanisms. Recent evidence suggests metabolic changes correlate with the disease state. To increase ATP demand within motor neurons, we treated wildtype and ALS-mutant flies with the seizure-inducing GABA(A) receptor antagonist picrotoxin (PTX). Heterozygous *SOD1* mutants H71Y and G85R were exposed to PTX and morbundity was assessed over time and compared to *loxP* wildtype controls. These experiments revealed dominant sensitivity to PTX in G85R and H71Y heterozygotes. In addition, we assessed phenotypes in response to starvation and dehydration, perturbations which alter metabolism. Aged homozygous and heterozygous *loxP*, G85R, H71Y and *sod*-null flies were deprived of food and/or water, and time until lethality was quantified. Homozygous *sod* mutants displayed dramatic sensitivity to dehydration when compared to corresponding heterozygotes. These results may suggest that energy demand and alterations in metabolism affect phenotypic outcomes in ALS *Drosophila* mutant models.

Characterizing *IDH* Mutant Glioma Phenotype in *Drosophila*

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Metabolic reprogramming is a common hallmark shared by nearly all proliferating cancer cells. Gliomas, the most common tumors of the central nervous system, are known to have such metabolic shifts. The metabolic enzymes Isocitrate Dehydrogenase (IDH) 1 and 2 have been identified as drivers of glial cell tumorigenesis. These genes were found to be mutated in up to 70% of low and medium-grade gliomas. We are interested in using a *Drosophila* model to observe how glial cells in these *IDH* mutants differ from wild type cells. *Drosophila* is a suitable system as their glial cells are homologous to human glial cells in function, development, and gene expression. We have recently observed that when our *IDH* mutant allele is expressed with a repo glial cell specific promoter, there is a distinct phenotype of a large circular void of glial cells in the lobes of the 3rd instar larval brains. We suspect this phenotype could result from, 1) a decreased rate of glial cell proliferation resulting in other cells, such as neurons, outcompeting the glial cells in the brain lobe, or 2) apoptosis of glial cells in this region. We are currently investigating these hypotheses using immunostaining and confocal microscopy. We also are interested in observing how the metabolism of these mutants brains compare to wild type brains by utilizing qPCR to analyze expression of metabolic enzymes. Finally, we are in the process of creating flip recombinase induced clones of the *IDH* mutant allele in larval brains. To generate these clones, we must create recombinant flies harboring chromosomes with both our *IDH* mutant allele and a Flippase Recognition Target (*FRT*) allele. To facilitate this process we are optimizing a method of screening for recombinant flies via PCR from wings. These clones will then be used to model a more realistic setting, where tumorgenic cells are neighbors with wildtype cells within a tissue. This work, collectively, will characterize the metabolic and cellular phenotype of *IDH* mutant Gliomas and identify potential new targets for treatment at the metabolic level.

Developmental Lead Exposure in a Tau Knockout Mouse Model; Implication on Behavior and AD-related Biomarkers

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Though Alzheimer's disease (AD) is one of the leading causes of death in the United States, we currently have no disease modifying therapies, and little is known about its etiology. AD is characterized by the presence of tangles and plaques in the brain, which are respectively composed of hyperphosphorylated tau and Aβ aggregates. The Zawia lab has already shown that mice exposed to lead have learning deficits and a significant increase in AD related biomarkers. This study wanted to examine if Tau knockout mice and lead exposed mice would develop AD-like behavior and pathology even though they lacked tau completely. In order to study differences in memory and spatial learning between the groups, our study used wild type (WT) mice, Tau knockout mice and, Tau knockout mice that were postnatally exposed to lead. When the mice were 3-10 months old, behavior tests were performed, which did not show significant changes of memory and learning between the three groups. A second behavior test was performed on the mice again about a year later, and here we found a significant difference in memory and learning of the transgenic mice. Furthermore these deficiencies were more significant in the group exposed to lead. On a molecular level, we wanted to know how the absence of tau (with or without lead exposure) could affect Tau-related kinases, particularly CDK5, as well as expression of APP and levels of Aβ. Our results thus far have showed that CDK5 level is significantly lower in Tau knockout mice than WT mice, and we are still working on APP and Aβ expression. Lower levels of CDK5 suggest that Tau has a regulatory effect on the expression or degradation of CDK5.