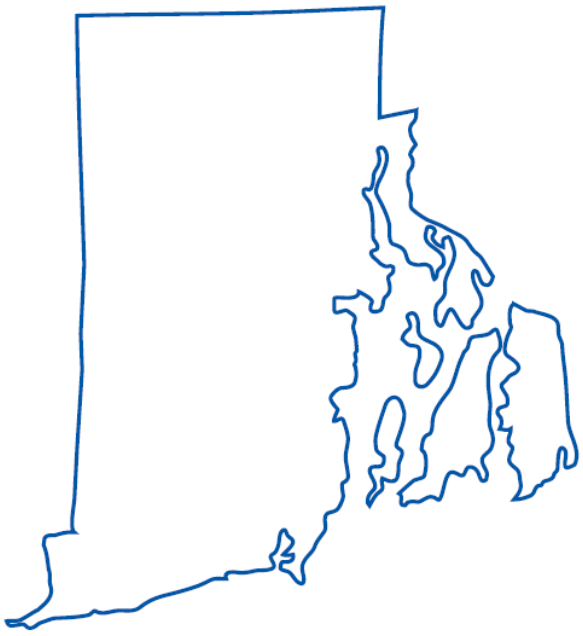
2015 Rhode Island Summer Undergraduate Research Fellowship Conference



Friday, July 31, 2015
8:00 AM

College of Pharmacy
and
Center for Biotechnology & Life Sciences
University of Rhode Island

Supported by

Rhode Island NSF EPSCoR
Experimental Program to Stimulate Competitive Research

RI-INBRE & RI NSF EPSCoR

8TH ANNUAL RHODE ISLAND SUMMER UNDERGRADUATE RESEARCH FELLOWS CONFERENCE

FRIDAY, July 31, 2015
COLLEGE OF PHARMACY AND CENTER FOR BIOTECHNOLOGY & LIFE 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. DONALD DEHAYES, PROVOST, UNIVERSITY OF RHODE ISLAND
	– THE HONORABLE GOVERNOR GINA M. RAIMONDO, GOVERNOR OF RHODE ISLAND
	– DR. ZAHIR SHAIKH, RI- INBRE PRINCIPAL INVESTIGATOR & PROGRAM DIRECTOR, UNIVERSITY OF RHODE ISLAND
	– DR. CAROL THORNBER, RI NSF EPSCOR PRINCIPAL INVESTIGATOR, UNIVERSITY OF RHODE ISLAND
	– CRAIG IRVING, GRADUATE STUDENT, UNIVERSITY OF RHODE ISLAND
9:30 – 12:30 PM	**SURF POSTER SESSION**
12:30 PM	**Lunch**

EXHIBITORS

Located in the Lobby near Check-In on the 1st Floor of the Pharmacy Building

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Cores RI

A directory of core research facilities, services, and instrumentation in Rhode Island.

www.coresri.org

Graduate Programs in Pharmaceutical Sciences, College of Pharmacy, University of Rhode Island

As a leader in Pharmaceutical Sciences graduate education, the URI College of Pharmacy offers you programs and specializations in highly sought after fields available at few other universities in the country.

http://web.uri.edu/pharmacy/academics/graduate/

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www.brown.edu/about/administration/biomed/graduate-postdoctoral-studies

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www.uri.edu/gsadmis

Outreach Center, College of the Environment and Life Sciences, University of Rhode Island

The Outreach Center offers a variety of programs and services and also fields requests for assistance from College and Cooperative Extension experts.

http://web.uri.edu/ceoc/

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.*

Presentation Times	**Research Theme to be Manned**	**Location**
9:30 AM – 11:00 AM	Chemistry	1st Floor Hallway, Pharmacy
	Marine Sciences	1st Floor Hallway, CBLS
	Molecular Biology	Room 105, Pharmacy
	Neuroscience	Room 130, Pharmacy
11:00 AM – 12:30 PM	Cell Biology	Room 240, Pharmacy
	Environmental Sciences	1st Floor Lobby, CBLS
	Genetics	1st Floor, Central Stairway, CBLS
	Microbiology	2nd Floor, Central Stairway, Pharmacy

DEMONSTRATIONS

Microscopy Demonstration
Dr. Al Bach, Ph.D.
in the Centralized Research Core Facility

Meet at 10:00 AM

Room 405
4th Floor of the Pharmacy Building

3D Visualization of Drug Action
Dr. Bongsup Cho, Ph.D.
Steven Mathews (Pharmacy)
Stephen Norris (Computer Engineering)

Meet at 10:30 AM

Room 170
1st Floor of the
Pharmacy Building

TOUR

Medicinal Garden

Meet at 12:30 PM
Near the signs at the doors to the Medicinal Garden.

They are located near the Central Staircase on the 1st Floor of the Pharmacy Building.

CELL BIOLOGY

LOCATED IN ROOM 240 ON THE 2ND FLOOR OF THE PHARMACY BUILDING

POSTERS ARE TO BE MANNED FROM 11:00 AM – 12:30 PM

PROTEIN A TAGGING OF HSP70 SSA1 IN SACCHAROMYCES CEREVISIAE [PSI+] CELLS

Jeremy Boutin, William Holmes

Department of Biology, Rhode Island College, Providence, RI

RI-INBRE Summer Undergraduate Research Fellowship Program

In order for proteins to carryout 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 co-chaperone Sis1 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.

ABERRANT SODIUM CURRENT CONTRIBUTES TO CONSTITUTIVE MTOR ACTIVITY IN MALIGNANT MELANOMA CELLS

Benjamin Gallant[1], Nicole Lizza[1], Jeanine Justiniano[1], Alfredo Gonzalez[1], An Xie[2], Yali Cui[3], Yinsheng Wan[1]

[1]*Department of Biology*, Providence College, Providence, RI
[2]*Cardiovascular Institute*, Rhode Island Hospital, Providence, RI
[3]*Department of Biology*, Northwest University, Xi'an, China

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Transformation from disciplined melanocytes to untamed melanoma cells remains an enigma. Our previous studies have demonstrated that melanoma cells are more resistant to oxidative stress and melanoma cells exhibit constitutive mTOR activity. Surprisingly, further studies have failed to present the expression and activity of EGFR using conventional anti-EGFR antibodies. We hypothesized that constitutive mTOR activity in melanoma cells may be due to mutated EGFR variants and membrane channel activities. Using patch clamping technique, we have shown that melanoma cells (WM 266-4) but not human skin melanocytes exhibit Na+ current which is blocked by TTX. Interestingly, mTOR inhibitor, rapamycin, blocks Na+ current. Western blot and confocal microspcopy data further revealed that Na+/Ca2+ exchanger blocker KB-R7943 (KBR), and L-type Ca2+ channel blocker Nifedipine (NIF) inhibits mTOR activity in melanoma cells in a dose dependent manner. To further characterize Na+ channels, we used commercially available channel antibodies. Western blot analysis data showed that melanoma cells but not human melanocytes express Na+ v1.5 and Na+ v1.6 and NCX3. Functional studies also indicated that KBR and NIF inhibit melanoma cell proliferation and migration. Taken all together, our data suggest that aberrant Na+ current contributes to constitutive mTOR activity in melanoma cells and channel blockers may be potential for the treatment of melanoma.

POTENTIAL APPLICATIONS OF NANOZYMES IN TREATMENT OF PIGMENTOUS SKIN DISEASES

Nicole Lizza, Benjamin Gallant, Justiniano Jeanine, Calianese David, Gammaratta Garret, Minglin Peng, Yali Cui, Yinsheng Wan,

Department of Biology, Providence College, Providence, RI

RI-INBRE Summer Undergraduate Research Fellowship Program

Vitiligo and melanoma are two pigment skin diseases with opposite causes. Vitiligo results from the loss of healthy melanocytes and melanoma is caused by overproliferation of mutated or transformed melanocytes. Our studies have shown that human melanocytes are sensitive to oxidative stress due to lack of antioxidase activity, whereas melanoma cells are more resistant to oxidative stress due to constitutive activity of mTOR and overexpression of antioxidases such as Catalases and SODs. Recent studies have indicated that some nanoparticles exhibit activities of anti-oxidases such as peroxidase, catalase and SODs. In this study, we investigated three available nanoparticles including their anti-oxidase activities, and effects on cultured human melanocytes and melanoma cells. We found that iron oxide nanoparticles from NN-Labs and Goldmag Biotech and gold nanoparticles have peroxidase and SOD activity in test tubes. However, none of the nanoparticles shows catalase activity. In vitro tests showed that the iron oxide nanoparticles at lower concentration have no effects on the proliferation and metabolism of human melanocytes and melanoma cells. Fe3O4 nanoparticle pretreatment protects against H2O2-induced apoptosis. Higher concentration of nanoparticles could induce apoptosis of human melanocytes or melanoma cells. Further biochemical studies demonstrated that nanoparticles have no effect of mTOR activity and expression of catalase, SOD1 or SOD2. We also observed that nanoparticles have marginal effect on the cellular physical behavior. Future tests will concentrate on the protective effects of nanoparticles on melanocytes and inductive effects of apoptosis on melanoma cells.

GALLIC ACID AND ITS ANTIPROLIFERATIVE EFFECT ON GASTRIC CANCER CELL LINE MKN-28 ANALYZED THROUGH FLOW CYTOMETRY

Felicia Talone, Heather Axen, JD Swanson

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

RI-INBRE Summer Undergraduate Research Fellowship Program

Cancer is the second leading cause of death in the United States, accounting for 22.92% of all fatalities. Gastrointestinal cancers are especially lethal leaving the 5-year survival rate at less than 30%. The course of treatment for gastrointestinal cancer includes chemotherapy and resection of the primary tumor, both of which are particularly aggressive, non-specific, and can have many deleterious side effects to the patient. Gallic acid is a plant phenolic found in raspberries and blackberries that has been shown to specifically target cancer cells, halting cellular proliferation. Through this study, gallic acid's effect on the immortal cancer cell line MKN-28 was observed by serum-starving cells for 48 hours and exposing them to differential dosages and time points of gallic acid treatment. Thus far, MKN-28 has been treated with both 0uM and 100uM of gallic acid for 0, 3, 6, 12, 24, 36 and 48 hours. The results of these experiments were evaluated by flow cytometry, a method of cell cycle analysis. This displayed that MKN-28 has a regular doubling time of 36 hours. However, when gallic acid is present MKN-28 undergoes cell cycle arrest in the G1/S phase and displays a delayed G2 peak, suggesting a longer doubling time. Gallic acid, when used as a neutraceutical can potentially reduce the negative consequences of common cancer treatments and promote a better quality of life for cancer patients while also ceasing the proliferation of these cancerous cells.

DELIVERY OF OPTIMIZED MITOXANTRONE TEMPORAL PROFILES USING ELECTRICALLY RESPONSIVE CRYOGELS

Tanner Barnes[1], Anita Tolouie[2], Rosa Rhatee[1], Stephen Kennedy[1,2]

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

RI-INBRE Summer Undergraduate Research Fellowship Program

It is expected that 1.5 million Americans will be diagnosed with cancer in 2015, and nearly 500,000 are expected to succumb to the disease. While recent advances in medical technology have greatly increased the 5-year survival rate for many types of cancer, there is still a need for more advanced therapy. Many current treatments involve the systemic delivery of cytotoxins to combat the disease. A problem with systemic drug delivery is that much higher concentrations of drug must be administered to ensure that therapeutic concentrations are present at the target site. This exposes cytotoxins to healthy tissues and exacerbates off-target effects. An alternative to systemic delivery is to implant a drug-laden biomaterial at the target site, which can reduce damage to healthy tissue and increase drug concentrations at the target site. While promising, most current biomaterials rely on diffusive release, meaning there is no control over dosage over time, even though more complex chemotherapeutic delivery profiles have been shown to improve therapeutic effect. We propose a responsive biomaterials approach that utilizes electrically responsive poly(acrylic acid) cryogels. We have shown that these cryogels are capable of delivering mitoxantrone, a common chemotherapeutic, with full control over timing and release rate. Optimization of the temporal delivery profile of mitoxantrone on B16-F10 melanoma cells revealed that a pulsatile delivery profile results in the lowest cell viability as well as live cell count.

VALIDATION OF IN SILICO PREDICTED HUMAN GENOME CROSS-CONSERVED T CELL EPITOPES ON REGULATORY T CELL RESPONSE

Joe Silva, Jacob Spinale, Mohab Ali, Ryan Tassone, Lenny Moise, Annie De Groot, Rui Liu

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

Independent Research

During thymic development, a variety of self-peptides are presented to T cell precursors to establish tolerance through positive and negative selection. A subset of T cells that recognize the self-peptides escape deletion and are converted to natural T regulatory cells (nTregs). In previous work, we suggested that sequences from pathogens that resemble self-peptides may consequently activate nTregs as a mechanism of immune escape termed “immune camouflage.” JanusMatrix is an algorithm that predicts cross-reactivity between input peptides and self-peptides (or peptides from any sequence database) by matching the specific amino acid sidechains that interact with the T cell receptor (TCR) when bound to HLA-DR molecules on the surface of antigen-presenting cells. Input peptides with many matches in the self-peptide database may be more likely to induce an nTreg response in human PBMCs.

To test this hypothesis, the five TCR-facing amino acids of a promiscuous HLA-binding peptide from tetanus toxin were sequentially mutated to leucine, an amino acid commonly found in human proteins. The number of potentially cross-reactive human peptides identified by JanusMatrix increased with the number of mutations. When all five TCR-facing residues were replaced with leucine, JanusMatrix reported over 650 self-peptides with the same TCR profile. The wild-type and series of mutated peptides were tested in PBMCs from healthy donors to determine their effect on Treg activation. In several donors, the frequency of Tregs expanded by each peptide was significantly correlated with the peptide’s similarity to self-peptides at its TCR-facing residues. The presence of human-like peptides in the proteins of viral, bacterial, or parasite genomes has implications for infectious disease and vaccine design. Further experiments are required to validate these findings.

GALLIC ACID TREATMENT ON GASTROINTESTINAL CANCER CELL LINES WITH IMPEDANCE MEASUREMENT ANALYSIS

Gwen Beaman, Heather Axen, JD Swanson

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

RI-INBRE Summer Undergraduate Research Fellowship Program

Cancer is a worldwide health problem with no reliably effective treatment method. In the United States cancer is the second leading cause of death and accounts for 22.92% of all fatalities. Due to their late detection rates and difficulty in treatment, gastrointestinal (GI) cancers, alone, make up 19.7% of all cancer cases; and with a 30% five-year survival rate, they are a significant health concern. The difficulty in treating cancer comes in targeting cancer cells while avoiding the surrounding healthy tissue. Common treatments, chemotherapy and resection of the primary tumor, are deleterious to both healthy and cancerous tissue. Gallic acid (GA) has been demonstrated to produce an anti-proliferative effect specifically on human cancer cells, without harming healthy tissue, for this reason GA has applications as a nutraceutical cancer treatment. GA is a phenolic compound found in the prickles of Rubus plants, such as raspberries, strawberries and blackberries. If GA arrests the cell cycle in multiple cell lines consistently, then GA can be effectively used to treat cancer throughout the GI tract. The xCELLigence system was implemented to measure the response of cell lines to GA treatment in real time through impedance measurement. This is a method of continuous, real-time and label free cell monitoring that allows observation of cell growth and decay. Impedance experiments have been conducted on gastrointestinal cancer cell lines AGS, MKN28, HT29, DLD1 and Caco2 at concentrations of 10µm, 20µm, 40µm and 100µm GA. The cells were plated in wells equipped with electrodes and measured once per minute throughout 170 hours of growth and treatment. These data will be used to determine proper exposure time and dosage to affect cell cycle arrest and apoptosis in cancer cells.

EXPRESSING THE N-TERMINAL ACETYLASE COMPLEX NATA IN ESCHERICHIA COLI

Kate Sollecito, William Holmes

Department of Biology, Rhode Island College, Providence, RI

RI-INBRE Summer Undergraduate Research Fellowship Program

The function of a protein is directly linked to its three-dimensional fold, which is determined by the amino acid sequence. Changing a proteins structure leads to a loss of function, and potentially the formation of toxic protein aggregates. If a protein can be isolated and purified, it can be studied in inaccessibility from other proteins and the effects of its surrounding environment. Escherichia coli are commonly used to produce large quantities of protein for purification and further study in vitro, however they do not perform the numerous post translational modifications (PTM) found in Eukaryotic cells. E. coli are modified to perform such PTMs, but one modification has eluded study, N-terminal acetylation. This N-terminus is more often than not acetylated in the eukaryotic system adding an acetyl group to approximately 80% of proteins. The inserted acetyl group effectively protects the protein strand from N-terminal protease degradation and potentially plays a significant role in the three-dimensional fold of the protein. The NatA complex is composed of two proteins, Ard1 and Nat1. By cloning both Ard1 and Nat1 genes into a co-expression vector, this Eukaryotic specific complex will potentially modify the N-terminus of proteins expressed in E. coli. The creation of this system will draw from the numerous benefits of protein expression in E. coli, while studying the effects of eukaryotic specific modifications on protein fold and function. The expression of NatA in E. coli can then be utilized to study aggregate prone proteins that are the root cause for neurodegenerative diseases.

INVESTIGATING THE EFFECTS OF THE NATURAL PLANT PRODUCT, GALLIC ACID, ON THE EXPRESSION OF GENES INVOLVED IN APOPTOSIS AND CELL CYCLE ARREST PATHWAYS IN GASTRIC ADENOCARCINOMA CELLS

Meaghan Trzasko[1], Heather Axen[1], Rhiannon Morrissey[1], Felicia Talone[1], Songhua Zhang[2], Steve Moss[2], JD Swanson[1]

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[2]*Warren Alpert Medical School*, Brown University, Providence, RI

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Gastric cancer is a substantial global health burden that does not respond well to common therapeutic treatments. Gallic acid (GA) is a secondary plant metabolite found naturally in plants such as raspberries, blackberries and strawberries. In plant cells, GA promotes rapid cell proliferation; however in cancer cells, GA is proposed to contribute to cell cycle arrest, without affecting normal, healthy cells. To evaluate the effects of GA on cell cycle, we treated cells from the immortal gastric adenocarcinoma cancer line AGS, an epithelial cancer of the stomach lining. We investigated the mechanistic responses of AGS to GA by evaluating changes in gene expression of the cells treated with GA at varying concentration of dosages (0, 5, 10, 15, and 20 µM) over a 24 hour time period. Following treatment we extracted RNA, converted RNA to cDNA and quantified changes in gene expression using qPCR. Specifically we investigated eight different genes associated with apoptosis (BAX2, BCL2 and RhoB) and cell cycle arrest pathways (MMP9, P21, CDK4, CDK6 and Cyclin D1). We found the genes BAX2 and MMP9 were overall up regulated in cells treated with GA, while the genes BCL2 and P21 had an overall down regulated. While the results for the BAX2 and BCL2 genes were expected, the MMP9 and P21 results were unexpected. The results suggest that GA does affect AGS cell proliferation by inducing apoptosis and arresting the cell cycle, and therefore may be an enticing therapeutic compound for gastric cancer that can be delivered via the consumption of natural products, such as fruit.

THE EFFECT OF DIFFERING CONCENTRATIONS OF GALLIC ACID ON CELL CYCLE AND APOPTOSIS GENE EXPRESSION IN THE GASTRIC CANCER CELL LINE MKN28

Michelle Gregoire[1], JD Swanson[1], Felicia Talone[1], Songhua Zhang[2], Steven Moss[2], Heather Axen[1]

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[2]*Warren Alpert Medical School*, Brown University, Providence, RI

RI-INBRE Summer Undergraduate Research Fellowship Program

Gastric cancer is a deadly form of cancer that is most commonly treated with chemotherapy. Chemotherapy is known to have harsh side effects thus alternative treatment for gastric cancer is being investigated in the form of nutraceuticals, natural compounds utilized to prevent and treat diseases. Gallic acid is one such nutraceutical, commonly found in berries, that has shown anti-proliferative effects selectively on cancerous cells. This study investigated the effect of different concentrations of gallic acid on the expression levels of genes important in the progression through the cell cycle (P21, MMP9, Cdk4, Cdk6, CyclinD1) as well as genes involved in apoptosis (Bax, Bcl2, RhoB) in the immortal gastric cancer cell line MKN28. Cells were treated with gallic acid in varied concentrations of 0, 5, 10, 15, 20, 40, 60, and 100 uM. Gene expression changes were then assessed using qCPR, wherein fold changes were evaluated by comparing genes of interest against a constitutively expressed housekeeping gene, RPL29. Despite our predictions that some genes would be up regulated in response to treatment with gallic acid and that others would be down regulated, we found that when the cells were treated with the lower dosages of gallic acid (10, 15, and 20 uM), their gene expression was up regulated. This suggests that gallic acid has the greatest effect on the gastric cancer cells between the concentrations of 10 and 20 uM.

IDENTIFICATION OF SECRETORY LIPASE GENE IN LEISHMANIA MAJOR AND LEISHMANIA TARENTOLE

Syeda Sultana, Alison Shakarian

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

Independent Research

Leishmania is a trypansomatid protozoan parasite responsible for cutaneous, visceral, and mucocutaneous leishmaniases. Symptoms of the disease are species specific. The parasites are opportunistic facultative lipids scavengers, further, we hypothesize that lipase may be responsible in part for the tissue destruction associated with the infection, acquisition of resources for parasite metabolism, and/or the altering of the signaling and functional capability of the host macrophage membrane. The experimental goal of this study is to determine if the secretory lipase gene (LdLip3), previously identified in L.donovani, is present in L.major and L.tarentolae. The methods used for this study include parasite culture, designing primers based on the LdLip3 gene from L.donovani, PCR amplification, gel electrophoresis, and sequence analysis. Expected results should indicate a PCR product of approximately 900bp, similar to the size of the LdLip3 in L.donovani and based on searches of the L,major genome. Further experiments will include cloning the PCR products and subsequent comparison to the LdLip3 lipase gene from L.donovani. We hope to use the lipase gene as a model for identifying a target Leishmania secretory protein molecule that could potentially be used for the treatment of this important human pathogen.

DEVELOPMENT AND EVALUATION OF AIR-GROWN LUNG CANCER SPHEROIDS

Nicholas Fraunfelter, Elisa Torrico, Samantha Meenach

Department of Chemical Engineering, University of Rhode Island, Kingston, RI

RI-INBRE Summer Undergraduate Research Fellowship Program

Lung cancer has the highest mortality rate of any type of cancer, with an estimated 160,000 deaths in the U.S. alone in 2014. This number can be partially attributed to the inability to properly evaluate chemotherapeutics in vitro, which leads to high costs in drug development and an unsatisfactory success rate of drug candidates. Three-dimensional (3D) multicellular spheroid models (MSC) have recently been used in testing chemotherapeutics since they more accurately portray tumors by exhibiting intrinsic physiological and morphological characteristics of tumor tissue in vivo. Currently, all anti-cancer agents are evaluated on two-dimensional cancer cells in liquid culture during the drug development process. Aerosol therapeutics targeting tumors in the air pathways of the lungs are not accurately evaluated in liquid culture and thus there is an urgent need for an in vitro model to overcome this limitation.

A more appropriate model has been developed here; it will allow for 3D lung cancer MSC to be grown in air interface culture in order to evaluate aerosol anti-cancer agents in a high-throughput fashion. Cells are seeded into a hydrogel mold comprised of a non-interactive, degradable biopolymer, alginate, within a Transwell. The alginate is degraded using ethylenediaminetetraacetic acid (EDTA) after spheroids have formed in the alginate molds, leaving the lung cancer spheroids in contact with media on their basolateral side only and the rest of the spheroids exposed to air.

The alginate hydrogels used were optimized to achieve a Young's modulus mimicking lung tissue. Varying the molecular weight and concentration of crosslinker allows for the control of the mechanical properties of alginate hydrogels. Solid spheroids were shown to form quickly and uniformly, via gravity, on the alginate hydrogels. Degradation was successfully performed on cell-seeded alginate, leaving spheroids on the Transwells with media only in contact on the basolateral side, thus showing the efficacy of this novel model. Overall, air-grown lung cancer MCS would be an excellent tool for the screening aerosolized anti-cancer agents. This new in vitro model can decrease the cost and time involved in the drug development process.

HIV-1 T CELL EPITOPES THAT MIMIC HUMAN SEQUENCES INDUCE REGULATORY T CELL RESPONSE IN NAÏVE PBMCS

Jacob Spinale, Mohab Ali, Joe Silva, Ryan Tassone, Lenny Moise, Annie De Groot, Rui Liu

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

Independent Research

Background and Hypothesis: Human immunodeficiency virustype 1(HIV-1) has adopted several strategies for evading host immunity over the course of its evolution. As a consequence, developing a vaccine against HIV-1 has been a difficult task. In previous work, we have identified a novel mechanism by which viruses that cause chronic infection like HIV might escape the human immune response by mutating their epitopes to present "human-like" amino acid sequences to the T cell receptor (TCR) when displayed on antigen-presenting cells. As T cells that bear TCR that recognize autologous epitopes with high affinity are either deleted in the thymus or converted to regulatory T cells (Tregs), viruses that incorporate human-like epitopes may exploit host tolerance to avoid or suppress effector responses. To search more rapidly for such epitopes, we developed an immunoinformatics tool, JanusMatrix. We have identified several human-like T cell epitopes in the envelope (Env) protein of HIV-1, one of which was included in both the HIV-1 E and HIV-1 B Env antigens that were used in the 'moderately effective' HIV RV144 trial in Thailand. We hypothesize that HIV-1 T cell epitopes that mimic human sequences may induce functional Treg response to the viral proteins, resulting in the suppression of effector T cell response. Therefore, we propose to characterize the phenotype and function of T cells that expand or react when stimulated by human-like epitopes contained in HIV-1.

Methods and Results: Ten epitopes from HIV Env were selected and synthesized for validation studies based on their degree of cross-conservation with self. Among them, 5 are human-like and the other 5 are non-human-like by virtue of their TCR-facing residues. For each of the human-like epitopes, a corresponding human homolog epitope from the human proteome is also selected.The proliferation and phenotype of T cells that respond to these peptides are characterized by Flow cytometry. In preliminary studies using naïve subject PBMCs (n=3), we found that the human-like HIV-1 epitopes and human homologs induced proliferation of Tregs. This may be an important means by which HIV-1 evades immune detection, and modulation of these human-like epitopes would improve the efficacy of Env-based HIV-1 vaccine.

DEVELOPING A YEAST MODEL FOR SOLID TUMOR GROWTH WITH SACCHAROMYCES CEREVISIAE

Alexandra Chasse, Stephen Rogers, Nicanor Austriaco, O.P.

Department of Biology, Providence College, Providence, RI

RI-INBRE Summer Undergraduate Research Fellowship Program

Cancer is most associated with the abnormal growth of cells that give rise to solid tumors. We are developing a yeast model for solid tumors by taking advantage of the yeast display system to coat yeast cells with human E-cadherin, a transmembrane protein that functions in cell adhesion junctions in mammalian cells in a calcium-dependent manner. We hypothesized that expression of this adhesion protein on the surface of yeast cells would enhance the adhesion of yeast cells to each other, forming a solid cell mass akin to a tumor. We have completed construction of the relevant plasmid constructs and are in the process of testing our model system to determine if expression of E-cadherin does lead to yeast clumps that are dependent upon calcium levels in the media.

THE EFFECTS OF GALLIC ACID ON PRIMARY CULTURES OF NORMAL HUMAN GASTRIC EPITHELIAL CELLS

Carla Pineyro[1], Songhua Zhang[2], Heather Axen[1], John David Swanson[1], Steven F. Moss[2]

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

RI-INBRE Summer Undergraduate Research Fellowship Program

Gastric adenocarcinoma (better known as stomach cancer) is the third leading cause of cancer deaths in the world. Because gastric cancer has a very poor response to conventional chemotherapy, it is important to investigate novel approaches for this disease. Gallic acid, a phenolic compound found in raspberries and blackberries, has been observed to selectively cause apoptosis in cancer cells in vitro. Gallic acid may serve as a promising anti-cancer agent if it inhibits gastric cancer cell proliferation selectively, doing no harm to normal gastric epithelial cells. The aim of this study is to compare the effects of gallic acid on normal versus malignant primary cultured human gastric epithelial cells. Patients undergoing diagnostic upper gastrointestinal tract endoscopy at Rhode Island Hospital were recruited into this study after informed consent. The study was approved by Rhode Island Hospital's IRB. Human gastric biopsy samples were digested enzymatically in vitro and approximately a total of 2.0 ~ 4.16 x10^6 normal gastric epithelial cells were isolated from each patient. When the cells reached about 80% confluence, 20 µM gallic acid in DMSO or DMSO only (vehicle) were added to the cultures. Cells were collected after 24 hours and fixed with ice cold 70% ethanol, then stained with Propidium Iodide (PI) for cell cycle analysis by using flow cytometry. To evaluate and characterize the isolated primary gastric epithelial cells in culture, cells were stained with anti-pancytokeratin and anti-Ki67 either for immunofluorescent staining or flow cytometric analysis. A primary gastric epithelial cell culture system was successfully established, using normal human gastric cells extracted from endoscopic biopsies. Immunofluorescent staining of anti-pan cytokeratin and anti-Ki67 confirmed that the cells in culture are healthy, proliferating gastric epithelial cells. Initial results indicate that in comparison with untreated control cells, gallic acid did not cause apoptosis and may instead promote cell proliferation in healthy primary gastric epithelial cells. We intend to recruit more patients, including gastric cancer patients to investigate if gallic acid has opposite effects on gastric cancer cells and whether gallic acid affects apoptotic gene expression in either cell type.

GENETIC CHARACTERIZATION OF PROGRAMMED CELL DEATH IN ANEUPLOID YEAST CELLS

Matthew Sanborn, Seth Pinches, Alexandra Chasse, Ryan Frazier, Jessie Barrios, Joel Hauerwas, Nicanor Austriaco, O.P.

Department of Biology, Providence College, Providence, RI

RI-INBRE Summer Undergraduate Research Fellowship Program

Aneuploidy is a genetic state of a cell that has a chromosomal number that is not an exact multiple of the haploid complement. It is a leading cause of spontaneous abortions and of mental retardation in humans, and is also a characteristic defect in cancer. Yeast cells that are aneuploidy manifest a diversity of phenotypes including cell cycle defects, genomic instability, protein imbalance, chaperone stress and proteotoxicity. We are investigating the links between aneuploidy in the yeast S. cerevisiae and programmed cell death. We have shown that aneuploid yeast cells are more sensitive to ethanol-induced cell death. Characteristics of programmed cell death include cellular production of reactive oxygen species (ROS) and an increased caspase activity level. We have noticed that aneuploid yeast cells appear to have an elevated level of basal cell death as measured by dihydrorhodamine 123 (DHR) and fluorochrome-labeled inhibitors of caspases (FLICA) staining, which reflect ROS and caspase activities, respectively. We are generating aneuploid cells with both an elevated copy number of the YCA1 gene that encodes the single yeast metacaspase, Yca1p, and a knock out of YCA1 to determine the effect of this gene on programmed cell death in aneuploid yeast cells.

IDENTIFICATION OF THE SECRETORY LIPASE GENE IN LEISHMANIA MEXICANA

Victoria Wood, Alison Shakarian

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Independent Research

Leishmania is a protozoan parasite that serves as an opportunistic lipid scavenger and causes the disease leishmaniasis. Leishmania mexicana is responsible for the clinical manifestation of cutaneous leishmaniasis, which results in skin sores and lesions on the body of the host. Previous research in our lab showed the presence of the lipase gene as LdLip3 in L. donovani, we thus hypothesize that the LdLip3 gene is partly accountable for the tissue destruction seen in human infection with this parasite. The research goal for this study is identifying the presence of a secretory lipase gene in the species L. mexicana. The experimental design used in this study involved culturing parasites, isolating gDNA, performing a PCR with specific primers based on the LdLip3 gene in L. donovani, and gel electrophoresis. Results indicated bands at approximately 0.9 kb for the positive control of the LdLip3 clones in L. donovani. Future research includes cloning and sequencing of the lipase gene from L. mexicana. Once obtained, we will compare the lipase gene from L. mexicana and L. donovani to gain a better understanding of the role this enzyme has in the biology of Leishmania.

IDENTIFICATION OF THE PRESENCE OF THE SECRETORY LIPASE GENE IN LEISHMANIA TROPICA

Nick Spitz, Syeda Sultana, Victoria Wood

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

Independent Research

Leishmania is a trypanosomatid protozoan parasite responsible for the human disease leishmaniasis. Twenty-one of thirty species are infectious to humans with specific host symptoms according to the species. The Leishmania parasite is an opportunistic lipid scavenger that is introduced to the host macrophage when it is transmitted via the bite of a phlebotomine sand fly. We hypothesize that an expressed lipase gene is correlated to the tissue damage of patients, a physiological dependency on fatty acids, and/or a mechanism to modifying the membrane of the host macrophage. Past studies in this lab showed a gene responsible for lipase activity was discovered in Leishmania donovani, and characterized as LdLip3. The current study is to identify a lipase gene in Leishmania tropica with the following experimental design: culture parasites and isolate gDNA from log phase cells. Primers based on Leishmania donovani were constructed and used in PCR reactions containing Leishmania tropica gDNA and LdLip3 cloned plasmid DNA as template.PCR was followed by gel electrophoresis analysis. Once obtained, the Leishmania tropica lipase gene will be cloned and sequenced for comparison to the LdLip3 gene.

AGS GASTRIC CANCER PROLIFERATION ARREST AFTER GALLIC ACID TREATMENT

Rhiannon Morrissey[1], Heather Axen[1], Felicia Talone[1], Meaghan Trzasko[1], Songhua Zhang[2], Steven F. Moss[2], JD Swanson[1]

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RI-INBRE Summer Undergraduate Research Fellowship Program

Gastric cancer is the fifth most common cancer in the world today. Current treatment methods include chemotherapy, which causes many negative side effects. Nutraceuticals are sought after as a way to combat cancer without also succumbing to the negative side effects associated with traditional therapies. Gallic acid (GA) is a secondary plant metabolite found most potently in raspberries, blackberries, strawberries, and green tea. This compound has been shown to cause cell cycle arrest and apoptosis in lung, breast, and colon cancer. AGS, an immortal line of gastric adenocarcinoma, was cultured and treated with 0, 20, and 100 uM solutions of GA. Cell samples were harvested at 0, 3, 6, 12, 24, 36, and 48 hours post-treatment. Cells were stained with propidium iodide and run through flow cytometry to investigate their functional response to treatment. Following GA treatment, cells were arrested in the G0/G1 phase of proliferation. These findings present GA as a promising natural alternative to chemotherapy as a way to treat gastric cancer.

ELISA SANDWICH ON PAPER

Constantine Anagnostopoloulos, Mohammad Faghri

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RI-INBRE Summer Undergraduate Research Fellowship Program

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. The “Lab on a Chip” project being conducted at the University of Rhode Island seeks to develop 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 more inexpensive and require less 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 was 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 can be used in the future to help detect Dengue Fever for patients who need a simpler and cheaper way.

USING SACCHAROMYCES CEREVISIAE AS A MODEL ORGANISM TO STUDY N-TERMINAL ACETYLATION OF TAU

Phillip Ashkar, William Holmes

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RI-INBRE Summer Undergraduate Research Fellowship Program

Neurodegenerative diseases like Alzheimer's disease and Chronic Traumatic Encephalopathy (CTE) share a common toxic protein, Tau. Tau is a mammalian protein that promotes the assembly and stabilization of microtubules. In both diseases, Tau is known to aggregate, blocking the neuronal highways leading to neuronal cell death. Post-translational modifications play a significant role in Tau aggregation, the most commonly studied is, hyperphosphorylation. Hyperphosphorylation of Tau increases its propensity to aggregate, in turn becoming more toxic to the cell. Recent work demonstrates that Tau is also acetylated at multiple lysine residues, and this modification also leads to a greater readiness to aggregate. These studies highlight the significance of post-translational modifications on Tau aggregation and toxicity, yet Tau has not been fully characterized for all post-translational modifications. The goal of this project is to determine if Tau is acetylated at its N-terminus and how this modification alters Tau aggregation and toxicity in cells. N-terminus acetylation is the most common modification to proteins, as 80% of mammalian proteins are N-terminally acetylated, and based on Tau's amino acid sequence, Tau is also predicted to be acetylated. Previous reports demonstrate minimal truncations to the N-terminus of Tau leads to 50 fold increases in aggregation propensity, suggesting the N-terminus plays a role in Tau aggregation. This study utilizes Saccharomyces cerevisiae as a model organism to study Tau because it provides a simple, yet powerful system to study post-translational modifications, cellular toxicity and, aggregation. If Tau is in fact N-terminally acetylated, a therapeutic agent could be generated to bind to the N-terminus acetylated region of Tau reducing the aggregation and toxicity of Tau.

CHEMISTRY

LOCATED ALONG THE 1ST FLOOR HALLWAY OF THE PHARMACY BUILDING

POSTERS ARE TO BE MANNED FROM 9:30 – 11:00 AM

GOLD-CATALYZED PHENYLATION OF HETEROCYCLES VIA C–H BOND ACTIVATION

Riley Davis, John Rhoat, Louis Marchetti, Brenton DeBoef

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RI-INBRE Summer Undergraduate Research Fellowship Program

This research aims for the regioselective, intermolecular addition of C-C bonds to heterocycles such as pyrroles, thiophenes, and furans, via C-H bond activation. Using a novel I(III) phenyl-source in the presence of a Au(I) catalyst results in a greener and more direct synthetic route for regioselectively accessing a variety of biarly compounds. This is exemplified by the successful synthesis of three heterocyclic biaryls. Further studies hope to increase yields, prove a wider scope of application, and elucidate the mechanism of this novel reaction.

NON-EVASIVE TRANSDERMAL ALCOHOL SENSOR

Rolando Rios, Kunal Mankodiya

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RI-INBRE Summer Undergraduate Research Fellowship Program

According to the CDC between the years of 2006 – 2010 the number of people who have lost their lives because of alcohol is estimated to be around 88,000. To minimize the loss of life and monitor potential health risk involved with alcohol use new non-evasive technology is needed to determine if an individual has been drinking. The applications of such a device has many uses. The data can be used by people who study alcoholism, giving them deeper insight into the behavior pattern associated with drinking. It can also be used to insure people do not get behind the wheel of a vehicle that could potentially endanger themselves or to the public, or used for severe alcohol abuser under house arrest. It can also be used voluntarily. The objective for this project is to develop an alcohol sensor that can detect and monitor alcohol consumption non-evasively through the skin. Researching existing technology available and finding a medium that can be used for creating a small wearable sensor that can respond when the presence of alcohol is detected. Transmitting this data via an android device such as smart phone, smart watch, or tablet to concerning family members.

INHIBITORY EFFECTS OF COMMON EDIBLE BERRY EXTRACTS ON THE FORMATION OF ADVANCED GLYCATION ENDPRODUCTS

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RI-INBRE Summer Undergraduate Research Fellowship Program

Glycation is a spontaneous process between reducing sugars and proteins that leads to the formation of Advanced Glycation Endproducts (AGEs). The formation and in vivo accumulation of AGEs have been linked to several chronic human diseases such as diabetes, inflammation, and neurodegenerative diseases. Current data suggests that phenolic-rich fruit, such as berries, show great promise as natural anti-AGE agents. Moreover, recent studies have shown that wild berries exert anti-glycation activity, which correlates with their antioxidant activities and total phenolic content. However, there is no similar data on commonly cultivated edible berries including blackberry (Rubus sp.), black raspberry (Rubus occidentalis), blueberry (Vaccinium angustifolium), cranberry (Vaccinium macrocarpon), red raspberry (Rubus idaeus), and strawberry (Fragaria ananassa). Therefore, these six berry powders were dissolved and extracted using an XAD-16 column to yield anthocyanin-free (ACF) and anthocyanin-rich (ACR) fractions which were subjected to DPPH (antioxidant), Total Phenolic Content, Total Anthocyanin Content and anti-AGE assays. The fractions were evaluated for anti-AGE effects by an intrinsic fluorescent assay using bovine serum albumin (as the model protein) and D-fructose (as the glycating agent) and compared to aminoguanidine, a synthetic anti-AGE agent (56 % inhibitory effect at 100 µg/mL). At equivalent concentrations of 100 µg/mL, the ACR extracts of blackberry, black raspberry, blueberry, cranberry, red raspberry and strawberry were 25, 85, 66, 25, 4, and 42 %, respectively. The ACR fractions had stronger inhibitory effects on AGE formation compared to their respective ACF fractions. This study suggests that anthocyanins are the major contributors to the anti-AGE activities of these common edible berries.

MUCUS-PENETRATING AEROSOL NANOCOMPOSITE MICROPARTICLES FOR THE TREATMENT OF CYSTIC FIBROSIS

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Independent Research

Pulmonary infection caused by Pseudomonas aeruginosa is a typical complication of cystic fibrosis (CF), which will accelerate the decline in lung function and potentially result in the early mortality of CF patients. Antibiotic delivery directly into the lung is increasingly recommended as maintenance therapy for CF patients with lung infections due to the minimized risk of systemic toxicity and resulting reduced drug resistance. However, the abnormally thick and sticky mucus present in the respiratory tract of CF patients impairs efficient mucus penetration and limits the range of antibiotics for inhalation treatment. Particle size of 1 - 5 μm is required to achieve deep lung deposition. Meanwhile, particles should be smaller than 260 nm to ensure efficient mucus penetration. Nanocomposite microparticles (nCmP) can solve the problem of particle size. However, the desirable properties of nCmP are significantly affected by the added surfactant during formation. On one hand, the surfactant can prevent agglomeration of nanoparticles in freeze-drying and ensure effective re-dispersity of them. On the other hand, excessive surfactant may result in crystallization in spray drying leading to failure of nCmP formation. In this project, we studied the influence of type and amount of surfactant added in making nCmP to develop an optimal method capable of achieving successful formation and redispersion of nCmPs.

NANOPARTICLE THERAPEUTICS: USING AEROSOLIZED TUMOR-PENETRATING NANOCOMPOSITE MICROPARTICLES FOR THE LOCAL TREATMENT OF LUNG CANCER

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RI-INBRE Summer Undergraduate Research Fellowship Program

The second leading cause of death in the world is cancer. Within the United States, lung and bronchus cancers are the leading causes of cancer death with an estimated 158,040 deaths expected in 2015. Additionally, only 17.4% of people survive five years after being diagnosed with Lung or Bronchus cancer.

The objective of this research was to synthesize and characterize a nanocomposite microparticle (nCmP) system to be used for the local treatment of lung cancer. This system involved a dry powder aerosol containing paclitaxel-loaded tumor-penetrating peptide conjugated nanoparticles that were encapsulated into microparticles via spray drying. By using nanoparticle therapeutics, the delivery of paclitaxel (PTX) directly to the tumor site can be achieved, circumventing the adverse side effects seen with systemic delivery. The polymer used to create the nanoparticles was acetalated dextran (Ac-Dex). The nanoparticles were characterized for their size, surface charge, and polydisperisty via dynamic light scattering, and drug encapsulation efficiency via high-performance liquid chromatography (HPLC). The tumor-penetrating peptide iRGD was conjugated via oxime bonding between the aldehyde groups on Ac-Dex and the alkoxyamine groups on iRGD. iRGD is used to target αv integrins expressed on tumors while simultaneously increasing the penetrating capability of the nanoparticles. Once conjugated, the nanoparticles were suspended in a solution of mannitol and spray-dried into microparticles. The resulting nCmP were then characterized for their water content using a Karl Fischer titration and their aerodynamic diameter using a Next Generation Impactor.

SEM indicated the presence of nanoparticles encapsulated into microparticles in the proper size range for lung deposition, and HPLC verified the presence of PTX inside the nanoparticles. Further cell viability studies will be conducted to understand how nCmP interact with cancer cells, and how iRGD enhances the penetrating capabilities of nanoparticles into cancerous cells and tissues. Results have shown the efficacy of nCmP as a potential therapeutic for lung cancer.

ARRAY-BASED DETECTION OF CARCINOGENS AND CARCINOGEN METABOLITES IN URINE

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RI-INBRE Summer Undergraduate Research Fellowship Program

When an anthropogenic event occurs, such as a chemical leak or an oil spill, many toxicants are released en masse into the environment. These toxicants are hazardous and potentially carcinogenic; they can be difficult to identify rapidly, sensitively, and selectively in complex environments, such as environmental and biological matrices. This precise detection is critical for first responders, medical professionals, and biomedical researchers to assess the extent of contamination, potential health risks, and long term environmental and physiological damage. Reported herein is the use of γ-cyclodextrin, an 8-membered cyclic sugar oligosaccharide, to accomplish precisely this kind of rapid detection by binding both the toxicant and a small molecule fluorophore simultaneously in the cavity of the cyclodextrin. The formation of these complexes, termed ternary complexes, allows the toxicant to participate directly in energy transfer with the fluorophore. Energy transfer occurs upon excitation of the toxicant which transfers energy to the fluorophore, resulting in a unique emission signal from the fluorophore. The modular nature of this energy transfer allows the emission signal of the analyte to be tuned through a choice of a fluorophore. This enables the development of an array, wherein each toxicant has a unique response pattern with a variety of fluorophores bound in the cyclodextrin cavity. Exposure of the array to an unknown carcinogen, followed by matching the response pattern to those of known carcinogens, enables the selective detection of highly toxic small-molecule carcinogens. In order to detect these carcinogens in biological systems, urine was chosen as an array matrix. Urine, is one of the most useful biological systems given that most of the metabolites, including those that are carcinogenic, are flushed through the body and can be traced within urine. These toxic analytes are found to be identified in urine in a variety of lifestyles including smoking habits.

Given that urine is the easiest to collect, and less complex than other bodily systems, its use as an array matrix will allow medical professionals to rapidly screen an individual's urine to assess the presence of carcinogens as well as their levels of carcinogen exposure.

SYNTHESES OF PARTIALLY AND FULLY SATURATED EUDISTOMIN ANALOGS

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RI-INBRE Summer Undergraduate Research Fellowship Program

β-carbolines are high-interest molecules due to their diverse biological activity. These molecules are naturally occurring tricyclic, aromatic indole alkaloids. A subclass of β-carbolines, known as the eudistomins, is reported to have a high binding affinity to DNA. In our research, fully and partially saturated eudistomin derivatives were synthesized and their DNA binding capabilities tested and compared. Two synthetic approaches were attempted to establish the most efficient method of making these compounds. It was determined that neither the fully saturated nor the partially saturated analogs showed any specific binding to DNA. Currently, our focus has shifted back to synthesizing and investigating fully aromatic eudistomin analogs.

RATE ACCELERATED ORGANOCATALYTIC RING-OPENING POLYMERIZATION VIA THE APPLICATION OF A BISTHIOUREA H-BOND DONATING COCATALYST

Samuel Spink, Matthew Kiesewetter, Elizabeth Kiesewetter

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RI-INBRE Summer Undergraduate Research Fellowship Program

The organocatalytic ring-opening polymerization of cyclic ester monomers, including lactide, δ-valerolactone, and ε-caprolactone, was performed using H-bond mediated catalysis. The cocatalysts were an H-bond donating bisthiourea (bisTU-3C) paired with various H-bond accepting bases. Polymerization reactions implementing this bisthiourea cocatalyst exhibit significantly increased reaction rates and productivities compared with the monothiourea, Cy-TU, yet these polymerizations retain the well-defined characteristics of a "living" polymerization: low polydispersity, predictable molecular weight and linear evolution of molecular weight with conversion. Increasing the activity and productivity of H-bond mediated transformations is vital to the efficient construction of well-defined and highly-functionalized materials for biological applications. The source of the observed rate enhancement is hypothesized and a mechanism is proposed.

DNA BINDING PROPERTIES OF METAL TERPYRIDINE-PHOSPHONIUM COMPLEXES

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RI-INBRE Summer Undergraduate Research Fellowship Program

Metal-based complexes have been widely used as chemotherapy agents for several decades and have produced significant results in mitigating the effects of cancer cells.The adversity to the aforementioned drugs is selectivity and acquired resistance. It has been documented that metal complexes with the tolyl terpyridine (TTPy) ligand have successfully bound to DNA and cleaved it. In our study, several different metal complexes were synthesized with a tolyl terpyridine phosphonium motif. With the addition of the phosphonium group, the TTPy complexes should become more selective to the mitochondria, and cellular uptake could be more efficient. Selectivity in addition to efficiency is achieved because of the delocalized positive charge on the phosphonium "end" of the complex, which is better absorbed by the more negative cancer cell. This study is to investigate the DNA binding properties of different metal complexes. Several novel metal complexes have been synthesized, and are being studied for their DNA binding properties. These include Copper(II), Gold(III), Ir(III), and Pd(II) with triphenyl, tricyclohexyl or tris-(4-fluorotriphenyl) phosphines incorporated as the phosphonium group. The interaction between these metal complexes and various forms of DNA are being investigated using UV spectroscopy and fluorescence assays. Future work could entail investigating the cleaving abilities of these metal complexes.

DEVELOPMENT OF AN ANALYTICAL METHOD FOR DETERMINATION OF METFORMIN IN PLASMA

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RI-INBRE Summer Undergraduate Research Fellowship Program

Metformin hydrochloride (N,N-dimethylimidodicarbonimidic diamide hydrochloride) is an oral hypoglycemic agent that is commonly used to lower blood glucose levels in patients with type 2 diabetes. There is a need to measure the concentration of metformin in plasma in order to better understand the pharmacokinetic characteristics of metformin. Several assays were published that were reviewed to initiate this project. The concentration of metformin was measured using a Hitachi Elite Lachrome high performance liquid chromatograph (HPLC). An Xterra reverse phase C18 column (4.60 x 5.00 mm) was used with a detector wavelength of 234 nm and an injection volume of 10 µl. This project used an isocratic mode of HPLC analysis with a flow rate of 1.00 ml/min. The mobile phase consisted of a 90/10 mix of aqueous and organic solutions. The aqueous component was prepared by mixing 0.05 moles of KH2PO4, 500 ml of a 20% sodium dodecyl sulfate solution, and 400ml of a 99% triethylamine. The final volume of the aqueous phase was brought to 1 liter with deionized water, and the pH was adjusted to 3.5 with phosphoric acid. The organic component was acetonitrile. The retention time for metformin was approximately 7 minutes. The lower limit of quantification was 500 ng/ml and the higher limit of quantification was 5000 ng/ml. This isocratic method gave a linear response of metformin in aqueous solutions and provides a foundation for future analyses of the drug in blood plasma samples.

INHIBITING THE QUORUM SENSING ACTIVITY OF B-KETO ESTERS

Emily Poulin, Susan Meschwitz, Stephanie Forschner-Dancause

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RI-INBRE Summer Undergraduate Research Fellowship Program

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 b-keto esters were tested in two bacteria: C. violaceum and V. harveyi to further investigate their QS inhibitory properties. Through the use of disc diffusion and broth assays, this Structure Activity Relationship study concluded that a directly attached phenyl ring is necessary on the b-keto ester structure to inhibit QS. The most promising compounds inhibited QS phenotypes without killing the bacteria and include: ethyl (4-methoxybenzoyl) acetate, ethyl (3-methoxybenzoyl) acetate, ethyl (3-methylbenzoyl) acetate, and methyl 3 (4-hydroxyphenyl) 3-oxopropanoate.

BREAKING DOWN THE WALL III: THE N-ACETYLGLUCOSAMINIDASE LYTG FROM BACILLUS SUBTILIS IS A TARGET OF UGI-DERIVED DIAMIDES

Jennifer Brewster[1], Amit Basu[2], Christopher Reid[1]

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RI-INBRE Summer Undergraduate Research Fellowship Program

The use of antibiotics in modern medicine has allowed many of whom would have succumbed to the same infection years ago to survive their infection. However, due to the overconsumption of antibiotics, resistance to clinically useful antibiotics is now common place. The discovery of new antibiotics has not kept pace with the growing threat to public health and the economy, therefore there is a crucial need for new chemical entities exploiting new antibiotic targets. Recently, we were the first to report that bacterial N-acetylglucosaminidases can serve as an antibiotic target. We have previously identified compounds from a panel of Ugi-derived diamides that inhibit growth of the Gram-positive organism Bacillus subtilis. Here, we report the in vitro characterization of the lead compounds fgka (MIC 22 uM) and fgkc (MIC 2.9 uM) against the GlcNAcase LytG from Bacillus subtilis. LytG is an exo-acting GlcNAcase that acts on PG as implicated in cell elongation and division.

Turbidometric assays with purified PG were used to determine the IC50 values of the two most promising compounds. The IC50 for fgka was 146 uM while the IC50 for fgkc was 74.67 uM. This data does not correlate with the observed MIC value for fgka and fgkc and are being assessed. In order to rule out non-specific inhibition via aggregation of the compounds, IC50 assays were repeated in the presence of 0.01% Triton X-100. Results indicate that fgka does not act as a non-specific inhibitor. While the in vitro inhibition is weak, they provide the first successful attempt to utilize GlcNAcases as an antimicrobial target.

MODIFICATIONS OF PLANAR LIGANDS WITH PHOSPHONIUM SALT MOIETIES AS POTENTIAL ANTI-CANCER STRATEGIES

Dave Robinson, Gary Marqus

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RI-INBRE Summer Undergraduate Research Fellowship Program

Phosphonium salts are known to selectively target and penetrate cancer cells. Incorporating these functional groups into established DNA binding molecules is a potentially effective anti-cancer strategy. Herein we report the synthesis, characterization, and DNA binding studies of several metal complexes with various geometries of the form L3[M], where [M] is Ru, Pd, Pt, Rh, or Cu. L3 is a large, planar, aromatic, meridinal tridentate ligand, being either of the tolylterpyridine (ttpy), or 2,2'-(4-p-tolylpyridine-2,6-diyl)bis(1-methyl-1H-benzo[d]imidazole)) (tMebip) motif. These ligands have been modified to contain a phosphonium salt group of triphenyl (TPP), tricyclohexyl (PCy3), or tris(4-fluorophenyl) (tfpp), phosphine. The affinity of these complexes for CT-DNA in aqueous tris-HCl buffer has been studied using UV-VIS spectroscopy. DNA binding has been observed using micromolar concentrations of our complexes. Furthermore, FRET melting assays were performed to study recognition and stabilization properties of these molecules for the G4-quadruplex, present in fluorescently tagged F-21T DNA.

DEVELOPMENT OF A PHOTO AND ELECTROCHEMICAL DETECTOR OF THIOCYANATE IN MARINE ENVIRONMENTS

Amanda McCabe, Craig Rockwell

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RI NSF EPSCoR Summer Undergraduate Research Fellowship Program

As long as the million-dollar aquarium trade flourishes, cyanide fishing poses a threat to coral-reef systems around the world. Fish that have been caught by cyanide fishing will produce thiocyanate ions as a metabolic product that could serve as a marker for this activity. There needs to be a chemosensor device that is both sensitive and selective to thiocyanate ions to aid in the identification of fish obtained by cyanide fishing. Porphyrins with metal centers present good candidates for chemosensors due to their ability to bind thiocyanate ions and be analyzed photo- and electrochemically. Here we present synthetic strategies to synthesize functionalized porphyrins to incorporate into a solid-state chemosensor device. UV-Visible absorption spectra characterize the response of copper (II) and manganese (III) porphyrins to thiocyanate exposure. We will explore additional metal centers (ruthenium (II), cobalt (III)) to apply a combined response approach to determining thiocyanate concentration in the presence of interferent anions in seawater.

EXPLORING STRUCTURE FUNCTIONS RELATIONSHIPS IN KMTR FROM MYCOBACTERIUM TUBERCULOSIS

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RI-INBRE Summer Undergraduate Research Fellowship Program

Mycobacterium tuberculosis (Mtb), the causative agent of tuberculosis, infects close to one-third of the world's population and kills nearly two million people annually. 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. The objective of this project is to explore the structure function relationships in KmtR, a Ni(II)- and Co(II)-responsive transcriptional regulator, from Mtb. The specific aims were to determine 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 Mtb genomic DNA. A plasmid was constructed for expression of the protein and the identity of the gene was confirmed by DNA sequencing. The protein has been expressed in E. coli and work is currently underway to purify the protein.

STUDIES TOWARD THE DEVELOPMENT OF A RAPID GOLD NANOPARTICLE MODIFIED SCREEN-PRINTED CARBON ELECTROCHEMICAL IMMUNOSENSOR ARRAY FOR CANCER MARKERS

Kathleen Gamez

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RI-INBRE Summer Undergraduate Research Fellowship Program

The detection of cancer protein biomarkers is a quick and effective means of early diagnosis and disease monitoring for improved patient health. Interleukin 6 (IL-6) and Interleukin 8 (IL-8) cancer biomarkers are found in high levels in patients with Cutaneous T cell lymphoma and can be measured as a means to detect cancer. Herein, we report on a rapid microfluidic immunosensor based on glutathione-gold nanoparticles (GSH-AuNPs) modified screen printed carbon electrochemical coupled to novel multi-labeled magnetic beads, (HRP/MB/Ab2)-PEG, with specially designed polyethylene glycol polymer brushes (PEG). PEG polymer chains prevent non-specific binding and the magnetic bead bioconjugate (MB) aggregation due to repulsive electrostatic forces. PEG was shown to lessen particle aggregation by dynamic light scattering (DLS). The magnetic bead bioconjugate was used for amplified detection in a multi-channeled electrical immunosensor. In this system, the IL-6 and IL-8 antigens were offline captured onto the multi-labeled magnetic beads, (HRP/MB/Ab2)-PEG and allowed to specifically bind to primary antibodies (Ab1) attached to GSH-AuNPs on the electrode array in a microfluidic channel. Catalytic reduction of hydrogen peroxide flowed over the immunoassay complex was used to generate the electrical signal. Results show that the bioconjugate had the largest sensitivity when HRP and Ab2 were added at 1000:1 ratio in a step-by-step process at 37°C. Non-specific binding events (NSB) which often controls the sensitivity and detection limit was optimized at 0.1%. The immunosensor array show great promise for point-of-care cancer diagnosis.

LIPID-COATED MAGNETIC NANOPARTICLES (L-MNPS) FOR GENE THERAPY AND MAGNETIC RESONANCE IMAGING

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RI-INBRE Summer Undergraduate Research Fellowship Program

Multifunctional nanoparticles engineered with combined cellular targeting, therapeutic, and diagnostic capabilities have the potential to revolutionize biomedicine. To achieve this, the particles must be monodispersed and reproducibly synthesized, chemically and physically stable in complex media, and amenable to tailoring surface chemistry. We are currently developing lipid-coated magnetic nanoparticles (L-MNPs) to meet these criteria by coating 30 nm iron oxide (magnetite, Fe3O4) nanoparticles with a self-assembled layer of phospholipids. Our objective is to design L-MNPs that will be effective for combined cancer gene therapy and drug delivery, and as contrast agents for magnetic resonance imaging (MRI). A cationic lipid (DOTAP) and a 2000 MW polyethylene glycol lipid (PEG-lipid) were used at different DOTAP: PEG-lipid ratios to coat the nanoparticles, providing a range of surface charge densities for siRNA binding and differing hydration shell thicknesses for MRI. L-MNPs were prepared in deionized water using a dual solvent exchange method employing chloroform, dimethyl sulfoxide combined with sonication and centrifugal filtration. Using dynamic light scattering (DLS), our results show that the L-MNPs have a hydrodynamic diameter between 60-90 nm and are extremely stable in water and phosphate buffered saline over the temperature range 25-55oC. For the MRI studies, L-MNPs were prepared in agarose gel and analyzed at the MRI Research Facility in the Institute for Brain Science at Brown University. The results show that the L-MNPs yield exceptionally high T2 and T2* signals for negative contrast imaging.

SYNTHESIS OF MOLECULAR PROBE BIOSENSORS FOR MRI AND TREATMENTS FOR ALZHEIMER'S DISEASE

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RI-INBRE Summer Undergraduate Research Fellowship Program

A cyclotriveratrylene (CTV) compound has been identified as a novel molecular probe for 129Xe magnetic resonance imaging (MRI) and spectroscopy. Past work in this field has relied on the use of Cryptophanes, cage shaped molecules whose syntheses are difficult and low yielding. CTV is a bowl-shaped compound that is also capable of reversibly binding xenon; this event can be detected in 129Xe NMR spectrum. Due to the ease of synthesis of this novel CTV, we hypothesize that it can be a superior molecular probe for functionalization and eventual use as a targeted 129Xe molecular probe.

PS48, (Z)-5-(4-chlorophenyl)-3-phenylpent-2-enoic acid, is known to bind phosphoinositide-dependent protein kinase 1 (PDK1a) which is involved in insulin signaling pathways. The complete pathogenesis of Alzheimer's disease (AD) is still unknown, but one emerging hypothesis is it may be a "type III diabetes," due to the association of insulin depletion in the brain with early stages of AD. Synthesis of PS48 has been achieved in our lab in 4 steps. We hope to use this compound as a key component in a collaborative effort towards the development of a library of novel kinase inhibitors for potential treatment of AD.

SYNTHESIS OF A PANEL OF PYRIMIDINONES AND EVALUATION OF THEIR BIOLOGICAL ACTIVITY

Alexander Hulme, Stephanie Dancause, Susan Meschwitz

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RI-INBRE Summer Undergraduate Research Fellowship Program

The abuse and misuse of antibiotics have caused outbreaks of drug resistant bacterial strains leading to infections becoming increasingly more difficult to treat. Quorum sensing (QS) is a process that bacterial species use to communicate with each other in order to act in a coordinated fashion. Autoinducers released by bacteria accumulate and ultimately control the expression of virulence factors when the population of bacteria reaches its quorum. The focus of this project is to synthesize compounds structurally similar to the quinolone autoinducers used in the Pseudomonas aeruginosa QS system. A facile, one-step synthesis using a microwave technology to prepare the desired scaffold has proven successful and the synthesis of a small focused library of analogs is underway. So far, eight pyrimidinone derivatives have been synthesized and are being tested in Chrombacterium violaceum and Vibrio harveyi for their ability to inhibit quorum sensing. In addition the inhibitory effects of different alkyl chain lengths as well as the effect of the nature and position of substituents on a phenyl ring are being investigated. Several compounds have exhibited quorum sensing inhibition and others have displayed antibiotic activity The compounds synthesized during this investigation are anticipated to serve as valuable tools in the study of quorum sensing and provide potential new leads in the development of anti-infective agents.

EVALUATION OF ANTIBIOTIC AND QUORUM SENSING INHIBITORY ACTIVITY OF VARIOUS MONOFLORAL HONEYS

Joshua Ng, Susan Meschwitz, Siobhan Meeken

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RI-INBRE Summer Undergraduate Research Fellowship Program

Honey is been known since ancient times for its healing properties and antibacterial activity. More recently, the anti-quorum sensing properties of various honeys have been investigated. Quorum sensing is a chemical signaling system that is regulated by small complex molecules called autoinducers which allows bacteria to communicate. Recent studies have identified Manuka honey as the most therapeutically potent honey. In this study, the activities of several monofloral honeys, including Tupelo, Holly, Apple Blossom, American Basswood, Buckwheat, Gallberry, and Texas Tallow were examined and compared to Manuka honey. The quorum sensing inhibitory activity of the honeys was evaluated using the bacterial model, Chromobacterium violaceum. Of the tested honeys, Texas Tallow, Tupelo, Apple Blossom, and American Basswood honeys exhibited quorum sensing inhibition using an agar-well diffusion test. A flask incubation assay was carried out on these quorum sensing inhibitory honeys in order to quantify the percent inhibition and demonstrated a concentration-dependent effect, as the inhibition activity increased with increasing honey concentration. Both quorum sensing inhibition and antibiotic activity was observed in these assays. Manuka honey was used as a model for antibacterial activity based on the optical density growth curve. Texas Tallow exhibited the most active quorum sensing inhibition. These studies demonstrate the potential of honey to inhibit cell-to-cell communication in bacteria and warrant further investigation.

SYNTHESIS AND BACTERIAL QUORUM SENSING INHIBITION OF PHEVALIN AND DERIVATIVES

Mark Grande, Kaylee Miller, Stephanie Dancause, Susan Meshwitz

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RI-INBRE Summer Undergraduate Research Fellowship Program

Infectious diseases are traditionally treated with compounds that either kill or inhibit bacterial growth. Antibiotic resistance is a growing concern among the development of new drugs. Quorum sensing (QS) is the process by which bacteria communicate with one another through chemical signals known as autoinducers. This communication allows the bacteria to coordinate their behavior and function as a multicellular organism rather than individual cells. Autoinducers are small molecules that are released by bacteria and bind to and stabilize receptor proteins, causing the ligand-protein complex to initiate transcription of quorum sensing genes. QS plays a role in regulating virulence and pathogenicity in bacteria. This creates an opportunity to control infectious bacteria without interfering with growth, making it less likely for bacteria to develop resistance. Our long-term goal is to optimize the synthesis of small molecules that have the capability to inhibit QS. We have successfully synthesized in four steps the pyrazinone, phevalin, a known regulator of virulence factor expression in Staphylococcus aureus. Bioassays utilizing Vibrio harveyi demonstrated the ability of phevalin to inhibit bioluminescence, a QS-controlled phenotype. By varying the amino acid starting materials, we are developing a small library of phevalin derivatives to further investigate the ability of these compounds to be potent quorum sensing inhibitors. To date, four derivatives have been synthesized and the structure-activity relationships have been investigated.

ANTIBIOTICS INSPIRED FROM NATURE: TOTAL SYNTHESIS OF BENZOISOXAZOLONE DERIVATIVES FROM BLASTOBACTER DINITRIFICANS

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RI-INBRE Summer Undergraduate Research Fellowship Program

Antibiotic resistance is a global problem. Antibiotic overuse and misuse has led to bacteria that have become resistant to certain antibiotic drugs. This has created a critical need for novel antibiotics to treat resistant infections. The goal of this investigation is to synthesize the natural product 2-hydroxy-benzisoxazolone and determine its antibiotic properties. Related natural products have demonstrated potent antibiotic effects against problematic gram-negative bacteria. A six-step synthesis was devised to form the compound. Highlights of the synthetic strategy include conversion of an ester to a hydroxyamide and then formation of an isoxazolne ring. Progress toward the natural product and analogs will be presented.

MOLECULAR IMAGING USING MASS SPECTROMETRY AND DESORPTION ELECTROSPRAY IONIZATION

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RI-INBRE Summer Undergraduate Research Fellowship Program

Desorption electrospray ionization (DESI), is one of the newest mass spectrometry (MS) ionization techniques that has greatly expanded the use of mass spectrometry to examine less treated samples found within the environment. The investigation of the spatial distributions of chemical components in solid and tissue samples has become very important in biology and chemistry today. The DESI source and specialized software enable this spatial information to be displayed as an image.

To learn how to create this type of MS image, we used rhodamine 6G (MW 443.5 m/z), the dye in red sharpie markers as a test molecule. The following test images were drawn on microscope slides and imaged. 1) Vertical line with a triangular base; 2) Horizontal line with varying thickness; 3) The letters SURF (Summer Undergraduate Research Fellowship).

Before we could collect MS images, the DESI source had to be assembled and several parameters optimized for best performance when connected to an AB Sciex QTRAP 4500 mass spectrometer. These parameters will be described.
This type of MS imaging requires several software programs to produce a two dimensional image from a traditional MS spectrum. These include Analyst, to control the mass spectrometer, Omni spray, to control the DESI source, Firefly, to create the image file, and Bio map to visualize the image.
We will present the test images collected.

HIGH SCHOOL RESEARCHERS AT THE UNIVERSITY OF RHODE ISLAND

Julia Mendoca, Alexandrea Pouliot, Ezra Feder, Hossam Zaki, Mindy Levine

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RI-INBRE Summer Undergraduate Research Fellowship Program

High school internships in the chemistry department at the University of Rhode Island have focused on studying interesting problems in supramolecular organic chemistry that can impact public health, national security, and environmental remediation. We herein report our research efforts towards these important goals, made during the summer of 2015.

ISOLATION OF ENTAMOEBA HISTOLYTICA ALCOHOL DEHYDROGENASE 2 (EHADH2) THROUGH FAST PROTEIN LIQUID CHROMATOGRAPHY WITH ION EXCHANGE

Katie Lowerre, Dr. Avelina Espinosa

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RI-INBRE Summer Undergraduate Research Fellowship Program

Entamoeba histolytica's EhADH2 enzyme (95 kDa) is essential for Entamoeba histolytica's growth and survival. Purification of EhADH2 is necessary for determining kinetic activity, affinity constants, and effects of various inhibitors on the protein's activity that could be useful in future treatments of amebiasis. The protein was cleaned through fast protein liquid chromatography through ion exchange. Ion exchange is based on the protein's ability to bind to the column resin that isolates EhADH2 according to its charge. The ÄKTA FPLC, GE life sciences fraction collector 920, and a 5 mL HiTrap Q XL anion exchange column were used in purification. The 5 mL HiTrap Q XL anion exchange column includes a positively charged quaternary amine that acts as a strong anion exchanger. Albumin concentrations that replicated those of previous runs of EhADH2 (around 1500 ug/ mL) were able to bind to the column and elute off into fractionalization. Specific conditions required to create a negatively charged EhADH2 are still in progress. Variability in the purification process before the use of FPLC may have contributed to lack of binding to the enzyme, where factors including net protein charge, amount of remaining salt concentration after dialysis procedures, and pH of buffers may have contributed to a lack of binding of the protein to the column. More research should improve the conditions so ion exchange can be effective in purifying EhADH2. Affinity chromatography may be a better option. HiTrap Blue affinity columns that contain Cibacron Blue media can effectively bind to proteins based on potential hydrophilic or hydrogen bond interactions. Also, there is potential for EhADH2 to possess a dinucleotide fold contributing its specific NAD binding site. Future studies include the use of the enzyme in a HiTrap Blue affinity column and NADH as an eluent.

SYNTHESIS OF NOVEL SELECTIVE ANTI-CANCER AGENTS

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RI-INBRE Summer Undergraduate Research Fellowship Program

The synthesis of cinnamyl arylphosphonium salts (CAPS) coupled with an RGD (Arg-Gly-Asp) sequence was attempted utilizing bench top organic procedures as well as microwave assisted solid phase peptide synthesis. The arylphosphonium salt (APS) moiety, a lipophilic cation, has been shown to selectively target cancer cells due to their overall negative membrane potential. From this class of compounds, APS with a cinnamyl group bound has been shown to have the highest affinity and toxicity towards malignant cells and in DNA binding studies. RGD is a recognition sequence for surface receptors in some cancers. The hypothesis is that APS enter mitochondria in tumor cells where they can act as DNA complexing agents and/or interfere with electron transport. The peptide sequence RGD (Arg-Gly-Asp) has been shown to be the recognition sequence for α, β integrins. These intergrins are only present in cells undergoing angiogenesis. Studies of co-administration of these compounds have been shown to decrease the viability of malignant cells while showing low toxicity to non-malignant cells. The current study attempts to develop a synthesis of CAPS and their RGD conjugates in order to investigate selective toxicity by both co-administration and administration of CAPS-RGD conjugates to normal and malignant cells.

HEAT SHOCK PROTEINS AS A POTENTIAL GAUGE OF CLIMATE CHANGE IN NARRAGANSETT BAY.

Jennifer Rodriguez, Franchesca Cruz, John Williams, Jr.

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RI NSF EPSCoR Summer Undergraduate Research Fellowship Program

Functional proteins must have 1) a specific primary sequence and 2) be able to assume a particular higher order structure. Hsp70 is a chaperone protein. Chaperone proteins aid in the correct re-folding of damaged proteins so they may re-acquire the necessary overall structure to retain their function. Hsp70 is found in all cells. However, cells that have undergone stress have a far greater expression of Hsp70. Stress factors include hypoxia, hyper and hypothermia, and deviations in pH. This project involves measuring Hsp70 levels induced by heat stress on live organisms. The overall hypothesis is that Hsp70 levels in marine organisms will correlate with climate change. The organism being studied is Geukensia demissa, a species of mussel native to Narragansett Bay. Samples were collected from Watchemocket, Passeonkquis, and Fox Hill, located in East Providence, Warwick, and Jamestown, respectively. The organisms were dissected upon collection, after being acclimated in a 20 C bath, or after exposure to 40 C for 20-30 minutes. Hsp70 was extracted from dissected gill tissue, isolated by gel electrophoresis, imaged by a western blot and antibody protocol and analyzed using "Image J" software. Isolation of Hsp 70 was substantial in nearly all trials of the experiment. We have seen correlation with the temperature exposure of the organisms and the collection location.

SELECTIVE TOXICITY OF SMALL MOLECULES IN CANCER VS. NORMAL CELLS IN CULTURE; EXPERIMENTAL DESIGN

Kayla Marcello, John Williams, Jr.

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RI-INBRE Summer Undergraduate Research Fellowship Program

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. 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, or APS + RGE or APS-RGE (conjugates) for observation by flow cytometry. We will determine EC50's and SAR's for a library of APS and modify the most promising leads to increase toxicity and selectivity.

NAD+ BIOSYNTHESIS IN MARINE MICROORGANISMS

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RI NSF EPSCoR Summer Undergraduate Research Fellowship Program

The coenzyme nicotinamide adenine dinucleotide (NAD) is an effective electron carrier found in all living things. In its oxidized (NAD+) or reduced (NADH) form, it is required for many fundamental cell processes including respiration, metabolism, and cell signaling. There are three main pathways for the biosynthesis of NAD; in most organisms, NAD can be synthesized from tryptophan or aspartic acid in what is known as the NAD de novo pathway; it can also be recycled from nicotinamide (NAM) in the two-step salvage pathway, or from nicotinic acid (NA) in the four-step Preiss-Handler pathway. NAM is found endogenously as the product of NAD consuming enzymes, and is then restored to NAD via multiple strategies. NAM and NA may also be present in the environment and readily diffuse through cell membranes. All three pathways are found within the general classification of marine microbes, however the strategies present within each genera vary greatly and seem to have no evolutionary pattern. Using known bacterial, algae, and fungal NAD pathway protein sequences, the NCBI BLAST database of the seven fully sequenced marine phytoplanktons was analyzed. The results were compiled and will be presented. Marine phytoplankton provide a primary role in removing carbon dioxide out of the atmosphere near surface waters, releasing atmospheric oxygen and storing carbon. Thus, a better understanding of bioenergetics within these organisms may provide insights to global climate phenomena.

SPR STUDIES OF NICOTINAMIDE PHOSPHORIBOSYLTRANSFERASE

Ashlee Sturtevant, Silvia Yanez, Lisbeth Avalos, Kristen Chauvin, Steven Berardinelli, Karen Almeida

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RI-INBRE Summer Undergraduate Research Fellowship Program

Nicotinamide phosphoribosyltransferase (NAMPT) is the rate limiting enzyme in the nicotinamide adenine dinucleotide (NAD) salvage pathway, which clinical studies have identified as a therapeutic target of interest. Our lab has identified two small molecule activators that lie on NAMPT's dimerization plane and show a significant increase in the enzyme's activity. To confirm the small molecules interaction with NAMPT, surface plasmon resonance (SPR) was used. This detection method allows for real-time monitoring, and high sensitivity while requiring only small sample volumes. Direct binding via amine coupling was used to immobilize NAMPT on the chips surface, which was then followed by a surface performance test involving injections of NAM over the sensor chip surface and monitoring the interaction. Bound NAM was stripped off during regeneration leaving only NAMPT on the chips surface. The same process can be used to monitor the interaction between the two small activating molecules with NAMPT. From this process, preliminary binding between NAMPT and an activating molecule was observed. Future studies using SPR will confirm the physical interaction between the small activating molecules, which will further our overall knowledge of the cellular balance of the critical metabolite, NAD.

RECONSTITUTION OF THE HUMAN NAD+ SALVAGE PATHWAY

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RI-INBRE Summer Undergraduate Research Fellowship Program

Nicotinamide adenine dinucleotide (NAD+) is a coenzyme found in all living cells. It is central to numerous metabolic pathways and many diseases related to aging, stress responses, inflammation and immunomodulatory responses. NAD+ plays a vital role in metabolism and functions to assist in electron transfer during oxidation/reduction reactions. It also acts as a substrate in a growing number of NAD+ consuming reactions. For example, NAD+ acts as a donor of ADP-ribose groups for the DNA damage response enzyme, poly [ADP-ribose] polymerase 1 (PARP-1). Hyper activation of PARP-1 depletes NAD+ levels requiring the cells to restore the consumed NAD+. Two novel carbon skeletons have been identified as activators of the NAD+ salvage pathway. In order to understand the effects of these small molecules, a new expression plasmid pDEST 17 NAMPT-ZEO was made using subcloning methods. The salvage pathway was reconstituted by transforming BL21 cells with expression plasmids for NAMPT, NMNAT1, and both. Expression of salvage pathway enzymes was confirmed by immunoblot analysis. The transformed cells were grown in media supplemented with salvage pathway metabolites. Validation of this reconstituted system will be presented along preliminary results. Reconstituting the NAD+ salvage pathway will provide insight into the effects of the two known activator molecules and how they affect the overall NAD+ regeneration pathway.

INVESTIGATIONS OF THE EXTENDED LIPID HYPOTHESIS WITH D_PG LIPIDS

Brianna Abbott, J.J. Breen

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The Walsh Student Research Fellowship Program at Providence College

Based on an extensive collection of work monitoring the interactions between cytochrome c and a variety of natural and synthetic lipids, P.K.J. Kinnunen and co-workers proposed the existence of two distinct binding modes for cytochrome c adsorbed on acidic lipid surfaces. One mode is purely a result of the electrostatic attraction of the positively charged protein with the negatively charged surface. The second mode involves an extended lipid conformation in which the non-polar tails of a lipid extend in opposite directions and interact with the hydrophobic domains of peripheral proteins. The extended lipid conformation is a unified way of explaining the molecular dynamics that occurs during membrane association with a peripheral protein, membrane fusion and lamellar-HII phase change, collision-dependent transfer of phospholipids between vesicles, and phospholipid flip-flop. In this poster, a series of luminescence experiments involving synthetic lipids and cytochrome c will be presented that further test and support this hypothesis. Phosphatidylglycerol lipids of various acyl chain lengths were fabricated into vesicles and labeled with a fluorescent dye. The adsorption and desorption of cytochrome c was monitored by the changes in relative fluorescence intensity resulting from energy transfer experiments between the dye and the paramagnetic center in cyt c. Our experiments show a general trend with the extent of desorption being dependent on the acyl chain lengths of the lipids with desorption being greater for shorter acyl chain lipids. Temperature dependent desorption experiments over a narrow range indicate that the observed trends are a result of the increased packing strain for the longer chain lipids in the vesicle bilayer and not due to any differences in the binding energy between the lipid and cyt c.

ALTERNATIVE-METAL FUEL CELL CATALYSTS: DRIVING CLEAN ENERGY AUTOMOBILE PRICES DOWN

Sean Flanagan, Thomas Arruda

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RI Space Grant Consortium Fellowship Program

Fuel cells are attractive energy conversion devices that generally produce electricity with no or little carbon pollution. One type of fuel is the H2/O2 (proton exchange membrane FC or PEMFC), where hydrogen and oxygen are reacted to produce electric power and water. There are no hazardous products or greenhouse gasses, making this technology the greenest possible option. The PEMFC is applicable for use in cars, having the potential to replace conventional gasoline combustion vehicles with zero-carbon automobiles. This would greatly reduce CO2 emissions produced by gas powered automotives, mitigating effects of atmospheric heating and changes to Earth's climate and ecosystems.

While this technology has been successfully proven as a viable, efficient system, it is costly due to the platinum catalysts utilized to facilitate the hydrogen-oxygen redox reactions powering the vehicle. We are investigating materials that can potentially be used as fuel cell catalysts that are based on inexpensive materials such as copper, cobalt, iron and others. Our research focuses on electrochemical and spectroscopic characterization of these non-platinum catalysts.

Electrochemical methods tell us how good the catalysts perform and spectroscopic methods reveal the chemical structure of these materials. The spectroscopic investigations we perform are known as X-ray Absorption Spectroscopy or XAS, and are completed in machines known as synchrotrons. In these machines, the materials are assembled into a specific "in situ" electrochemical cell where we apply specific voltages and measure the XAS spectrum. From the input of XAS spectra into specialized computer programs, we can make attempts to determine the chemical structure which is available by no other conventional means (e.g. NMR, IR Mass Spec). Structural determination can only be made when the experimental spectrum closely resembles the computer generated spectrum of the modeled compounds. If a successful determination is made, chemical adjustments could be made to the catalysts to improve activity and hopefully produce a better, cheaper and longer lasting fuel cell.

TOXICANT DETECTION IN COMPLEX BIOLOGICAL ENVIRONMENTS

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RI-INBRE Summer Undergraduate Research Fellowship Program

Providing both first responders and scientists with a facile and rapid tool that can detect carcinogenic toxicants in complex environments can be very beneficial to both monitor and detect exposure. Gamma-cyclodextrin, an eight-member cyclic oligosaccharide, can simultaneously bind a fluorophore and carcinogen through ternary complex formation. In this system, γ-cyclodextrin facilitates proximity-induced energy transfer. Energy transfer to and emission from the fluorophore (energy acceptor) occurs upon excitation of the analyte (energy donor) to produce an emission peak unique to each fluorophore-carcinogen combination. These unique signals can lead to the array-based detection of the analytes. Analytes of interest, including environmentally-persistent PAHs (ex: anthracene, benzo(a)pyrene, and pyrene) and their metabolites were tested with three fluorophores in breast milk, plasma, and urine. The method requires minimal sample preparation; however specific components such as lipids and proteins were systematically removed using commercially available agents to determine if and how they participate in energy transfer. In addition, an array was developed in urine and thoroughly investigated using both parent PAHs and their metabolites. The detection of different PAH metabolites can be used to determine how long a patient may have been exposed to the toxicants. The results of these experiments will provide a new tool for the rapid detection of toxicants in biological fluids, to accurately quantify individuals' level of toxicant exposure, and to study, predict, and treat individuals who are at risk of developing diseases from such exposure.

GOLDEN NANOSCALE LIGHTNING RODS: WATER QUALITY MONITORING USING SURFACED ENHANCED RAMAN SPECTROSCOPY (SERS)

Catherine Linh, Buddini Karawdeniya, Y. M. Nuwan D. Y. Bandara, Julie Whelan, Jason Dwyer

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RI NSF EPSCoR Northeast Water Resource Network Summer Internship Program

Water quality monitoring is a vital part of our stewardship. A water sample can contain a wide range of chemicals, both beneficial and harmful, present over wide ranges of concentration, and the task of determining the water quality in such a complex sample requires high-performance instrumentation. We are using a technique called surface enhanced Raman spectroscopy (SERS) to take spectroscopic “fingerprints” of molecules, with the aim to apply SERS to water quality monitoring in the field. To make the approach viable, we grow gold nanoparticles on a solid surface, and use these “lightning rods” to enhance the Raman signal by up to a million-fold. Real-world water samples present tremendous practical challenges, including biofouling of the sensor surface. Conventional water sensors attempt to overcome this problem with integrated wiper blades, but our SERS devices are too delicate. We are therefore developing disposable films to protect the device surfaces.

SYNTHESIS OF 2-AMINO ALPHA CARBOLINE, AND OTHER ALPHA CARBOLINE DERIVATIVES; PRECURSORS TO DNA ADDUCTS

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RI-INBRE Summer Undergraduate Research Fellowship Program

2-Amino-alpha-carboline (2-AαC) is a heterocyclic aromatic amine, which has been found in high concentration in the liver hepatocytes of carcinoma patients. 2-AαC has been found in cigarette smoke and in overcooked meat at concentrations far greater than that derived from 4-aminobiphenyl, a well human bladder carcinogen. 2-AαC forms DNA adducts by binding at the C8 position of guanine base. The 2-AαC DNA adducts are believed to be responsible for cancer initiation in the liver and urinary bladder as well as cecum and colon. In order to conduct laboratory tests to clarify whether or not 2-AαC DNA adducts have mutagenic or carcinogenic effects, it is important to be able to synthesize the molecules in the lab. Previous synthesis of 2-AαC is inefficient and costly. In this poster we describe a new, shorter, and more efficient synthesis of 2-AαC and its nitro and fluoro analogs, which are critical precursors for preparation of 2-AαC-DNA adducts.

DEVELOPMENT OF A CANCER BIOMARKER DETECTION SYSTEM UTILIZING THE ELECTROGENERATED CHEMILUMINESCENCE OF LUMINOL

Thomas Stracensky, Munique de Oliveira, Bernard Munge

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RI-INBRE Summer Undergraduate Research Fellowship Program

This poster proposes the fabrication of an electrogenerated chemiluminescence (ECL) biosensor array for the detection of caner biomarkers in serum. This array features layer by layer assembled capture antibodies attached to non-oriented multi-walled carbon nanotube (MWNT) at the bottom of micro wells with hydrophobic barriers built on a pyrolytic graphite (PG) block. Analyte detection was carried out by a sandwich assay between the PG array and the bioconjugate. Two types of bioconjugate were fabricated using magnetic beads, one bioconjugate with glucose oxidase (GOx) and secondary antibodies and the other bioconjugate with directly attached luminol and secondary antibodies. Interlukin-8 has been shown to be a cancer biomarker for head and neck cancers and was used as the analyte for detection. ECL was initiated by the conversion of glucose by GOx to produce hydrogen peroxide, which in turn reacts with luminol in solution to produce light when a potential is applied. The light was captured using a charged coupled device (CCD) camera and the signal was directly proportional to the amount of analyte present. This device is in the early stages of development but would serve to be a sensitive, quick, cheap, and easy way of measuring biomarkers in the point of care setting for clinical diagnostics.

ENVIRONMENTAL SCIENCES

LOCATED IN THE SOUTH LOBBY ON THE 1ST FLOOR OF THE CENTER FOR BIOTECHNOLOGY & LIFE SCIENCES

POSTERS ARE TO BE MANNED FROM 11:00 AM – 12:30 PM

CHANGING ANION NUTRIENTS AND POLLUTION INFLUX TO THE PROVIDENCE RIVER AND NARRAGANSETT BAY FROM THE BLACKSTONE RIVER WATERSHED.

Christina Watts, Alexandra Dickey, Julia Crowley-Parmentier, Dan McNally

Department of Science & Technology, Bryant University, Smithfield, RI

RI NSF EPSCoR Summer Undergraduate Research Fellowship Program

Dead zones, or hypoxic/ anoxic regions in bodies of water causing fish kills, are caused by the eutrophication process. What triggers the eutrophication process is excess nutrients, Nitrogen and Phosphorus, in the form of nitrate and phosphate anions. The sources of these nutrients are reported to be from point sources (e.g. waste water treatment plants) and non-point sources (e.g. run-off from septic systems, fertilized lawn and crop fields, and impervious surfaces). We believe that the Blackstone River Watershed contributes these nutrients to Narragansett Bay in increasing amounts throughout the summer and causes hypoxic/anoxic conditions in the water column. Water samples were collected three times during June and July at eight sites along the Blackstone River and Providence River, leading to Narragansett Bay. We recorded the temperature, dissolved oxygen (DO), pH and conductivity. Each water sample was analyzed in triplicate using a Thermo Scientific (Dionex) Ion Chromatography System 2100 for fluoride (F-), chloride (Cl-), nitrite (NO2-), bromide (Br-), nitrate (NO3-), sulfate (SO42-), and phosphate (PO43-) anions. Our results indicate a slight increase in nitrate over the summer and down river with a high of 8.1ppm at our Providence River site. Phosphate levels also appeared to increase down river with a high of 0.08ppm at a site before the Providence River, but there was no discernable detection of phosphate in the Providence River. Fluoride, bromide, and sulfate had noticeable higher levels at the Providence River site than the Blackstone River sites at 4-5x (0.33ppm), 1000x (44.3ppm), and 100x (866ppm), respectively. The data suggests that run-off from the Blackstone River Watershed contributes anion nutrients and pollution to Narragansett Bay throughout the summer, but a larger contributing role may come from run-off of the immediate Providence area.

DISTRIBUTION OF ANT SPECIES OVER PROVIDENCE COLLEGE CAMPUS

Joseph Burt, James Waters

Department of Biology, Providence College, Providence, RI

RI-INBRE Summer Undergraduate Research Fellowship Program

Species abundance and distribution data are required to understand the impacts of urbanization on biodiversity and the relationships within ecological systems. The purpose of our experiment is to create a database of ant species found in Providence, focusing first on sampling the biodiversity on campus at Providence College. Although the diversity of ants across many regions of New England have already been sampled, Providence and Rhode Island have only ever been the subject of two historical studies and are are not represented well in the scientific literature. Our methods involved using pitfall traps to systematically collect ants in a spatio-temporal longitudinal study spanning ten weeks and covering a range of different abiotic and biotic gradients on campus. This design will make it possible to test hypotheses about whether or not there are correlations between ant diversity and tree species distributions and campus land-use patterns. We have also started analyzing the time-series data for trends associated with seasonality and variation in weather. A total of 1338 ants have been collected so-far, with an average of 223 (+/- 132 SD) ants per week. Intriguingly, the abundance of ants dominates the abundance of all soil arthropods combined (on average 279 +/- 156 SD total arthropods per week). We are in the process of curating a collection of the ants so that the data can be analyzed by species. We have positively identified species Camponotus pennsylvanicus, Formica subsericea, Prenolepis imparis, Lasius neoniger, and Temnothorax caespitum. In addition, we have confirmed the presence Nylanderia pubens, the first time this invasive species has been reported in Providence. Thanks to the Department of Biology at Providence College for supporting this research.

GREENHOUSE GAS FLUXES VARY WITH NITROGEN PULSES ALONG A VEGETATION-DEFINED GRADIENT IN A NEW ENGLAND SALT MARSH

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RI NSF EPSCoR Summer Undergraduate Research Fellowship Program

Salt marshes are important carbon (C) sinks due to high levels of productivity, slow decomposition, and minimal emission of the greenhouse gases (GHGs) carbon dioxide (CO2) and methane (CH4). While this ecosystem service is well known, patterns of salt marsh C cycling are less well understood. Generally, New England salt marshes are characterized by vegetation zones that reflect gradients in edaphic stress and interspecific competition, and so GHG fluxes between zones may differ. Further, responses of these zones to anthropogenic impacts such as eutrophication may vary, with implications for responses of marsh C cycling to drivers of global change. While some studies have explored differences in GHG fluxes between vegetation zones, none report GHG fluxes along the entire gradient of marsh vegetation communities, from intertidal zone to upland edge. The objectives of this experiment were to characterize GHG fluxes along a New England marsh vegetation-defined gradient, and to test responses of different vegetation-defined zones to nitrogen enrichment. Using a cavity ringdown spectroscopy (CRDS) gas analyzer (Picarro Labs), GHG fluxes were measured from 5 plots along a transect spanning 5 vegetation communities including S. alterniflora, S. patens, Phragmites, and the transition areas between each zone. On 3 measurement dates, GHG fluxes were measured pre- and post- enrichment with pulses of nitrogen-enriched seawater. CO2 fluxes varied significantly between vegetation zones, with substantially more CO2 uptake at plots with greater aboveground biomass. All zones, except S. patens, displayed CO2 uptake. CH4 emissions were greatest from the S. alterniflora zone. GHG fluxes were also affected by N enrichment, and responses varied between vegetation zones. Unexpectedly, CH4 emissions tended to increase after the pulse N additions. This was especially evident in the S. alterniflora (low-marsh) zones. Results of this study suggest that GHG fluxes from salt marshes are heterogeneous and vary with vegetation zone, and that net GHG emissions may be ameliorated by N enrichment under some conditions. To better understand between-zone differences in GHG flux dynamics, fluxes should be measured over diel and annual cycles. This will determine if the role of salt marshes in C sequestration changes with seasonal variability along with variability in vegetation characteristics.

EFFECTS OF NITROGEN ENRICHMENT AND RIBBED MUSSEL PRESENCE ON SALT MARSH GREENHOUSE GAS FLUXES

Ryan Quinn, Rose Martin, Serena Moseman-Valtierra

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RI NSF EPSCoR Summer Undergraduate Research Fellowship Program

The ribbed mussel, Geukensia demissa, is a filter feeder whose presence is associated with increased Spartina alterniflora biomass and is responsible for depositing large quantities of nitrogen (N) from tidal water into marsh sediment through waste excretion as ammonia and through gamete and tissue production which in turn feeds marsh decomposers. Because of G. demissa interactions with N, primary producing communities, and decomposers, ribbed mussel presence may be associated with both increased CO2 uptake and CH4 production. Greenhouse gas (GHG) fluxes were measured in plot sites with regular occurring mussel densities and in plot sites void of mussel presence before and after pulse additions of potassium nitrate (KNO3). Mussel presence and KNO3 additions did not exert immediate differences in GHG fluxes compared to plot sites with mussels but soil variables were significantly associated with GHG flux trends. CO2 and CH4 both were significantly affected by soil temperature ($F(1,16)=16.97$, $p< 0.001$, $R2=0.48$; $F(1,16)=7.55$ $p= 0.01$, $R2= 0.28$). CO2 was significantly affected by soil oxidation-reduction potential ($F(1,16)=5.04$, $p= 0.04$, $R2 =0.19$). CH4 increased with increasing pore water salinity ($F(1,15)=11.98$, p

MACROINVERTEBRATE BIOLOGICAL ASSESSMENT OF WATER QUALITY IN RHODE ISLAND

Jennifer Kane[1], Kristin McDermott[1], Britta Anderson[2], Marissa Simpson[3], Jameson Chace[1]

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RI NSF EPSCoR Northeast Water Resource Network Summer Internship Program

The water quality of our local watersheds is of high importance due to their role as primary sources of drinking water for the residents of Newport and Middletown, Rhode Island. Water quality of a watershed is an integrated ecological assessment of physical, chemical and biological properties. Macroinvertebrates live the entirety of their life cycles in these streams and are thus extremely sensitive to physical and chemical changes in the watershed. They are therefore used as a biological indicator of ecosystem health. For our study in 2015 the EPT (Ephemeroptera, Plecoptera, Trichoptera), Order richness, and EPT/Diptera scores where determined to give each stream a Macroinvetebrate-based quality index. We found that EPT scores were highest at Cork Brook in Scituate Resevior, the most pristine and forested site, and lowest at Maidford River which is mostly an agricultural landscape. Order richness was fairly constant across all sites, with averages of 13 orders present at Bailey's and Maidford and 10 at Cork Brook. EPT/Diptera scores were used to determine the ratio between pollution intolerant and pollution tolerant macroinvetebrates. Lowest ratios were found along the Maidford River with an average ratio of 3.5 EPT/Diptera followed by Bailey's Brook (7.6 EPT/Diptera) then Cork Brook (16.89 EPT/Diptera). To conclude if macroinvertebrate communities can be used as accurate biological indicators of water quality we compared our results to our chemical analysis. A negative correlation was found between high nutrient readings in the Maidford River (nitrates 1.94 ppm, phosphates 0.25 ppm) and low EPT and EPT/Diptera ratios. These results indicate that macroinvertebrates can be used to provide an accurate water quality assessment compared with chemical and physical analysis. On Aquidneck Island both Bailey's Brook and Maidford River watersheds are biologically impoverished compared to Cork Brook, indicating lower water quality.

LINKING SOCIAL AND ENVIRONMENTAL DETERMINANTS TO HEALTH: A LOOK INTO RESILIENCY USING A HEALTH EQUITY APPROACH

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Independent Research

Public health efforts are constantly striving to prevent, protect against, and mitigate the impact of emergencies. However, a focus on environmental justice that works to identify and break down the cultural and environmental barriers that separate communities is often overlooked. Health preparedness can be defined as the actions taken in anticipation of an emergency with consequences to public health and to facilitate rapid, effective, and appropriate response. Such actions are relative to the degree of municipal organization and community competence (WHO). Geographically, health preparedness and community health resilience are not evenly distributed; barriers leave vulnerable populations especially at-risk before, during, and after an emergency. In order to eliminate disparities (i.e., the inequalities that exist when various groups do not benefit from the same health statues) (Healthy People 2020), emergency preparedness and environmental justice has to advocate for vulnerable or at-risk groups in order to promote health equity. We aimed to identify the most vulnerable geographical areas not covered under the Environmental Protection Agency's (EPA) Safe Water Drinking Act. This research analyzes data from the Federal Emergency Management Association (FEMA) Preparedness in America: Research Insights to Increase Individual, Organizational, and Community Action, U.S. National Census Data and Behavioral Risk Factor Surveillance System data. A joint coalition between the Rhode Island Department of Health's Center for Drinking Water Quality and the Center of Emergency Preparedness and Response submitted a grant opportunity entitled SafeWell Collaborates. Private well testing is generally recognized as voluntary and requires own funding and education, leaving lower income residents unable to self-advocate. Demographic Census data was collected to assign equity factors to volume factors in funding formulas using demographic information and risk assessment (e.g., age, gender, median income, education attainment, etc.). Further knowledge on environmental justice factors affecting at-risk populations needs attention to understand health resiliency. Increasing surveillance activities and social capital is the crucial as the SafeWell Collaborative strives to coordinate community planning and engagement by implementing well workshops, enforcing municipal well testing, and sampling water.

DIFFERENCES IN METHANE AND CARBON DIOXIDE FLUXES BETWEEN IFAS BNR TANKS AT FIELDS POINT WASTEWATER TREATMENT PLANT

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RI NSF EPSCoR Summer Undergraduate Research Fellowship Program

In 2004, RI passed legislation that placed nitrogen removal requirements on wastewater treatment plants (WWTPs). In response to this, ten tanks at the Fields Point WWTP were upgraded in 2013 to perform integrated fixed film activated sludge (IFAS) biological nitrogen removal (BNR). With these upgrades, Fields Point has met the new nitrogen requirements, however, this process may also release potent greenhouse gases (GHGs) including methane and carbon dioxide that contribute to global climate change. Due to budget and time constraints only tank one of the ten IFAS BNR tanks at Fields Point has been measured for methane and carbon dioxide fluxes on a bimonthly basis. The purpose of this project is to determine if the methane and carbon dioxide fluxes from tank one are representative of all ten tanks. On three different dates in June and July of 2015, methane and carbon dioxide fluxes were measured from two additional, randomly selected, IFAS BNR tanks at Fields Point. Measurements were taken in the re-aeration zone of each tank using a floating chamber connected via tubing to the Picarro G5208, which measures methane and carbon dioxide simultaneously real time. Due to the potential for large hourly variability in fluxes, tank one was measured before and after the measurements in the two additional tanks to determine if the fluxes had significantly changed over the measurement period. Gas fluxes were calculated using Matlab and R to plot the change in concentration over time. All statistical analysis were performed in JMP and consisted of a paired t-test test for each gas to determine if the pre and post tank one fluxes significantly differed and a mixed model was used for each gas to determine if there was a significant difference in fluxes from the three tanks. There was no significant difference between pre and post carbon dioxide and methane fluxes in tank one. There was a significant difference in methane and carbon dioxide fluxes from the three tanks. However, on average, tank one had the lowest carbon dioxide and methane fluxes out of the three tanks. Therefore, tank one data can be scaled up to determine a conservative estimate of the total carbon dioxide and methane fluxes from all ten tanks. In the future, additional tanks could be measured during the winter months to ensure that these results are not season dependent.

THE BLACKSTONE RIVER AS A SOURCE OF HEAVY METAL CONTAMINATION TO NARRAGANSETT BAY.

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RI NSF EPSCoR Summer Undergraduate Research Fellowship Program

The Blackstone River has a long history of pollution from industries such as textiles, metal fabrication, and woodworking that used the river for power and waste disposal. As recently as 1990, the Environmental Protection Agency (EPA) called the Blackstone, “the most polluted river in the country with respect to toxic sediments”. Metal contaminates remain trapped in sediment behind the many dams on the river. This “reservoir” of heavy metals continues to pollute the water and affect ecosystems today. These toxic metals can become bioavailable to aquatic organisms and potentially enter food webs from the Blackstone River to Narragansett Bay. The extent of heavy metal leaching from these sediments to water and the river’s contribution to metal contamination to Narragansett Bay requires further research. We analyzed water samples from the Providence River, which leads into Narragansett Bay and from 7 sites along the Blackstone River to determine the contribution of metal pollution to Narragansett Bay. We analyzed these water samples for Arsenic (As), Barium (Ba), Beryllium (Be), Cadmium (Cd), Chromium (Cr), Cobalt (Co), Copper (Cu), Iron (Fe), Lead (Pb), Manganese (Mn), Nickel (Ni), Thallium (Tl), Selenium (Se), Silver (Ag), Strontium (Sr), Uranium (U), Vanadium (V), and Zinc (Zn) using an Inductively Coupled Plasma - Mass Spectrometry (Agilent ICP-MS). Our results indicate the Providence River site is highly contaminated with metals such as V, Zn, Se, Sr, Ag, and U. Metals like As and Pb are found throughout the Blackstone River and there appears to be occasional “hot spots”. In addition to contaminated sediments, our results suggest that run-off, especially from the city of Providence, also plays a role in metal contamination in Narragansett Bay waters.

CHEMICAL AND GIS LAND-USE ANALYSIS REVEALS POLLUTION HOTSPOTS IN AQUIDNECK ISLAND

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RI NSF EPSCoR Northeast Water Network Summer Internship Program

The Bailey Brook and Maidford River watersheds in Aquidneck Island, Rhode Island, are the sources of Newport's drinking water and are the estuaries of Newport and Middletown public beaches. Newport is known for its beaches and residents are dependent on tourist revenue. However, due to poor watershed management Newport's beaches have been subject to several beach closures. The purpose of this study was to examine the chemical and physical characteristics of Bailey Brook, Maidford River, and Cork Brook, a forested control in Scituate Reservoir watershed, in order to determine hot spots, areas of high pollution, and hot moments, times of high pollution. Focused management efforts on hot spots and moments will increase cost effectiveness and potentially reduce or eliminate beach closures. Chemical analysis of the streams included investigation of temperature, pH, conductivity, dissolved oxygen (DO), nitrogen concentrations, and phosphate concentrations. Physical analysis of the stream was scored by a habitat assessment and definition of land-use. Geographic Information Systems (GIS) was then used as an analytical tool to determine where the pollution is entering the watershed. Seven study sites were identified as hotspots. Five Maidford River sites were hotspots for dissolved oxygen (0.05ppm) and nitrogen (>1ppm) concentrations above outside of EPA standards. Two Bailey Brook sites were hotspots for dissolved oxygen and phosphate concentrations outside of EPA standards. All sites were within EPA standards for pH and conductivity. The map created in GIS provides a spatial and temporal scientific analysis as well as a visual means of effectively communicate watershed issues to the community and policy makers. This GIS analysis combined with real-time sensor information will more precisely, effectively, and soundly focus watershed management efforts.

MODELING CARBONATION OF PERIDOTITE UNDER BIOTIC AND ABIOTIC MARINE CONDITIONS

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RI NSF EPSCoR Summer Undergraduate Research Fellowship Program

Peridotites are ultramafic rocks (i.e., rocks rich in Fe and Mg) from Earth's mantle, that underlay the crust and react readily with water and CO2 to produce magnetite, antigorite, serpentine, clays, and carbonates (e.g., magnesite, calcite, dolomite) in a process called serpentinization. In 2008, Kelemen and Matter studied mantle peridotites in the Samail Ophiolite, Oman and found that serpentinization of the geologic structure naturally converts over 104 tons of atmospheric CO2 per year into solid carbonates. Research into increasing the rate of carbonation of peridotites has focused primarily on conditions such pressure of the gas, temperature, concentration, and improving methods of hydraulic fracturing and injecting (refs?). This study investigated the impact of microbes present in seawater on the carbonation rate of peridotites exposed to various concentrations of CO2. The reaction was conducted in 50 airtight 22 mL serum vials, each containing 16g of autoclaved, pulverized peridotite and 16 mL of either live or autoclaved seawater. Once sealed, the vials were injected with specific concentrations of CO2 (in increments from 500 ppm to 2500 ppm, and placed on a shaker running at 350 rpm for 5 days. After shaking, the samples settled for one day before being opened, dried for 30 minutes at 80 oC, and ground into a 150µm powder for X-ray diffraction (XRD) analysis. A model mineral composition for the reacted peridotite was derived from XRD data using XPowder Software, to establish a semi-quantitative measurement for the carbonates formed. The initial composition of the peridotite prior to reaction was less than 6wt % calcite and magnesite. It was found that for both of the water conditions and all concentrations of CO2 tested, carbonates were formed. Vials containing the highest concentrations of CO2, 2500 ppm, reacted to form the most carbonate minerals: 30wt % ± 5 wt % in the autoclaved water, and 38wt % ± 6 wt % in the water containing microbes. At lower concentrations, 500 ppm to 1000 ppm, carbonates formed at similar rates under both biotic and abiotic conditions, averaging 24 wt %±5 wt %. These results provide the groundwork for investigating the effects of microbes in a serpentinizing environment. A follow up study with longer incubation periods, larger reaction vessels, different cell densities, and smaller grains of peridotite could allow differences in carbonation at low CO2 concentrations to be better resolved.

COASTAL RESTORATION CASE STUDIES FROM THE OCEAN STATE

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RI NSF EPSCoR Summer Undergraduate Research Fellowship Program

In coastal urban environments, effective information circulation between stakeholders is a necessary component in defining the broad array of costs and benefits associated with restoration. Four case studies were synthesized using a combination of interviews with restoration managers and quantitative metrics to inform policy makers and the general public of monetary, environmental, educational, and community outcomes that arise during restoration efforts. Among the Rhode Island projects chosen for case study are a greenway restoration along the Woonasquatucket River in Olneyville, a suite of Shannock Dam restorations on the Lower Pawcatuck River, a dam removal on the Pawtuxet River and a saltmarsh restoration in the Town of Bristol. The documents seek to explicitly outline the benefits, challenges, solutions, and takeaways of each project in a manner that combines both number driven results and nuanced social issues. Additionally, these studies serve as a factual report containing information about partners, funding, habitat, and public stakeholders associated with each project.

GENETICS

LOCATED IN THE NORTH LOBBY ON THE 1ST FLOOR OF THE CENTER FOR BIOTECHNOLOGY & LIFE SCIENCES

POSTERS ARE TO BE MANNED FROM 11:00 AM – 12:30 PM

GENOME ASSEMBLY AND ANNOTATION OF PROBIOTIC MICROBE BACILLUS PUMILUS RI06-95

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RI-INBRE Summer Undergraduate Research Fellowship Program

Bacillus pumilus RI06-95 is a marine bacterium isolated in Narragansett, Rhode Island, which has shown probiotic activity in larval shellfish against marine pathogens. The genome of this organism provides insight into the microbe's probiotic ability and may be used in future studies of the probiotic mechanism. DNA was isolated from an overnight culture using the Wizard genomic DNA purification kit (Promega) following the manufacturer's instructions, except DNA was eluted using 100 µL of Type I water. Sequencing was then performed using an Illumina MiSeq sequencer at the Rhode Island Genomics and Sequencing Center. The read library contained 8,784,938 reads that averaged 238.79 bp in length. De novo assembly was performed and resulted in 16 contigs with an average coverage of 913×. The total size of the draft genome is 3,643,624 bp with an average contig length of 227,727 bp. All contigs were submitted to RAST (Rapid Annotation using Subsystem Technology), which identified several putative subsystems which may be related to the probiotic activity, including: a siderophore assembly subsystem, sialic acid synthesis genes, chemotaxis regulator genes, and exopolysaccharide genes. After RAST annotation, the contigs were submitted to antiSMASH 3.0.1 (Antibiotics and Secondary Metabolite Analysis Shell), which identified a cluster that shows structural similarity to the published amicoumacin gene cluster. Because amicoumacin is a known antibiotic, we are now investigating this compound's role in the probiotic ability of RI06-95 through a gene knock-out experiment.

METABOLIC ANALYSIS OF IDH MUTANT GLIOMAS IN DROSOPHILA

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RI-INBRE Summer Undergraduate Research Fellowship Program

Isocitrate dehydrogenase (IDH) is mutated in low and medium grade gliomas, however the mechanism of IDH mutant behavior is undetermined. Through a series of molecular cloning techniques an IDH1 mutant model has been established in both Drosophila cell lines using the inducible pMT vector, and in vivo using the Gal4 regulated pattB-UASt vector. Similarly, an SBP-tagged mutant model was created to look at protein interactions. The tagged mutants were inserted into the pMT vector to be analyzed in cell lines. Using the pMK33 vector the mutated cells lines were transfected into stable cell lines. Through western blot analysis of transfected S3 cells our GFP-IDH mutant and SBP-IDH mutant constructs were validated. The constructs that were transfected into cell lines are being used to examine the protein interaction and knockdown function of the IDH gene of interest. Furthermore, the mutated fly lines are being crossed to different driver lines to examine how the mutation is affecting cell proliferation in the wings, cuticle, brain, eyes, and the whole development of the flies.

SNP DRIFT: TOWARDS A GENOMIC ESTIMATE OF POPULATION SIZES

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RI NSF EPSCoR Summer Undergraduate Research Fellowship Program

The ability to calculate effective population sizes is an essential tool for the population geneticist. There are multiple avenues for acquiring this number and useful applications for it. One way to predict the effective population size of a population is to calculate the genetic drift of said population over time. We used DNA samples of two different species of cichlid fish native to Lake Malawi in Africa: Melanochromis auratus (MA) and Labeotropheus fuelleborni (LF). These samples were collected in two regions of the lake: Mumbo Island (MUM) and Harbour Island (HI). The samples were collected on one trip in 1996 and again in 2002. We used Single Nucleotide Polymorphisms (SNPs) to quantify the genetic drift that occurred between both populations of both species. We found that the typically more social LF went through genetic drift slower than the more territorial MA based on FST analysis. This implies that the LF populations are larger than the MA populations. With this knowledge, we can continue our efforts to estimate effective population sizes. With the climate changing, and wildlife challenged as a result, population geneticists need reliable tools to gauge effective population sizes, including using genetic drift to be able to do so.

INVESTIGATING THE METABOLIC PHENOTYPE OF GLIOMA MODELS IN DROSOPHILA

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Gliomas are the most common tumors of the central nervous system. Drosophila glial cells seem to be homologous to human glial cells as the function, development, and gene expression are similar in both organisms. The most common genetic lesions in gliomas are associated with mutation or amplification of the tyrosine kinase EGFR or mutations in Ras and PI3K genes. These mutant forms result in continuous kinase activity causing cellular proliferation. Recently the metabolic enzyme Isocitrate Dehydrogenase (IDH) has also been identified as a driver of glial cell tumorigenesis. We are interested in observing how the metabolism of glial cells differs in these mutants when compared to wild type flies. We will further investigate whether a synergistic effect is observed when established oncogenes, EGFR, Ras and dp110 (PI3K), are combined with IDH mutations. In order to observe these phenotypes, we optimized an immunostaining protocol for brains from late 3rd instar larvae using anti-repo antibody, which is glial cell specific. We observed that there was an overproliferation of glial cells and/or disproportionate brain lobes in EGFR, Ras, IDH, and dp110 mutant flies. Along with the visualization of glial cells, we will work to understand how metabolism changes from wild type growth to proliferation, and from proliferation to metastasis. We can then identify potential new targets for treatment of these malignant tumors at the cellular metabolic level.

CHARACTERIZATION OF YEAST BAX INHIBITOR, BXI1, FUNCTION IN CELL DEATH, THE UNFOLDED PROTEIN RESPONSE, AND CALCIUM SIGNALING IN SACCHAROMYCES CEREVISIAE.

Savannah Benko, William Cavedon, Melissa Brown, Morgan McCarthy, James O'Brien, Lukas Ritzer, Victoria Hallisey, B. Michael Berry, Nicanor Austriaco, O.P

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RI-INBRE Summer Undergraduate Research Fellowship Program

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 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. With DTT induction experiments using Δbxi1 mutants and different UPR GFP reporters, we have confirmed our published data that suggests that BXI1 is involved in the UPR though our analysis suggests that the deletion of BXI1 does not alter the Ire1p signaling mechanism. We have also shown that calcium triggers the UPR independently of Ire1p and Bxi1p. We are in the process of determining the redox state and calcium levels in cells lacking BXI1 to determine if knocking out the gene alters the physiology of the ER.

A GENETIC SCREEN TO ISOLATE NOVEL ENHANCERS OF SOD1 TOXICITY IN A DROSOPHILA MODEL OF AMYOTROPHIC LATERAL SCLEROSIS

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RI-INBRE Summer Undergraduate Research Fellowship Program

Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disease that causes progressive motor neuron degeneration leading to respiratory failure in afflicted individuals 3 to 5 years after diagnosis. Approximately 10% of ALS cases are familial (fALS), and 10% of these instances are due to point mutations in Cu/Zn superoxide dismutase (SOD1). SOD1 is a ubiquitously expressed free radical scavenger and mutations in the gene cause selective death of motor neurons by mechanisms which are only partially understood. To systematically identify novel genes influencing ALS pathology, we conducted a genome-wide screen to identify enhancers of SOD1 toxicity using the model organism Drosophila melanogaster . The sodG85R mutation causes ALS in humans and the corresponding mutation produces a recessive adult-lethal phenotype in flies. To identify enhancers, the phenotype of heterozygous sodG85R/sod+ was assessed in trans with deficiencies spanning regions of the 2nd and 3rd chromosomes. Two regions were identified which produced larval or pupal lethality in a sodG85R/sod+ heterozygous background. Further deficiency mapping refined each region to a relatively small number of candidate genes. To determine whether mutant sod-related phenotypes were enhanced specifically, each enhancer line was tested in a Drosophila model of Parkinson's disease. Preliminary results show no interaction based on lethality and wing phenotype tests. By analyzing smaller deficiencies and transposable element insertions we hope to identify the ALS-enhancing gene within each respective region.

CREATING A MODEL OF SOD1 PROTEIN AGGREGATION IN DROSOPHILA MELANOGASTER

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RI-INBRE Summer Undergraduate Research Fellowship Program

Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disease affecting motor neurons selectively leading to paralysis and eventual death. About 10% of familial cases of ALS are associated with mutations in superoxide dismutase 1 (SOD1), an enzyme that scavenges superoxide radicals. Over 150 point mutations in SOD1 gene cause ALS and toxicity is correlated with protein aggregation, leading to cell death. Previous work showed a cysteine 111 residue within human SOD1 to be critical for protein aggregation in cell lines. SOD1 is highly conserved in metazoans and most species except humans contain a serine at the corresponding 111 position. To test the effect of this amino acid in an animal model system, sodS111C mutants were created through homologous recombination in Drosophila melanogaster. The phenotypes of sodS111C flies will be characterized and compared to wildtype. Further studies will examine the effect of this mutation in ALS-associated sodA4V and sodG85R backgrounds.

A GENETIC DIVERSITY ANALYSIS OF CARCINUS MAENAS AND HEMIGRAPSUS SANGUINEUS IN THE NARRAGANSETT BAY

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RI NSF EPSCoR Summer Undergraduate Research Fellowship Program

Both the European green crab (Carcinus maenas) and the Asian shore crab (Hemigrapsus sanguineus) are known invasive species in North America. However recent studies have indicated that a decline in the green crab population is occurring in the Narragansett Bay while the Asian shore crab population remains unaffected. Our previous studies have shown that while the genetic diversity of the green crab population in the Narragansett Bay remains within Hardy-Weinberg equilibrium (P=0.05, d.f. = ranging from 9 to 67), the population is experiencing a decrease in heterozygosity and an increase in homozygosity. However, a larger sample size is required to verify the decrease of genetic diversity previously seen in the Narragansett Bay green crab population. Therefore, this study aims to further investigate the genetic diversity of both the European green crab and Asian shore crab populations in the Narragansett Bay as an early indicator of the future identity of the populations. Specifically, it is hypothesized that competition between the Asian shore crab and the European green crab is leading to a decrease in the heterozygosity of the green crab population. Both Asian shore crab and green crab tissue samples were collected from different locations throughout Narragansett Bay. The genetic diversity of the two crab species were analyzed using six previously described microsatellite markers. In future studies we will continue to increase the sample sizes of both crab species to gain a more accurate understanding of the genetic diversity of both invasive species in the Narragansett Bay.

NEW DISCOVERED DIVERSITY IN RED ALGAL GENERA IN TEMPERATE AND TROPICAL CLIMATES

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RI NSF EPSCoR Summer Undergraduate Research Fellowship Program

Global climate change is predicted to have significant consequences for the world's oceans. Some of the greatest effects of a changing ocean will be on sessile organisms, such as seaweed. Before we understand how seaweeds will react to change, we need to determine the diversity in regions likely to be most affected to create a baseline for future comparison. Here we collected species of algae found in tropical and sub-tropical western Atlantic and compared them to collections from temperate environments. Using the polymerase chain reaction (PCR), we are able to amplify DNA barcode markers (rbcL) and (COX1) from two algal genera with species throughout the western Atlantic, Champia and Dasya. With these data we created phylogenetic trees comparing species identity along the known range. These two genera were selected because previous studies have suggested undescribed genetic diversity within their species. In addition to genetic data, portions of each sample were pressed and preserved in a herbarium to allow us to examine morphological traits and leave a permanent record for the future. Molecular data demonstrate that there are multiple new species within these two genera that have not previously been described. Within Dasya we believe to have at least two new species, which were only classified as Dasya sp. previously. In the genera Champia, C. parvula was previously reported in temperate waters and tropical waters. Our molecular results show that C. parvula is not found in the western Atlantic. Specimen previously identified as C. parvula from RI form a unique species, and tropical specimen resolve as three additional new species. Our data expand the number of recognized Champia and Dasya species in the western Atlantic and provide a baseline for future change.

MARINE SCIENCES

LOCATED ALONG THE HALLWAY ON THE 1ST FLOOR OF THE CENTER FOR BIOTECHNOLOGY & LIFE SCIENCES

POSTERS ARE TO BE MANNED FROM 9:30 – 11:00 AM

ANALYZING BURIAL RATES OF QUAHOGS IN VARIOUS SEDIMENTS AT DIFFERENT TEMPERATURES

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RI NSF EPSCoR Summer Undergraduate Research Fellowship Program

Bivalves are highly productive aquatic species that have positive economic and environmental impacts. Many are harvested commercially or grown on farms. The quahog (Mercenaria mercenaria) is a popular bivalve that is wild-harvested commercially in RI and used in aquaculture. People have little understanding of its capacity to protect itself through burrowing in the sediment following release from a farming containment system, as is commonly seen in fisheries enhancement and/or aquaculture. The rate at which they bury affects their vulnerability toward predators and the harsh conditions in which they reside. This research analyzes the burial rates of quahogs based on size, sediment, and temperature with the use of a GoPro camera. The two sites selected to conduct the analysis were Mt. Hope Bay (hard, sand substrate) and Town Pond (soft, mud substrate).Juvenile quahogs, reared in a land-based upweller nursery, were released within the footprint of a frame mounted GoPro camera and monitored for their ability to dig into the sediment. The time of burial relative to the size of the juvenile was monitored to assess their capacity to bury. This will give a better understanding of how size, sediment type, and temperature (time of year) affect the quahog's burial rate. This knowledge will allow shellfish managers and farmers to optimize planting schedules and will contribute to an assessment of predicted changes to quahog life history characteristics in the light or projected climate change.

SCIENCE COMMUNICATION OUTREACH ABOUT COASTAL STORM IMPACTS IN NATIONAL PARKS

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RI NSF EPSCoR Summer Undergraduate Research Fellowship Program

Hurricane Sandy devastated the East Coast when it struck in October 2012. After the storm, it was determined that a greater understanding of Sandy's impact and the implications of future coastal storm events related to climate change were needed. The U.S. Department of the Interior allocated funds to the National Park Service (NPS) to investigate the storm's impact on coastal parks. A variety of studies are currently being carried out at Fire Island National Seashore, Gateway National Recreation Area, and Assateague Island National Seashore, among other parks, to assess the current and future status of the parks' natural resources in light of the storm.

Information gathered through the research will inform park management protocols and strategies to increase park resilience, but there is a lack of readily accessible public information regarding these Post-Hurricane Sandy efforts. This project aims to fill the gap by developing a series of resource briefs that highlight NPS research in clearly accessible terms to public stakeholders and decision-makers.

Briefs were developed by 1. Categorizing the different types of project based upon their proposals; 2. Providing a basic overview of the different types of projects; 3. Filling in details and verifying accuracy by speaking to project principal investigators; 4. Requesting photos from project team members; and 5. Securing approval for each brief from the NPS.

Briefs will eventually be posted on the NPS website and distributed to park rangers and visitors. Briefs will increase awareness and understanding of the vital research and restoration projects that are taking place at the three parks. This work will also inform interested parties about Sandy's impact, future coastal storm impacts, and strategies to increase park resilience in the face of climate change.

FATTY ACID PROFILES OF MARINE FISHES FROM RHODE ISLAND COASTAL WATERS

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RI-INBRE Summer Undergraduate Research Fellowship Program

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 (n = 10); black sea bass, Centropristis striata (n = 10); striped bass, Morone saxatilis (n = 6); scup, Stenotomus chrysops (n = 11); winter flounder, Pseudopleuronectes americanus (n = 10); and bluefish, Pomatomus saltatrix (n = 11). 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). Irrespective of fish species, mono-saturated fatty acids had the highest [FA] and %FA (mean [FA] = 183.5 mg/100 g; %FA = 46.2%), followed by saturated ([FA] = 146.6 mg/100 g; %FA = 32.7%), omega-3 ([FA] = 44.3 mg/100 g; %FA = 18.6%), and omega-6 fatty acids ([FA] = 7.5 mg/100 g; %FA = 2.5%). Fatty acid profiles also demonstrated significant inter-species differences. With respect to %FA, mono-saturated fatty acids were significantly higher in scup and bluefish relative to summer flounder and striped bass (SCP = 54.6%, BF = 48.8%, SF = 40.1%, SB = 39.3%). Conversely, omega-3 fatty acids were significantly higher in both flounder in comparison to black sea bass and scup (SF = 31.1%, WF = 26.3%, BSB = 12.1%, SCP = 8.3%). With respect to [FA], bluefish had significantly higher concentrations of mono-saturated and saturated fatty acids relative to summer flounder (BF = 245.4-307.4 mg/100 g, SF = 52.5-81.3 mg/100 g). Ratios of omega-6-to-omega-3 (n6:n3) fatty acids were reduced in flounder and striped bass (n6:n3 = 0.14-0.23) relative to scup, bluefish, and black sea bass (n6:n3 = 0.30-0.36); hence suggesting the former species provide greater health benefits for human consumers. 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.

PHYTOPLANKTON ABUNDANCE ALONG AN ESTUARINE GRADIENT IN THE NARRAGANSETT BAY

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RI NSF EPSCoR Summer Undergraduate Research Fellowship Program

Phytoplankton are the dominant primary producers in most marine food webs and variation in their abundance and diversity can influence the abundance and diversity of the organisms occupying higher trophic levels. As part of a multiyear project on the effects of climate change and sea level rise effects on the distribution of intertidal invertebrates and shorebirds, I quantified the distribution of phytoplankton and water quality at nine sites in Narragansett Bay, RI. The nine sites spanned the estuarine gradient from the upper Bay (Conimicut Point) to Dutch Harbor. The goal was to describe variation in phytoplankton abundance and species composition along the West Passage gradient. At each site, water samples were taken at the surface, fixed in Lugol's iodine, and brought back to the lab for phytoplankton species identification and enumeration. Phytoplankton were identified using phase contrast microscopy, with counts made using a 1mL Sedgwick-Rafter chamber. We also measured salinity with a YSI 556 mulitparameter water quality meter at each site. The phytoplankton were numerically dominated by

THE APPLICATION OF MICROSATELLITE ANALYSIS TO DETERMINE BLOOM IDENTITY OF ULVA COMPRESSA AND ULVA RIGIDA IN NARRAGANSETT BAY, RI

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RI NSF EPSCoR Summer Undergraduate Research Fellowship Program

Two closely related green microalgae species, Ulva compressa and Ulva rigida have been found in increasing amounts in Narragansett Bay, RI over the last 2 decades. These species have been found to form harmful algal blooms (HAB) that have the ability to cause widespread ecological and economical damage in the area of occurrence. We seek to determine if these HABs are due to the proliferation of one or multiple individuals. In order to determine the possible changes in the bloom population identity, microsatellite analysis was performed. Specific microsatellite primers were designed by searching NGS data for di, tri, tetra, penta and hexa- nucleotide repeats using Microsatellite Commander V2. These primers were then screened using genomic DNA from U. compressa and U. rigida samples in a PCR. With this amplified DNA, we then ran a 2% agarose gel electrophoresis to visualize polymorphism. Primers that showed polymorphic banding patterns were then tested on a wide range of Ulva compressa and Ulva rigida samples collected across Narragansett Bay at relevant stages of the bloom cycle. With the analyzation of the banding patterns, we can determine bloom identity due to the gel revealing if the algal samples are from the same or multiple individuals.

DIFFERENTIAL GENE EXPRESSION IN ULVA COMPRESSA UNDER SUMMER AND WINTER PHOTOPERIODS

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RI NSF EPSCoR Summer Undergraduate Research Fellowship Program

Ulva compressa is one of the most abundant species in harmful macroalgal blooms in Narragansett Bay, RI. These aggregations of algal biomass can have significant impact on coastal communities and have increased in size and duration over the past several decades. This study seeks to identify a link between temporal changes and gene expression potentially causing bloom behavior. This was done by collecting U. compressa at Oakland Beach in Warwick, RI and growing the samples in culture. Cultures were grown under typical summer and winter photoperiods, and then tested for differential expression of light response genes using qPCR. Gene candidates were identified through computational screens that showed differential gene expression in NGS data from U. compressa samples collected at relevant stages in the bloom cycle. Candidates include the genes for stress related light harvesting complex (lhcSR), malate dehydrogenase, Rieske iron-sulfur protein gene, carotene biosynthesis related gene, and heat shock protein 90c. The qPCR data displays differential gene expression of these light response genes in response to exposure to different photoperiods; sixteen hours of light simulating the summer and eight hours of light simulating the winter. These data support previous findings of differential gene expression of the 5 genes in samples collected from Narragansett Bay throughout the U. compressa bloom cycle.

DISTRIBUTION OF INTERTIDAL ORGANISMS ALONG NARRAGANSETT BAY AND NEWPORT NECK IN RESPONSE TO SEA LEVEL RISE

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RI NSF EPSCoR Summer Undergraduate Research Fellowship Program

Human activity is influencing marine environment in multiple ways, including climate warming-mediated sea level rise. Estimating intertidal organism abundance in relation to current environmental conditions is essential to accurate modeling of species responses to future environmental changes. This multiyear study focused on how sea level rise will affect abundance and distribution of near shore invertebrate and fish populations along Newport Neck and Narragansett Bay, Rhode Island. Intertidal substrate and organism abundance where quantified by 0.25m2 quadrat surveys along Newport Neck (n= 41) and Narragansett Bay (n = 29; Bay n= 20 , Coves n = 9). There are species specific substrate preferences among Neck, Bay and Cove sites. Asian shore crabs (Hemigraspus sanguineus) were more abundant at the Neck compared to the Bay and were absent in muddy Coves. Similarly, mussels (Mytilis edulis and Geukensia demissa) were more abundant at the Neck sites compared to the Bay and Cove sites with a shift from M. edulis at Neck sites to G. demissa at Bay sites. Two gastropod mollusks showed different spatial patterns. Mud snails (Ilyanassa obsoleta) were most abundant at Cove sites than at the Bay and were absent at Neck sites, while periwinkles (Littorina littorea) were most abundant at Neck sites and at moderate abundance in the Bay and reduced at Cove sites. Barnacles (Cirripedia sp.) were more abundant at Bay and Cove sites relative to Neck sites. These intertidal organism abundance data will be used to develop substrate –habitat models to project intertidal organism distributions in response to sea level rise. Projected 2 m sea level by 2100 will result in an intertidal zone that has a greater abundance of large substrates and less finer substrates favoring the upward expansion barnacles, mussels and Asian shore crabs and reduced abundance of mud snails and periwinkles.

FORAGING ECOLOGY OF BLUE CRABS (CALLINECTES SAPIDUS) AND THEIR POTENTIAL IMPACT ON WINTER FLOUNDER (PSEUDOPLEURONECTES AMERICANUS)

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RI NSF EPSCoR Summer Undergraduate Research Fellowship Program

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 2012-2015, 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 food habits of blue crabs via visual/genetic analysis of stomach contents and measurements of stable nitrogen and carbon isotope signatures in chelae muscle tissue.

BLUE CRAB PREDATION ON JUVENILE WINTER FLOUNDER IN NEW ENGLAND WATERS ASSESSED THROUGH PCR-BASED METHODS

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RI NSF EPSCoR Summer Undergraduate Research Fellowship Program

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 crabs 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.

SPATIAL VARIATIONS IN MERCURY AND SELENIUM CONCENTRATIONS IN MARINE FISHES OF RHODE ISLAND: RISKS AND BENEFITS TO HUMAN HEALTH

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RI-INBRE Summer Undergraduate Research Fellowship Program

Mercury (Hg) is a prevalent environmental contaminant that poses risk to human health, and exposure occurs mainly by consuming fish. Therefore, 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, thus increasing their health benefits. In this study, total Hg and Se concentrations were measured in the muscle tissue of four 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), and black sea bass (Centropristis striata) (offshore: n = 8-10 per species, inshore: n = 19-20 per species) . Data were analyzed and compared based on spatial variations (inshore and offshore) relative to 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, whereas total Se concentrations did not vary spatially. Conversely, scup showed no spatial variation in total Hg or total Se concentrations. 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 (exception = summer flounder). In contrast to Hg bioaccumulation patterns, Se concentrations were relatively constant across fish size. HBVs were inversely related to fish 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.

THE BIODIVERSITY AND REVISED TAXONOMY OF THE RED ALGAL GENERA CRYPTONEMIA AND WRANGELIA BY MOLECULAR ANALYSIS OF RBCL AND SSU MARKERS

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RI NSF EPSCoR Summer Undergraduate Research Fellowship Program

Rhodophyta contains the greatest species diversity of all macroalgal phyla and are globally distributed from North to South Pole. The biodiversity patterns of red algal species can be correlated to the geographic regions where they were collected. However, ocean temperatures are expected to change in response to global climate change. Here we targeted two tropical (St. Croix and Key West) areas for comparison to Bermuda, which is located at a subtropical/temperate boundary. Bermuda is an important location as its temperate winter temperature (~180°C) currently excludes many tropical algae. Increasing sea temperature will have a greater influence on species composition at climatic boundary sites, such as Bermuda. This study describes the biodiversity of the tropical and temperate genera Wrangelia and Cryptonemia in the western Atlantic using molecular data to supplement morphological. Both rbcL and SSU genetic markers were used to examine 76 samples of Cryptonemia and 12 samples of Wrangelia, respectively. These data reveal six species of Wrangelia in the western Atlantic, where only three were previously described. We resolve four distinct clusters under what had previously been recognized as W. penicillata. Within Cryptonemia we have identified five species, three of which are new, and excluded C. crenulata from the western Atlantic. As marine environmental conditions fluctuate the distribution of red algae will correspond, and regional diversity will continually change. Baseline data, such as those described here, will be critical to identify species shifts in a changing ocean.

COORDINATION OF A MULTI-JET SYSTEM BY NANOMIA BIJUGA

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RI NSF EPSCoR Summer Undergraduate Research Fellowship Program

Nanomia bijuga, a cosmopolitan siphonophore, is a colonial organism composed of a variety of clonal individuals. One type of clonal individual, termed a nectophore (multiple nectophores are collectively termed the nectosome), produces propulsive jets that power whole-colony movement through the water. The nectophores change size and fluid jet orientation during development. The goal of this project was to determine whether individual nectophores exhibited coordinated contractions during forward propulsion. In situ videos of two individuals were analyzed by calculating contraction length and frequency for each nectophore during a contraction sequence. The anterior-most nectophores were often either inactive or contracted so minimally that their movements could not be detected within the analyzed video sequences. Out of sixteen contraction sequences analyzed, eight demonstrated contraction initiated at the fifth, sixth, or seventh, nectophore within their nectosomes. This is most likely due to the fact that these nectophores provide more power for movement, while the anterior nectophores provide directional control of the colony. These findings suggest that coordination within the nectosome may be essential for efficient movement in this colonial multi-jet system.

PREDICTED JET VELOCITIES OF THE HYDROMEDUSA SARSIA TUBULOSA DURING BELL CONTRACTION

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RI NSF EPSCoR Summer Undergraduate Research Fellowship Program

Bell lengths of the hydromedusa Sarsia tubulosa characteristically typically range from 6-20 mm in length and propel the medusa by jet propulsion. This involves the ejection of water in the opposite direction of their desired pathway through their velar apertures. The flow of the ejected water is seen as thrust maximizing and energy minimizing vortex rings. The aim of this research was to study bell volume changes throughout jet propulsion in order to determine whether actual jet velocities compared with model predictions for evacuation of a simple volume. A full contraction was defined as when the medusa was at a resting state until the resting state was reached again. The images of three previously collected and recorded medusae were analyzed. Using a freehand tracing method, the bell was traced and the volume of bell was found in mm3 using X and Y coordinates. Velar apertures of the medusae were also recorded in mm using a straight line. The volumes were used to calculate the velocity of the water that would be ejected through the velar opening throughout contraction. It was found that this method of predicting velocity resulted in a relatively smooth pattern and would be useful in future studies.

DISTRIBUTION OF MACROINVERTEBRATES IN THE NARRAGANSETT BAY ALONG A GRADIENT OF ANTHROPOGENIC INFLUENCE

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RI NSF EPSCoR Summer Undergraduate Research Fellowship Program

The Narragansett Bay is one of the most studied estuaries in the Unites States and is known to have a north to south declining gradient of anthropogenic influence due to runoff (ie: septic waste and industry). Benthic macrofauna are important indicators to the environment as they ingest particles in the water column and sediments, which affects their distribution and health. The bay has undergone changes in the recent years, such as the addition nitrogen removal from wastewater treatment plants as well as continued anthropogenic influence that may affect the distributions of the benthic macrofauna. The purpose of this study was to update the macroinvertebrate distribution knowledge within the Narragansett Bay through an abundance survey. We set up ~6 10m transects per site and recorded species abundance with a 0.25 m2 quadrat. These surveys were performed at the following sites along a north to south declining gradient of anthropogenic influence: Conimicut Point, Greenwich Bay, North Jamestown, Fort Wetherill, and at the Graduate School of Oceanography (GSO) pier. We hypothesized that there would be higher species evenness and richness at the lower anthropogenically influenced sites (southern sites), and lowest at more anthropogenically influenced sites (northern sites). It was also predicted that the species abundances would differ from site to site because of the north-south anthropogenic gradient. We found that species abundance differed significantly between sites, excluding the species Crassostrea virginica, Busycotypus canaliculatus, and Microciona prolifera. The Shannon's index showed that the diversity and evenness of species was highest in the southern part of the bay, Fort Wetherill (H = 1.41, Eh = 0.79) and the GSO Pier and (H = 1.13, Eh = 0.70), while it was lowest at Conimicut Point (H = 0.99, Eh = 0.40), supporting our hypothesis. This could be due to the high anthropogenic influence of the northern sites leading to more pollutants and higher nutrient concentrations in the water column, allowing varying species richness and evenness between sites. Previous studies have also shown high species richness at Conimicut Point, but differed in which species were most abundant at each site. Our study shows that there may still be potential anthropogenic influences within the Narragansett Bay affecting the marine benthos, though further studies need to be deployed.

COUNTING QUAHOGS: USING THE BULL RAKE AS A STOCK ASSESSMENT TOOL.

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RI NSF EPSCoR Summer Undergraduate Research Fellowship Program

With a focus on Greenwich Bay, in the northwestern section of Narragansett Bay, we assessed the stocks of the hard clam (Mercenaria mercenaria), locally known as a quahog, using a commercial harvesting tool. Within Rhode Island waters, commercial quahoggers are limited to the use of a tool called a bullrake to harvest the quahogs. Quahog management, under the purview of RI-Department of Environmental Management (RIDEM), relies on an annual assessment of the standing stock of quahogs in Approved waters. Currently theRIDEM uses a hydraulic dredge to sample open areas of the Bay. However in certain areas, the dredge is unable to maneuver to sample quahog stocks. Working with commercial quahoggers, a method to use the bullrake was developed and evaluated to aid RIDEM in stock assessment. Data were collected on quahoggers'catch efficiency of raking and used to measure the actual number of quahogs per square meter in both open and closed areas. To calibrate the use of the bullrake as a stock assessment tool, the quahogger would rake as they normally would. A person on the boat tracks the movement of the rake with the use of handheld GPS units. To assess efficiency and accuracy of the distance measurement, divers in the water would follow the rake collecting any possible missed or undersized quahogs and measuring the distance raked. Total area sampled and total catch, adjusted for the bullrake catch efficiency can be calculated from the deckside observations. The observed stock density is compared to diver collected quadrat-based density estimates collected adjacent to the raked tracks. Based on the calibrations demonstrated with this project, RIDEM can use quahogger-based stock assessments as a component to their quahog management responsibilities.

FIGHT, FLIGHT OR FEED? MYSID BEHAVIOR IS AFFECTED BY CONSPECIFICS, PREY, AND PREDATOR CUES

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RI NSF EPSCoR Summer Undergraduate Research Fellowship Program

Mysid shrimp are crutial members of our local Narragansett Bay ecosystem as important prey for game fish. We are interested in looking at behavioral factors that may affect feeding efficiency in these crustaceans, which may in turn affect numbers of fishes available for human markets. When food is available, mysids need to choose among a number of possible actions: pursuing prey, repelling other mysid competitors, and avoiding predators. Which behaviors dominate may depend on the presence/absence of food items, conspecifics, or predator cues such as visual stimuli or kairomones as well as circadian patterns. Local mysids are thought to participate in diel vertical migration in which they feed solitarily at night in the epipelagic zone but return to mesopelagic zone swarms during the day. Because of these daily rhythms, mysids may switch between competitive feeding and swarming vigilance modes depending on time of day or light cues. We used two sets of approaches to assess trade-offs between these basic behaviors in lab adapted Americamysis bahia. First, we compared the effects of light levels and predator cue (water from grass shrimp tanks) on feeding rates. Second, we looked at pairwise behavior in the presence/absence of predator cue and prey. We found that mysids fed more in the dark than light, and more in the presence of predator cue than in its absence. Conversely, pairwise aggression was highly variable with no clear pattern associated with predator cue or prey availability. In future work, we hope to refine our system to test reactions of wild-caught mysids under more realistic environmental cues.

DO PCBS CAUSE DEVELOPMENTAL DEFORMITIES IN CHONDRICHTHYES? OPTIMIZATION OF THE SKATE EXCASING MODEL

Nicholas Andreozzi, Sara Lazar, Keya Thakkar, Timothy Bock, Rebeka Merson

Department of Biology, Rhode Island College, Providence, RI

RI NSF EPSCoR Summer Undergraduate Research Fellowship Program and RI Science & Technology Advisory Council

Increases in precipitation attributed to global climate change can introduce anthropogenic chemicals into marine habitats. This may have direct consequences on the health of Narragansett Bay. Apex predators are critical to the stability of food webs, but are inherently susceptible to environmental pollutants through bioaccumulation and biomagnification. A legacy chemical and known teratogen, 3, 3', 4, 4', 5- pentachlorinated biphenyl (PCB126) is present in Narragansett Bay. To assess the effects of dioxin-like PCBs like this on embryonic development of fishes in the class Chondrichthyes, we modified the little skate (Leucoraja erinacea) egg case model. Given the long embryonic development time (6 months in 18 °C), we hypothesize that the skate will be more sensitive to PCB toxicity than bony fish models. Our model involves "excasing" the embryo and placing it into an experimental chamber to continue development. To optimize this model prior to PCB exposure, we monitored temperature, dissolved oxygen (DO), pH, nitrate, and salinity. We evaluated embryos held in static water conditions and assessed embryonic growth during post-oviposition weeks 2 to 12, which corresponds to sensitive stages in well-characterized toxicology models, Fundulus heteroclitus (killifish) and Danio rerio (zebrafish). All embryos removed from their egg cases survived through those stages for more than 5 weeks. The minimum holding volume with negligible reduction of DO in a sealed container was 1.7 liters seawater, salinity 33 ppt, 18 °C, for up to 5 days. These procedures will allow us to safely evaluate PCB embryotoxicity in the little skate.

HOLE-Y ULVA! EXAMINING THE ROLE OF PERFORATIONS IN SPECIES OF BLOOM-FORMING MACROALGAE

Ivy Burns, Lindsay Green, Carol Thornber

Department of Biological Sciences, University of Rhode Island, Kingston, RI

RI NSF EPSCoR Summer Undergraduate Research Fellowship Program

Blade-forming species of the genus Ulva are key contributors to macroalgal blooms and generally propagate asexually via fragmentation. The dominant blade-forming species of Ulva in Narragansett Bay are Ulva rigida (blade contains perforations) and Ulva compressa (blade lacks perforations). These perforations are naturally occurring and seem counter-intuitive since they decrease the photosynthetic area of the algal blade. We formed two hypotheses to test the role of perforations in Ulva rigida. First, we hypothesized that the holes in Ulva rigida aid in propagation via fragmentation. To test this hypothesis, we compared tissue toughness and tensile strength of Ulva rigida and Ulva compressa. Ulva rigida had higher tensile strength than U. compressa and observations indicated that tearing in U. rigida generally occurred near the perforations. There was also a slight difference between the tissue toughness of the species, although it was not statistically different. Our second hypothesis was that the perforations in Ulva rigida may increase turbulent flow over the blade surface, thus increasing nutrient uptake and growth in low water flow environments where Ulva blooms generally occur. For this hypothesis, both species were grown at three sites in Greenwich Bay that have varying flow rates. Blades were also grown in outdoor flow at URI's Graduate School of Oceanography (GSO) through tanks using ambient seawater with two treatments: high and low flow. Both species exhibited an increase in the percent of surface area comprised of perforations under high flow conditions at the GSO, most likely due to their rapid growth rate. Interestingly, the surface area to mass ratio of the blades decreased from start to finish in all treatments. This indicates that the blades were getting thicker. The decrease in surface area to mass was more dramatic under high flow rates for U. compressa and was almost equal for U. rigida.

USING BOAT MOORINGS AS A NURSERY SITE FOR CORAL RESTORATION

Steven Tadros, Graham Forrester

Department of Natural Resources Science, University of Rhode Island, Kingston, RI

RI NSF EPSCoR Summer Undergraduate Research Fellowship Program

Coral reefs are one of the most biodiverse ecosystems containing about 25% of all marine species and offer billions of dollars per year in the fishing and tourism industries. They act as coastal barriers to storms and erosions and are an indicator of the health of the ocean. Unfortunately, due to climate change and increased human activity, the health of these ecosystems is declining rapidly. A proposed solution to deal with the decline of reefs is coral restoration. One of the most highly used methodologies for coral restoration, and the one used in this study, is the coral gardening technique, which involves taking small fragmented pieces of coral and transplanting them to a nursery at a protected site where the coral can survive and grow at a higher rate than if on the reef, and when they grow to a size with higher survival rates they are out-transplanted back onto the reefs. In some countries, obtaining sites for nurseries is difficult legally because it requires a seabed lease. In others with marine parks, regulators have not approved sites for nursery use close to conservation areas. Existing boat moorings are an alternative as nurseries because legal permission for their installation is already in place, and many marine parks use them as a way of preventing damage from boat anchors. Boat moorings were used as a nursery site off the coast of Guana Island, British Virgin Islands. This study focuses on the restoration of the Staghorn coral, a key species in the structure of reefs. Mid-water nurseries offer advantages such as faster coral growth and reduced damage from sedimentation compared to nurseries attached to the seabed. In this study 4 nursery designs were used across 9 mooring lines. Over the course of the deployment of the nurseries some designs seemed more durable and reliable than others, and these designs were then chosen to be the permanent nurseries for the four months required for proper coral restoration. Over the four months, the success of the nurseries and corals were monitored and after that time the corals were out-transplanted back onto reefs. The success of the designs is determined by the success of the corals, which is determined by the survival and growth rate. The growth rate of corals is determined by comparing photographs taken pre- and post-nursery using imaging software. Creating a time effective and cost efficient technique for coral restoration may lead to higher nursery success and, therefore, higher coral success.

SPECIES ABUNDANCE AND THE OCCURRENCE OF EPIPHYTES ON BLOOM-FORMING MACROALGAE IN GREENWICH BAY, WARWICK, RHODE ISLAND

Anne Filteau[1], Lindsay Green[2], Carol Thornber[2]

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RI NSF EPSCoR Summer Undergraduate Research Fellowship Program

Large blooms of Ulva have ecological and economic consequences making the study and identification of each species important. Examining species abundance and fitness provides useful data on differences within this important genus. Epiphytism lowers the host's fitness through competition for light and resources. The susceptibility of species to epiphytism has important consequences for it's overall success. To determine the abundance of epiphytes, 5-10 blades of U. rigida and U. compressa were cleaned and placed in mesocosms at three different field sites (Chepiwanoxet Point, Greenwich Cove, and Sandy Point in Warwick, RI) for one week. At the end of the week, the blades were examined and epiphytes were identified and counted. To determine the abundance of the three main Ulva species (U. lactuca, U. rigida, and U. compressa), two 10 m transects were conducted at two sites (Chepiwanoxet Point and Warwick City Park) in both June and July 2015. A 0.25 m2 quadrat was placed at every meter along each transect and the percent cover of blade-forming Ulva was recorded. All Ulva material was then collected and identified using microscopy. Our results show that U. compressa had significantly more epiphytes than U. rigida, with an average epiphyte load of 25.67 and 2.25 per blade, respectively. We also found that Ulva blades grew significantly less when epipytized; species identity did not effect this pattern. Of the Ulva collected in June and July at Chepiwanoxet Point and Warwick City Park, U. rigida accounted for >83%. There was no difference in growth rate between Ulva species so this may be because U. compressa is more heavily epiphytized than U. rigida, which reduced its fitness. The red algal genera Ceramium and Champia were the most commonly found epiphytes. Overall, our results suggest that U. rigida may have a mechanism to avoid epiphytism, leading to its dominance in Greenwich Bay.

MICROBIOLOGY

LOCATED ON THE 2ND FLOOR OF THE PHARMACY BUILDING

POSTERS ARE TO BE MANNED FROM 11:00 AM – 12:30 PM

INVESTIGATION OF DEEP ATLANTIC SEDIMENTS FOR NEW ANTIBIOTICS

Renata Torres Rego[1], Robert Deering[2], Jiadong Sun, Meagan Hamblin, David C. Smith[3], David C. Rowley[2]

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RI-INBRE Summer Undergraduate Research Fellowship Program

According to the Centers for Disease Control and Prevention, at least 23,000 people die each year in the Unites States as a result of bacterial infections resistant to antibiotics. The majority of our antibiotics are derivatives of secondary metabolites produced by terrestrial bacteria. Because of the need for new sources of compounds with antibiotic action, the present study aims to discover novel antibiotics produced by deep-sea bacteria. Marine sediments were collected during the ocean drilling expedition Knorr Cruise 223 from 17 sites in the North Atlantic Ocean at depths up to 5500 meters. Bacteria from these sediments were isolated and cryopreserved. In this study, 37 strains were cultivated in a marine medium at 25 °C using both static and shaking (175 rpm) conditions. Secondary metabolites were extracted with Amberlite XAD resin and sequentially eluted with water, 10% aqueous methanol, and 100% methanol. The resulting 74 culture extracts from the 100% methanol fractions were assayed against Escherichia coli and Staphylococcus aureus using a disk-diffusion method. 13 extracts showed activity against S. aureus. The extract with the greatest growth inhibition halo was analyzed using high performance liquid chromatography (HPLC) and the isolated peaks were assayed for antibiotic activity. A single active compound was revealed by this experiment. More intensive chemical analysis is underway to identify the antibiotic as well as molecular genetic assays to classify the taxonomy of these bacteria.

BREAKING DOWN THE WALL II: UGI-DERIVED DIAMIDES TARGET THE PEPTIDOGLYCAN METABOLISM

Keyana Roohani[1], Mary O'Connor[2], Ethan Magno[2], Amit Basu[2], Christopher Reid[1]

[1]*Department of Science & Technology*, Bryant University, Smithfield, RI
[2]*Department of Chemistry*, Brown University, Providence, RI

RI-INBRE Summer Undergraduate Research Fellowship Program

Recent years have seen a rise in bacteria resistant to antibiotics, leading to increasingly dangerous infections from previously non-fatal pathogens. Gram-positive bacteria are characterized by the thick peptidoglycan (PG) layer and teichoic acid in their cell wall. The PG layer is composed of a polysaccharide backbone comprised of N-acetylglucosamine (GlcNAc) and N-acetyl muramic acid (MurNAc). The adjacent polysaccharide strands are cross-linked via the pentapeptide side chains attached to the MurNAc. Bacteria can produce enzymes known as autolysins, which have the capability of breaking down the peptidoglycan of cell walls. These enzymes are involved in processes such as cell growth, division and motility. A panel of Ugi-derived diamides based off of previously identified inhibitors was screened against several Gram-positive organisms. From this screening, 2 compounds (fgka and fgkc) showed antimicrobial activity against Bacillus subtilis (B. subtilis) with MIC values of 22 & 2.9 µM respectively. Previous results have suggested the ability of these compounds to inhibit bacterial N-acetlglucosaminidase activity. Here we demonstrate that the mode of action of fgka and fgkc in B. subtilis is peptidoglycan metabolism using targeted metabolomics. B. subtilis cells treated with fgka or fgkc show similar activity to cells treated with vancomycin. In the presence of fgka or fgkc B. subtilis accumulates nucleotide sugar precursors of PG synthesis such as UDP-MurNAc-pentapeptide. Additionally, phenotypic analysis by microscopy indicates that fgka and fgkc interfere with cell elongation and division.

ANALYSES OF HFQ FUNCTION IN GROWTH AND STRESS RESPONSES IN THE METAL REDUCING BACTERIUM SHEWANELLA ONEIDENSIS

Emma Hodges, Ally Luongo, Matt Cupelo, Dan McGrath, Brett Pellock

Department of Biology, Providence College, Providence, RI

RI-INBRE Summer Undergraduate Research Fellowship Program

The RNA chaperone protein Hfq has been broadly implicated in bacterial sRNA function in Gram-negative bacteria. We have used a genetic approach to understand the role of Hfq in the dissimilatory metal reducing bacterium Shewanella oneidensis. Loss of Hfq in S. oneidensis results in slow exponential phase growth, reduced terminal cell density in stationary phase, a striking loss of colony forming units in extended stationary phase, and an exquisite sensitivity to oxidative stress.

We have found that the exponential phase growth defect of the hfq mutant is the result of reduced heme levels. Both heme levels and exponential phase growth of the hfq mutant are completely restored by supplementing the growth medium with 5-aminolevulinic acid, the first committed intermediate synthesized during heme biosynthesis. Increasing gtrA expression via an inducible plasmid vector also restores heme levels and exponential phase growth of the hfq mutant. Our data indicate that reduced heme levels are solely responsible for the exponential growth defect of the S. oneidensis hfq mutant.

We are currently investigating the etiology of the hfq mutant's defects in oxidative stress resistance and stationary phase survival. Pre-treatment with sub-lethal doses of hydrogen peroxide restores survivorship of hfq mutant cells to wild type levels, suggesting that the hfq mutant is capable of adapting to oxidative stress conditions, but is less capable than wild type S. oneidensis at handling lethal doses of hydrogen peroxide. Our data suggests that delayed expression of the catalase gene katB is responsible for the hfq mutant's oxidative stress sensitivity. Finally, we have found that increasing expression of the stationary phase sigma factor rpoS rescues the stationary phase survival defect of the hfq mutant, suggesting that Hfq regulates rpoS expression in S. oneidensis and that rpoS misregulation in the hfq mutant contributes to its catastrophic death in stationary phase.

SURVEYING DISTRIBUTION AND PREY RANGE OF PREDATORY BACTERIA IN RHODE ISLAND

Nadiva Brown, Nicole Cullen, Brett Enos, Sean O'Donnell, Laura Williams

Department of Biology, Providence College, Providence, RI

RI-INBRE Summer Undergraduate Research Fellowship Program

Predatory bacteria are bacteria that attack and digest other bacteria in order to propagate. Predatory bacteria have been isolated from soil, marine and freshwater environments. The most well-studied predatory bacteria belong to two closely related families in the class Deltaproteobacteria. Certain species within these families, such as Bdellovibrio bacteriovorus, are found in a wide range of environments, whereas other species, such as Halobacteriovorax marinus, are restricted to marine environments. Both of these species are obligate predators that attack Gram-negative bacteria, including animal and plant pathogens. This trait makes predatory bacteria an attractive alternative to antibiotics, which are losing effectiveness with the rise in antibiotic resistance.

We aimed to survey the diversity of predatory bacteria occurring in different sites around and near Rhode Island, including soil, estuary, freshwater and built environment sites. We also aimed to characterize the prey range of these predatory bacteria, which is the range of prey species that are susceptible to attack. We used both culture-dependent and -independent approaches. In culture-dependent approaches, we isolated potential prey bacteria from three environments (soil, estuary, freshwater stream) and used 16S rRNA gene sequencing to classify the potential prey. We validated growth and microscopy protocols using a type strain of Bdellovibrio bacteriovorus. We observed predatory bacteria by microscopy in an estuary sample, but have not yet detected predators in soil and freshwater samples. In culture-independent approaches, we validated genus-specific PCR to assay the presence of two genera of predatory bacteria. Using this PCR, we tested metagenomic DNA from swab samples of surfaces and soil and water samples from environmental sites.

Moving forward, we will continue isolation of predatory bacteria from chosen environmental sites. We will classify recovered predators using 16S rRNA gene sequencing and test their prey range by challenging them with potential prey described here. This work will contribute to our understanding of predation in bacteria and explore the potential for therapeutic applications of predatory bacteria in the fight against pathogens and antibiotic resistance.

INHIBITORY EFFECTS OF SUBSTITUTED PYRAZOLINE DERIVATIVES ON ENTAMOEBA HISTOLYTICA

Alexander Tight[1], Hien Ngo[1], Lauren Rossi[2], Avelina Espinosa[1]

[1]*Department of Biology, Marine Biology & Environmental Science*, Roger Williams University, Bristol, RI
[2]*Department of Chemistry & Physics*, Roger Williams University, Bristol, RI

RI-INBRE Summer Undergraduate Research Fellowship Program

Entamoeba histolytica is an intestinal parasite that causes disease in humans. Amoebiasis is a major public health risk, with about 50 million cases, and 100,000 deaths per year worldwide. The disease is primarily treated with metronidazole, which is effective but has adverse side effects such as neurological complications. Previous work in our laboratory generated 25 compounds within three series of pyrazoline derivatives that block the bifunctional enzyme alcohol dehydrogranse 2 (EhADH2). We tested these series of compounds in their ability to inhibit growth and survival of Entamoeba histolytica trophozoites. The inhibitors were tested at 60 and 120 uM concentrations and compared to the drug of choice metronidazole. Our results demonstrate that series 1a is the most effective. Compared to series 2, series 1a is significantly less bulky which could give the compound a greater affinity to bind to the enzyme. Series 1b is the least bulky, but the propyl group may hinder its effectiveness although more data is needed on this series to establish the effect of each structural moiety. Future studies will test these compounds efficiency at enzyme inhibition and toxicity in human cells in comparison to metronidazole.

BREAKING DOWN THE WALL I: UGI-DERIVED DIAMIDES AS NARROW SPECTRUM ANTIBIOTICS AGAINST GRAM-POSITIVE PATHOGENS.

James Gravier[1], Mary O'Connor[2], Ethan Mango[2], Amit Basu[2], Christopher Reid[1]

[1]*Department of Science & Technology*, Bryant University, Smithfield, RI
[2]*Department of Chemistry*, Brown University, Providence, RI

RI-INBRE Summer Undergraduate Research Fellowship Program

Despite the increased prevalence of multidrug resistant (MDR) Gram-positive pathogens, the development of new antimicrobials targeting these organisms has lagged behind. We have previously demonstrated that glycosyl-triazoles inhibit bacterial N-acetylglucosaminidases with antibacterial activity.We have characterized a panel of Ugi-derived diamides based on this lead compound. The panel was screened against Bacillus subtilis, Staphylococcus aureus, Streptococcus pneumoniae, Enterococcus faecalis, and Peptoclostridium difficile in a resazurin microtiter MIC assay. Results from the MIC assay identified several potent and narrow spectrum inhibitors. Top hits for S. pneumoniae included fgbc, fgbb, fgkb, and fgkc with MIC values in the range of 2.34 to 4.69 uM. Top hits for P. difficile were fgna and BI. fhba at 46.87 to 23.44 uM. Top hits for S. aureus included fgia, fgqa, and fgta at 9.375 uM, 4.6875 uM, and 18.75 uM respectively. In order to rule out nonspecific inhibition due to compound aggregation, MIC assays with B. subtilis were run in the presence of 0.001% Triton X-100. In order to confirm the mode-of-action in S. aureus as peptidoglycan metabolism, an autolysin-deficient (lyt-) strain of S. aureus was used in the resazurin microtiter MIC assay. Antimicrobial activity of fgia, fgqa, and fgta, were completely attenuated in the lyt-, confirming that the molecular target is an autolysin.

FILAMENTATION PROTECTS CANDIDA ALBICANS FROM AMPHOTERICIN B-INDUCED PROGRAMMED CELL DEATH VIA A MECHANISM INVOLVING THE YEAST METACASPASE, MCA1.

Melissa Brown, Morgan McCarthy, David Laprade, Nicanor Austriaco

Department of Biology, Providence College, Providence, RI

RI-INBRE Summer Undergraduate Research Fellowship Program

The budding yeast Candida albicans is one of the most significant fungal pathogens worldwide. It proliferates in two distinct cell types: blastopores and filaments. Only cells that are able to transform from one cell type into the other are virulent in mouse disease models. Apoptosis, or programmed cell death, is a controlled form of cell suicide that occurs when C. albicans cells are exposed to fungicidal drugs like amphotericin B and caspofungin, and to other stressful conditions. We now provide evidence that suggests that programmed cell death is cell-type specific in yeast: Filamentous C. albicans cells are more resistant to apoptosis induced by either amphotericin B or caspofungin than their blastospore counterparts. Finally, our data suggests that this phenomenon is mediated by a mechanism involving the yeast metacaspase, MCA1.

IMPACT OF LEGACY POLLUTION ON THE PROVIDENCE RIVER ESTUARY MICROBIOME

Sarah Falco, Christopher W. Reid, Julia Crowley-Parmentier

Department of Science & Technology, Bryant University, Smithfield, RI

RI NSF EPSCoR Summer Undergraduate Research Fellowship Program

The Providence River resides in an urban-industrial setting with an extensive history of contamination. Having been surrounded by industrial sites for many years there is a legacy of contaminants. The Oxford street sampling site has been used in the past to house large oil storage units and is currently home to a metal recycling facility. The exposure to these legacy pollutants has impacted the microbiome of the estuary. Our goal is to present an initial assessment of the site and begin to identify the members of the microbial community. We assessed abiotic conditions at the water's edge and the site 6 feet inland during low tide. We estimated total organic carbon (TOC) at the water's edge to be 0.95 ± 0.78% and 6 feet away from the water to be 1.47 ± 1.20%. We estimated total inorganic carbon (TIC) at the water's edge to be 6.74 ± 5.01% and 6 feet away from the water to be 9.03 ± 1.75%. An anion analysis of pore water indicated levels of fluoride (1.02±0.24ppm at water's edge and 0.96±0.16ppm 6 feet from water), bromide (37.02±20.35ppm at water's edge and 48.57±4.73ppm 6 feet from water), and sulfate (2019.45±204.75ppm at water's edge and 1985.46±132.89ppm 6 feet from water). Members of the microbial community were identified using 16s rDNA sequencing. Bacteria identified at the Oxford street sampling site included Desulfotomaculum thermocisternum (a sulfate reducer), Lewinella lutea, and Thermus caliditerrae. High levels of sulfate correlate with the presence of sulfate-reducing bacteria.

ENTAMOEBA SPP AS MODELS OF ENVIRONMENTAL STRESSES IN MARINE AND FRESHWATER PROTISTS

Meagan Hackey, Joshua Leitao, Avelina Espinosa

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

RI NSF EPSCoR Summer Undergraduate Research Fellowship Program

Entamoeba spp. are protistan parasites that infect a variety of larger organisms including komodo dragons, turtles, snakes, reptilian species and humans upon ingestion from contaminated water sources. Environmental factors including temperature and pH variation, which result from global warming and climate change have a significant impact on the growth of Entamoeba spp. Acid rain is causing a gradual decrease in aquatic pH and global warming is increasing air and water temperature worldwide. These species are important for biodiversity and they may have implications for animal behavior in the larger organisms they inhabit. In vitro assays were performed to test the environmental conditions that lead to differential viability in Entamoeba spp. after 1 week of growth. Upon statistical analysis, it was identified that cell surface area is significantly different in cells incubated at high temperatures and extreme pH values when compared to healthy cultures. The release of smaller molecular weight proteins in cultures incubated above 37°C was identified through SDS PAGE protein gel electrophoresis, which may indicate that Entamoeba spp. release 70kDa heat shock proteins (HSP70) and chaperonin to maintain homeostasis by decreasing aggregation and maintaining proper protein folding. These proteins are potentially similar to those identified in E. invadens, E. histolytica, and related species including Trichomonas vaginalis, Encephalitozoon cuniculi, and Giardia lamblia as heat shock identifiers and other characteristic down regulated proteins. Slightly increased temperature and decreased pH values resulted in little change or slightly improved cell health observed through cell shape, confluency, cell density, aggregation, waste, and media conditions. This indicates that environmental change may result in increased Entamoeba spp. growth which poses a threat to human and animal health in the future. Further research is required to identify possible mechanisms to contain Entamoeba spp. growth in the changing environment and to understand the role of heat shock proteins in the glycolytic pathway of these species under environmental stress.

LIFE ON THE EDGE: MICROBIAL COMMUNITIES IN AN URBAN- INDUSTRIAL ESTUARY

Drew Phelan, Julia Crowley-Parmentier, Christopher Reid

Department of Science & Technology, Bryant University, Smithfield, RI

RI NSF EPSCoR Summer Undergraduate Research Fellowship Program

The Providence River has been subjected to centuries of industrialization exposing the river to high levels of heavy metal and organic contamination impacting the ecosystem immensely. Bold Point Park (BPP) began as a shipyard from the 1780's to the 1840's where it served as both a terminal for the Providence and Boston Railroad which further increased industrial pollution. During the American Revolution and War of 1812, BPP also served as part of Fort Hill, a military defense base. Now, the public's increased use has added to the estimated 27,000 cubic yards of shoreline debris to have polluted the river by 1980. This legacy pollution has had a significant impact on the estuarian microbial ecosystem. Heavy metal contamination was observed in clams found at BPP. High levels of Arsenic (14.5mg/kg), Copper (90.8mg/kg), Lead (52.5mg/kg) and Zinc (163.4mg/kg) were present. Sediment samples were analyzed for Total Organic/ Inorganic Carbon (TOC/TIC) by FT-IR analysis and were found to 1.67% +/-0.68 and 9.06% +/- 5.50, respectively. Identification of the microbial community was carried out using 16SrDNA sequencing which revealed strains such as Desulfotomaculum thermocisternum and Stanieria cyanosphaera. A large presence of sulfate-reducing and halophillic microorganisms were also recorded. Despite decades of urban redevelopment, BPP remains a significantly polluted estuary which has an increasingly higher impact on the public life as the heavy metal and organic pollutants travel up to high orders of life.

MOLECULAR BIOLOGY

LOCATED IN ROOM 105 ON THE 1ST FLOOR OF THE PHARMACY BUILDING

POSTERS ARE TO BE MANNED FROM 9:30 – 11:00 AM

DEVELOPMENT AND DISEASE: INVESTIGATIONS INTO METABOLIC CHANGES IN DROSOPHILA MELANOGASTER

Michelle Ouellette, Julia Fabiano, Marla Tipping

Department of Biology, Providence College, Providence, RI

RI-INBRE Summer Undergraduate Research Fellowship Program

Shifts in metabolism are critically involved in a variety of organismal processes including development and onset of disease. For example, the mammalian estrogen-related receptor (ERR) plays an important role in coordinating growth and metabolism. By regulating specific metabolic genes, ERRs can direct subsequent metabolic transitions in developing organisms such as inducing the onset of glycolysis. Additionally, metabolic changes are prominent during cancer cell growth and proliferation. For example, the metabolic enzyme isocitrate dehydrogenase (IDH) is involved in the most common type of human gliomas when mutated. Drosophila melanogaster provides an outstanding model for the study of these metabolic changes in development and disease due to the numerous genetic tools and metabolic assays available as well as its conservation of human disease genes. First, we aim to identify additional proteins associated with the Drosophila analog of ERR (dERR) that might also be involved in directing glycolytic metabolic shifts during development. Second, we are working to create fruit flies that express a GFP-tagged version of wild-type or mutant IDH under the control of their endogenous promoters. The development and manipulation of an IDH mutant phenotype in the fly will assist in the elucidation of its enzymatic activity and protein interactions in the formation of gliomas.

TARGETING OF INFLAMMATORY PROTEINS USING HOLLOW GOLD NANOSPHERES

Heather Conboy, Wei Lu

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

RI-INBRE Summer Undergraduate Research Fellowship Program

Inflammatory disorders such as rheumatoid arthritis pose a serious burden to patients due to debilitating symptoms including painful swelling that can eventually erode bones and cause joint deformity. Current treatments focus on controlling swelling flares, but fail to give patients long term relief. The purpose of this research is to design a drug delivery system which targets localized inflammatory diseases. To do so, we are investigating gold nanoparticles and their unique protein absorption properties as a potential treatment for inflammatory disorders. We utilize SDS-PAGE and 1H-NMR techniques to determine whether or not mouse inflammatory factor mIL-1β is bound to our hollow gold nanospheres (HAuNS) following laser treatment. The SDS-PAGE results show mIl-1β is only bound to the nanospheres after laser treatment, but not without laser treatment. The phenomenon is also confirmed through our 1H-NMR experiments. Likewise, mIL-1β is only detected on our HAuNS after laser treatment. Using this data, we can continue to study the unique chemical properties of the gold nanoparticle and make further strides to use nanotechnology to treat inflammatory diseases.

CIMRF SUPPRESSES ENDOGENOUS TISSUE DEVELOPMENT DURING TRANS-DIFFERENTIATION OF NON-MUSCLE TISSUE TO MUSCLE

Emmanuel Asiedu, Lindsay Ratcliffe, Taylor Ferrare, Thomas H. Meedel

Department of Biology, Rhode Island College, Providence, RI

RI-INBRE Summer Undergraduate Research Fellowship Program

Myogenic Regulatory Factors (MRFs) are a unique class of basic-helix-loop-helix (bhlh) transcription factors that regulate muscle development in animals. Much of what is known about the properties of these genes stems from research conducted with vertebrate MRFs. For example, vertebrate MRFs have been shown to direct trans-differentiation of non-muscle tissues to muscle tissues, and in the case of the vertebrate MRF, MyoD, this trans-differentiation is accompanied by down-regulation of the endogenous program of gene expression. Our lab is interested in the functional evolution of the MRF gene family, and uses the chordate, Ciona intestinalis as a focal organism. Our previous work showed that similar to vertebrate MRFs, the MRF of Ciona intestinalis, CiMRF, has the ability to elicit trans-differentiation of non-muscle tissues to muscle tissues. These studies were done by misexpressing CiMRF in the notochord and endoderm of Ciona embryos, two non-muscle cell types, and examining the expression of cell-specific markers using in situ hybridization and histochemical assays. The current project was designed to test whether, like MyoD, trans-differentiation to muscle by CiMRF is accompanied by down regulation of endogenous gene activity. To do this we examined the expression of cell-specific markers of notochord and endoderm development. In both cell types marker gene expression was decreased as a result of CiMRF expression. Moreover, expressing CiMRF in the notochord of Ciona embryos produced larvae with abnormal tails, which is consistent with published work indicating that tail development requires normal notochord formation, which, in turn, depends on the expression of Brachyury, a notochord-specific transcription factor whose activity we show is decreased when CiMRF is active in the notochord. Together with other work from our lab, we conclude that CiMRF not only has the ability to direct muscle-specific gene activity in non-muscle tissues but that it does so in a manner that involves suppression of the endogenous gene activity of the non-muscle cell type. These results indicate that the mechanism by which chordate MRFs function to regulate muscle development is highly conserved.

FUNCTION AND CONSERVATION OF THE N-TERMINUS OF THE CIONA INTESTINALIS MYOGENIC REGULATORY FACTOR

Lindsay Ratcliffe, Taylor Ferrare, Emmanuel Asiedu, Thomas H. Meedel

Department of Biology, Rhode Island College, Providence, RI

RI-INBRE Summer Undergraduate Research Fellowship Program

Our lab studies Myogenic Regulatory Factors (MRFs), which are functionally conserved basic helix-loop-helix (bHLH) transcription factors that regulate metazoan muscle development. A distinguishing feature of MRFs is their ability to stimulate muscle development when they are expressed in non-muscle cell types. We took advantage of this feature in order to study the properties of MRFs by expressing them in the notochord and endoderm of embryos of the ascidian, Ciona intestinalis. Published work has shown that MRFs exhibit a high degree of functional conservation. However, when we tested their activity in Ciona embryos, only MRFs of C. intestinalis and the closely related ascidian, C. savignyi, were able to stimulate muscle gene expression. We also found that the MRFs of both Ciona species possessed a large N-terminal domain that is not present in the non-ascidian MRFs that we tested, and that this domain was essential for promoting muscle development in our assay. We then examined whether similar N-termini are found in the MRFs of other ascidians, and whether they might function by interacting with other factors in the embryo, most likely proteins, to direct muscle development. Our results show that N-termini with comparable activities are found only in the MRFs of Ciona and other closely related ascidians. Analyses of whether the N-terminus interacts with other factors to stimulate myogenesis were less definitive, but were consistent with that possibility. Together with other studies from our lab (e.g. see the Poster by Aseidu et al), we conclude that ascidian MRFs function in a manner that is typical of this family of transcription factors. However, at least some ascidian MRFs are distinct in that they possess an unusual and large N-terminal domain that is essential for promoting myogenesis in Ciona embryos. This domain is most likely an evolutionary novelty that is restricted to a limited number of ascidian species.

ONCOMETABOLITE 2-HYDROXYGLUTARATE INHIBITION OF ALKB FAMILY DNA REPAIR ENZYMES

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Mutant isocitrate dehydrogenase (IDH) proteins are found in a variety of human cancers including low grade gliomas, secondary glioblastomas, acute myeloid leukemia, chondrosarcomas, and T-cell lymphomas. Wild type IDH proteins normally function in the TCA cycle to produce alpha-ketoglutarate (αKG), a ketoacid that plays a pivotal cofactor role in a multitude of human enzymes. The mutant IDH proteins, however, gain the ability to produce the oncometabolite 2-hydroxyglutarate which has been observed to competitively inhibit αKG/iron dependent enzymes. The AlkB family DNA repair enzymes, in particular E. Coli AlkB and its two human homologues ABH2 and ABH3, play a crucial role in the catalytic repair of mutagenic and toxic DNA base lesions. All three aforementioned enzymes are also αKG/Fe dependent dioxygenases, and since the repair ability of the AlkB family proteins is dependent on αKG, it is very likely that all three of these DNA repair proteins are inhibited by 2-HG similarly to other mammalian αKG/Fe dependent enzymes. The purpose of this work was to examine if 2-HG does exhibit an inhibitory effect on the function of ABH2, ABH3 and AlkB, especially at the concentrations of 2-HG to αKG found in tumor cells. First, Michaelis–Menten enzymatic parameters such as Km and kcat of each of the three proteins were ascertained using various concentrations of m1A and m3C, the optimal DNA lesion substrates for the three enzymes, and AHB2 was determined to be the most catalytic enzyme of the three. Then inhibition reactions of the AlkB and its human homologues were carried out with 2-HG in vitro. The results thus far in our study have illustrated that 2-HG does inhibit the enzymes we have analyzed up to this point.

THE EFFECT OF DENGUE VIRUS INFECTION ON MITOCHONDRIAL MORPHOLOGY

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RI-INBRE Summer Undergraduate Research Fellowship Program

Dengue virus (DENV) is a mosquito-borne human pathogen of global medical importance. DENV causes an acute febrile illness that is sometimes associated with a life-threatening plasma leakage syndrome, dengue hemorrhagic fever (DHF). Previous work in the laboratory has shown that cells infected with DENV show significant changes in mitochondrial morphology. Mitochondria are dynamic organelles and change shape in response to their environment in the cell to induce innate immunity, cell death or increase ATP production. Many proteins are involved in each of these processes. The focus of this project was to analyze proteins involved in mitochondrial fusion and fission. DENV infected and uninfected human liver cells were fractionated to separate cytosolic and mitochondria fractions and compare to total cell lysates. Fusion and fission proteins were analyzed using western blot. Continuing with this project would require further analysis of different mitochondrial proteins, specifically those involved in fusion and fission. The depletion of these proteins ex vivo will also help to further characterize the effect of mitochondrial dynamics on DENV infection. Understanding viral-host interactions can identify mechanisms that DENV uses to propagate. In turn, therapeutics can be designed to inhibit these viral targets and impair DENV replication.

A NOVEL DIPROLINE SEGMENT IN THE SQUALUS ACANTHIAS AHR1 LIGAND BINDING DOMAIN DOES NOT PREVENT PCB126-DEPENDENT ACTIVATION

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RI NSF EPSCoR Summer Undergraduate Research Fellowship Program and RI Science & Technology Advisory Council

Environmental chemicals can cause many deleterious health effects on organisms, from molecular to ecological levels. These chemicals can cause toxicity through multiple biological pathways. One pathway is through the aryl hydrocarbon receptor (AHR). The AHR is a ligand-activated protein that binds to many chemicals including some polycyclic aromatic hydrocarbons (PAHs) and halogenated aromatic hydrocarbons (HAHs). AHR is present in all vertebrates and some invertebrates, and has evolved independently in these diverse lineages. The structure of the AHR's ligand binding domain (LBD) determines binding and chemical-dependent transcriptional activation. Characterization of the amino acid residues that determine binding may allow identification of at-risk species exposed to known AHR agonists. AHR1 in spiny dogfish shark (Squalus acanthias) does not bind to typical AHR ligands. Previous mutagenesis experiments have been unsuccessful in restoring S. acanthias AHR1 binding affinity to PCB126, a potent AHR agonist. Further investigation of the AHR1 LBD revealed a novel amino acid motif, a diproline segment, which we hypothesized to impair binding by changing the overall structure of the domain. Proline, unlike other amino acids, imposes rigidity, and thus restricts rotation. RNA was isolated from multiple individual sharks, and reverse transcription carried out to produce cDNA. Polymerase chain reaction was performed to amplify cDNA segments containing the diproline codons, and the amplicons sequenced to assess the frequency of the diproline motif. Results from 3 individuals suggest that this motif may be encoded by a rare single nucleotide polymorphism. Site-directed mutagenesis (c1142t, P381L) produced a leucine residue in place of the proline, which is present in the same position in the AHR1 of other vertebrates. This mutation did not rescue PCB126-induced activation in reporter gene assays. Additional homology modeling, coupled with site-directed mutagenesis and creation of chimeric AHRs, will assess determinants of binding and activation of S. acanthias AHR1. Population level assessment of the diproline allele frequency may reveal multiple roles in both xenobiotic response and endogenous functions of AHR1.

INVESTIGATION OF D2R BIOCHEMICAL AGGREGATION

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RI-INBRE Summer Undergraduate Research Fellowship Program

D2 dopamine receptor (D2R) is a 7 pass transmembrane domain G-protein coupled receptor. Dysfunction of this receptor within the brain is thought to be linked to behavioral, psychological and movement disorders. As such the D2R has been a target for many types of antipsychotic drugs. We have previously reported that D2R is localized to two distinct biochemical cellular pool; either soluble or insoluble in the non-ionic detergent Triton X-100. Both D2R fractions are found both endogenously expressed in mouse brain and transiently expressed in HEK-293 cells. SDS-PAGE and subsequent western blotting reveals D2R protein specific signal streaking at high molecular weights. Together, the insolubility and streaking could indicate intracellular protein aggregates which have been shown to be in cytotoxic. Previous research shows that protein chaperones, like heat shock protein 104(Hsp 104), can disaggregate proteins by assisting in their refolding and resolubilization (Lashuel, 2010). Therefore the goal of this project was to clone Hsp104 in the mammalian expression vector, for co-expression with D2R. Thus we can elucidate the effects of Hsp 104 on D2R's biochemical properties.

THE EFFECTS OF PERFLUOROOCTANESULFONIC ACID (PFOS) ON NON-ALCOHOLIC FATTY LIVER DISEASE (NAFLD) AND OBESITY.

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RI-INBRE Summer Undergraduate Research Fellowship Program

Background: Non-alcoholic fatty liver disease (NAFLD) is characterized by increased fat content within the liver in the absence of alcohol use. NAFLD is most often related to obesity; the recommended way to treat NAFLD is through diet and exercise. If left untreated, fatty liver can worsen and progress to cirrhosis, which can irreversibly damage the liver. Previous studies have shown that, in mice, PFOS administration increases liver weight and induces lipid accumulations in hepatocytes (Wan et al., 2012; Bijland et al., 2011). Growing evidence indicate that PFOS may interfere with the benefits of weight loss in mice by increasing fat content in liver. (Salter, 2015). Objectives: This project will determine whether PFOS exposure interferes with weight loss and gain in mice with a standard and high fat diet. It is hypothesized that mice administered a low dose exposure to PFOS in food (0.003%; ~360 µg/kg/day) will be slightly resistant to weight loss-induced improvement of NAFLD and more sensitive to high fat diet-induced fatty liver. Methods: Mice were fed a standard chow low-fat diet (LFD) or 60% Kcal high-fat diet (HFD) for 4 weeks and a diabetes phenotype was confirmed by fasting blood glucose concentrations. At four weeks the mice were divided into two main groups – diet alone or diet containing 0.003% PFOS (~360 ug/kg/day). Then, for each group they were subdivided into 3 groups –mice fed LFD, mice fed HFD, or mice fed HFD that were switched to a LFD, to mimic dietary changes to combat NAFLD. This resulted in a total of 6 treatment groups as follows: i) LFD, ii) HFD-LFD, iii) HFD, iv) LFD + PFOS, v) HFD-LFD + PFOS, and vi) HFD+PFOS. Mice were fed control or PFOS–containing diet for 10 weeks. Body weight was monitored and fasting blood glucose levels were collected throughout the study. Serum lipid levels, glucose tolerance test (GTT) and pyruvate tolerance test (PTT) were administered to assess treatment. At time of necropsy, body, liver, and white adipose weight were determined. Conclusions: The data suggests that PFOS exposure acts on lipid metabolism, lowering serum lipids, decreasing white adipose tissue mass, and increasing lipid content in the liver; thus, increasing the resistance to weight loss-induced improvement of NAFLD. Further studies on the tissues collected at necropsy will be utilized to confirm our physiological findings, and assist in determining the mechanism by which PFOS contributes to hepatic lipid accumulation.

EFFECTS OF 3'UTR LENGTH OF MALE GERM CELL TRANSCRIPTS ON TRANSLATIONAL EFFICIENCY

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RI-INBRE Summer Undergraduate Research Fellowship Program

Use of alternative polyadenylation sites located in the 3' untranslated region (UTR) of messenger RNA (mRNA) can result in transcripts that code for the same protein, but have different length 3'UTRs, including short and long isoforms. Compared to the long 3'UTR isoform, the short isoform has a loss of sequence, including microRNA binding sites, which may impact translational efficiency and mRNA stability. Short 3'UTR isoforms are characteristic of highly proliferative cells, such as cancer cells. Similarly, male germ cells appear to have transcripts with alternative polyadenylation sites which result in short 3'UTR isoforms. In male germ cells this characteristic short 3'UTR isoform may be necessary for proper development of fertile spermatozoa. We hypothesize that the short 3'UTR of specific male germ cell transcripts increases the translational efficiency of the transcript resu lting in increased protein production. For this study, we investigated the male germ cell transcripts Cpsf6, Bzw1, and Dazap1 and the somatic control Timp2. These transcripts have alternative polyadenylation sites in the 3'UTR which result in both short and long 3'UTR isoforms. To test the hypothesis, we attempted to individually sequence and clone the short and long 3'UTRs of the transcripts into a pmirGLO vector (Promega, E1330) to compare the translational efficiency in a male germ cell line (Gc-4spc) and somatic cell line (3T3). 3'RACE was used to identify a short testis specific 3'UTR isoform, and a mid and long 3'UTR isoforms for Bzw1. The short 3'UTR isoform of Timp2 was identified by 3'RACE. 3'UTR isoform sequences of Cpsf6 and Dazap1 were previously published and utilized. The short and long 3'UTRs of Cpsf6, Bzw1, and Dazap1 were amplified through polymerase chain reaction to add XbaI restriction enzymes sites for cloning. The short 3'UTR isoform of Timp2 has been a mplified and efforts towards amplifying the long 3'UTR isoform is underway. After amplification, 3'UTR isoforms of Cpsf6 and Bzw1 were cloned into the pmirGLO vector and screened for proper insertion. To date, we were able to successfully clone the alternatively polyadenylated isoforms of Cpsf6. Sequenced plasmids containing the short and long 3'UTR of Cpsf6 were transfected into the Gc-4spc and 3T3 cell line and protein production was measured by luciferase assay (Promega, E1910). The short Cpsf6 3'UTR had higher protein production when compared to the long Cpsf6 3'UTR isoform supporting our hypothesis.

EFFORTS TOWARDS PROTEOMICS IN CIONA INTESTINALIS

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RI NSF EPSCoR Summer Undergraduate Research Fellowship Program

Ciona intestinalis, an invasive tunicate species in Rhode Island, feed on plankton and other suspended organic matter, and significantly influence the Narragansett Bay ecosystem. The purpose of this experiment was to project the hidden effects of climate change on the reproductive fitness of this species. C. intestinalis are being reared in tanks simulating current and projected ocean temperatures. After gonads have been dissected, protein will be extracted and sent to the Brown EPSCoR Proteomics Center to be analyzed using liquid chromatography/tandem mass spectrometry, which can identify over a thousand proteins in one sample. This is the first time that protein mass spectrometry method has been applied in this way to marine invertebrates. The data will be analyzed to determine which proteins are up or down-regulated in the different conditions. Protein expression changes, if detected, will be used to infer possible physiological stresses on the reproductive system of the animals.

A preliminary siphon regeneration investigation allowed for practice of protein extraction techniques and data analysis while waiting for growth of the temperature experiment animals. Ciona intestinalis has the ability to regenerate amputated siphons, and the proteins involved in this regeneration are unknown. The purpose of this preliminary experiment was to determine what proteins are involved in siphon regeneration in order to provide further insight on how stem cells function. Siphons were surgically removed, and re-amputated after regeneration periods of 2 and 5 days. We observed the regeneration process by whole mount Nomarski and fluorescence microscopy. Meanwhile protein was extracted by SDS-PAGE fractionation and tryptic digest and then sent to the Brown EPSCoR Proteomics Center to be analyzed using liquid chromatography/mass spectroscopy. The data will then be analyzed to determine which proteins were up or down-regulated during regeneration.

INTER ASSAY VARIABILITY OF T CELL RECEPTOR SPECTRATYPE ANALYSIS

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T cell receptor (TCR) spectratype analysis is a method to evaluate the size and diversity of the T lymphocyte repertoire, and has been used as an assessment of the overall function of the immune system. Our long-term goal is to apply TCR spectratype analysis to compare umbilical cord blood samples from HIV-exposed or –unexposed infants. The objective of this project was to investigate the magnitude and sources of variability within the assay. The purpose of this is to be able to eliminate assay variability so that only the variability within the sample would be observed. We analyzed TCR Vβ gene usage using a protocol based on the method established by Balamurgan et al (The Journal of Immunology, 2010). Total cellular RNA was isolated from healthy control donor peripheral blood mononuclear cells (PBMCs), and cDNA was prepared by reverse transcription. TCR Vβ gene usage was then measured by real-time polymerase chain reaction (PCR) using one labeled 3’ primer, and twenty four 5’ primers. Replicates were performed using the same input cDNA. Data from this analysis will be presented showing assay variation subtracted from sample variation.

NEUROSCIENCE

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SIMULTANEOUS WEIGHT INTERVENTION TO STOP SMOKING: PROJECT SWISS

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RI-INBRE Summer Undergraduate Research Fellowship Program

Smoking and obesity are the first and second leading causes of preventable deaths in the United States (Centers for Disease Control and Prevention). Standard intervention for both behavior risks is either behavioral, pharmacologic or a combination of both. Acceptance and Commitment Therapy (ACT) is a behavioral intervention and empirically supported treatment that focuses on acceptance and mindfulness that has proven efficacy for both smoking cessation and weight loss as separate behaviors. However, to date, there are no ACT studies that simultaneously target both smoking cessation and weight loss. Therefore, the aims of this project are to 1) pilot test a novel ACT intervention that simultaneously targets smoking cessation and weight loss having refined two existing ACT treatment protocols with proven efficacy for both smoking and excess weight and 2) conduct a preliminary randomized controlled trial (RCT) to compare this novel ACT intervention to a general health control that equates for intervention time.

CAUSAL LEARNING IN CHILDREN THROUGH PRETEND PLAY

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RI-INBRE Summer Undergraduate Research Fellowship Program

Children are often taught rules, skills, and other cognitive tasks through pretend play. However, evidence that children truly learn from pretense has been mixed. This study builds on recent research showing that preschoolers did not learn a novel causal rule through pretend play. To test whether multiple people pretending helped children transfer novel causal rules from pretense to reality, two different experimenters demonstrated the novel causal rule. The first experimenter introduced a box as a pretend "blicket" detector and four blocks to preschoolers (N = 58). The experimenter pretended that two of the blocks (either two red blocks or two square blocks) were "blickets" by pretending that whenever the "blickets" were placed on the detector, a bell sound was made. The second experimenter repeated this exact procedure. Finally, after completing a distractor task, children were given a real "blicket" detector with four new blocks and were asked if they could "make the detector go". Results show that children were more likely to pick a blicket on the first try when the blickets were the same color, rather than the same shape. Further, older children learned better with two experimenters compared to just one, while there was no difference between one or two experimenters for younger children. Overall, this data suggests that children do not easily learn novel causal rules through pretend play.

ASSESSING DOMINANCE HIERARCHIES THROUGHOUT DEVELOPMENT IN SOCIALLY-HOUSED RATS

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RI-INBRE Summer Undergraduate Research Fellowship Program

Past research lacks robust conclusions about whether or not rats form social hierarchies as few research groups socially house rats. Investigating the social nature of rats is critical as they are the most widely used animal model for translation neuroscience and, like humans, are highly social. The present study assessed social hierarchies in ten group-housed Long-Evans rats throughout five stages of development: juvenility, early adolescence, late adolescence, early adulthood and late adulthood. In order to determine if a dominance hierarchy exists, social behavior was analyzed through two measures. First social observations of naturally occurring dominant-submissive interactions were observed in the home cage using focal sampling for at least five minutes per subject and the outcome of aggressive and submissive behaviors were recorded. Dominant behaviors included biting, pinning, mounting, and stealing food; submissive behaviors included retreating, and laying down on one's back in a submissive posture when approached by another rat. Second, a dominance based skill task, known as the tunnel task was implemented on every two-animal pair. 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 the naturally-occurring dominant and submissive behaviors and the tunnel task, social hierarchy orders were determined using Elo-ratings to reproduce a representative number which dynamically decreases or increases based upon the outcome of interactions. In order to obtain an objective level of overall sociality, animals were observed using scan sampling three times a day to determine which rats were close in proximity to each other during each scanning period. The extent to which dominance hierarchies are stable or shifting throughout development will be discussed and the relationship between dominance hierarchies and overall levels of sociality will also be evaluated.

THE EFFECT OF STRESS CONTROLLABILITY ON ANXIETY AND NOCICEPTION IN LATE ADOLESCENCE AND ADULTHOOD

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RI-INBRE Summer Undergraduate Research Fellowship Program

Previous anxiety models take into account the effect of adolescent stress on anxiety but have overlooked the importance of stressor control as a facilitator of anxiety reduction in late adolescence. Twenty-four rats were randomly selected and divided evenly into two conditions titled control and yoked--control rats had the ability to escape and avoid shock within an operant chamber. Yoked rats were subject to the same operant chamber outcomes as their paired control rat, but without said control over their stressor. We predicted that rats lacking control over their stressor would exhibit a greater amount of anxiety-like behavior in late adolescence through adulthood, when tested on an elevated plus maze (EPM). Yoked rats spent more time in a closed area and were not as explorative as control rats, demonstrating a greater amount of anxious behavior. Similarly, we predicted and observed a trend that rats with stressor control would exhibit increased pain tolerance as measured by the Hargreaves plantar test.

CIRCADIAN RHYTHM EFFECTS ON CELLULAR RESPIRATION IN NEURAL CELLS OF MALE FISHER RATS

Michael DeCapua, Pamela Snodgrass-Belt

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Independent Research

Circadian rhythms are approximately 24 hour internally generated oscillations that allow organisms to anticipate their environment and predict events rather than simply respond to them. These clocks can be measured using different methods, including behavioral changes that can be quantified by output rhythms like wheel running data, or molecular changes within individual cells that can be measured by quantifying gene regulation. Here we have chosen a novel approach to investigate circadian oscillations by measuring metabolic activity. The Seahorse Bioscience Flux Analyzer measures cell respiration. Comparing two groups of male fisher rats entrained to opposite light dark cycles, we expect different measurements of activity in different brain regions at different times of day or circadian times. Extensive research over the last 20 years has shown that every cell in the body has a clock and these molecular clocks are organized in a hierarchical manner with the master pacemaker found in the hypothalamus, the suprachiasmatic nucleus (SCN).1 We set about to measure oxygen consumption in the SCN with the extracellular flux analyzer (Seahorse Bioscience). Using intact cells, rather than isolated mitochondria, is a reasonable approach to directly evaluate cellular activity in this specific region and other regions within the brain.2 We have worked at developing and optimizing a procedure to investigate the differences in cellular metabolism of various brain regions at different circadian times in the rat model. Our efforts have led to improved techniques in isolating brain regions, sustaining brain tissue, standardizing tissue size, maximizing output readings from the Seahorse analyzer, and developing a method to get reproducible results.

ASSESSING OXIDATIVE DAMAGE TO RNA IN A DROSOPHILA MODEL OF ALS

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RI-INBRE Summer Undergraduate Research Fellowship Program

Amyotrophic lateral sclerosis (ALS) is an adult-onset neurodegenerative disease affecting upper and lower motor neurons. ALS is characterized by progressive paralysis and death by respiratory failure, usually within 2-3 years of symptom onset. Most cases of ALS are sporadic, occurring in people with no prior known family history, but 10% of ALS cases are familial (fALS) and inherited. Over 150 point mutations in the Cu/Zn superoxide dismutase 1 (SOD1) gene cause fALS resulting in a toxic gain-of-function of the SOD1 protein. Numerous cellular and molecular processes contribute to disease pathogenesis and oxidative stress is a prominent feature implicated as an early pathological event. While major consequences of oxidative stress are established, including DNA damage, lipid peroxidation and protein misfolding, the effects of oxidative damage on RNA have not been well characterized. A Drosophila model has been developed in which a point mutation associated with human fALS (H71Y) has been introduced using homologous recombination. Using a monoclonal antibody (15A3) that recognizes hydroxylated guanine, a nucleotide modification resulting from free radical toxicity, oxidative damage to RNA was assessed. Preliminary results show increased levels of cytoplasmic hydroxylated guanine in neurons of sodH71Y mutants compared to neurons in wild-type flies. Further experimentation will assess total oxidative species as well as ratios of oxidized and reduced glutathione in Drosophila.

ASSESSING ADOLESCENTS' STRESS RESPONSE TO A PARENT-ADOLESCENT VISUAL STIMULUS

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RI-INBRE Summer Undergraduate Research Fellowship Program

Transitions within important interpersonal relationships confront adolescents with new challenges that impact their regulatory abilities and may increase reactivity of underlying physiological and biological systems. Despite the stress that these transitions may induce, few studies have examined the effect that interpersonal stressors, particularly within the family, have on adolescents' neurobiological stress response. Related research has suggested that acute stress causes functional and structural changes in the prefrontal cortex and amygdala, areas associated with cognitive control and emotion, as well as increases in physiological markers of stress associated with HPA-axis and ANS arousal. Thus the purpose of the current study was to examine the effect of a visual stimulus designed to portray parent-adolescent conflict on neural and physiological indices associated with the way adolescents' bodies handle stress. This visual stimulus represented varying levels of conflict between a mother and adolescent (positive, normal, and hostile) and heart rate was obtained during this task. Furthermore, we examined if the visual stimulus we developed demonstrated ecological validity from the perspective of the participants in the study. To date, data has been collected from 15 participants (57.1% females, M age =14.7, SD =0.91) to examine the effect of the parent-adolescent stimuli on adolescents' neurobiological response to stress as measured by heart rate, blood pressure and self-report of affect. Quantitative data also has been gathered from participants to assess their perceptions of the ecological validity of the parent-adolescent stimulus. Results suggest that there are slight increases in physiological measures for adolescents' systolic and diastolic blood pressure as well as decreases in their positive affect after the visual stimuli, however, heart rate does not appear to show the same increases. During the parent-adolescent stimulus, there were also increases in heart rate during the hostile visual stimulus with comparison to the positive and normal stimuli. As we are just in the initial stages of data collection no firm conclusions may be drawn but results are promising and generally support the association of the parent-adolescent stimulus with neural and physiological indices of stress response. Responses from participants suggest that the developed stimulus appears to be realistic and mirrors conflict that they experience with their parents.

CIRCADIAN RHYTHMS AND FEEDING ENTRAINMENT

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Independent Research

To increase their ability to efficiently internally and externally use resources, organisms rely on their internal biological clocks to predict daily environmental events. Light, the predominant internal signal, controls oscillations in locomotors activity as well as endocrine and cellular activity. Most cells in the body have been shown to contain molecular clocks that are synchronized to the master clock found in the suprachiasmatic nucleus of the hypothalamus (SCN). Most animals move around and feed during their wake cycle. Self-reinforcing entraining signals are created to help set peripheral clocks in cells outside the SCN. Extensive research shows restricting food to short periods of time can also entrain circadian rhythms. Here we investigate what would happen if feeding were not reinforced by the light dark cycle. This is done by only providing food for some groups outside the normal reinforced light dark cycle, similar to the effects of mixed entraining signals. In one group, animals were given food for eight hours in the middle of a twelve-hour light cycle; in another group, animals were given food for eight hours in the middle of a twelve-hour dark cycle. These data was compared to animals that were allowed to feed when they wanted on the same 12/12 light dark cycles. Using changes to the molecular clock in the live, changes in BMAL, PER using real time qPCR were measured.

EXAMINING THE LOWER BOUNDS OF VERB COMPREHENSION

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RI-INBRE Summer Undergraduate Research Fellowship Program

In the early stages of learning to talk, children acquiring English produce many words for objects (nouns) but few words for actions (verbs). This discrepancy may be due to input factors such as frequency, saliency, and the kinds of words that parents elicit and reward, and/or differences in the cognitive and semantic complexity of nouns versus verbs. One approach to this problem examines whether children also lag in their comprehension of verbs. The goal of our research is to examine the course of verb comprehension across the second year. We use the Preferential Looking Task (PLT) to assess comprehension. The PLT measures visual attention to a target versus a distracter image before (baseline trial) and after (test trial) the target image is labelled. Nouns are tested using photographs of objects; verbs are tested using videos of actors performing actions. Comprehension is defined as an increase in visual attention to the target image during test compared to baseline. Previous research using the PLT with 3-4 sec baseline and test trials has found that children comprehend nouns as early as 6 months, but no verbs until 18 months of age, suggesting late onset of verb comprehension which parallels the data on production. However, recent work on the development and neurological foundation of visual attention suggests an alternative hypothesis: dynamic stimuli elicit longer bouts of visual attention and may be mediated by different neurological processes than attention to static displays. This suggests that children may require trials of longer duration to inspect and interpret the dynamic stimuli
used to test verbs. We test the hypothesis that children assessed with increased baseline and test trials will demonstrate verb comprehension. We tested 12, 14, 16, 18, and 20-month-olds on 12 verbs using baseline trials of 10 sec and test trials of 5 sec. Visual attention to target and distracter images during baseline and test trials was recorded by a Tobii T60XL eye tracker system. Results suggest that verb comprehension begins well before 18 months of age and increases significantly across the second year.

METAMEMORY IN RATS

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RI-INBRE Summer Undergraduate Research Fellowship Program

Metamemory is the ability to cognitively assess the strength of one's own memories. There is strong evidence for the existence of metamemory in non-human primates and although researchers have investigated metamemory in rodents, results are not robust. Metamemory can be operationally defined as improved performance on choice trials when there is an option to decline tests when memory is weak as compared to forced trials in which there is no such option. We tested the ability of ten Long-Evans rats to distinguish between remembering and forgetting by presenting a decline option that allows the four-choice match to sample (MTS) to be by-passed. If animals have metamemory, they should choose to take tests in which they remember the correct response as this choice leads to a preferred reward. Correspondingly, they should decline tests when they forget as it leads to a guaranteed but less preferred reward. To establish these reward contingencies associated with correct MTS selection and the decline response, preference tests were implemented. After demonstrating a preference towards one of two food rewards, rats learned a four-choice MTS task in which the sample was a sand-filled and scented cup. After sampling the odor in one room, rats entered the MTS room where they were rewarded with the preferred reward after digging in one of four cups that contained the matching odor from the sample phase. Retention intervals were then titrated for each animal until the rate of successful DMTS trials fell in the range of 40%-70% so that subjects had experience with both remembering and forgetting, answering successfully and unsuccessfully. Subjects were also introduced to a decline response in the decline room, in which an unscented, sand-filled cup contained a less preferred reward. Finally, the rodents entered the metacognitive testing phase where they experienced sessions that were comprised of 25% forced trials in which the decline room was not open. The remaining 75% of trials were choice trials in which both the doors to the MTS room and decline room were open. Preliminary results will be discussed.

TODDLERS DO NOT APPRECIATE THE ROLE OF THE MIND IN PRETENSE

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RI-INBRE Summer Undergraduate Research Fellowship Program

Young children are known to pretend and understand others pretense around two years of age. However, research has shown that children do not correctly understand pretense as a mental state until age six, instead claiming that pretense is solely a behavior. The current study tested 17-30 month olds (N = 69) on whether or not they implicitly understand pretense as a mental-based or action-based state. Children witnessed an experimenter correctly pretend with both a familiar and a novel object in one of two conditions. In the acting-as-if condition, the experimenter claimed the novel object was unknown to her, but she pretended with it correctly (an unexpected event). For the knowledge condition, the experimenter claimed to know what the novel object was and pretended with it correctly (expected event). The amount of time children spent looking at the experimenter while pretending with each object was measured. If children look longer in the acting-as-if condition, it would suggest that toddlers appreciate pretense as a mental state. Children also participated in a comprehension of pretense assessment and age-appropriate executive function tasks (inhibitory control, delay of gratification, and working memory). In addition, parents were asked to complete two parent measures regarding their children's pretense and temperament. Results show that children looked more at the familiar object than the novel object, but there was no difference between conditions. Additionally, children with higher executive function scores scored higher in the comprehension of pretense assessment. These data suggest toddlers do not appreciate the role of knowledge in pretend play.

GENETIC ASSOCIATION OF GABRG2 AND BODY DYSMORPHIC DISORDER IN A MOUSE MODEL

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Body dysmorphic disorder (BDD), marked by preoccupation with a perceived defect in physical appearance, is associated with high rates of functional impairment, suicide ideation and suicide attempts (Phillips, Mernard et al. 2002). Symptoms of BDD often manifest in patients during adolescence (Phillips, Didie et al. 2005). 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). Exploring the genetic impact of this mutation, GABRG2 mutant mice underwent home cage monitoring and behavioral assays to assess anxiety, depression and fear conditioning. Further, a cohort of p30 mice was exposed to TMT to investigate the impact of an adolescent stressor.

EXPRESSION OF VOLTAGE-SENSITIVE SODIUM CHANNELS IN DEVELOPING RAT BRAINS

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Voltage-sensitive sodium channels (VSSCs), are multisubunit ion channels that are responsible for conducting electrical impulses in neurons. VSSCs are composed of the pore-forming α-subunit and two β-subunits that regulate the kinetics of the channel. In these experiments, we used automated western blotting to characterize the level of expression of VSSC α- and β-subunit isoforms in post-natal day (PND) 15 and 90 rat brain tissue. Results indicate that Nav1.2 was the most abundantly expressed isoform in both the PND15 and PND90 samples. Nav1.6 appeared to be uniquely expressed in PND90. Preliminary results with the β-subunits indicate that the β1 and β2 subunits are the most-expressed subunits in the PND15 and PND90 rat brain tissues and that the β-subunit in the PND90 tissues was also heavily glycosylated compared to the PND15. Future studies will investigate the hypothesis that pyrethroid insecticides (VSSC agonists) will exhibit age-dependent toxicity due to the difference in VSSC expression.

CHARACTERIZATION OF RAT BRAIN TISSUE MICROTRANSPLANTED IN XENOPUS LAVEIS OOCYTES

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Recently, the National Research Council has called for a new vision of toxicity testing that integrates cellular response pathways. New toxicity testing should be capable of assessing chemicals and mixtures in a comprehensive manner that is efficient and reliable. New approaches should be able to assess different species, life stages, and genders. To this end, we evaluated microtransplantation of rat brain tissue isolated from different life stages into Xenopus oocytes. This approach allows for the examination of native proteins and receptors that have been post transcriptionally and translationally modified by the host system. In these experiments, we characterized rhodamine labeled tissue isolated from post-natal day 15 and 90 rats. Results indicated that rhodamine labeled tissue for both developmental ages is nontoxic to the oocytes. Furthermore, there was no difference in frequency of incorporation for PND15 or PND90 tissues. The results confirm that microtransplantation is a viable method of toxicology testing in which native receptors are successfully microtransplanted.

EXPRESSION OF HUMAN N-TYPE VOLTAGE-SENSITIVE CALCIUM CHANNEL INTO XENOPUS LAEVIS OOCYTES

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N-type voltage-sensitive calcium channels are membrane proteins found primarily at presynaptic nerve terminals, and are responsible for the control of neurotransmitter release. Evidence suggests that voltage-sensitive calcium channels are involved with the development of the acute neurotoxic response caused by pyrethroids, a class of synthetic insecticides widely utilized in agriculture and urban environments. The goal of the present study was to co-express the α and β subunits of human N-type voltage-sensitive calcium channel (Cav2.2) into Xenopus laevis oocytes in order to assess the effects of the pyrethroid deltamethrin on the operation of the expressed channels. We obtained ultrapure plasmid DNA for both α and β genes, as well as linearized those plasmids utilizing restriction enzymes. We used the linearized α and β subunit genes as a template for the in vitro transcription of cRNAs. cRNAs were then injected into defolliculated Xenopus laevis oocytes to examine the effects of pyrethroid insecticides the functional Cav2.2 channel using two-electrode voltage clamp. It is expected that pyrethroids will be agonists of human Cav2.2.

THE EFFECT OF LEAD EXPOSURE ON THE SPATIAL LEARNING OF TAU KNOCKOUT MICE

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Alzheimer's disease (AD) is a neurodegenerative disease that results in severe loss of memory and other intellectual abilities. It is the sixth leading cause of death in the United States and has no known cure or cause at this point. AD is pathologically characterized by the presence of amyloid beta (Aβ) plaques and neurofibrillary tangles (NFT) composed of hyperphosphorylated tau, a protein that stabilizes neuronal microtubules in the brain.

Previous work by the lab has shown that primates and mice exposed to lead (Pb) early on in life have an increase in AD related biomarkers such as Aβ and tau (Bihaqi and Zawia, 2013; Bihaqi et al., 2014), as well as learning deficits (Bihaqi et al., 2013). These spatial learning deficits were shown in the Pb exposed mice. In addition, the lab has shown that early developmental exposure to Pb increases the amount of tau in a human tau transgenic mouse model (Dash et al., unpublished data). These mice have the human tau gene and lack the mouse tau gene.

To further confirm if an increase in tau results in memory deficits, Pb exposed mice that do not contain the human or mouse tau gene were used in this study. Our study compared the transgenic Pb exposed mice to two controls: mice not exposed to Pb (non-carrier) and wild-type mice that are not genetically altered. We looked to see if these Pb exposed mice would develop AD-like behavior even though they lacked tau completely. The control and Pb exposed mice were compared using behavioral tests designed to assess memory and spatial learning.

Our results showed that both the control knockout mice and the Pb exposed mice performed worse in behavior tests when compared to the wild type mice. The performance of the Pb exposed mice was significantly different from the wild type mice; however, the unexposed non-carrier mice exhibited a trend of lower performance that was not statistically significant. The appearance of a trend this early in life suggests that these mice lacking tau would probably be highly compromised, as they get older. At this stage, we were unable to determine how much of the difference in cognitive test can be attributed to the additional Pb exposure rather than the lack of tau. Data from the lab reported wild type mice exposed to Pb do not show deficits until much later on in life (Bihaqi et al., 2013). Therefore the combination of a genetic deficit with and an environmental challenge may have a greater impact than either alone.

COMPONENTIAL ANALYSIS OF VISUAL ATTENTION AND JUDGMENT DATA: WHITES' PERCEPTIONS OF BLACKS

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Visual attention is a necessary first step in the processing of information; if a stimulus is not attended to, it will likely have no effect on judgments or behavior. For this reason, a theoretical and statistical analysis of visual attention is important. Most research on visual attention assumes that the stimulus (e.g., a person's facial features, gender or race) is the prime determinant of visual attention. Presumably, features of the stimulus capture attention and this permits further processing in memory. We argue that visual attention is much more complicated, and propose that there are individual differences in visual attention among perceivers when exposed to the same stimuli (i.e., the perceiver effect), differences among stimuli in the visual attention they elicit (i.e., the target effect), and unique attention to specific stimuli by specific perceivers. Each of these effects are estimated using variance component analysis. Result show that visual attention measurements have a complicated componential structure that should not be ignored.

AMYLOID PRECURSOR PROTEIN LOCALIZES TO THE POST-SYNAPTIC TERMINAL

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Published studies on the distribution of the amyloid precursor protein have reported APP to localize to the presynaptic terminal and to directly associate with synaptic vesicles through the co-localization of APP and synaptic vesicle markers as well as through immuno-electron microscopy. However, in the current study we find APP to localize to a unique domain adjacent to the synaptic vesicle pool providing evidence that contradicts the published work. In lamprey spinal cord APP and the synaptic vesicle marker SV2 are in close proximity, but show distinct staining patterns. To help determine whether APP is in the pre or post-synaptic terminal, we studied APP distribution in the well-organized vertebrate retina. Here we discover that APP is further from the cleft than the post-synaptic density protein PSD-93. Studies at the EM level will be needed to determine the structures associated with this disease causing protein.

THE AMYLOID PRECURSOR PROTEIN OF ALZHEIMER'S DISEASE IS ABUNDANT AT THE TRIPARTITE SYNAPSE

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The amyloid precursor protein, a causative agent in Alzheimer's disease, has been and remains a molecule under intense investigation, however the wildtype function of APP and its role in causing disease is not well understood. Here, we find that APP localizes to a discrete domain near the synaptic cleft in rare synapses of yet unknown parent cells of the vertebrate retina. These synapses occur in both the inner and outer plexiform layers. Using markers against APP and astrocytes as well as pre and post-synaptic proteins, we find APP on the presynaptic side of the cleft and close to astrocytes in the tripartite synapse. At the light level, we are unable to determine whether APP is in the presynaptic terminal or with the astrocyte. Localizing APP at the EM level is ongoing.