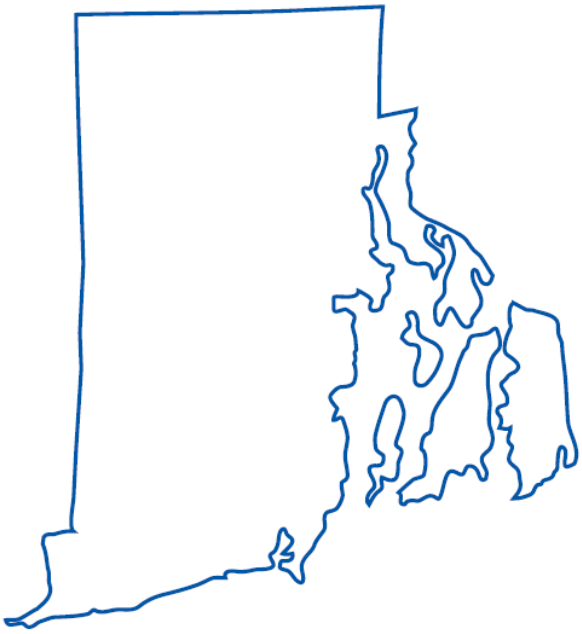
2014 Rhode Island Summer Undergraduate Research Fellowship Conference



Friday, August 1, 2014
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

7TH ANNUAL RHODE ISLAND SUMMER UNDERGRADUATE RESEARCH FELLOWS CONFERENCE

FRIDAY, AUGUST 1, 2014
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. DAVID DOOLEY, PRESIDENT, UNIVERSITY 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
	– DR. BREEA GOVENAR, DEPARTMENT OF BIOLOGY, RHODE ISLAND COLLEGE
	– KELSEY LUCAS, GRADUATE STUDENT, HARVARD UNIVERSITY
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

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.

web.uri.edu/pharmacy/academics/graduate

The Office of Graduate & Postdoctoral Studies, Division of Biology & Medicine, Brown University

The mission of our office is to provide an outstanding training environment that fosters learning and development for our biomedical scholars.

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

Graduate School, University of Rhode Island

A community of innovative scholars committed to creating new knowledge by bridging the realm of the present with the realm of the possible.

www.uri.edu/gsadmis

Metcalf Institute for Marine & Environmental Reporting, Graduate School of Oceanography, University of Rhode Island

The Metcalf Institute for Marine & Environmental Reporting is an international leader in providing science training for journalists.

metcalfinstitute.org

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.

web.uri.edu/ceoc

Tours

Medicinal Garden Tours	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.

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**	**Poster List on Page #**
9:30 AM – 11:00 AM	Molecular Biology	19
	Genetics	11
	Marine Sciences	12
	Neuroscience	23
11:00 AM – 12:30 PM	Cell Biology	1
	Chemistry	4
	Environmental Sciences	8
	Microbiology	17

CELL BIOLOGY

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

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

THE DISTRIBUTION OF CELL TYPES IN NANOMIA BIJUGA (HYDROZOA: SIPHONOPHORA)

Samuel Church, Stefan Siebert, Pathikrit Bhattacharyya, Casey Dunn, *Department of Ecology and Evolutionary Biology*, Brown University, Providence, RI; Steve Haddock, *Research Institute*, Monterey Bay Aquarium, Moss Landing, CA

RI NSF EPSCoR Summer Undergraduate Research Fellowship Program

The siphonophore Nanomia bijuga is a pelagic hydrozoan (Cnidaria) with a complex colonial organization. The colony is composed of genetically identical yet morphologically and functionally distinct zooid types, which predominantly arise in two growth zones. We describe the cellular anatomy of these zooid types as well as the stem and gas-filled float, called the pneumatophore. In particular we focus on the distribution of cell morphologies across zooids and relate those to hypotheses about zooid function. We pay specific attention to the organization of these cells into tissue layers and highlight areas of epithelial complexity. This work provides a foundational framework for gene expression studies and further understanding of the division of labor within a cnidarian colony.

DIET COMPOSITION AFFECTS THE GLYCOCONJUGATE COMPOSITION OF SUMMER FLOUNDER TISSUES

John Da Lomba, *Department of Biology*, Community College of Rhode Island, Warwick, RI;
Bruno Soffientino, Graduate School of Oceanography, University of Rhode Island, Kingston, RI;
Marta Gomez-Chiarri, Department of Fisheries, Animal and Veterinary Science, University of Rhode Island, Kingston, RI

RI NSF EPSCoR Summer Undergraduate Research Fellowship Program

Summer flounder (Paralichthysdentatus), a popular carnivorous fish in New England, is an important candidate for aquaculture development. Two of the major challenges of marine aquaculture are the cost of feeds and disease. The inclusion of plant (e.g. soybean) proteins as a replacement for fish meal in the diets of marine carnivorous fish may lead to economical advantages and increased sustainability.Anti-nutritional factors in soybean meal (SBM), such as saponins and oligosaccharides, can lead to decreased growth, but interestingly, previous research has shown that these products may also lead to increased disease resistance in summer flounder.The objective of this research is to further evaluate the mechanism by which oligosaccharides and saponinsdecrease growth and increase disease resistance in summer flounder. Glycoconjugates are known to act as binding sites for disease causing bacteria, so I looked whether glycoconjugate composition of the cells in summer flounder tissues were associated to changes in dietary composition.Lectins, proteins of plant origin which bind specifically to different glycoconjugateson cells, can be used to monitor changes in glycolysation of summer flounder cells. Tissues from 2 feeding trials were evaluated. Experimental diets included: 1) fish meal control diet (FM); 2) purified soy protein concentrate (SPC) with no antinutritional factors; 3) SPC diet supplemented with the water fraction of SBM (rich in oligosaccharides); 4) SPC diet supplemented with the butanol fraction of SBM (rich insaponins); and 5) SPC diet supplemented with 0.4% of purified oligosaccharides.No changes in staining was observed in the liver and spleen of fish fed the different diets. Differences in lectin(UEA 1, SBA, and RCA 120) binding to cells in summer flounder intestines were noted between fish fed the FM control and both the saponinandthe oligosaccharide-containing diets. For UEA I, staining decreased in goblet cells of fish fed saponins and oligosaccharides compared to fish fed the FM diet. For RCA 120, staining was highest in brush border and lamina propria of fish fed the FM and the SPC diet from one of the trials.Major differences in SBA staining intensitywere noted in fish fed saponin between thebrush border and goblet cells of the anterior intestines.These complex changes might have implication in the increased disease resistance associated with oligosaccharide-containing diets.

MTOR AND PKM2 ARE CONSTITUTIVELY UP-REGULATED IN HUMAN MELANOMA CELLS BUT NOT IN MELANOCYTES

Jeanine Justiano, Ryan Garrity, *Department of Biology*, Providence College, Providence, RI; Alfredo Gonzalez, *Department of Chemistry and Biochemistry*, Providence College, Providence, RI; Diana Jacques, *Department of Biology*, Northeastern University, Boston, MA; Daniel Louis, Elizabeth Myrus, Classical High School, Providence, RI

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Melanoma is considered one of the most aggressive and less treatable cancers. While various models and mechanisms have been proposed, the transformation from tranquil melanocytes to invasive melanoma cells remains an enigma. Pyruvate kinase M2 has been shown to be an important constituent of the Warburg effect (a hallmark of cancer) and an increase of this kinase correlates with increased glycolysis and an up-regulated mTOR pathway. Despite constituent activation of the mTOR/AKT pathway in melanoma cells, it is still not known whether pKM2 is up-regulated upon melanocyte transformation to melanoma. In order to study PKM2, we compared the cellular response to α-melanocyte stimulating hormone (or α-MSH) in both primary human melanocytes and melanoma (WM266-4 Cell line). Both types of cells were treated with α-MSH. Immunoblots and confocal microscopy were use d to visualize the activity of mTOR and pKM2 at different time points after treatment. Our results showed that α-MSH activates mTOR pathway in both human melanocytes and melanoma cells, while melanoma cells exhibit constitutive activation of mTOR. We found that PKM2 is also constitutively up-regulated in melanoma cells. Interestingly, our experiments did not show a change in the level of phosphorylation of pKM2 in both types of cells in response to α-MSH treatment.

GALLIC ACID AS A NUTRACEUTICAL TREATMENT FOR GASTRIC ADENOCARCINOMA MKN28 CELLS

Gwen Beaman, Rhiannon Morrissey, Meaghan Trzasko, JD Swanson, Kari Clifton, *Department of Biology and Biomedical Sciences,* Salve Regina University, Newport, RI; Steven Moss, *Warren Alpert School of Medicine,* Brown University, Providence, RI

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Gallic acid (GA) is a phenolic compound found in the prickles of Rubus plants, such as raspberries, strawberries and blackberries, which has shown anti-cancer properties and may have applications as a nutraceutical cancer treatment. GA is a secondary cell metabolite stored in the glandular trichome head of Rubus plant prickles that seems to have proliferative effects. However, GA has anti-proliferative effects on human cells, specifically cancer cells, seemingly without harming healthy cells. GA has previously been tested on AGS cells, a gastric adenocarcinoma cell line, in this lab. An additional cell line (MKN28) has been introduced to examine and compare the affect of GA on this morphologically different adenocarcinoma. Its morphology classifies it as well-differentiated adenocarcinoma, whereas AGS is classified as poorly differentiated; MKN28 cells are predicted to react differently than AGS cells, making it a good comparison cell line for GA treatment. To examine the determine ideal GA exposure to arrest the cell cycle, several doses and time points are measured. GA dosages that have been examined in AGS are as follows: 0uM, 25uM, 40uM and 60uM; cells treated at each dose were then harvested at time points of 6 hours, 12 hours and 24 hours, a time 0 control was also used for comparison to treated cells. Flow cytometry was then used to determine the effect of GA on the growing cells by examining the proportion of cells in G1/G0 versus G2/M phase. These tests have been done on AGS cells but MKN28 have not been used in this context. To provide a viable comparison between the two levels of differentiation, these dosages and time points will be kept consistent across both AGS and MKN28 experiments. We predict that GA will arrest the cell cycle of MKN28 as it has been shown to do in AGS cells indicating its effect on gastric adenocarcinomas is not isolated to one specific morphology and may have applications as a nutraceutical cancer treatment.

MODULATION OF OVARIAN CANCER CELL METABOLISM VIA PYRUVATE KINASE M2 (PKM2) AND GLUCOSE DEPRIVATION

Jeanine Justiniano, Ryan Garrity, Marla Tipping, Yinsheng Wan, *Department of Biology*, Providence College, Providence, RI; Alfredo Gonzalez, *Department of Biochemistry and Chemistry*, Providence College, Providence, RI; Diana Jacques, *Department of Biology*, Northeastern University, Boston, MA; Daniel Louis, Elizabeth Myrus, Classical High School, Providence, RI

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Ovarian cancer remains one of the most aggressive types of cancer in women. While the molecular mechanisms of the invasiveness of ovarian cancer have been extensively studied, the treatments for ovarian cancer are still limited. Understanding the metabolism of ovarian cancer cells has been of great interest to many scientists. Cancer cells express a higher consumption rate of glucose which results in accumulation of lactate even in an oxygenized environment. This alteration in metabolism is known as the Warburg effect and is necessary for the growth and survival of cancer cells. Pyruvate kinase M2 or PKM2 has been shown to be a regulator enzyme responsible for the conversion of PEP to pyruvate. Recent studies have demonstrated that PKM2 is over-expressed in tumor cells. However, its functions are yet to be elucidated. In this project, we investigated the eff ects of glucose deprivation on ovarian cancer cells (CaOV3 cell line). The results showed that reduction of glucose supply in culture medium affects cells morphologically and metabolically. MTT assay data showed that glucose alters cellular proliferation in a dose dependent manner. Lower glucose supply results in alkalinization of culture medium. Seahorse data indicated that glucose deprivation reduces oxygen consumption rate of ovarian cancer cells. Western blot analysis and confocal microscopy demonstrated that PKM2 expression is altered in the cytoplasm in response to glucose deprivation. PKM2 level starts to decrease 30 minutes post 1 mM glucose treatment, reaches to the lowest levels at 1 hour, and recovers within 2 hours. Collectively, our data suggest that PKM2 may be a molecular target for ovarian cancer treatment and glucose deprivation may be a potential approach for modulation of PKM2.

UV RADIATION AND OXIDATIVE STRESS ALTER CELLULAR METABOLISM IN CULTURED HUMAN SKIN KERATINOCYTES

Ryan Garrity, Alfredo Gonzalez, Jeanine Justiniano, Diana Jacques, Marla Tipping, *Department of Biology*, Providence College, Providence, RI; Daniel Louis, Elizabeth Myrus, Classical High School, Providence, RI

RI-INBRE Summer Undergraduate Research Fellowship Program

UV radiation and oxidative stress have been shown to be related to skin aging and skin cancer. Our previous studies have demonstrated that UV radiation induces generation of reactive oxygen species that leads to MAP kinase activation and MMP expression with a result of collagen reduction in cultured human skin keratinocytes in vitro and in human skin in vivo. We have also found that UV radiation and oxidative stress induces dehydration in human skin cells. Existing data have indicated that oxidative stress may alter cellular metabolism. We hypothesized that UV radiation and oxidative stress may also change metabolism in skin cells. Here we examined the effects of UV radiation and hydrogen peroxide on the metabolism of cultured human skin keratinocytes (HaCat cells). Western blot analysis showed that UV radiation inactivates PKM2 in keratinocytes in a time dependent manner. Seahorse data showed that UV radiation and H2O2 treatments decrease oxygen consumption rate (OCR) in HaC at cells. mTOR inhibitor Rapamycin increases oxygen consumption in both untreated and H2O2 treated cells. However, ERK inhibitor U0126 increases OCR in untreated cells, but decreases in H2O2-treated cells. PKM2 activator DASA increases OCR in untreated cells but decreases OCR in H2O2 treated cells. Given that H2O2 is a recognized inhibitor of PKM2, we conclude that inhibition of PKM2 by UV radiation and oxidative stress may directly affect oxygen consumption and glycolysis in human skin cells.

UNDERSTANDING THE ROLE OF UBE4B IN SKELETAL MUSCLE DEVELOPMENT

Kayla Hersey, Sarah Spinette, *Department of Biology*, Rhode Island College, Providence, RI

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Ube4b is an E3 ligase that functions to degrade specific proteins. Studies suggest that Ube4b functions as either a tumor suppressor or oncogene, and is expressed throughout the development of skeletal muscle, or myogenesis. Myogenesis involves proliferation, apoptosis, and differentiation, processes that can all be mis-regulated during cellular transformation. We may better understand the role of Ube4b by inhibiting its activity in cells of the skeletal muscle lineage during development. In order to do this a line of transgenic mice was developed in which the U-box domain of Ube4b was removed only in skeletal muscle through expression of iCre-recombinase, which is controlled by the MyoD promotor. Mice with the mutant genotype, ΔU/ΔU, MyoDiCre+, appear much smaller in size compared to control mice approximately four days after birth. These observations led us to question whether muscles of mutant mice may contain a reduced number of myofibers, or myofibers that are smaller in size. A reduced number of fibers could be due to fewer satellite cells during embryogenesis and early postnatal development. Smaller myofibers may indicate that individual fibers are not growing normally postnatally due to reduced hypertrophy. This could be due to a defect in myoblast fusion, fewer activated myoblasts resulting from decreased numbers of activated satellite cells or reduced proliferation. In order to test these hypotheses mutant and control littermates were dissected at different ages and hind limb muscles were snap frozen. Serial transverse sections of these muscles were cut and stained. Analysis of these cross sections showed that mutant mice have a larger number of smaller myofibers and a decreased number of large myofibers compared to control mice four days after birth. In order to determine the number of activated satellite cells and myoblasts per myofiber, immunohistochemistry was performed using an antibody to Pax-7 in order to mark satellite cells , DAPI to mark the nuclei, and WGA-Texas Red to mark the sarcoplasmic membranes. Results showed no difference in the number of Pax-7+ cells per myofiber between mutant and control mice. Additional experiments have been performed using antibodies to Ki-67 and MyoD in order to determine if these satellite cells are proliferating into myoblasts.

ANALYSIS OF ISOCITRATE DEHYDROGENASE (IDH) GENE FUNCTION IN MANIPULATING GLIAL CELL METABOLISM

John Mills, Marla Tipping, *Department of Biology*, Providence College, Providence, RI

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We are interested in the metabolic changes that occur in various disease states. To investigate this we are studying the isocitrate dehydrogenase (IDH) gene. Mutations in IDH lead to glial cell tumors, which switch metabolic program and become highly glycolytic. Our goal is to analyze these changes using both the Seahorse Biosciences XF Flux Analyzer to measure basic metabolism, and real time polymerase chain reaction (RT-PCR) to measure the levels of metabolic gene expression. The metabolic affects due to IDH point mutations, or loss of function (LOF) or gain of function (GOF) of IDH have not been fully studied. To study this Drosophila S2R+ and S3 cells were optimized for metabolic analysis using the XF flux analyzer. We will continue to optimize this technique for Drosophila tissues as well. We have also generated three dsRNAs that target different regions of the IDH mRNA to use in both the XF flux analysis and metabolic gene expression analysis. We have begun investigating metabolic gene expression in S3 cells treated with these IDH dsRNA, as well as in S3 cells overexpressing a mutant form of IDH. Future continuation of these studies will provide insight into the metabolic changes that occur in IDH mutant tissues.

IMMUNO-LOCALIZATION OF THE AMYLOID PRECURSOR PROTEIN OF ALZHEIMER'S DISEASE IN THE GOLDFISH SPINAL CORD AND RETINA

Sean Moran, Juliana Granato, Joseph DeGiorgis, *Department of Cellular Dynamics*, Providence College, Providence, RI

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The amyloid precursor protein of Alzheimer's disease contains a single transmembrane domain and associates with membrane bound vesicles. APP has been implicated in the adhesion of growth cones of developing neurons and has been hypothesized to have a role in synaptic formation. While a number of studies suggest that APP is found in the presynaptic terminal and to associate with synaptic vesicles, here we find that APP localizes to an adjacent but distinct region to the presynaptic vesicle pool labeled by the synaptic vesicle marker SV2. In addition, experiments using markers against the postsynaptic protein PSD95 show overlap with APP, thus suggesting that APP may be in the postsynaptic terminal, however the exact location of APP remains to be determined. Recently, we have begun to address this issue using pre-embedding immuno-electron microscopy where the synaptic regions can be defined and visualized. Our preliminary studies show that our fixation protocol is adequate for visualizing synapses and for identifying the pre and postsynaptic terminals while maintaining the antigenicity required for the detecting the APP molecule.

ADENOCARCINOMA CELL CYCLE ARREST AFTER GALLIC ACID TREATMENT

Rhiannon Morrissey, Gwen Beaman, Meaghan Trsasko, Adrianna Enxuto, JD Swanson, Kari Clifton, *Department of Biology and Biomedical Sciences*, Salve Regina University, Newport, RI; Songhua Zhang, Steven Moss, *Warren Alpert Medical School*, Brown University, Providence, RI

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Gallic acid (GA) is a secondary plant metabolite found in naturally occurring products, such as raspberries, blackberries, strawberries, and green tea. In plant cells, GA induces rapid cell proliferation; while in cancer cells, GA is suggested to effectuate cell cycle arrest. AGS is a commercial line of gastric adenocarcinoma; an epithelial cancer of the stomach lining. This makes AGS a suitable model system to test with GA, since GA would theoretically come into direct contact with AGS cells in vivo. Cells are cultured in F-12K medium under standard conditions until they reach 70-80% confluence, and are then passaged into 6-well plates at a seeding density of 2.5 x 105 cells per well. Each well of the plate receives a variable amount of GA solution for treatment, in the amounts of 0 uM, 40 uM, and 60 uM, respectively. The cells are harvested at time po ints of 0 hours, 6 hours, 12 hours, and 24 hours post-treatment and subjected to flow cytometry for cell cycle analysis. Flow cytometry indicates which phase of the cell cycle each cell in the sample is in, based on the cell's DNA content. This information gives an idea of where the GA is impacting the cycle of proliferation for these carcinoma cells. It has been shown in other cancer cell lines (ranging from esophageal cancer to colon cancer) that GA halts the cell cycle in the G2/M phase, which prevents cell proliferation and thus stops the growth of the cancer cells. Similarly, we found that there was a dose-dependent increase in the proportion of cells in the G2/M phase. This effect was attributed to cell cycle arrest induced by GA. Importantly, GA does not have this effect on healthy somatic cells. This selective cytotoxicity makes GA a potential candidate for use as a nutraceutical in the treatment of cancer.

DISTRIBUTION OF THE AMYLOID PRECURSOR PROTEIN IN THE SQUID GIANT SYNAPSE AND GIANT AXON: A LIGHT AND ELECTRON MICROSCOPY APPROACH

Erin O'Donnell, James Stevenson, Joseph DeGiorgis, *Department of Biology*, Providence College, Providence, RI

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The Amyloid Precursor Protein is a causative agent in Alzheimer's disease as mutations in the APP gene lead to familial forms of this disorder and a fragment of the APP protein builds up in brain lesions of afflicted individuals. APP is a type 1 transmembrane protein that spans the lipid bilayer of membrane bound vesicles and contains a highly conserved sequence at the C-terminal that extends into the neuronal cytoplasm. Here, we show that APP labels as puncta along the length of microtubules in extruded axoplasm of the squid giant axon by confocal microscopy. At the EM level, we find that APP distributes to the surfaces of isolated axoplasmic organelles and localizes to the organelle/microtubule interface suggesting that APP may be involved in axonal transport. By Western blot of sucrose density gradient fractions of isolated axoplasm all detectable APP is found in the organelle isolate. To determine the number of APP molecules in axoplasm and in the organelle fraction we have setout to use a quantitative Western blot approach. To this end, we have cloned squid APP and are currently in the process of overexpressing APP in E. coli. In addition, we have conducted preliminary studies to understand the distribution of APP at the squid giant synapse and have used a cryosection/immuno-confocal approach as a precursor to immuno-EM.

IN VITRO DEGRADATION AND CELLULAR UPTAKE STUDY OF HOLLOW COPPER SULFIDE NANOPARTICLES

Julie Scott, Liangran Guo, Wei Lu, *Department of Biomedical and Pharmaceutical Sciences*, University of Rhode Island, Kingston, RI

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Hollow copper nanoparticles (HCuNPs) have been used for cancer photothermal therapy in mouse models. Studies from our lab showed the nanoparticles can be metabolized. However, there is little information about the mechanisms of HCuSNPs' degradation. To explore this mechanism, we investigated the degradation behavior of pegylated HCuSNPs (PEG-HCuSNPs) in phosphate buffer saline (PBS, pH 7.4) and acetated buffer (50 mM, pH 4.9, a lysosome-mimic pH) for up to 1 week. We also tested the cellular uptake of PEG-HCuSNPs by primarily cultured mouse hepatocytes in vitro. Transmission electron microscopy (TEM) and energy dispersive spectrometry (EDS) results showed that PEG-HCuSNPs disintegrated into small CuS nanoparticles in both PBS and acetate buffer. The amount of copper ions in acetate buffer was 8.3-fold higher than that in PBS, suggesting accelerated degradat ion of the nanoparticles in acidic environment. The uptake of PEG-HCuSNPs by mouse hepatocytes depended on the nanoparticle concentration and incubation time. Taken together, our preliminary results revealed that the PEG-HCuSNPs can degrade in both PBS and acidic media, and be internalized by primarily cultured mouse hepatocytes in a concentration- and time-dependent manner.

DETERMINING WHETHER UBE4B ACTIVITY EFFECTS SKELETAL MUSCLE PERFORMANCE

Jeremy Boutin, Sarah Spinette, *Department of Biology*, Rhode Island College, Providence, RI

RI-INBRE Summer Undergraduate Research Fellowship Program

Skeletal muscle tissue develops through a complex process called myogenesis that is controlled in part by the regulated degradation of specific proteins. These substrates are degraded by the proteasome after being marked by the cell's ubiquitin conjugation system that is composed of three levels of enzymes (E1, E2 and E3). Ube4b is an E3 ligase enzyme that mediates ubiquitin conjugation through its U-box domain. To determine whether or not Ube4b plays a crucial role in skeletal muscle development and function, a transgenic line of mice expressing inactivated Ube4b was created. When iCre-recombinase is expressed it cleaves exon 26 of Ube4b that encodes for the catalytic domain (U-box) of Ube4B. A MyoD promoter is used to express iCre-recombinase in skeletal muscle causing the expression of inactivated Ube4b proteins in skeletal muscle cells. Mice lacking active Ube4b in their skeletal muscle exhibit reduced postnatal growth, skeletal muscle size and loss of sarcomere structur e within many skeletal muscle fibers. These observations have led to the hypothesis that Ube4b mutant mice should have diminished performance during strength assays. In order to test this hypothesis, mutant and control littermates were subjected to two strength assays: the hanging wire and grip strength tests. Reverse Transcriptase Polymerase Chain Reaction (RT-PCR) and Western Blots were used on skeletal muscle samples to see if an inactivated Ube4b was being expressed, as expected Ube4b mutants. The two strength assays showed that mice lacking Ube4b performed worse at two and four weeks old, but better at three weeks old. The data collected so far has not underlined an overall trend. Given the high variability in these assays there is not a sufficient sample size to reach a conclusion.

INVESTIGATING THE EFFECTS OF UBE4B INACTIVATION ON THE FORMATION OF SKELETAL MUSCLE IN MICE

Irina Maglysh, Maeghan Sullivan, Sara Spinette, *Department of Physical Sciences*, Rhode Island College, Providence, RI

RI-INBRE Summer Undergraduate Research Fellowship Program

There is currently significant evidence that abnormal regulation of E3 ligase enzymes, components of protein turnover mediated by the Ubiquitin-proteasome system, are involved in cellular transformation and cancer. New experiments have shown that this method of protein degradation also plays an important role in myogenesis which involves the same fundamental processes important for transformation, specifically, proliferation, apoptosis and differentiation. Ube4b, a ubiquitin E3 ligase has been implicated in both cancer and myogenesis, though the specific molecular mechanisms through which it acts are unclear. We hypothesized that we might better understand the role of Ube4b in cancer by determining how it may regulate these processes in normal cells during muscle development. To determine how inactivation of Ube4b affects the formation of skeletal muscle in v ivo, an iCre-dependent conditional knock-out mouse model was developed in which the U-box domain of Ube4b was removed only in skeletal muscle through expression of iCre-recombinase controlled by the MyoD promoter. It has been observed that mice lacking active Ube4b in their skeletal muscle have smaller muscles composed of smaller muscle cells and die prematurely. Our previous research suggested that NMIIa may be a substrate of Ube4b because of its increased and prolonged presence in the muscle of Ube4b mutants. Since NMIIa, a protein essential for myoblast fusion, is expressed transiently in vivo during the early postnatal muscle growth phase, it could be that the NMIIa is not getting degraded due to expression of inactive Ube4b and is therefore still expressed in the myotubes of mutant animals while it is degraded in those of control littermates. Tissue staining was performed to visualize NMIIa in the cell, however, further staining needs to be done to distinguish betwe en myoblasts and mature myofibers. Another possible explanation for the overall mutant phenotype is that the loss of Ube4b activity may cause muscle degeneration or result in increased apoptosis of myoblasts during postnatal hypertrophy. Degeneration would trigger muscle repair through increased activation and proliferation of satellite cells and myoblasts followed by increased myoblast fusion. This hypothesis was investigated through western blotting for PCNA, a marker of cell proliferation, and Cleaved Caspase-3, a facilitator of apoptosis.

TRACKING PHENOLIC ACIDS IN GLANDULAR TRICHOMES OF RASPBERRIES, ROSES, AND ROSE HIPS

Meaghan Trzasko, JD Swanson, *Department of Biology and Biomedical Sciences*, Salve Regina University

RI-INBRE Summer Undergraduate Research Fellowship Program

One of the features found in a number of plants in the genus Rubus is the development of prickles along their stems. Prickles are outgrowths of protective epidermal or cortical tissue that can be used in the deterrent of herbivores, structural support for plants that climb and even cell growth. According to our previous studies prickles develop throughout four stages. Of particular interest is stage II of prickle development where a glandular trichome forms consisting of a stalk connecting a glandular head to the rest of the plant. The glandular head is thought to store phenolics. Phenolics are secondary plant metabolites that are potentially used by the plant in cell growth and development, and being able to track these phenolic acids will provide a greater understanding of the exact roles they play in these types of plants. It is hypothesized that these ph enolic acids are sent as signals from the trichome head down the rest of the stalk, causing the epidermal tissue to produce more cells. Furthermore we hypothesize that if the trichome heads are removed, these signals can no longer be sent and the prickles will not continue to develop. By using diphenylboric acid 2-aminoethyl ester (DPBA) to stain the phenolics in the prickles of roses, raspberries and rose hips, we were able to track their movements under blue light with a dissecting fluorescence microscope. The length of the trichomes and distances the phenolics traveled down the prickle were recorded for both normal prickles and prickles with their glandular heads removed. The results were supportive of the original hypothesis that in all plants studied, phenolics travel from the glandular trichome head down the stalk of the prickle to the rest of the plant tissue causing cell proliferation to occur. Additionally, when the trichome heads are removed, the signals are no lo nger sent to the plant tissue and cell production slows by half. These results suggest that phenolics are sent as signals from the glandular head down the stalk of the trichome and these compounds may play a major part in prickle development.

EVALUATION OF THE CANCER-PREVENTIVE EFFECT OF RESVERATROL-LOADED NANOPARTICLES ON THE FORMATION OF TUMOR SPHEROIDS

Alexandra Tsoras, *Department of Chemical and Materials Engineering*, University of Kentucky, Lexington, KY; Samantha Meenach, *Department of Chemical Engineering and Department of Biomedical and Pharmaceutical Sciences*, University of Rhode Island, Kingston, RI

RI-INBRE Summer Undergraduate Research Fellowship Program

Lung cancer is the most common type of cancer worldwide, accounting for 13% of all deaths annually. Survivors of this cancer nearly always live with the possibility of cancer recurrence. The objective of this research was to develop a drug-loaded nanoparticle that decreases the likelihood of lung cancer recurrence by preventing the reformation of lung tumors. Nanoparticle encapsulation has been shown to increase drug bioavailability, enhance tumor targeting for localized delivery, and allow for more sustained release of drugs compared to free drug administration. The nanoparticles used for this project were resveratrol-loaded acetalated dextran nanoparticles (RSV:Ac-Dex NPs). The NPs were formed using a single emulsion process, encapsulating the hydrophobic drug RSV inside the tunable degradable polymer Ac-Dex. These particles were characterized in terms of morphology, size and zeta potential, drug loading, in vitro drug release and degradation. Particles loaded with RSV showed successful encapsulation of the drug, with particle size ranging from 150 - 250 nm in diameter. Particles also demonstrated sustained release of drug at both pH 7.4 and pH 6.5 to represent release in normal physiological conditions and local tumor environment conditions, respectively. The efficacy of RSV in A549 lung adenocarcinoma cell line was evaluated in both two-dimensional (2D) and three-dimensional (3D) cell culture conditions. Dose-response curves from the drug applications was compared with the cellular response to application of the RSV:Ac-Dex NPs. The diameters of 3D lung cancer multicellular spheroids (MCS) were measured when RSV was applied either before or after MCS formation in order to compare the effects of RSV as a preventive or treatment drug. Cellular response in A549 cells exhibited significant cell death with concentrations of RSV r anging from 50 - 250 μM, and yielded significantly smaller or nonexistent MCS when RSV was applied before formation. The delivery of the actual NPs for in vitro cellular response tests will be performed for comparison to free drug application. Overall RSV:Ac-Dex NPs are expected to be a useful and efficient chemotherapy to prevent recurrence of lung tumors for cancer patients in remission.

REGULATION OF MITOCHONDRIAL MORPHOLOGY DURING DENGUE VIRAL INFECTION

Sierra Valois, Diane Lang, Alan Rothman, Carey Medin, *Department of Cell and Molecular Biology*, University of Rhode Island, Kingston, RI

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). Previously we developed a plasmid-based reporter to allow robust non-destructive identification of DENV-infected cells and used this technology to look at organelle changes in DENV infected cells. Over time, we observed a significant increase in mitochondrial lengths in DENV infected cells at 48hr post infection. To understand the factors that play a role in mitochondrial elongation in DENV infected cells, we analyzed proteins involved in fusion (Mfn1, Mfn2 and Opa1) and fission (Drp1) of mitochondria. .Dynamin-related protein 1 (Drp1), a member of the Dynamin family of large GTPases, is a predominantly cytosolic protein that is recruited to mitochondria during fission. Drp1 is regulated by two serine phosphorylation sites; S616 and S637. Drp1 S616 phosphorylation activates fusion, whereas phosphorylation of Drp1 S637 inhibits mitochondrial binding and impairs fission. Our results show that Drp1 protein levels do not change during DENV infection. However, the level of phosphorylated Drp1 at S616 was decreased during DENV infection. Cells transfected with plasmids expressing Drp1 phosphorylation mutants show changes in mitochondrial morphology during DENV infection when compared to uninfected cells. The results of this project have provided preliminary evidence that mitochondrial elongation seen during DENV infection involve phosphorylation of Drp1 at serine 616.

AUTOMATIC QUANTIFICATION OF FUSION-FROM-WITHOUT BY DENGUE VIRUS

Patrick Brehio, Carey Medin, Alan Rothman, *Department*, Institution, City, State

Laboratory of Viral Immunity and Pathogenesis

Dengue virus (DENV) envelope protein is responsible for fusion to the endosome membrane in the cell, causing the viral positive sense single stranded RNA to be released into the cytoplasm. The envelope protein (E protein) changes conformation in the acidic conditions of the endosome in order to bring its fusion peptide to the endosome membrane. It has been documented in the literature that this type of fusion in a cell endosome, referred to as fusion-from-within, can be replicated on the cell surface as fusion-from-without. Fusion-from-without (FFWO) occurs when infected cells are treated with acidic media, mimicking the acidic conditions within an endosome. However, there needs to be a method of automatically quantifying FFWO activity in order to measure the effects of candidate fusion inhibitors. We developed a method to quantify FFWO using a combination of the cell analysis programs ImageJ and CellProfiler. Aedes albopictus cells (C6/36) were infected with DENV-2 . At 24 and 48 h post-infection, cells were treated with medium at pH 5.0-7.4 and observed by phase-contrast and fluorescence microscopy. Images were analyzed to count both fused and unfused cells. This method can be utilized in future experiments to measure fusion inhibition.

ROLE OF MITOCHONDRIA IN INNATE IMMUNITY DURING DENGUE VIRUS INFECTION

Hassan Janoudi, Diane Lang, Carey Medin, *Department of Cell and Molecular Biology*, Institute for Immunology and Informatics, University of Rhode Island, Providence, RI

Independent Research

Dengue virus causes an acute febrile illness that, in some patients, is associated with a life threatening plasma leakage syndrome, Dengue hemorrhagic fever (DHF). High viral levels in serum can increase the number of infected antigen presenting cells and subsequently increase the pool of activated naïve and memory T-Cells leading to over production of inflammatory cytokines and pathogenesis. The innate immune response plays an important role in the early immune response to inhibit the amount of virus produced in the infected cell, which can impact the activation of the adaptive immune response. Mitochondrial antiviral signaling (MAVS) protein resides on mitochondria and it is important for innate immune response against RNA viruses. RIG-I like receptor is activated through recognition of viral replication intermediates and will bind to MAVS, which leads to downstream signaling cascades. Ultimately, activated transcription factors will localize to the nucleus and initiate the expression of proinflammatory cytokines such as type I interferon (IFN). Type I IFNs play an important role in inducing an antiviral state and inhibiting viral replication in infected cells. Previous work in the lab has shown an increase in the mitochondrial tubulation and elongation in DENV infected cells. Therefore, we hypothesize that increased mitochondrial lengths in DENV-infected cells would increase the level of MAVS on mitochondria and subsequently increase the induction of the antiviral response. To analyze MAVS on mitochondria, cells were fractionated to separate mitochondria from the cytosol. Western blots were performed to analyze the level of MAVS expression in DENV-infected cells versus uninfected. Our results showed an increase in MAVS protein levels in total cell lysates in DENV infected cells when compared to uninfected cells. Contrary to our original hypothesis, the mitochondrial fraction in infected cells showed a decrease in MAVS protein levels in the DENV-infected cells at the mitochondrial level. The results of this project shows a change in availability of MAVS on mitochondria for innate immune signaling suggesting an inhibition of the cellular antiviral mechanism during DENV infection.

GENERATION OF GENOMICALLY TAGGED IDH FOR IN VIVO ANALYSIS OF GLIOMAS IN DROSOPHILA MELANOGASTER

Michelle Ouellette, Marla Tipping, *Department of Biology*, Providence College, Providence, RI

Walsh Fellowship

Isocitrate dehydrogenase (IDH) is an important enzyme involved in the formation of gliomas, or tumors in human glial cells. Gliomas are the most prevalent form of human brain cancer and are devastating to human health because of their highly invasive nature and accelerated proliferation rate. Since little is known about the mechanism by which IDH induces gliomal disease phenotype, establishing a model that will provide insight into the changes in cellular metabolism and signaling resulting from IDH mutations remains important. To investigate this, a specific and efficient method will be used to create a fruit fly (Drosophila melanogaster) that expresses a tagged version of wild-type or mutant IDH under the control of its endogenous promoter. This method, called “recombineering,” or recombination mediated genetic engineering, is a gene tagging strategy tha t utilizes endogenous promoters for the in vivo study of protein function and allows for easy manipulation of genomic constructs by fostering site directed integration of protein tags into the Drosophila genome. A Green Fluorescent Protein (GFP) tag will be integrated in the genome of IDH, which will allow for future localization and protein interaction studies in Drosophila melanogaster.

CHEMISTRY

LOCATED IN ROOM 130 ON THE 1ST FLOOR OF THE PHARMACY BUILDING

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

MICRO- AND NANOFLUIDICS FOR MOLECULAR SENSING

Joshua Doyle, Jason Dwyer, *Department of Chemistry*, University of Rhode Island, Kingston, RI

RI NSF EPSCoR Summer Undergraduate Research Fellowship Program

The pursuit of higher sensitivity in molecular detection is a significant driver of the development of single molecule detection devices. The widespread adoption and use of such new devices can frequently be hindered by cost and operational complexity, especially sample handling challenges. Nanopore-based devices and methods offer the potential to address these application challenges while delivering reliable single-molecule sensing. Nanopores are nanometer-scale flow channels in thin membranes which can, through careful design, be integrated with micro- and nanofluidic sample channels for ease of sample handling. In the typical mode of nanopore sensing, the entry of a molecule into a nanopore modulates the ionic current that would otherwise pass through the open nanopore. This current blockage is characteristic of the molecule and of the nanopore size and sh ape. Nanopores can be challenging structures to fabricate by traditional means, and we are thus exploring alternative fabrication schemes that remove barriers to fabrication while easing the integration of nanopore elements with fluid control systems.

SYNTHESIS OF 2-PYRAZOLINE DERIVATIVES AND EVALUATION AS ANTIAMOEBIC AGENTS

David Lam, Lauren Rossi, *Department of Chemistry*, Roger Williams University, Bristol, RI;
Joseph Tashjian, Avelina Espinosa, *Department of Biology*, Roger Williams University, Bristol, RI

RI NSF EPSCoR Summer Undergraduate Research Fellowship Program

Entamoeba histolytica, an amitochondriate parasite, has been shown to cause amoebiasis in humans. Previous studies have also shown substituted pyrazoline derivatives to exhibit antiamebic growth properties and inhibition of E. histolytica alcohol dehydrogenase 2 (EhADH2), a crucial enzyme of the parasite. Di- and tri-substituted 2-pyrazolines were synthesized and tested for amebic growth inhibition activity. Comparisons between these compounds suggest that the substitution pattern of the pyrazoline ring affects E. histolytica growth.

OPTIMIZATION OF NAMPT PURIFICATION PROTOCOL

Lisbeth Avalos, Sophia Almeida, Karen Almeida, *Department of Physical Sciences*, Rhode Island College, Providence, RI

RI-INBRE Summer Undergraduate Research Fellowship Program

As a major constituent of cellular activity, nicotinamide adenine dinucleotide (NAD+) is of critical relevance in studies involving cell longevity. Gene transcription, stress response and cell metabolism are among the plethora of cellular processes that utilize NAD+ to maintain cellular stability. While in cellular respiration, NAD+ is protonated and used as an electron acceptor, in other cellular processes, such as that of DNA repair signaling by Poly-ADP ribosylase polymerases (PARP1), NAD+ is consumed through cleavage and converted into nicotinamide (NAM). The NAD+ salvage pathway restores depleted levels of NAD+ through the recycling of NAM. The rate-limiting enzyme of this salvage pathway, nicotinamide phosphoribosyltransferase (NAMPT), converts NAM into nicotinamide mononucleotide (NMN), a direct precursor of NAD+. Correlations between cell longevity an d increased cellular concentrations of NAD+ in yeast cells target NAMPT as a potential regulator of metabolic and age related diseases. To obtain the purest possible level of protein, a low pressure protein purification system was used. Initial enrichment was executed via affinity chromatography using Ni2+-NTA. Further NAMPT isolation was accomplished on a HiPrep 16/60 size exclusion column. Two variants of 6x His epitope tagged NAMPT were compared. Preliminary results from initial affinity chromatography suggest C-terminal His tagged NAMPT is significantly more pure than N-terminal His tagged NAMPT. The additional polishing on a column yields a highly purified protein suitable for downstream analysis such as X-ray crystallography and Surface Plasmon Resonance analysis to study small molecule NAMPT binding.

VIRTUAL SCREENING OF POTENTIAL QUORUM SENSING ANTAGONISTS

Monaf Awwa, Susan Meschwitz, *Department of Chemistry*, Salve Regina University, Newport, RI

RI-INBRE Summer Undergraduate Research Fellowship Program

Protein function is often modified in natural systems by the presence of ligands in regions pivotal to the role of a protein. One system of importance is the quorum sensing system present in many species of bacteria since approximately 350 genes for virulence are encoded through quorum sensing (Bottomley et al 2007). The opportunistic bacterial pathogen, Pseudomonas aeruginosa, uses three transcriptional regulators, LasR, RhlR, and PqsR, to control the transcription of many of its virulence genes. Two protein domains with widespread homology, LasR and PqsR, were investigated via virtual screening to determine how several natural and synthetic ligands interact with their binding sites. Evidence already shows that quorum sensing inhibitors targeting LasR can attenuate the pathogenicity of Pseudomonas aeruginosa (Bottomley et al 2007). In order to investigate th e characteristics of the binding sites, the protein structures were first downloaded from Research Collaboratory for Structural Bioinformatics (RCSB) Protein Data Bank. The proteins were then modified for analysis by the program Autodock Vina (Trott et al 2010) and several ligands of interest were docked and compared for analysis. The quorum sensing proteins shared distinct hydrophobic regions and regions of hydrogen bonding, which is predicted by the presence of an alkyl chain and carbonyl groups present in the natural ligands, which is N-3-oxo-dodecanoyl-L-homoserinelactone for LasR and 2-heptyl-3-hydroxy-4(1H)-quinolone for PqsR. The cyclic dipeptide and pyrimidinone derivatives with the best binding affinity suggest that aromatic pi-pi interactions with the multiple tyrosine residues provide significant stability to the ligand interaction and hold promise as potential antagonists.

SYNTHESIS OF PS48 FOR POTENTIAL TREATMENT OF ALZHEIMER'S DISEASE

Joelle Bitar, *Chemical Technology*, Community College of Rhode Island, Warwick, RI; John Sirios, Brenton DeBoef, *Department of Chemistry*, University of Rhode Island, Kingston, RI

RI-INBRE Summer Undergraduate Research Fellowship Program

While the pathogenesis of Alzheimer's disease (AD) is still unknown, one burgeoning hypothesis is that it is a kind of "type III diabetes" because depletion of insulin in the brain is associated with early stage AD. We are currently testing the hypothesis that a small molecule can reverse the negative effects of insulin insensitivity that occurs in the brain. The compound PS48, (2Z)-5-(4-Chlorophenyl)-3-phenyl-2-pentenoic acid, has been found to be a potential treatment in cell-based assays. This product is commercially available, costing $117 per ten milligrams (or $11,700 for one gram!) for each of the E and Z isomers. We have synthesized PS48 in the lab in four reactions: aldol condensation, alkene hydrogenation, Horner-Wadsworth-Emmons olefination, and ester hydrolysis. The goals of the project were to produce sufficient quantities of PS48 for in vivo experiments and to successfully synthesize a library of derivatives for subsequent testing.

ENZYMATIC STUDIES OF NICOTINAMIDE PHOSPHORIBOSYL TRANSFERASE (NAMPT)

Bopha Chan, Silvia Yanez, Karen Almeida, *Department of Chemistry*, Rhode Island College, Providence, RI

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 the 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+. The rate-limiting enzyme to NAD+ salvage pathway is nicotinamide phosporibosyltransferase (NAMPT). Thus, clinical studies have identified NAMPT as a therapeutic target of interest. NAMPT catalyzes the transformation of nicotinamide (NAM) and 5-phosphoribosyl pyrophosphate (PRPP) to nicotinamide mononucleotide (NMN) and inor ganic pyrophosphate (PPi) and becomes functional in its homodimeric form. To elucidate the biological and physiological relevance, studies on the enzymatic activity of NAMPT were conducted with either purified recombinant human NAMPT or with crude cell lysate from E. coli expressing human recombinant NAMPT. NAMPT activity was measured by converting NMN into a quantifiable fluorescence derivative that was measured spectroscopically. Preliminary results of crude cell lysate activity studies revealed detectable and reproducible enzymatic activity, suggesting that reconstitution of the NAD+ salvage pathway in E. coli for future studies is possible. Results optimizing the NAMPT assay will be presented.

SELECTIVE TARGETING AND KILLING OF BREAST CANCER CELLS BY ARYLPHOSPHONIUM SALT-PEPTIDE COMPLEXES

Daniel DeSimone, Chris Gemski, Evan McCabe, *Department of Physical Sciences*, Rhode Island College, Providence, RI

RI-INBRE Summer Undergraduate Research Fellowship Program

Polypeptides and arylphosphonium salt (APS) were synthesized utilizing microwave assisted solid state synthesis and confirmed using mass spectroscopy. The peptide sequence RGD (Arg-Gly-Asp) has been previously shown to be the recognition sequence for α,β integrins. These intergrins are only present in cells undergoing angiogenesis. Arylphosphonium salts have shown in previous research literature to be possible anti-cancer agents. Arylphosphonium salts are lipophilic cations and as such they have been found to accumulate preferentially in tumor cells due to the high membrane potential of tumor mitochondria. These characteristics also make them ideal phase transfer catalysts. APS bound via an ester link to RGD has a greater affinity to cancer cells than do APS alone. Further, APS accompanied by co-administered RGD "escort" molecules is expected to have an even greater affinity for α,β integrin receptors. The hypothesis is that APS is carried 1) as covalently bound cargo to integrin proteins embedded in the cells' plasma membrane, and once there the APS is endocytized along with the polypeptide into the cell by neurophilin-1 proteins which are also located on the cell surface and have a greater affinity for RGD than do integrins, and 2) RGD acts as an escort molecule for the APS possibility by a phase transition catalytic mechanism. This greater affinity is due to the "CendR" rule, polypeptide sequences following the motif known as "CendR", meaning the carboxylic acid end of the polypeptide contains either a lysine or arginine.

IMPROVING CELL MEMBRANE TRANSPORT OF CISPLATIN ANALOGS

Cristina Encarnacion, Gary Marqus, David Robinson, Chin Hin Leung, *Department of Physical Sciences*, Rhode Island College, Providence, RI

RI-INBRE Summer Undergraduate Research Fellowship Program

Phosphonium salts are known to penetrate well through cell membranes. Incorporating phosphonium salts to cisplatin (cis-Pt(NH3)2Cl2), a well known anticancer drug, will improve the passage of the drug into the cell membrane which could in turn improve its efficiency and selectivity. To make the salts, we monosubstitute a dihaloalkane with triphenylphosphine. The phosphonium salts are added to amine ligands by substitution of the remaining halogen. The resulting molecules are then coordinated to platinum precursors. The coordination chemistry of these amine ligands are explored. These platinum complexes are expected to enter the cell membrane more efficiently and bind to the DNA.

CYTOTOXICITY AND DNA BINDING STUDIES OF THE NATURAL PRODUCT EUDISTOMIN U

Jennifer Giulietti, Chad Roggero, Seann Mulcahy, *Department of Biochemistry and Chemistry*, Providence College, Providence, RI; Bongsup Cho, *Department of Biomedical and Pharmaceutical Sciences*, College of Pharmacy, University of Rhode Island Kingston, RI

RI-INBRE Summer Undergraduate Research Fellowship Program

β-carbolines are naturally occurring aromatic indole alkaloids that have been used to probe a broad range of ailments. A subclass of β-carbolines, known as the eudistomins, is reported to have diverse biological activity, as well as a high binding affinity to DNA. Here, we characterize eudistomin U's cytotoxic activity through growth inhibition assays on prokaryotic, eukaryotic, and cancer cell lines. Secondly, we report the compound's interaction with DNA, which was evaluated through a range of biophysical and spectroscopic techniques. We have determined that eudistomin U weakly binds to DNA in a nonspecific fashion, thus DNA binding is most likely not the cause of toxicity observed in the bacterial and cancer cell lines. We hope that these experiments will elucidate how eudistomin U disrupts biochemical pathways.

STUDIES DIRECTED TOWARD THE SYNTHESIS OF PYRAZINONES AS POTENTIAL QUORUM SENSING INHIBITORS

Mark Grande, Lindsey Coates, Susan Meschwitz, *Department of Chemistry*, Salve Regina University, Newport, RI

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 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. Quorum sensing 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 resis tance. Our long-term goal is to optimize the synthesis of small molecules that have the capability to inhibit quorum sensing. We have successfully synthesized in four steps the pyrazinone phevalin, a known regulator of virulence factor expression in S. aureus. By varying the amino acid starting materials, it will be possible to create a library of phevalin derivatives to further investigate the ability of these compounds as quorum sensing inhibitors.

SYNTHESIS AND EVALUATION OF THE QUORUM SENSING INHIBITORY EFFECTS OF SUBSTITUTED PYRIMIDINONES

Alexander Hulme, Kaitlin Chambers, Susan Meschwitz, *Department of Chemistry*, Salve Regina University, Newport, RI

RI-INBRE Summer Undergraduate Research Fellowship Program

The misuse of antibiotics has 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. These compounds will have the potential to inhibit quorum sensing by acting as competitive inhibitors. A one-step synthesis to prepare the desired scaffold has proven successful and the synthesis of a small focused library of analogs is underway. Each analog is being tested for quorum sensing inhibition to determine the inhibitory effects of different alkyl chain lengths as well as the effect of the nature and position of substituents on a phenyl ring. 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.

SYNTHESIS OF PLANAR POLYCATIONIC METAL COMPLEXES FOR G4-QUADRUPLEX STABILIZATION

Gary Marqus, Cristina Encarnacion, David Robinson, Chin Hin Leung, *Department of Physical Sciences*, Rhode Island College, Providence, RI

RI-INBRE Summer Undergraduate Research Fellowship Program

The G4-quadruplex is a guanine-rich nucleic acid formation that primarily occurs in the telomeric region of DNA. When stabilized, many crucial functions involving the stability of DNA strands are disrupted. Stabilizing these quadruplex formations may prove to be a valuable approach towards eliminating cancer cells. Past studies concerning the stabilization of G4-Quadruplex regions have revolved around large planar molecules that are bound to a central metal cation. Through a series of synthetic steps including condensation, bromination, and Suzuki reactions, we have incorporated phosphonium salts into these large planar structures. Various metals such as palladium, ruthenium, and platinum will then be bound to these molecules. The phosphonium moiety should increase the permeability of these metal complexes and in turn increase their cellular distribution thro ughout cancerous cells. Our primary goal is to test the effect of incorporating phosphonium salts into these established G4-binding complexes.

MICROFLUIDIC-IMMUNOSENSOR ARRAY FOR MULTIPLEX ELECTROCHEMICAL DETECTION OF TWO CANCER BIOMARKER PROTEINS IN SERUM

Clarissa Morganti, Kathleen Gamez, Bernard Munge, *Department of Chemistry,* Salve Regina University, Newport, RI

RI-INBRE Summer Undergraduate Research Fellowship Program

Cancer is the leading cause of death in the developing world. Early detection is the best means to minimize the damage cancer takes on a patient. Tumor suppressor p53, vascular endothelial growth factor (VEGF), and Interleukin 6 (IL-6) and 8 (IL-8) are biomarkers found in high levels in patients with head and neck squamous cell carcinoma (HNSCC) and can be measured as a means to detect cancer. Accurate, quick, and cost effective protein cancer biomarker detection is a promising means of early detection and disease monitoring for better patient outcomes. Herein we report on an ultrasensitive microfluidic immunosensor based on novel multi-labeled magnetic beads, (HRP/MB/Ab2)-PEG, with specially designed polyethylene glycol polymer brushes (PEG) to minimize non-specific binding and particle aggregation. PEG was shown to lessen particle aggregation by dynamic light scattering (DLS). The DLS results showed a narrow size distribution of (HRP/MB/Ab2)-PEG particles with an average particle diameter of 1060 nm. These PEG-covered particle results were indicative on a monodisperse size distribution as compared to the results of a HRP/MB/Ab2 without PEG, which shows a broad size distribution with an average particle diameter 2526 nm, suggesting high aggregation level. Using BCA and ABTS procedures, it was found that the average amount of Ab2 bound to the (HRP/MB/Ab2)-PEG particles was around 115,000 molecules and the average amount of bound HRP was around 50,000 molecules. 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 bound to the multi-labeled magnetic beads, and primary antibodies (Ab1) were attached to glutathione-coated gold nanoparticles (GSH-AuNPs) specific to the biomarkers on the electrode array. The antigen-bou nd MBs were then allowed to bind to the GSH-AuNP-Ab1 immunoassay within the microfluidic channel. Hydrogen peroxide flowed over the immunoassay while voltage was applied to create a reduction peak with a measurable current. Using this set-up, it was found that the bioconjugate had the largest sensitivity when HRP and Ab2 were added 1000:1 in a step-by-step process at 37°C. This microfluidic immunoarray based on a panel of four biomarkers for the fabrication of ultrasensitive biosensor microarrays is promising for point of care diagnosis, as it provides a rapid, low-cost system for the detection of multiple cancer biomarkers.

ANTIOXIDANT AND QUORUM SENSING INHIBITORY ACTIVITIES OF HONEYS IN COMPARISON TO MANUKA HONEY

Joshua Ng, Susan Meschwitz, *Department of Chemistry*, Salve Regina University, Newport, RI; Jacqueline Soscia, Lincoln School, Providence, RI

RI-INBRE Summer Undergraduate Research Fellowship Program

Honey is been known since ancient times for its healing properties and antibacterial activity. More recently, the antioxidant and anti-quorum sensing properties of various honeys have been investigated. Recent studies have identified Manuka honey as the most therapeutically potent honey. In this study, the activities of several monofloral honeys, including Tupelo, Buckwheat, Gallberry, and Texas Tallow were examined and compared to Manuka honey. Column chromatography with Amberlite and elution with methanol was used to isolate the polyphenol compounds from honey and the antioxidant potential of the methanol extract measured using the DPPH assay. Of the honeys tested, Tupelo honey was found to have the highest antioxidant activity with an EC50 of 119 microgram/mL. The quorum sensing inhibitory activity of the honeys was evaluated using the bacterial model C hromobacterium violaceum. Of the tested honeys, both Texas Tallow and Tupelo honeys exhibited quorum sensing inhibition using an agar-well diffusion test. A flask incubation assay was carried out on Texas Tallow honey to quantify the inhibition and demonstrated a concentration-dependent effect, as the inhibition activity increased with increasing honey concentration. These studies demonstrate the potential of honey to inhibit cell-to-cell communication in bacteria and warrant further investigation.

SYNTHESIS OF MULTI-FUNCTIONALIZED ANNULATED B-CARBOLINES VIA INTRAMOLECULAR [2+2+2] CYCLIZATION

Michael ODonnell, Jonathan Varelas, Satyam, Khanal, *Department of Chemistry*, Providence College, Providence, RI

RI-INBRE Summer Undergraduate Research Fellowship Program

β-Carbolines are a class of indolopyridine molecules that have been shown to exhibit interesting biological properties. While their synthesis is a mature field, new methods are desirable to produce carbolines more efficiently. We will describe a five step synthetic strategy to synthesize structurally unique annulated β-carbolines, which bear an additional ring fused to the core scaffold. We will describe our attempts to diversify the cyclized molecules by changing both the coupling linker in a Sonogashira cross-coupling reaction and also varying the structure of our starting material. We will also explain our findings concerning the synthesis of β-carbolines in a one-pot procedure via a novel palladium-catalyzed one-pot Sonogashira coupling/intramolecular [2+2+2] cyclization.

MODIFICATION OF CISPLATIN ALTERNATIVES TO INCREASE CELLULAR AND MITOCHONDRIAL DISTRIBUTION

David Robinson, Cristina Encarnacion, Gary Marqus, Chin Hin Leung, *Department of Physical Sciences*, Rhode Island College, Providence, RI

RI-INBRE Summer Undergraduate Research Fellowship Program

Herein is reported the synthesis of multiple novel DNA binding and potentially anti-cancer metal complexes. These compounds attempt to address the shortcomings of widely used Pt-based cancer drugs (i.e. cisplatin, oxaliplatin, etc.) which include lack of selectivity to cancerous cells, as well as evolved drug resistance. The issue of selectivity is addressed by incorporating lipophilic phosphonium salt moieties to metal complexes with established anti-tumor activity. It is known that phosphonium salts selectively pass through the membranes of cancerous cells and mitochondria. The modification of compounds such as 2,2'-(4-p-tolylpyridine-2,6-diyl)bis(1-methyl-1H-benzo[d]imidazole)palladium(II) chloride (Pd(tMebip)), a G-quadruplex binding complex, and auranofin, with phosphonium salt moieties is being investigated.

LIBRARY SYNTHESIS OF β -CARBOLINES VIA SUZUKI CROSS- COUPLING REACTIONS

Chad Roggero, Jen Giulietti, Patrick Tate, *Department of Chemistry*, Providence College, Providence, RI

RI-INBRE Summer Undergraduate Research Fellowship Program

Heterocyclic amines known as carbolines have attracted interest within the field of organic chemistry due to their diverse biological activity. Carbolines are indole alkaloids consisting of a three-ring fused system. Our research has focused primarily on substitution at the 1-position of the ring. We will describe a 5-step synthesis for the naturally occurring β–carboline eudistomin U, employing 2 key reactions: a Bischler-Napieralski ring closure and a Suzuki cross coupling. This synthetic methodology was then extended by coupling various boronic acids with a common triflate intermediate in an attempt to build a diverse library of structural analogs.

ORGANOCATALYTIC RING-OPENING POLYMERIZATION OF D-VALEROLACTONE WITH TETHERED CO-CATALYSTS

Samuel Spink, Elizabeth Kiesewetter, Matthew Kiesewetter, *Department of Chemistry*, University of Rhode Island, Kingston, RI

RI-INBRE Summer Undergraduate Research Fellowship Program

The organocatalytic ring-opening polymerization of d-valerolactone (VL) was performed using hydrogen bonding cocatalysts. The cocatalysts, a sym-bisthiourea (BisTU-3C) and sym-bisguanidine base (p-bisTBD), exhibit considerably faster ROP kinetics versus their monomeric analogues (Cy-TU and BnTBD) as well as the characteristics of a 'living' polymerization. The length of the linker between the thiourea moieties had little effect on the ROP rate. The source of the increased rate is hypothesized.

ULTRASENSITIVE DETECTION OF CANCER BIOMARKERS USING ELECTROCHEMILUMINESCENCE

Thomas Stracensky, Bernard Munge, *Department of Chemistry*, Salve Regina University, Newport, RI

RI-INBRE Summer Undergraduate Research Fellowship Program

L wells with hydrophobic polymer walls, Silica nanoparticles containing Ru(bpy)32+ and secondary antibodies (RuBPY-silica-Ab2) were used in this system for highly sensitive analyte detection using interleukin-8 (IL-8). The array was fabricated by forming wells on a conductive pyrolytic graphite chip connected to a potentiostat which generated an ECL signal. The sandwich immunoassay protocol employed antibodies attached to SWCNTs in the wells to capture analyte proteins. Then RuBPY-silica-Ab2 weμThis poster describes fabrication of electrochemiluminescence (ECL) immunosensor array for ultra-sensitive detection of cancerous biomarkers of head and neck squamous cell carcinomas (HNSCC) and also correlated with Cutaneous T cell lymphoma (CTCL). The array featured capture-antibody attached to functionalized single-wall carbon nanotube (SWCNT) forests in small 10 we added to bind to the captured proteins. ECL was initiated in the microwells by the electrochemical reaction of n-tripropyl amine (TprA), and Ru(bpy)32+ , and was measured with a Charge-Coupled Device (CCD) camera. Separation of the analytical spots by the hydrophobic wall barriers allow for simultaneous immuno-optoelectrochemical detection of multiple proteins in a single sample without cross-contamination, a future major goal of this project. Using a large amount of Ru(bpy)32+ per silica nanoparticle means this system can give a much larger signal for each analyte which in turn gives a much lower detection limit than other ECL devices. This SWCNT immunoarrays conjugated with RuBPY doped silica nanoparticles provide a simple, yet sensitive approach to the detection of proteins.

CHARACTERIZATION OF GOLD NANOPARTICLES AND MULTI-LABELED MAGNETIC BEADS FOR IMMUNO-ELECTROCHEMICAL DETECTION OF CANCER BIOMARKER PROTEINS

Vinicius Silva Tanganeli, Bernard Munge, *Department of Chemistry*, Salve Regina University, Newport, RI

RI-INBRE Summer Undergraduate Research Fellowship Program

Early detection is the best approach to reduce the effects of cancer, which is the leading cause of death in the developed world. Protein arrays used to measure multiple cancer biomarkers have a considerable promise for early detection. The array promises cheap, accurate and fast detection for patients with head and neck squamous cell carcinoma (HNSSC). Biomarkers such as Tumor suppressor p53, vascular endothelial growth factor (VEGF), Interleukin 6 (IL-6), and Interleukin 8 (IL-8) are found in high levels in patients with HNSSC. Such immunosensor devices feature capture antibody attached to Glutathione protect gold nanoparticles (GSH-AuNP) and coupled to novel multi-labeled magnetic beads, (HRP/MB/Ab2)-PEG. Characterization of Magnetic beads bioconjugate and GSH-AuNP is critically important for successful immunosensing. Information about the GSH-AuPs particl e size (Position of maximum wavelength (λmax)), was obtained from the optical spectra of the buffer solution containing gold nanoparticles using UV/Vis spectrophotometer showing a characteristic surface plasmon absorption center around 510 nm. The value of absorbance at the maximum wavelength was used to calculate the particle size following a literature report (Haiss et al, (2007). The size determined was 4.20 nm with a λmax of 517 nm. The glutathione molecule in the form of thiolate protects the AuNPs, which is confirmed by using Fourier Transform Infrared Spectroscopy (FT-IR). The absence of the S-H stretching vibrational at 2521 cm-1 and the presence of carboxylate group on the AuNPs spectrum at 1711 cm-1 provides evidence that the gold is attached to the glutathione. In addition, FT-IR was used to characterize the magnetics beads bioconjugate of the array. The bands at 1653, 1275 and 1260 cm-1 provide the evidences of HRP/Ab2 while the bands at 830, 1073 and 1108 cm-1 provide the evidences of PEG in the magnetic beads. The results of the characterization provides confirmation for successful fabrication of the GSH-AuNP and the novel (HRP/MB/Ab2)-PEG that offers minimal non-specific biding events and particle aggregation.

EVALUATING MUCUS-PENETRATING NANOCOMPOSITE MICROPARTICLES FOR THE DELIVERY OF THERAPEUTICS FOR PULMONARY ARTERIAL HYPERTENSION

Julie Cuddigan, *Department of Chemical Engineering*, University of Rhode Island, Kingston, RI; Samantha Meenach, *Department of Chemical Engineering and Department of Biomedical and Pharmaceutical Sciences*, University of Rhode Island, Kingston, RI

Independent Research

Pulmonary arterial hypertension (PAH) is a chronic, incurable, and life-threatening disease that is characterized by abnormally high blood pressure in the arteries leading from the heart to the lungs. Patients often suffer from chest pain, shortness of breath, fainting, dizziness, and in many cases heart failure and ultimately death. The therapies currently approved to treat PAH are most commonly available in oral or intravenous form, but often result in undesirable systemic side effects. An effective treatment needs to be designed to deliver targeted therapeutics to the lung periphery to ensure maximum localized bioavailability and to decrease the side effects associated with systemic delivery. The goal of our research is to develop and evaluate dry powder aerosols comprised of nanocomposite microparticles (nCmP) containing the newly-investigated PAH drug ta crolimus to be delivered directly to the lung and surrounding arteries. These particles are designed to overcome mucociliary clearance in the lung and to avoid alveolar macrophages to allow for longer retention time in the lungs. The process of forming these aerosols began with synthesizing the biodegradable polymer, acetalated dextran, with an appropriate cyclic acetalated conversion (65-75%). Then using a single emulsion evaporation method, the polymer was used to form nanoparticles which were characterized by size, charge, and drug encapsulation. The final step of synthesizing the nCmP was spray drying a solution of nanoparticles suspended in mannitol in water. The nanoparticles were successfully optimized to be of appropriate size for microencapsulation (150 - 230 nm). The drug loaded particles had an encapsulation efficiency of 32.7%, resulting in 1.31 mg tacrolimus/40mg acetalated dextran. The nanoparticles were loaded with the fluorescent dye FITC to verify their succ essful encapsulation into the mannitol excipient. We were able to prove our successful synthesis and the morphology of the dry nCmP using confocal fluorescence and scanning electron microscopy. The development of the nCmP could lead to the improved treatment of PAH for many patients afflicted by this life-threatening disease.

IRON CATALYZED DIRECTED ARYLATION BY C-H BOND ACTIVATION OF HETEROCYCLIC IMINES

Riley Davis, Brenton DeBoef, *Department of Chemistry*, University of Rhode Island, Kingston, RI

Independent Research

The catalytic directed arylation of pyridines at the 5- position has been achieved via C-H bond activation on ten unique substrates. Application of an iron catalyst accompanied by the C-H bond activation pathway makes this process inexpensive and “green” by eliminating the need for pre-functionalized substrates. The ability to arylate heterocycles through this method is useful for preparing important substrates in pharmaceutical and industrial processes.

ENVIRONMENTAL SCIENCES

LOCATED IN THE MAIN HALLWAY AT THE END CLOSEST TO PHARMACY ON THE 1ST FLOOR OF THE CENTER FOR BIOTECHNOLOGY & LIFE SCIENCES

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

HEAT SHOCK PROTEINS IN GEUKENSIA DEMISSA AS INDICATORS OF CLIMATE CHANGE AND ENVIRONMENTAL STRESS ON MARINE LIFE IN NARRAGANSETT BAY

Joe Burgess, Sean Grace, Victoria Themuda, John Williams Jr., *Department of Physical Sciences*, Rhode Island College, Providence, RI

RI-INBRE & RI NSF EPSCoR Summer Undergraduate Research Fellowship Program

This aim of this study was to create a reproducible procedure for using G. demissa as an indicator for global climate change and the overall health of the ecosystem of Narragansett Bay. The target organism, commonly known as the Atlantic ribbed mussel, is an intertidal bivalve native to Narragansett Bay and found along the coast from St. Lawrence to Texas. When exposed to heat stress, these organisms express heat shock proteins (hsp). Heat shock proteins are a class of chaperone proteins which refold and protect other proteins after heat damage. They also identify and dispose of irreparably damaged proteins. Specifically, this study looked at heat shock protein 70 (hsp70) of molecular weight 70kD. Organisms were collected from sites along the bay and either acclimated to room temperature, heat treated, or dissected on-site before being analyzed for protein ex pression. Collection sites were; Passeonquis, Warwick, RI; Watchemoket, East Providence, RI; and Fox Hill,Jamestown, RI. Western blotting was performed to quantify the expression of HSP70 in these different conditions for comparison. Once a protocol is established, an ongoing collection of data will be used to monitor the health of the ecosystem of the bay and the local progress of global climate change.

EVALUATION OF ANTIMICROBIAL RESISTANCE IN THE BLACKSTONE RIVER

Jennifer Brewster, Christopher Reid, *Department of Science and Technology*, Bryant University, Smithfield, RI

RI NSF EPSCoR Summer Undergraduate Research Fellowship Program

The Environmental Protection Agency reported in 1990 that the Blackstone River was the most polluted river in the United States given its highly toxic sediments. Industry along the river, from as early as the 1700's used the river to dispose of solvents, heavy metal waste, and dyes, which to this day influence the conditions of the river's ecosystem (1). There is evidence suggesting a link between industrial pollutants and the development of antibiotic resistance in the environment (2). These antibiotic resistance genes (ARGs) allow bacterial populations to survive exposure to particular antibiotics and the potential to pass them to other bacteria. This project evaluated and compared ARGs occurrence in the Blackstone River and pristine sites (Diamond Hill Reservoir, Lincoln Woods). DNA samples from water and soil at each site were and screened for the pr esence of genes conferring resistance to sulfonamides, tetracyclines, vancomycin, and various β-lactamases. Sixteen of our twenty confirmed ARG hits came from our Blackstone River samples, including those for extended spectrum β-lactamase, tetracycline, and sulfonamide families. Our other four ARG hits came from our pristine sites, which included β-lactamase and tetracycline ARGs. Interestingly, sulfonamide resistances were found in both Blackstone River and pristine samples. Additionally, ARGs were predominately found in soil samples as opposed to water samples. In total, we have discovered twenty hits for ARGs within our three Blackstone River sites and two pristine sites, some containing multiple hits with SHV β-lactamase and sulfonamide leading as the most prominent hits among our samples both pristine and along the Blackstone River. Cloning and sequencing of a SHV β-lactamase positive sample from the Blackstone River revealed it to be Aeromonas hydrophila, a fish a nd human opportunistic pathogen.

(1) Burt, L., Protection, C. B. O. R., Haas, G., & Commissioner, A. A. Blackstone River Watershed 2003-2007 Water Quality Assessment Report.
(2) McArthur, J. V., Tuckfield, R. C., Lindell, A. H., & Austin, B. C. (2011). When Rivers Become Reservoirs of Antibiotic Resistance: Industrial Effluents and Gene Nurseries. In Proceedings of the 2011 Georgia Water Resources Conference, held April (pp. 11-13).

TRACE ELEMENTS BIOACCUMULATION IN SEAWEED FROM INDUSTRIAL SITES ON THE PROVIDENCE RIVER, RHODE ISLAND

Jacqueline Kratch, Julia Crowley-Parmentier, Dan McNally, *Department Science and Technology*, Bryant University, Smithfield, RI

RI NSF EPSCoR Summer Undergraduate Research Fellowship Program

Concentrations of major and trace metals were determined in three different species of seaweed, Ulva prolifera, Ulva lactuca, and Fucus vesiculosus, collected from the intertidal zone at three locations on the north and west sides of the Providence River: India Point Park (IPP), Oxford Street (OX), and Public Way (PW). Sediment, surface and groundwater samples were also collected at these locations. These sites of historic and ongoing industrial activity include a former railroad yard (IPP), coal and petroleum storage sites (OX and PW) and metal recycling facilities (PW). Petroleum contamination is evident at each of these sites, and this study has confirmed previously identified elevated levels of Cu, Ni and Pb in the sediments.

Studies by others (Ryan et. al, 2012) have shown that seaweeds can concentrate trace metals, thus may act as bio-monitors in polluted areas. These metals are also available for uptake by the higher order organisms such as shorebirds and fish that use the seaweed as a food source. Seaweed and sediment samples were digested using HNO3 and H2O2 in a Milestone Ethos Microwave. Sediment, water and seaweed samples were then analyzed for major and trace metals using an Agilent 7700 ICP-MS. All of the seaweed had increased metal concentrations compared to the seawater (on the order of 1000x) confirming that the seaweeds bio-accumulate the metals. The sediments had higher metal concentrations than the seaweed. In general concentrations of certain trace metals (Zn, Cu, As, Cd) were higher in Fucus vesiculosus than in the other seaweed samples. Ulva prolifera from Public Way, the site with the most visible contamination, had significantly higher Pb concentrations compared to the other species and other locations.

DRAWING THE LINE: MORPHOLOGICAL AND MOLECULAR SPECIES DEFINITION OF AMERICAMYSIS

Jennifer Cyr, Anthony DelPizzo, Jeffrey Markert, Elisabeth Arévalo, *Department of Biology*, Providence College, Providence, RI; T.J. McGreevy, *Department of Natural Resources Science*, University of Rhode Island, Kingston, RI; Anne Kuhn, *Department of Population Biology*, U.S. Environmental Protection Agency, Narragansett, RI

RI NSF EPSCoR Summer Undergraduate Research Fellowship Program

Opossum shrimp of the genus Americamysis (Crustacea:Pericarida:Mysida:Mysidae) are marine organisms vastly distributed throughout coastal estuaries. They have been widely used as model organisms in environmental and toxicology studies for the past 30 years. In a former study, the genus Mysidopsis was reclassified into six species of the genus Americamysis based on 9 major morphological characters. However, the characters used in this study are too ambiguous to be used as a robust tool to clearly distinguish between species. In our investigation, we aimed to effectively outline Americamysis species parameters by combining morphological traits and mitochondrial (CO-1) and nuclear (18S) loci in a phylogenetic approach. From this combined data, we were unable to support the breakdown of Americamysis into six independent species as previously proposed. Although ad ditional loci and species must be sought in future studies to obtain better resolution, this project has provided new insight into the phylogeny within the genus Americamysis. Our research group is interested in using Americamysis as a model species to assess the effects of climate change along the Rhode Island coast via the completion of ecological, behavioral and conservation studies.

FEEDING RATES AND BEHAVIORS OF LOCAL MYSID SHRIMP

Michael Finnerty, Elizabeth Kawa, Maia Bailey, *Department of Biology*, Providence College, Providence, RI

RI NSF EPSCoR Summer Undergraduate Research Fellowship Program

Mysid shrimp are small crustaceans found locally in Narragansett Bay. They are important here and elsewhere as food sources for game fish. Under climate change, the ranges of zooplankton such as mysids are expected to shift towards the poles as waters warm. We focused our research on two local species, Americamysis bahia and A. bigelowi. While their ranges overlap in Rhode Island, the range of A. bahia extends southward into more tropical waters, while A. bigelowi is more temperate. In order to understand the current distribution of these species and more accurately predict how they will change in the future, we sought to establish baseline rates for two basic behaviors important in resource competition, feeding rates and aggression. To estimate feeding rates, we counted out exact numbers of prey (brine shrimp, Artemia salina) and placed them with a single my sid for 24 hours. Remaining prey and losses from control treatments were subtracted from the original number to calculate 24-hour feeding rates. For aggression, we observed pairs of shrimp in the laboratory taking general notes on behavior and counting how many times the pairs came into contact during 10-minute trials. We found that A. bahia eat significantly more than A. bigelowi and that, although not statistically significant, they tend to interact more, especially when in intraspecific pairs of females. These results may help us understand how A. bahia is already becoming more common our local ecosystem. We hope to follow up on this research with new trials using different prey species, direct feeding competitions and more behavioral observations.

REPRODUCTION STRATEGIES OF MYSID SHRIMP

Giovanni Illiano, Max Molina, Elisabeth Arévalo, Jeffrey Markert, *Department of Biology*, Providence College, Providence, RI

RI NSF EPSCoR Summer Undergraduate Research Fellowship Program

Americamysis bahia, a mysid shrimp, are used for ecotoxicology purposes and conservation genetic studies because they are easy to culture. Female mysids carry eggs in a brood pouch (marsupium) until larvae are mature, however, little else is currently known about their reproductive biology. Reproductive strategies vary widely in arthropods, and can include spermatophore deposition, broadcast spawning, sperm storage, and multiple paternity. Experiments were designed to test these possibilities. For most experiments, photos were taken periodically to analyze egg/larvae development. In one set of experiments, females were isolated after releasing their broods, and none of these produced additional broods, ruling out sperm storage. In another, females were placed in exclusion tanks and surrounded by males. These failed to produce additional broods, sugges ting that A. bahia males do not fertilize eggs using free-swimming sperm. To test whether females mate with more than one male at a time, microsatellites are being developed and are being used to analyze DNA of females and their offspring. These findings provide a base for more behavioral and genetic experiments with mysids.

MECHANISMS BEHIND THE PRODUCTION OF HARMFUL GREEN HOUSE GASES FROM MYTILUS EDULIS AND MERCENARIA MERCENARIA: IS IT REALLY THEIR FAULT?

Alexandra Moen, Melanie Garate, Serena Moseman-Valtierra, *Department of Biological Sciences*, University of Rhode Island, Kingston, RI

RI NSF EPSCoR Summer Undergraduate Research Fellowship Program

It is a scientific consensus that excess amounts of anthropogenic-derived nutrient overloading has impacted marine ecosystems and will continue to have an intensifying effect in the future. This consensus is demonstrated when looking at the heightened release of the green house gas (GHG), nitrous oxide (N2O), from local benthic invertebrates. Nitrous oxide is the third most important GHG and directly causes ozone destruction. The benthic invertebrates release N2O as a result of microbial processes including nitrification and denitrification processes. It has also been hypothesized that epiphytic organisms on these benthic invertebrates may increase their production of N2O. In our study, we used Mytilus edulis and Mercenaria mercenaria, collected from Narragansett Bay in two different experiments to test what variables impacted the release of nitrous oxide. Ex periment 1 involved two different nitrogen species, ammonium chloride and sodium nitrate with M. mercenaria, to discern if one had a larger impact than the other. The second experiment quantified the difference in the N2O output of M. edulis with epifauna as well as M. edulis without epifauna. We hypothesized that for experiment 1, the addition of nitrogen in the water will increase the production of N2O and for experiment 2 M. edulis individuals with epifauna will show significantly greater N2O production as compared to those without epifauna. Experiment 1 concluded that the form of nitrogen did not significantly affect N2O production rates, however both were greater than un-enriched controls with plain seawater. For experiment 2, N2O production was greatest for intact mussels with epifauna. Heightened levels of N2O production from live M. edulis with epifauna are most likely due to active filter feeding pulling in microbes that are also absorbed and digested by the epifaun a. Further studies will examine the interaction between the relationship between epifauna and live mussels as well as how excess nitrogen impacts this relationship.

LATERAL FURCULAR SPREADING IN THE ZEBRA FINCH (TAENIOPYGIA GUTTATA) WING BEAT CYCLE

Christina Perri, David Baier, *Department of Biology*, Providence College, Providence, RI

RI NSF EPSCoR Summer Undergraduate Research Fellowship Program

The furcula, commonly called the “wishbone”, represents the fused clavicles found in most birds. The physiological role played by this unique evolutionary feature is uncertain, but past studies have suggested that it may act as an energy-saving mechanism during long-distance flight, such as migration. As climate change expands some birds’ migration distances, understanding the furcula and these energetic savings becomes increasingly important. Relatively little study has been done of the shoulder girdle, which is deeply hidden by skin, feathers and muscle and thus requires x-ray video to capture its motion in vivo. Lateral furcular bending during flight, which indicates movement at the coracosternal joints, has so far been observed in only two species: the chukar partridge (Alectoris chukar), a basal neornithine, and the European starling (Sturnus vulg aris), a highly derived passerine. Surprisingly, the phasic pattern of furcular spreading is reversed for the two species: bending occurs during downstroke in the starling, but during upstroke in the chukar. Here we use biplanar x-ray videography to track furcular spreading through the wing beat cycle in zebra finches (Taeniopygia guttata), another highly derived passerine. Maximum spreading occurs at the downstroke-upstroke transition and is 15.324 ± 1.280 mm, and minimum spreading occurs at the upstroke-downstroke transition and is 12.030 ± 1.227 mm. This spreading pattern resembles that of the starling, which suggests that the timing of furcular spreading may be linked to differences in skeletal architecture between phylogenetic groups.

DO PHOTOSYNTHETIC ORGANISMS AFFECT GREENHOUSE GAS EMISSIONS IN BIOLOGICAL NUTRIENT REMOVAL TANKS AT FIELD'S POINT WASTEWATER TREATMENT PLANT?

Ryan Quinn, Serena Moseman-Valtierra, Elizabeth Brannon, *Department of Biological Sciences*, University of Rhode Island, Kingston, RI

RI NSF EPSCoR Summer Undergraduate Research Fellowship Program

Nitrous oxide (N2O) and methane (CH4) are greenhouse gases (GHGs) 300x and 20x more potent than carbon dioxide (CO2) respectively. The Biological Nutrient Removal (BNR) process at Field's Point wastewater treatment plant (WWTP) in Providence, RI decreases nitrogen levels in wastewater before it is discharged into Narragansett Bay, but it also releases GHGs. BNR is composed of a series of anoxic and aerated zones in which ammonium is transformed to nitrate and denitrified (mainly as N2) before being released to the bay. High N2O and CH4 fluxes have been observed as byproducts of the BNR process. Minimal algae growth (<10%) has been observed in the BNR tanks at the Field's Point WWTP but it is possible photosynthetic organisms in the wastewater are affecting observed GHG fluxes by assimilating nutrients or fixing CO2. An experiment was designed to compare differences in GHG production from wastewater incubated in conditions favorable to photosynthesis (light) versus conditions unfavorable to photosynthesis (dark) and with or without algal biomass. Gas samples were collected from sealed assay jars between 0 and 90 minutes and analyzed on a gas chromatograph (Shimadzu GC-2014). There were no significant differences in GHG production between treatments. However there was a significant decline in CO2 and N2O production (but not CH4) over a 90 minute time period, indicating that the assays became anoxic. Future research will investigate how algae presence affects dissolved oxygen levels in the wastewater because that strongly controls GHG emissions.

COMPARISON OF MACROINVERTEBRATE POPULATIONS IN RIFFLES AND POOLS OF THREE SMALL RHODE ISLAND WATERSHEDS

Meaghan Senack, Zoe Moskwa, *Department of Environmental Studies*, Salve Regina University, Newport, RI; Jacob Peterson, *Department of Natural Resources Science*, University of Rhode Island, Kingston, RI; Jameson Chace, *Department of Biology and Biomedical Sciences & Environmental Studies*, Salve Regina University, Newport, RI

RI NSF EPSCoR Summer Undergraduate Research Fellowship Program

The health of Rhode Island coastal urban watersheds is a pressing issue due to the risk of contamination from storm water runoff and leaking sewer systems that increase human exposure to pathogens at local beaches and degrade the quality of drinking water supply. Macroinvertebrate sampling is a method used to determine the overall health of a stream. Standard protocols involve sampling the macroinvertebrate community across a 100m reach. In large streams and rivers macroinvertebrates are sampled independently in different habitat types, however in smaller streams the macroinvertebrate communities are usually pooled across the riffles and pools. Even in a small stream riffles and pools may create very different species-species habitats. In this study, we tested the validity of pooling macroinvertebrates in small low-gradient watersheds. Macroinvertebrates were collected by kick netting at one station in each of three RI watersheds: Cork Brook in Scituate, RI, and Bailey Brook and Maidford River on Aquidneck Island. The macroinvertebrates were identified into scientific orders and sampling was conducted three different times at each location in June and July 2014. To control for effort, at least 15 kicks were made per habitat type in each stream and at least 100 total macroinvertebrates were sampled. Macroinvertebrate communities were similar at sampled sites on Aquidneck Island but differed from the community of Cork Brook. Within a site pool and riffle EPT Count was statistically similar, although typically higher in riffles than pools. Simpson's diversity index was similar across all sites; only the Maidford River had significantly higher diversity in riffles than pools. From these results, we determined that in these small watersheds, separating pool and riffle populations does not seem to be necessary in order to gain an ac curate macroinvertebrate assessment, provided that pools and riffles are surveyed equally and at least 100 individuals are collected. These results are preliminary as this study had a small sample size, short time frame (June-July), and small spatial coverage of the watersheds (1 sampling site per watershed). In addition to adjusting sample size, duration, and spatial coverage, the study would be enhanced by identification of macroinvertebrates to the species-level, to better determine accurate EPT and diversity indices.

HOW DO MICROBE-MINERAL INTERACTIONS IN EARTH'S OCEANS RESPOND TO OCEAN ACIDIFICATION?

Mahrukh Shaikh, Dawn Candace, *Department of Geoscience*, University of Rhode Island, Kingston, RI

RI NSF EPSCoR Summer Undergraduate Research Fellowship Program

Oceans are the largest sinks for the anthropogenic carbon dioxide, and the absorbed CO2 is distributed throughout the ocean. The increased CO2 concentration affects carbonate chemistry and causes a decrease of ocean pH. Given continually increasing atmospheric CO2, the ocean surface pH is expected to decrease by about 0.4 units by 2100, and is likely to stress marine microbial communities globally. Marine microbes make up the bottom of the food chain in the oceanic ecosystem, and it is imperative that we understand better the adaptive responses of these microbes to expected ocean acidification. With this aim in mind, unfiltered seawater samples were incubated in the presence of serpentinite (i.e., a rock composed primarily of the mineral serpentine, an important deep-sea bedrock) as a solid growth substrate. To assess microbial response to changing environmen ts, serpentinite was presented as sand-sized grains and thin wafers, and incubated with seawater under the following conditions: (1) light + ambient levels of CO2 (~400 ppm), (2) dark + ambient CO2, (3) dark + elevated CO2 (~700 ppm), and (4) dark + quasi-anaerobic atmosphere.

We hypothesized that there would be different responses in the suspended microbes vs. mineral-associated microbes. In particular, we expected greater biomass in water when compared to mineral adherence in condition 1. In conditions 2 and 3, greater adherence to the mineral was expected. In condition 4, few microbes were expected to be observed on the mineral bedrock, with the lowest biomass in the water. Serpentinite sands were tested for the microbial biomass over 5 consecutive days via FTIR spectroscopy, while SEM data were collected after 4 days to determine wafer surface colonization. Aqueous suspensions were analyzed using UV/VIS spectroscopy to monitor changes in optical density, a proxy for biomass. UV/VIS spectroscopy data confirmed our hypotheses: high biomass was observed in condition 1 and low biomass seen in condition 4. SEM data showed morphologically distinct cells apparently associated with rock wafers. FTIR profiles showed that there were features common to al l experimental conditions and at all times, while real differences emerged over time in all the conditions. Further studies are needed for more in depth analysis of these changing microbial communities, which will hold the key to our understanding of changing marine ecosystem diversity in the face of climate change.

PELLET STOVE ASH AND TAR AS NON-POINT POLLUTION SOURCES FOR POLYCYCLIC AROMATIC HYDROCARBONS AND TRACE HEAVY METALS

Leigha Tacey, Dan McNally, Julia Crowley-Parmentier, *Department of Science and Technology*, Bryant University, Smithfield, RI

RI NSF EPSCoR Summer Undergraduate Research Fellowship Program

Wood pellet stoves are increasing in popularity as an alternative source of heating. In the United States alone, approximately 1,000,000 residences/businesses use 4,700,000 tons of wood pellets annually. The resulting generation and improper disposal of ash and tar may contain trace metals and carcinogenic PAHs that impact the environment and human health. The presence and concentration of these contaminants may also be dependent on brands of pellets and stoves. In this study we identify and quantify PAHs and trace metals from three different pellet brands (Stove Chow, Greene Team, Green Supreme) burned in two different stoves (Cumberland and Vistaflame). Pellet stove ash and tar samples were analyzed for EPA's 16 priority PAHs using gas chromatography – mass spectrometry (GC-MS). Trace metals were analyzed using inductively coupled plasma-mass spectromet ry (ICP-MS). Our results show that all 16 PAHs were present, but concentrations were higher in tar than ash for all brands of pellets. Additionally, higher molecular weight PAHs, such as probable carcinogens Dibenzo[a.h]anthracene and Benzo[g,h,i]perylene, had higher concentrations than lower molecular weight PAHs for all brands. The presence and concentration of trace metals in tar and ash was pellet dependent, but Vanadium (V), Chromium (Cr), Cobalt (Co), Arsenic (As), and Lead (Pb) were consistently most abundant across all brands. Mercury (Hg) was only present in Stove Chow ash with a concentration of 242.5µg/g (Cumberland) and 711.49µg/g (Vistaflame). Our results indicate pellet stove ash and tar can potentially release toxic metals and PAHs to the environment and enter the food web. Our results show additional research is warranted and may be used to develop non-point source pollution management policy.

PFOS AND NON-ALCOHOLIC FATTY LIVER DISEASE

Kimberly Ezeama, Danna Salter, Angela Slitt, *Department of Biomedical and Pharmaceutical Sciences*, University of Rhode Island, Kingston, RI

RI-INBRE Summer Undergraduate Research Fellowship Program

PFOS, or perfluorooctanesulfonic acid, is a synthetic fluorosurfactant and an environmental pollutant that is abundant in society and has been shown to have hepatotoxic effects. This study intended to examine the mechanism by which PFOS modifies the response to caloric restriction through the lipogenesis pathway in C57BL/6 male mice, and its effects on the development of non-alcoholic fatty liver disease (NAFLD). The mice were administered PFOS (0.1mg/kg) or vehicle (water) daily and either fed ad libitum (freely) or placed on a caloric restriction (25% kcal reduction) for five weeks. Our primary results showed that PFOS administration caused an increase in lipid content in the liver, and the impaired loss of fat in response to caloric restriction from the liver. This lipid accumulation can result from increased fatty acid transport through several different mechanisms including fatty acid transport proteins (e.g. FATP-1), increased synthesis of fatty acid (e.g. ACC-1, FAS, and SCD-1), as well as altered upstream pathway modulation through decreasing enzymes such as p-AMPK. The purpose of this experiment was to establish whether or not the effects of PFOS to impair the effects of caloric restriction occurred through altering the expression of enzymes involved in lipogenic, glucogenic, and insulin pathways. Liver lysates of each of the mice were analyzed (with an n=4 or n=6 treatment group) through SDS-PAGE followed by Western Blot analysis using either LiCor™ infrared or chemiluminescent-tagged antibody detection methods. By looking at the protein concentrations of each of the mice that underwent caloric restriction, PFOS had decreased the expression of enzymes such as total AMPK, total AKT, and FATP-1, yet did not significantly alter the expression of PEPCK, ACC, phosphorylated ACC, phosphorylated AMPK, phosphorylated AKT, or SCD-1. More analysis of several other pathways must also be taken into account, and other alternative mechanisms that may further explain the abnormal fat retention in the liver that might lead to NAFLD.

COMPARISON OF HYDROPONICALLY AND CONVENTIONALLY GROWN LETTUCE AND SPINACH

Shelby Johnson, Maria Smith, Jameson Chace, *Department of Biology and Biomedical Sciences*, Salve Regina University, Newport, RI

Sustainability Fellowship

Maximizing agricultural output to keep pace with a growing global, especially urban, human population is an increasingly important concern. Indoor hydroponically grown produce has potential benefits that exceed conventional farming practices. In 2011, the Salve Regina University Hydroponic Research Lab began growing lettuce (Lactuca sativa) and spinach (Spinacia oleracea) in tower systems; we compared these data with literature on farming production to evaluate costs and benefits. We tested the hypothesis that indoor, year round, hydroponically grown lettuce and spinach would produce higher crop yields. Different varieties of lettuce: butter head, green bib and red leaf, and spinach: smooth leaf, corvair and savoid, have successfully been grown in the tower system. The tower drip system is kept on a 30-minute on/off watering schedule, under T-5 florescen t lights (12L: 12D). The plants were grown at various nutrient levels ranging between 400 and 1610ppm, and a 6.1 +/- 0.2 that were maintained daily. On average, harvested lettuce yielded 53.5 g per plant, and spinach 23.4 g per plant. Hydroponic harvest was partial and individual plants were grown for up to three months, with plants grown year round in the system. Yields were converted to lb/ft2 and tons/acre compared to conventionally grown spinach and lettuce found in the literature. The hydroponically grown lettuce yielded 1.7 lb/ft2 compared to 0.4 lb/ft2 and 1.2 lb/ft2 per harvest, which was 1.4% higher than conventionally farm grown. Spinach grown hydroponically yielded 0.7 lb/ft2 versus 0.3 lb/ft2 and 0.5 lb/ft2 per harvest, which was 1.44% higher yield than conventional agricultural systems. Based on the literature, conventionally grown lettuce yielded between 19.9 tons/acre and 20.7 tons/acre in California from 2007-2009, while hydroponically grown lettuce could yield 359.9 tons/acre. Hydroponically grown lettuce yields almost 18% more lettuce than the conventionally grown lettuce. Hydroponically grown crops can produce more crops per sq. ft2 and by acre due to the higher density of crops and vertical farming. Other benefits of hydroponics include minimized water loss, reduced fertilizer and insecticide use and year round growth. The key cost to hydroponics is that to maintain the systems, adequate lighting and power via electricity are necessities. If the benefits of production outweigh the costs, hydroponics may be a viable solution in urban areas where space is limited.

CORRELATING STREAM WATER QUALITY WITH POLLUTION TOLERANT AND INTOLERANT MACROINVERTEBRATES IN THREE RHODE ISLAND WATERSHEDS

Zoe Moskwa, Meaghan Senack, *Department of Environmental Studies*, Salve Regina University, Newport, RI; Jacob Peterson, *Department of Natural Resources Science*, University of Rhode Island, Kingston, RI; Jameson Chace, *Department of Biology and Biomedical Sciences*, Salve Regina University, Newport, RI

NEWRnet

Local urban and agricultural watersheds provide a range of essential ecosystem services including a habitat for vital pollution-indicator macroinvertebrates. Macroinvertebrates are ideal indicators of watershed heath due to the diversity of pollution tolerant and pollution sensitive taxa, vital position in the aquatic food chain, and overall ubiquity. This study aims to link overall water quality at each of the three sampling sites with its respective macroinvertebrate community to examine whether high or poor water quality is an accurate indicator of the species present. From 1 June to 25 July 2014 nine sites on Aquidneck Island were sampled, four along Bailey Brook (mostly urban), five along Maidford River (mostly agricultural), and one at Cork Brook in Scituate RI (undeveloped) as a reference point. Water samples were taken at each site using a YSI 556 MPS and FlowTracker Handheld ADV to collect temperature, pH, dissolved oxygen, and flow. Nitrate was measured with S::CAN measurements taken every 30 minutes from 28 June to 28 July 2014. Macroinvertebrates were sampled at one designated site per watershed on three different occasions using kick nets and 20 kicks per location. Based on the literature, pollution tolerance values were applied to the macroinvertebrates. There was a significant difference between the tolerance values averaged for each three locations: Cork had the lowest pollution tolerance followed by Bailey and Maidford that had the highest tolerance. Cork also had the most abundant and evenly distributed pollution sensitive species in the orders Ephemeroptera, Plecoptera, Trichoptera which form the EPT Index. PH was similar at all sites: Bailey 6.63 pH, Cork 7.06 and Maidford 7.05. Dissolved oxygen varied at all sites, but was in the aerobic zone at all sites: Maidford 99.05%, Cork 101.2% and Bailey 66.7%. Baile y had the lowest average temperature of 18.0 C, with Maidford 21.4 C and Cork 20.1 C. Nitrates for Cork were lowest at 0.33ppm, higher at Bailey 0.85ppm and Maidford 2.05ppm. The streams of Aquidneck Island are impaired by nitrate contamination. Correspondingly, macroinvertebrate community is the most diverse and pollution intolerant at the high water quality site. Macroinvertebrate bioassessments appear to be a good indicator of water quality in these small costal watersheds.

COMPARING YIELD AND CAROTENOID CONTENT BETWEEN ORGANIC AND INORGANIC HYDROPONICS AND TRADITIONAL ORGANIC FARMING

Maria Smith, Shelby Johnson, Jameson Chace, *Department of Biology and Biomedical Sciences*, Salve Regina University, Newport, RI; Susan Meschwitz, *Department of Chemistry*, Salve Regina University, Newport, RI

Sustainability Fellowship

A global emerging issue is how to feed a growing human population on limited available land while reducing environmental impacts. Recent agricultural developments include hydroponic farming (growing plants without soil) and the rapidly expanding local, organic farming market; both potentially reduce pollution and farmland destruction, conserve resources, and allow growth near urban centers. However, few studies have compared the production of these two growing methods. In 2014 we compared yield per plant and total carotenoid content of basil (Ocimum basilicum) and red romaine lettuce (Lactuca sativa) using three different growing methods. Two sets of five red romaine and eight basil plants were grown hydroponically using traditional inorganic hydroponic nutrients and organic hydroponic nutrients under T-5 florescent lights (12L:12D) in the hydroponic lab at S alve Regina University. Another set of ten red romaine and ten basil plants were grown on an organic farm plot in Middletown, RI. All plants were allowed to grow for forty-two days from May-July, then harvested, weighed and analyzed for carotenoids (both beta-carotene and lutein) using HPLC. The average yield of lettuce per plant for organic hydroponics was 132.18g, organic farming 256.7g, and non-organic hydroponics was 295.06g. The organic farm and inorganic hydroponic yields were similar, and the organic hydroponics had a much lower yield. The same trend was found with basil, organic hydroponics had an average yield of 18.91g, organic farming 40.33g, and non-organic hydroponics 47.25g. Carotenoids are important nutrients for the body, and are antioxidants, which absorb free radicals and prevent some cancers. It was found for both types of plants that organic hydroponics had the highest levels with inorganic hydroponics, and organic farming coming in second and third, resp ectively. Based on a comparison with USDA Agricultural Research Service lettuce and basil grown hydroponically had up to a three times higher carotenoid content than when conventionally grown. Hydroponically grown produce such as lettuce and basil can achieve equal to higher yields than organically grown produce and all three growing methods can provide an equal, if not higher, concentration of key nutrients such as carotenoids.

GENETICS

LOCATED NEAR THE CENTRAL STAIRWAY ON THE 1ST FLOOR OF THE PHARMACY BUILDING

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

CHARACTERIZING STICHODACTYLA HELIANTHUS TOXIN ACTION IN VIVO USING DROSOPHILA MELANOGASTER

José Hurtado, Victoria St. Amand, Geoff Stilwell, *Department of Biology*, Rhode Island College, Providence, RI

RI NSF EPSCoR Summer Undergraduate Research Fellowship Program

Many aquatic species of phytoplankton, invertebrates, and some fish produce effective marine neurotoxins for immobilizing prey. Genome sequencing and bioinformatics have revealed the cnidarian genomes N. vectensis and H. magnipapillata contain approximately 400 putative short peptide toxin genes. To better understand the biological action of these toxins, we will use the genetic model organism D. melanogaster as an in vivo expression system for analysis of toxin efficacy. As a proof of concept, we will first express the Stichodactyla helianthus toxin (STX), a previously characterized toxin potassium channel antagonist (Kv 1.1 and 1.3). Our study did not use a sea anemone organism for STX extraction. Instead, we synthesized our own toxins by primer engineering, with and without, a glutactin secretory tag then cloned into a pUAST expression vector to enable in ducible expression in flies. Further analysis will be discussed and will include P element mediated germline transformation in Drosophila and the effect of toxin action in wildtype and potassium channel mutants shaker, shal, and shab.

GENETIC DIVERSITY CHANGES OF THE GREEN CRAB (CARCINUS MAENAS) RESULTING FROM INVASIVE COMPETITION OF THE ASIAN SHORE CRAB (HEMIGRAPSUS SANGUINEUS)

Adriana Enxuto, JD Swanson, *Department of Biology and Biomedical Sciences*, Salve Regina University, Newport, RI

RI-INBRE Summer Undergraduate Research Fellowship Program

The Green Crab (Carcinus maenas) the Asian Shore Crab (Hemigrapsus sanguineus) are both considered significant invasive species in the United States. However, ecological studies in the Narragansett bay RI, have indicated that the Green crab population has recently declined whereas the Asian Shore crab population has increased. To this end, we hypothesize that if the Green Crabs are inbreeding due to competition with the Asian Shore crab decreasing their population size. This in turn will be manifested over time by a decrease in heterozygosity. Crab legs were removed from 5 coastal regions located around Aquidneck Island in Narragansett Bay, RI in order to extract their DNA. Six microsatellite markers were used to examine the genetic diversity of the Green Crab to determine what may be the possible genetic changes resulting from the decrease in their populat ion. Results suggest that although the population of Green Crab remains in Hardy-Weinberg equilibrium (P=0.05, d.f. = 9) there is some indication of possible underlying genetic mechanisms resulting from the observed population decrease. For example, some homozygous genotypes are more abundant compared to what we would expect (compared to Hardy-Weinberg) proposing positive selection for some genotypes. Furthermore, there's a noticeable decrease in heterozygosity potentially due to inbreeding depression over time (Fpop = 0.55 stdev = 0.212). My future work includes correlating inbreeding depression at each of the five study sites with an abundance of Asian Shore crab to determine if competition is a driver for the decrease in Green Crab populations due to their documented invasive behavior.

UNDERSTANDING THE EFFECTS OF GALLIC ACID ON STOMACH CANCER BY INVESTIGATING GENE EXPRESSION

Stephanie Liptak, Rhiannon Morrissey, JD Swanson, Kari Clifton, *Department of Biology and Biomedical Sciences,* Salve Regina University, Newport, RI

RI-INBRE Summer Undergraduate Research Fellowship Program

Gallic acid is a naturally derived secondary plant metabolite, involved in prickle development, originally derived from the Rubus family that has been shown to have inhibitory effects on multiple lines of cancer. It can be found in natural products such as grape seed extract, green tea, and raspberries, and has the potential to benefit medicine because it is natural and only cytotoxic to cancer cells while not harming healthy cells. This study aims to compare the expression of genes involved in the mechanisms of action of gallic acid on human gastric cancer (AGS) cell line after exposure to gallic acid in a time and dose dependent experiment. The genes and their subsequent pathways we are investigating include: p21 involved in cell cycle arrest in G1/S and G2/M; Bax/BCL-2 involved in apoptosis; CDK4 and Cyclin D1 involved in G1 cell cycle arrest; and MMPs whi ch are tumor-specific genes. We are also working to optimizing our seeding density of AGS cells so that the gene expression detected in treated cells are strictly from the effects of gallic acid and not from contact inhibition. Under optimal conditions we do not expect to see a change in the vehicle-treated cells across time points. Understanding the gene expression will help us understand the mechanism(s) by which gallic acid is effecting the AGS cells. With these results we can move forward with other cell lines and primary culture to develop gallic acid as a neutraceutical.

AMINO ACID SEQUENCE OF 25KD WATER SOLUBLE PROTEIN FROM GINGER ROOT (*ZINGIBER OFFICINALE*)

Tyler Perry, *Biotechnology Certificate Program*, Community College of Rhode Island, Warwick, RI; Alia Sadaf, Atiatul Wahab, Iqbal Choudhary, *International Center of Chemical and Biological Sciences*, University of Karachi, Karachi, Pakistan; Kim Andrews, Aftab Ahmed, *Department of Biomedical and Pharmaceutical Sciences*, College of Pharmacy, University of Rhode Island, Kingston, RI

RI-INBRE Summer Undergraduate Research Fellowship Program

The ginger plant, a member of the Zingiberaceae family, has a rich history dating back to the Roman Empire over 2000 years ago. While still found in many South Asian dishes today, ginger was used, historically, in Chinese and Indian folk medicines to combat inflammation, respiratory disorders, and arthritis. Limited studies have been reported on the bioactive proteins from ginger extract. In the current study, the ginger root was washed with distilled water, skinned, and ground using an electric blender. This mixture was stirred for four days at 4oC without a protease inhibitor. It was then filtered using cheese cloth, centrifuged at 26,000 x g, and lyophilized. A 25kD water soluble protein was successfully purified by HPLC using a BioBasic SEC-300 (4.6x250 mm) gel-filtration column and analyzed by SDS-PAGE electrophoresis. The purified 25kDa protein was oxid ized by performic acid and digested by TPCK-treated trypsin. The tryptic peptides were separated by a Prosphere C18 (4.6x250 mm) RP-HPLC column. The N-terminal amino acid sequence of three tryptic peptides was deduced by way of an automatic Edman protein sequencer. These amino acid sequences were aligned using the Basic Local Alignment Search Tool (BLAST) database and it was found to be Zingipain-2 (Cysteine proteinase GP-II).

MARINE SCIENCES

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

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

EVOLUTIONARY ADAPTATION TO CLIMATE CHANGE IN NEOMYSIS AMERICANA

Kelli Butler, Nathan Andrews, Gordon Ober, Jason Kolbe, Carol Thornber, *Department of Biological Sciences*, University of Rhode Island, Kingston, RI; Jason Grear, A*tlantic Ecology Division*, Environmental Protection Agency, Narragansett, RI

RI NSF EPSCoR Summer Undergraduate Research Fellowship Program

Anthropogenic activities have set global warming at a rate unprecedented for the earth and this may have a profound impact on marine environments. Projections for ocean warming range from 1.1-6.4°C by 2100 based on IPCC forecasts. When natural habitats are disrupted, populations will have one of three responses: relocate, adapt, or go extinct. Our experiment assesses the ability of a native mysid shrimp, Neomysis americana, to evolve in response to increased ocean temperatures. We sampled wild N. americana from Narragansett Bay and bred them to produce a lab-reared generation that will be exposed to two water temperature treatments, one at ambient 24°C, and the other at 28°C, a predicted summer maximum for Narragansett Bay. Both the parents, P1, and offspring, F1, are undergoing performance tests and morphological measurements to determine baseline cha racteristics and the genetic basis for this variation. Performance data (thermal tolerances and burst swimming speed) were collected for N. americana. Burst swimming speed data was obtained by video analysis. Thermal minima and maxima were determined by exposing individuals to warming or cooling and the temperature at death was recorded. Performance data was also collected on two ecologically similar species, Heteromysis formosa and Americamysis bahia. Morphological features (total body length, eyestalk length and width, antennal scale length and width, telson length and width, statocysts length and width) were measured on dead adults using image analysis software. Current work has found significant differences in thermal minimum and maximum between the three mysid species, and performance curves for the three species seem to follow a similar trend but further analysis is needed. N. americana individuals will again undergo performance and morphological testing after approxim ately 10 generations of exposure to warming treatments. This will determine if populations show an evolutionary adaptation response to increased ocean temperatures. The findings from the experimental populations will be compared with the initial baseline data for any observed changes. We expect to find N. americana to have an increased thermal maximum at the end of the study as well as some morphological adaptations.

THE EFFECT OF SALINITY AND POPULATION ON THE GROWTH OF THE GREEN ALGA ULVA COMPRESSA AT AMBIENT AND ELEVATED TEMPERATURES

Kyle Carpentier, Carol Thornber, *Department of Biological Sciences*, University of Rhode Island, Kingston, RI; Michelle Guidone, *Department of Biology*, Armstrong Atlantic State University, Savannah, GA; JD Swanson, *Department of Biology and Biomedical Sciences*, Salve Regina University, Newport, RI

RI NSF EPSCoR Summer Undergraduate Research Fellowship Program

Climate change is predicted to increase the frequency of precipitation events and seawater temperature. Ulva compressa is a sheet forming green alga that is a dominant contributor to algal blooms in Narragansett Bay. Algal blooms can be economically and ecologically harmful and impact recreation, tourism, fisheries, and native fauna and flora. The ability of Ulva compressa to tolerate salinity and temperature extremes as a result of climate change has yet to be tested. The objective of this study was to determine the response of U. compressa from two separate populations to a wide range of salinity levels at both ambient and elevated seawater temperatures. Blades of U. compressa from Connecticut and Rhode Island were grown in 2, 12, 22, and 32 PSU seawater at both ambient (day: 21.4°C ± 0.1, night: 19.5°C ± 0.04) and elevated (day: 28.3°C ± 0.4, night: 19.02°C ± 0.2) temperatures in separate flow-through seawater systems. Ulva compressa blades grown at ambient temperature with 2 PSU seawater were incapable of maintaining a positive net growth for more than two days; there was no difference in growth rates between all four salinity levels tested during the first two days. Salinity ranging from 12-32 PSU had no effect on growth of blades in either temperature treatment. Mass gain of blades from the Connecticut population decreased over time while blades from Rhode Island showed the opposite trend although there was no significant difference in growth over time in either population. Our results show that Ulva compressa will likely withstand low salinity environments produced as a result of increased precipitation events due to climate change. Further, we hypothesize that genetic variation may be responsible for the differential tolerances between Connecticut and Rhode Island material documented here. Interestingly, we also documented a general trend where elevated temperature may reduce the negative impact of low salinity. However, replication of the temperature treatment is necessary to confirm this hypothesis.

THE USE OF SOCIAL MEDIA TO ORGANIZE A GRASS-ROOTS CONSERVATION CAMPAIGN AIMED AT CONTROLLING AN INVASIVE SPECIES (LIONFISH IN THE VIRGIN ISLANDS)

David Gleeson, *Department of Biological Sciences*, University of Rhode Island, Kingston, RI;
Kristian Dzilenski, *Department of Marine Affairs*, University of Rhode Island, Kingston, RI;
Graham Forrester, *Department of Natural Resources Science*, University of Rhode Island, Kingston, RI

RI NSF EPSCoR Summer Undergraduate Research Fellowship Program

Lionfish are an invasive species in the Atlantic, and by consuming small fishes severely impact fish biodiversity on coral reefs. Efforts to control lionfish by using divers to spear them have been developed, usually by local government agencies. The purpose of this research is to examine the operation and effectiveness of a grass-roots lionfish removal program in the British Virgin Islands, Reef Guardians BVI. Local volunteer divers spear lionfish in their spare time and use a Facebook group to record and coordinate their activity. We first compiled 654 activity reports from Facebook, dating from the start of group activity in 2012 until present. Volunteers concentrate most of their efforts on a few dive sites of economic interest, while visiting other sites just a few times each year. Most removals are done by a core group of locals when weather conditions are best for diving (spring), whereas a much larger group of visitors to the islands contribute primarily by reporting lionfish sightings. Evidence for the effectiveness of removal programs is limited, so our current fieldwork is aimed at testing whether volunteer removals can reduce lionfish populations, and whether frequent spearing visits makes lionfish more evasive.

EFFECTS OF OCEAN ACIDIFICATION AND DIET ON LARVAL SIZE, SURVIVAL RATE, AND OTOLITH MORPHOLOGY OF AMPHIPRION CLARKII

Laura Anderson, Elizabeth Groover, *Department of Marine Biology*, Roger Williams University, Bristol, RI; Andrew Rhyne, Bradford Borque, *Center for Economic and Environmental Development*, Roger Williams University, Bristol, RI; Rober Holmberg, Robyn Hannigan, *Department of Environmental Science*, University of Massachusetts – Boston, Dorchester, MA; Dillon Post, *Department of Biology*, Community College of Rhode Island, Warwick, RI

RI NSF EPSCoR Summer Undergraduate Research Fellowship Program

Increasing concentrations of CO2 in the atmosphere due to anthropogenic sources results in the decrease in pH of our world's oceans through the process of Ocean Acidification (OA). This increase in CO2 disrupts the chemical system that produces carbonate, which is used by many marine organisms in conjunction with calcium or magnesium to synthesize compounds used to form crucial body structures. It is known that the decrease in the ocean's supply of carbonate negatively affects certain organisms' ability to form external calcium carbonate structures, however much less is known about its impact on internal calcium carbonate structures such as otoliths (inner ear stones of teleost fish). Teleost fish use these otoliths to sense orientation and acceleration, making them essential for navigation and predator evasion, which directly correlates to survival. Th is research aims to understand how decreasing pH effects the development of reef fish larvae, including body size and morphology of otoliths. Additionally, a diet component was added to the experiment, which looked at the effect of varying zooplankton food sources (copepods vs. rotifers) on larval development. Knowledge obtained through this study could provide insight into how larval survival is effected by ocean acidification through its impact on the formation of otolith structures.

For this research, 24 ten-gallon (38L) tanks were stocked with 28 Amphiprion clarkii (clarkii clownfish) larvae in pH environments ranging from 7.3 to 8.1. The larvae were raised for the remainder of their ten-day larval cycle, then euthanized using tricaine methanesulfonate (TMS) and stored in ethanol. The fish were photographed and their standard lengths measured using Photoshop. Sagittae otoliths were extracted from each fish using a polarizing stereomicroscope, which were then photographed, and mounted on scanning electron microscope (SEM) stubs for later morphological analysis.

USING LARVAL OCEAN ACIDIFICATION STUDIES AS A BASIS FOR OCEAN ACIDIFICATION CURRICULUM FOR ELEMENTARY EDUCATION

Laura Anderson, Elizabeth Groover Andrew Rhyne, *Department of Marine Biology*, Roger Williams University, Bristol, RI; Bradford Borque, *Center for Economic and Environmental Development*, Roger Williams University, Bristol, RI; Rober Holmberg, Robyn Hannigan, *Department of Environmental Science*, University of Massachusetts – Boston, Dorchester, MA; Dillon Post, *Department of Biology*, Community College of Rhode Island, Warwick, RI; Michael Tlustly, *John H. Prescott Marine Laboratory*, New England Aquarium, Boston, MA

RI NSF EPSCoR Summer Undergraduate Research Fellowship Program

An increase in atmospheric CO2 concentration results in increasing CO2 absorption by the ocean, in turn altering seawater carbonate chemistry in a process known as Ocean Acidification. Ocean Acidification most visibly effects external calcifiers, causing the dissolution of existing calcite structures and hindered development of new structures. Ocean Acidification also affects ionic balance in marine organisms, affecting the growth of otoliths (ear stones) in fish. The primary function of otoliths is gravisensing, a sense critical to fish in their three-dimensional world. However, the physical and chemical changes of otoliths has been overlooked due to the more visible ailments of hard-shelled organisms. Educating the public as to the effects of carbon dioxide in the atmosphere is key to public acceptance of greenhouse gas reduction. As a part of our Ocean Ac idification program, a lesson plan was created in conjunction with the Teacher Resource Center at the New England Aquarium in order to educate students and teachers about the unseen harm of Ocean Acidification and its treacherous effects on calcifying organisms. Additionally, a presentation explicating these effects will be instated at the New England Aquarium. Through this outreach, we hope to enhance public awareness about Ocean Acidification in the context of personal carbon footprints, thereby illuminating the dangerous effects of climate change on marine life.

HIGHLY SKEWED SEX RATIOS AMONG HOMARUS AMERICANUS, LIBINIA EMARGINATA AND CANCER IRRORATUS ALONG NEWPORT NECK

Katherine Jones, Jennifer Kane, Heather Nicholson, Jameson Chace, *Department of Biology and Biomedical Sciences*, Salve Regina University, Newport, RI; David Borkman, *Department of Biology*, University of Rhode Island, Kingston, RI

RI NSF EPSCoR Summer Undergraduate Research Fellowship Program

Accurate demographic information is required to build predictive models of future population growth, species responses to environmental change, and establish robust management plans for commercial fisheries. As part of a five-year research project examining how sea level rise will effect abundance and distribution of near shore invertebrate and fish populations along Newport Neck, Rhode Island, we ascertained age and sex distributions for several key crustacean species: American lobster (Homarus americanus), spider crab (Libinia emarginata) and rock crab (Cancer irroratus) and green crab (Carcinus meanas) at fifty-two subtidal sites. Crabs were captured in shallow (< 10 m) ventless lobster traps baited with mackerel and checked twice per week May-September, 2011-2014. Animals were captured, sexed, lengths of the carapace measured to approximate age then r eleased. Sex ratios were highly skewed towards males for three of the species, lobster (1 females: 3.69 males), spider crab (1: 3.31), rock crab (1: 6.21), but not green crab (1: 0.97). Sex ratios were consistent across all sites, even though abundance was highly variable. Lobsters were most abundant at the most prominent cliff faces, spider crabs were most abundant in sheltered sites with small substrate bottoms, rock crabs were most abundant in Gooseberry Cove, and green crabs most abundant in sheltered coves. While lobsters and rock and spider crabs have male biased sexual size dimorphism, we used size as an estimation of relative age. Larger individuals of both sexes for all species tended to be found more often in sites with greater wave exposure, whereas smaller, i.e., younger, individuals of all species tended to be found in protective coves. Coves along Newport Neck could serve as a nursery for these crustaceans. The skewed sex distribution could be due to several factors: biased sex ratio at birth, higher female mortality, and/or higher mobility and lower trap shyness of males. The fact that green crabs do not have a biased sex ratio suggests that there are important factors to consider when projecting future population growth or decay of lobster, spider and rock crab populations.

INVASIVE SPECIES COMPETITION AND HABITAT SELECTION AMONG THE ASIAN SHORE CRAB AND EUROPEAN GREEN CRAB ALONG NEWPORT NECK

Jennifer Kane, Jameson Chace, Katherine Jones, Heather Nicholson, *Department of Biology*, Salve Regina University, Newport, RI; David Borkman, *Department of Biology*, University of Rhode Island, Kingston, RI

RI NSF EPSCoR Summer Undergraduate Research Fellowship Program

The European green crab (Carcinus maenas) and the Asian shore crab (Hemigrapsus sanguineus) are two invasive species that are currently distributed along Newport Neck. European green crabs arrived in the U.S. in the mid-1800s while Asian shore crabs arrived in 1988. The intertidal abundances and distributions of green crabs and Asian shore crabs were surveyed during June and July from 2011-2014 as part of a five-year study on sea level rise and its effects on near shore invertebrate and fish populations in Rhode Island. At each intertidal site (n = 43), systematic timed crab abundance surveys were conducted along 20 m of shoreline at low tide. The species of crab and sex were observed and recorded during the survey to trace abundance and distribution at each site. Akakie's Information Criterion (AICc) was employed to determine substrate-habitat relations hips of crabs in the rocky intertidal zone. Asian shore crabs were most positively associated with large boulder (> 1.25m) and cobble (64 - 305mm), while green crabs were associated with small boulders (30.5cm - 1.25m) and gravel (4 - 64mm). Overall, Asian shore crabs and green crabs share the same rocky intertidal zone for habitat use, and therefore compete for the same space. However, as the habitat quality increases a threshold effect occurs as Asian shore crab density increase more rapidly relative to the changes in abundance of the green crabs. Green crabs abundance begins to decline at highest densities of Asian shore crabs. Interspecific dominance may be due to Asian shore crabs having a wider variety of resources in their diet to choose from, and they have also been known to prey on green crabs. As sea levels continue to rise the intertidal habitat these crabs share will start to change into larger substrate with less of the small substrate sizes they prefer. This change in substrate may cause changes in the abundance and distribution of both species along Newport Neck as individuals disperse and populations adapt to the new environment sea level rise will bring.

MARINE SPECIES ABUNDANCE AND RICHNESS ALONG NEWPORT NECK

Irene Luperon, Sarah Matarese, *Department of Biology*, St. Georges School, Middletown, RI;
Jameson Chace, *Department of Biology*, Salve Regina University, Newport, RI

RI NSF EPSCoR Summer Undergraduate Research Fellowship Program

Baseline abundance and richness of marine invertebrates is important for future comparisons to predict how a changing climate will impact species living within the intertidal and sub-tidal zones along Newport Neck. The goal of this study was to evaluate the distribution of marine species both in the intertidal and sub-tidal at nine sites along Newport Neck. Sessile intertidal organisms were surveyed using quadrat (0.5 m2) methods, and mobile animals were surveyed through systematic searches along 20 m transects. Subtidal (< 10 m depth) animals were surveyed with ventless lobster traps and minnow traps. Distribution and abundance of blue mussels (Mytilus edulis) and periwinkles (Littorina littorea) were highly nonrandom, while barnacles (Cirripedia sp.) were more evenly distributed across all sites. Among the six different crab species identified, Asian shor e crabs (Hemigrapsus sanguineus) were the most common and showed the greatest significant variation in distribution and abundance. In the subtidal zone lobsters (Homarus americanus), spider crabs (Libina sp.), and rock crabs showed a significant site-specific changes in abundance and distribution along Newport Neck. Small juvenile fish and eels captured in minnow traps were evenly distributed across Newport Neck but were most common in the sheltered coves. Species such as black sea bass (Centropristis striata), cunner (Tautogolabrus adspersus), summer flounder (Paralichthys oblongus) and American eel (Anguilla rostata) were common suggesting that areas of Newport Neck serve an important ecosystem service as a nursery to game species. While the rocky intertidal and sub-tidal zone of Newport appears largely homogenous, the species that inhabit this area exhibit fine-grained responses to underlying substrate variation. Establishing baseline understanding of the richness and abundances of marine organisms along the Rhode Island coast is important to document and understand distributional changes predicted sea level rise, increased temperature and reduced pH due to climate change. This information is particularly important for managing key commercial species (lobster, rock crab, game fish) in the face of a rapidly changing environment.

INVESTIGATING THE NATURE OF THE CAUSATIVE AGENT OF SEA STAR WASTING SYNDROME

Jillon McGreal, Caitlin DelSesto, Marta Gomez-Chiarri, *Department of Fisheries, Animal and Veterinary Science*, College of the Environment and Life Sciences, University of Rhode Island, Kingston, RI; Gary Wessel, *Department of Molecular Biology*, Cell Biology, and Biochemistry, Brown University, Providence, RI

RI NSF EPSCoR Summer Undergraduate Research Fellowship Program

Sea stars are keystone predators, maintaining ecosystem stability in marine coastal communities through food web interactions. In recent years, populations of Forbes sea stars, Asterias forbesi, in Rhode Island, Massachusetts, and Maine have been affected by what has been called the Sea Star Wasting Syndrome (SSWS), which has likely caused severe mortalities and a sharp drop in population numbers. Previous cohabitation challenge experiments between stars with SSWS and healthy-looking stars show that an infectious agent transmitted through water likely causes SSWS. The purpose of this study was to determine whether the pathogenic agent causing SSWS is viral, bacterial, or a toxin. Water from tanks in which sea stars experienced mortality due to SSWS (infected water) was used in challenge experiments. Sea stars collected from the bay were held in 4 liters of m filtration membrane (containing viruses or toxins but no bacteria or parasites); and 4) FSSW with the addition of filtered water treated with UV for 2.5 hours (to inactivate viruses). Each experimental group had 2 replicate tanks containing 2 stars each. Stars were monitored daily for 13 days for mortality and signs of SSWS. Mortalities (3/4) were only observed in stars treated with filtered infected water, suggesting that the pathogenic agentμfiltered and UV sterilized salt water (FSSW) in closed systems at constant temperature (20.4-23.6 °C) and salinity (28 – 32 psu). Healthy-looking sea stars were acclimated for 12-days after injection with a dosage of the antibiotic enrofloxacin (0.11 mL/kg of body weight) on day-1 of the acclimation period. Experimental groups for the challenge experiment included sea stars held in: 1) FSSW (control); 2) FSSW with the addition of infected water; 3) FSSW with the addition of the filtrate from infected water passed through a 0.22 is a virus. The absence of SSWS in stars treated with infected water was unexpected, and could be due to resistance of some sea stars to SSWS and/or low concentrations of pathogen in the non-filtered infected water. Future studies are needed to further investigate the pathogenic agent causing Sea Star Wasting Syndrome.

MARINE SPECIES-SPECIFIC HABITAT MODELS USED TO PREDICT FUTURE

Heather Nicholson, Katharine Jones, Jennifer Kane, Jameson Chace, *Department of Biology and Biomedical Sciences*, Salve Regina University, Newport, RI; David Borkman, *Graduate School of Oceanography*, University of Rhode Island, Kingston, RI

RI NSF EPSCoR Summer Undergraduate Research Fellowship Program

This study was undertaken to determine how sea level rise will affect the abundance and distribution of near shore invertebrate and fish populations. I tested the hypothesis that these marine species will be nonrandomly distributed along Newport Neck based on species-specific substrate preferences. In order to determine the current abundance and distribution of species in the subtidal zone of Newport Neck, ventless lobster traps were set at 45 sites from May-September 2011-2014. To determine substrate choice for lobsters, Homarus americanus; common spider crabs, Libinia emarginata; rock crabs, Cancer irroratus; and green crabs, Carcinus meanas; we estimated the percent coverage of bedrock, manmade materials, large and small boulders, cobble, gravel, sand, and mud on the ocean floor at each trap site. Additionally, in order to predict which areas will be h abitable for these species when sea level rises an estimated one to two meters, we also perform substrate surveys in the current intertidal zone. Akiakie's Information Criterion (AICc) was employed to determine the substrate-habitat relationships of lobsters, spider crabs, green crabs, and rock crabs in the nearshore subtidal environment. Lobsters were most positively associated with large boulders; spider crabs with mud and cobble; green crabs with sand, mud, and bedrock; and rock crabs with bedrock, sand, and manmade substrate. The substrate coverage of the current intertidal zone, which is expected to become the new subtidal zone as sea level continues to rise, is largely gravel, cobble, and small boulders compared to the current subtidal zone substrate which is largely sand and large boulders. This predicted change in the substrate availability for the fish and invertebrate species of Newport Neck is likely to impact their habitat choices, which could in turn alter the food web and influence the economy of local fisheries.

ALGAL BLOOM: DATA NARRATION THROUGH INTERACTIVE ANIMATION

Yidan Zeng, *Department of Glass and Computer Science*, Rhode Island School of Design and Brown University, Providence, RI; Oscar Robert, *Department of Graphic Design*, Rhode Island School of Design, Providence, RI

RI NSF EPSCoR Summer Undergraduate Research Fellowship Program

Effects of climate change are clearly evident in data collected from the Narragansett Bay over the past 50 years. However, engaging the general public with these results remains a challenge. There is often a loss of context from the presentation of the data to the subject being presented on. When data is communicated in the structure of separated and isolated variables, such as water temperature, dissolved oxygen, precipitation, and wind speed, the lay-person's understanding of the systemic relationships between variables is often missing. Drawing from immersive gameplay as inspiration, our investigation centered on presenting the integrated relationships between variables, employing animation and web-based interactions to facilitate the engagement between the viewer and scientific material.

Partnering with the University of Rhode Island Graduate School of Oceanography, we extracted a data narrative from the data sets accumulated over five decades of research conducted in the Narragansett Bay. Using Maya animation software, we began a two-part project that introduces the living space and relationships between marine organisms. On an animated trip deep into the Narragansett Bay, users are given the context of what happens in the event of an algal bloom. On the reverse trip back up, organisms respond to the user’s touch, allowing for an organism-specific interaction which provides further layers of information. Within the context of an algal bloom event, we hope to engage the public with multiple organisms affected, and in turn talk about the climate change factors and human actions that have exacerbated this problem.

SPECIFICITY AND SENSITIVITY OF A PCR-BASED APPROACH FOR DETECTING WINTER FLOUNDER IN BLUE CRAB STOMACHS

Abigail Scro, Kelly Cribari, David Taylor, *Department of Marine Biology*, Roger Williams University, Bristol, RI; Kathryn Markey, *Aquatic Diagnostic Laboratory*, Roger Williams University, Bristol, RI

RI NSF EPSCoR Summer Undergraduate Research Fellowship Program

Increasing water temperatures in the Northwest Atlantic have resulted in blue crabs (Callinectes sapidus) extending their geographic range northward to Southern New England coastal habitats, including the Narragansett Bay Estuary (RI, USA). The increased abundance of blue crabs in this area 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 blue crab predation on juvenile winter flounder. To evaluate the sensitivity and specificity of the approach, a winter flounder-specific (WF208) primer set was tested against winter flounder, blue crab, and alternative prey items. The effect of digestion time on detecting flounder DNA in crab stomachs was also determined in laboratory feeding experiments (0-10 hr post-feeding). DNA extractions of tissue and gut contents were carried out using a Qiagen DNeasy Blood and Tissue Kit and the 208 base-pair primer set. WF208 primers successfully and exclusively amplified winter flounder tissue (high sensitivity and specificity). The DNA concentration and quality of digested flounder tissue consistently declined as digestion time increased. PCR results were more variable, however, with flounder DNA being positively detected in 0-38% of crab stomachs examined between 0 and 8 hr post-feeding. In the future, additional feeding and spiking experiments will be conducted by manipulating the modes of sacrifice, crab preservation techniques, and DNA extraction protocols in order to optimize the PCR results.

LET ME TELL YOU A STORY: CURATING CHARISMA OF MARINE PLANKTON THROUGH PERSONAL NARRATIVES

Noah Schlottman, Beatrice Steinert, *Nature Lab*, Rhode Island School of Design, Providence, RI

RI NSF EPSCoR Summer Undergraduate Research Fellowship Program

The way that scientific information is usually communicated often fails to employ aesthetic, emotional, or physical attributes that make it relatable to the public. In this study we tested the hypothesis that personal narrative or voice can be used to present scientific information in a way that is accessible and engaging to audiences beyond those of the scientific community. Marine plankton provide rich subject matter for testing this approach due to their integral role in marine ecosystems, making them an important part of discussions surrounding global climate change. Additionally, because of their microscopic scale, not only are they relatively unknown and unseen, but they also fail to generate the same level of support or interest as charismatic megafauna.

Charisma results from the perception of characteristics of an organism, which provokes an emotional response, making it a human-created and human-centric phenomenon. In parallel with a personal exploration of marine plankton, we embraced the idea that we can curate the charisma of these organisms to make them more engaging and relatable. Our outputs included a video installation with microscopy footage of plankton and composed accompanying sound, an online blog containing illustrative text with integrated video, and a hand-printed, narrative-driven book about marine plankton. The final step involved testing our outputs to determine their effectiveness as vehicles for communicating scientific information.

EFFECTS OF TEMPERATURE ON COMMUNITY DYNAMICS IN THE ROCKY INTERTIDAL

Jake Swanson, Patrick Ewanchuk, *Department of Biology*, Providence College, Providence, RI

RI NSF EPSCoR Summer Undergraduate Research Fellowship Program

The 2007 IPCC report states that mean surface temperature in the North Eastern United States may rise between 2°C and 4°C by 2100. Rising temperatures will lead to myriad changes in the marine environment including increased ocean temperatures. Increased ocean temperatures will lead to, amongst other repercussions, marine species migrating poleward. These changes in species distribution will cause shifts in many marine ecosystems, as novel species are introduced community dynamics will change according to the niche filled by the new species. Elevated temperatures will not only cause species to extend their ranges, they will also have an effect on the organismal level. Feeding and growth rates can be positively or negatively affected, depending on the organism. Raised temperatures can also alter interactions such as predation and competition. A shift toward s or away from one species can disrupt the standard community dynamic, leading to a change in species composition in a certain habitat. This study examined the effect of temperature on competition for food between two predatory marine snails, the Dogwhelk (Nucella lapillus) and the Southern Oyster Drill (Urosalpinx cinerea). It was determined that higher temperatures lead Urosalpinx cinerea outcompeting Nucella lapillus for sources of food. During the course of the experiment, it was also determined that, during feeding, Urosalpinx cinerea showed a statistically significant preference to drill on the outermost edge of the mussel shell and that the difference in hole size drilled was statistically significant with Urosalpinx cinerea drilling larger holes than Nucella lapillus.

CHANGES IN GENE EXPRESSION OF MOLECULAR FUNCTIONS IN ULVA RIGIDA

Megan Sylvia, Stephanie Liptak, JD Swanson, *Department of Biology and Biomedical Sciences*, Salve Regina University, Newport, RI

RI NSF EPSCoR Summer Undergraduate Research Fellowship Program

The macroalgal genus Ulva, commonly known as “sea lettuce”, develops into harmful macroalgal blooms (dense, floating aggregations) in shallow coastal systems worldwide, including Narragansett Bay, RI. These blooms have significant negative ecological and economic impacts on coastal communities. However, little data exist on the genetic makeup and underling functional genetics that link bloom dynamics to environmental changes, especially with respect to global warming. To this end, we embarked on a one-year metagenomics study to begin to understand the link between gene expression changes, bloom dynamics and environment. This was completed by sampling two abundant bloom-forming Ulva populations at three sites around the Narragansett Bay, RI at relevant stages of the bloom cycle. After collection, samples were identified and RNA was extracted and sequenc ed using HiSeq 2000 NGS technology. The resulting sequence was assembled using the Geneious software, and was annotated using Blast2Go. These data combined indicated large gene expression changes through the year that could be indicative of bloom formation providing one of the first insights to the genomics of this behavior. In particular protein-binding transcription factor activity, receptor activity and electron carrier activity have shown to have greater change in gene expression in the functional pathways across the months of May, July and September. Moreover, some individual gene expression profiles be leveraged to act as markers to predict macroalgal blooms.

PROTEIN EXPRESSION OF ORAL SIPHON TISSUE REGENERATION IN CIONA INTESTINALIS

Shannon Aurigemma, *Department of Biology, Marine Biology, and Environmental Sciences,* Roger Williams University, Bristol, RI; James Tempest, *Department of Biology,* Community College of Rhode Island, Warwick, RI; Meg Wharburton, *Department of Biology,* Rhode Island College, Providence, RI; Steven Irvine, *Department of Biological Sciences,* University of Rhode Island, Kingston, RI

RI NSF EPSCoR Summer Undergraduate Research Fellowship Program

Ciona intestinalis are ascidians (sea squirts), which are in the most closely related group to vertebrates. Unlike most vertebrates, they have the ability to regenerate body parts after a piece of tissue has been severed from them. We hypothesize that different proteins will be expressed in the new regenerated tissue as compared to the unharmed tissue. In this study, we are working towards examining the protein expression of regenerating vs. control oral siphon tissue. The C. intestinalis individuals were collected from docks at Snug Harbor in Wakefield, Rhode Island and then were raised in a tank at 16°C both prior to and post dissection at the University of Rhode Island. The oral siphon was dissected below the pigment cells and the individuals were placed back in the tank to regenerate tissue for 3 and 6 days. The tissue collected served as the protein control. After the specified number of days the new regenerated tissue was dissected and underwent a protein extraction along with the control tissue. The concentration of the protein was then quantified and after a trypsin digestion the samples were sent to the EPSCoR Proteomics Facility at Brown University for liquid chromatography/mass spectrometer (LC/MS) analysis. We also analyzed previously collected LC/MS data from C. intestinalis maintained at different temperatures. These data show differences in protein expression, suggesting that our method is capable of detecting the hypothesized changes in protein expression in regenerating animals.

EFFECT OF PROJECTED OCEANIC TEMPERATURE INCREASE ON THE PROTEOME OF THE TUNICATE, CIONA INTESTINALIS

Megan Wharburton, Thomas Meedel, *Department of Biology*, Rhode Island College, Providence, RI; Shannon Aurigemma, *Department of Biology, Marine Biology, and Environmental Science*, Roger Williams University, Bristol, RI; James Tempest, *Department of Biology*, Community College of Rhode Island, Warwick, RI; Steven Irvine, *Department of Biological Sciences*, University of Rhode Island, Kingston, RI

RI NSF EPSCoR Summer Undergraduate Research Fellowship Program

Our labs have initiated a pilot project to study the effect of predicted rising ocean temperatures on the proteome of the model organism, Ciona intestinalis. In order to conduct this work we established experimental tanks of varying water temperatures at the Graduate School of Oceanography (GSO) at the University of Rhode Island's Bay Campus. Embryos were seeded onto acrylic panels and reared to post-larval stage zooids that attached to the panels, which were then hung in control and experimental tanks with flow through seawater maintained at 16° C and 21° C, respectively. When the animals in these tanks reach sexual maturity, at around two months post fertilization, their tissues will be dissected and their proteins extracted. Extracted proteins will be analyzed by liquid chromatography and mass spectroscopy at the Proteomics Shared Resources Facilit y at Brown University, which utilizes a bioinformatics approach in order to identify the proteins. Work this summer involved tank set up and maintenance and determining the protein extraction method that works best for this project. We also analyzed previously collected proteomic data from C. intestinalis maintained at different temperatures. These data show differential protein expression, suggesting that our method is sensitive enough to detect the hypothesized changes in protein profiles. Future studies include examining the effects of increased temperature on the reproductive success of Ciona, and studying how lowered pH, representative of predicted ocean acidification, affects the proteome and reproductive success of Ciona.

MERCURY CONTAMINATION IN BLUE CRABS FROM RHODE ISLAND COASTAL WATERS

Nicholas Clabrese, David Taylor, *Department of Marine Biology*, Roger Williams University, Bristol, RI

RI-INBRE Summer Undergraduate Research Fellowship Program

The blue crab (Callinectes sapidus) has supported lucrative commercial and recreational fisheries in the middle- and southern Atlantic Coast and Gulf of Mexico. Blue crab populations have also recently increased in southern New England coastal waters, which will likely elevate their fishery status in this region. With an emerging Rhode Island (RI) crab fishery, research is needed to quantify possible contaminants in this species. Most notably, mercury (Hg) is a pervasive environmental contaminant that adversely affects human health, and exposure occurs mainly by consuming contaminated fish and shellfish. In this study, blue crabs were collected from Narragansett Bay and associated coastal ponds and tidal rivers (Seekonk and Taunton Rivers). The claw muscle tissue of each crab was excised and analyzed for total Hg using automated atomic absorption spectroscopy. Results were subsequently analyzed relative to crab body size (carapace width, CW) and habitat use. Bioaccumul ation of Hg was evident in crabs from the Seekonk and Taunton Rivers, but not in conspecifics from the bay or ponds. Taunton River crabs also had a significantly higher mean Hg concentration relative to the other habitats (mean Hg: Taunton = 0.82 ppm dry wt; Other = 0.21 ppm dry wt). Spatial variations in crab Hg levels were attributed to habitat-specific Hg burdens in their prey, including shrimp, bivalves, and gastropods. Prey Hg concentrations were also significantly related to localized sediment Hg and methylmercury concentrations and grain size. With only 2.2% of legal-size blue crabs (> 127 mm CW) exceeding the U.S. Environmental Protection Agency action level, crabs from RI coastal waters present minimal risk to human consumers.

MERCURY AND SELENIUM CONCENTRATIONS IN COASTAL FISHES: RISKS AND BENEFITS TO HUMAN HEALTH

Joshua Jacques, Mary Yurkevicius, David Taylor, *Department of Marine Biology*, Roger Williams University, Bristol, RI

RI-INBRE & RI NSF EPSCoR 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 six fish species collected from RI coastal waters, including summer flounder (Paralichthys dentatus), striped bass (Morone saxatilis), tautog (Tautoga onitis), scup (Stenotomus chrysops), bluefish (Pomatomus saltatrix), and black sea bass (Centropristis striata) (n = 19-20 per species). Data were analyzed relative to fish body size to assess bioacc umulation patterns, and Se:Hg molar ratios and Health Benefit Values (HBV) were calculated to estimate the relative health risk vs. benefit of each species for human consumers. 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 (exception = summer flounder). In contrast to Hg bioaccumulation patterns, Se concentrations were relatively constant across fish size. Se:Hg molar ratios and 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 research will examine the fatty acid profiles (e.g., omega-3 concentrations) of each fish species to further evaluate their respective health benefit to humans.

FATTY ACID PROFILES OF MARINE FISHES FROM RHODE ISLAND COASTAL WATERS

Mary Yurkevicius, Joshua Jacques, David Taylor, *Department of Marine Biology*, Roger Williams University, Bristol, RI; Nancy Breen, *Department of Chemistry*, Roger Williams University, Bristol, RI

RI-INBRE Summer Undergraduate Research Fellowship Program

Marine fish are an excellent source of omega-3 fatty acids 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=15); black sea bass, Centropristis striata (n=17); and striped bass, Morone saxatilis (n=15). Fatty acid profiles of fish muscle tissue were determined by esterification and gas chromatography. Data were categorized as saturated, mono-saturated, omega-3 and omega-6 fatty acids, and results were expressed as concentrations (mg/100g wet weight; [FA]) and percent of total fatty acid content (%FA). Irrespective of fish species, saturated fatty acids had consistently higher concentrations relat ive to the other measured profiles (mean saturated [FA] = 15.6 mg/100 g; %FA = 47.6%), whereas omega-6 fatty acids were depleted (mean omega-6 [FA] = 1.5 mg/100 g; %FA = 3.4%). Inter-species comparisons further revealed that omega-3 fatty acids were lower in black sea bass relative to striped bass and summer flounder (BSB: [FA] = 3.9 mg/100 g; %FA = 13.9%, SB and SF: [FA] = 10-15 mg/100 g; %FA = 21-28%); hence suggesting the latter species provides greater health benefits for human consumers. Lastly, the fatty acid profiles of the coastal fishes examined in this study were qualitatively compared to measurements made on store bought (aquaculture and wild) Atlantic salmon (Salmo salar) and sockeye salmon (Oncorhynchus nerka).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.

FORAGING ECOLOGY OF BLUE CRABS (CALLINECTES SAPIDUS) AND THEIR POTENTIAL IMPACT ON LOCAL BENTHIC COMMUNITIES

Molly Fehon, David Taylor, *Department of Marine Biology*, Roger Williams University, Bristol, RI

Rhode Island Science and Technology Advisory Council

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 coastal habitats. This study examined the potential impact of blue crabs on local fauna by analyzing their diet and overall abundance and size structure. Potential predation by blue crabs on juvenile winter flounder (Pseudopleuronectes americanus) was of particular interest due to declining populations of this flatfish species. Blue crabs were collected from Narragansett Bay and associated coastal ponds and tidal rivers using beach seines. Collected crabs were enumerated, measured for carapace width (CW), and preserved in 95% ethanol for subsequent stomach content analysis. In the laboratory, crab stomach contents were extracted and prey were identified to lowest practical taxon with the aid of stereomicroscopes. Prey importance was quantified by their volumetric contribution to the total stomach contents (%V). The main prey of blue crabs across habitats were bivalves, crabs, and unidentified crustaceans (mean %V = 29%, 21%, and 19%, respectively), and ontogenetic shifts in diet were observed. Winter flounder were detected in < 2% of the crab stomachs analyzed, and all incidences of flounder predation occurred in tidal rivers. Size-frequency distributions were consistent across habitats (CW range = 11-253 mm), indicating that crabs utilize these habitats across several life history stages. Blue crab abundances also demonstrated considerable spatial and temporal variations, such that crab abundance was highest in the tidal rivers in May (3.5/100 m2), August in the coastal ponds (1.2/100 m2), and July in the bay (1.7/100 m2). Future research will further examine the foraging ecology of blue crabs via genetic analysis of stomach contents and measurements of stable carbon and nitrogen isotopes in crab muscle tissue.

INSIGHTS INTO BLOCK ISLAND SOUND PLANKTON DYNAMICS USING IN SITU AND SATELLITE DATASETS

Nathan Goff, Sarah Knowlton, *Department of Physical Sciences*, Rhode Island College, Providence, RI; Shuwen Zhang, Lew Rothstein, *Department of Physical Oceanography*, Graduate School of Oceanography, University of Rhode Island, Kingston, RI; Susanne Menden-Deuer, *Department of Biological Oceanography*, Graduate School of Oceanography, University of Rhode Island Kingston, RI

Rhode Island Science and Technology Advisory Council

The purpose of the project is to use a coupled biogeochemical-physical model for Rhode Island Sound (RIS) and Block Island Sound (BIS) to investigate biogeochemical cycling in waters off Rhode Island. Nutrient, chlorophyll-a, and plankton dynamics are used to initialize the model and chlorophyll-a is used to validate and interpret the model outputs. Circulation models have suggested that Long Island Sound (LIS) may impact the biogeochemistry of BIS. Thus, annual and interannual nutrient and chlorophyll-a variability are studied using moored station data in eastern LIS near its interface with BIS in order to investigate this interaction. A distinct seasonal cycle is observed at all stations in LIS, and moving east across LIS toward BIS there are decreasing values of nutrient and chlorophyll-a concentrations. Satellite chlorophyll-a data is compared to surfa ce in situ observations in the region to compare the temporal and spatial variability between the datasets. Although the in situ and satellite data differ temporally in their collection, the average spatial patterns are similar. Satellite and in situ chlorophyll-a concentrations averaged yearly over a decade displayed similar decreasing values moving from the mid-LIS to the BIS interface. In contrast, the monthly average in situ and satellite chlorophyll-a values can vary by as much as 71 percent and display different seasonal trends. This study shows that a significant gradient in biogeochemical measurements between LIS and BIS exists, which will aid in the development of the biogeochemical model.

SPATIAL AND TEMPORAL TRENDS IN NUTRIENT AND CHLOROPHYLL A CONCENTRATIONS ON THE COASTAL OCEAN OF RHODE ISLAND SOUND, BLOCK ISLAND SOUND AND THE ADJACENT INNER SHELF

Rachel Miller, Sarah Knowlton, *Department of Physical Sciences*, Rhode Island College, Providence, RI; Shuwen Zhang, Lewis Rothstein, *Department of Physical Oceanography*, Graduate School of Oceanography, University of Rhode Island, Kingston, RI; Susanne Menden-Deuer, *Department of Biological Oceanography*, Graduate School of Oceanography, University of Rhode Island, Kingston, RI

Rhode Island Science and Technology Advisory Council

To investigate biogeochemical cycling in Rhode Island coastal ocean waters, a coupled biophysical model is configured with four biological state variables, i.e. dissolved inorganic nutrients (N), non-living particulates (detritus: D), phytoplankton (P), and herbivorous zooplankton (Z). Monthly mean concentrations of nitrogen (nitrate, nitrite, ammonia) at the ocean surface, mid- and lower depths and surface chlorophyll-a concentrations from the Rhode Island Sound (RIS) and Block Island Sound (BIS) are necessary to initiate and verify the NPZD model for the region. There are, however, very few observational data for the RIS and BIS. To support initialization and validation of the model, this study focuses on analysis of observational data so far assembled. Available data from this region include a historical dataset from the 1970s and chlorophyll-a concentrati ons from 2008-2010. Extensive data sets are also available from adjacent bodies of water: Narragansett Bay (NB), Long Island Sound (LIS), Gulf of Maine (GoM) and Nantucket Shoals (NS). Data for the above-mentioned regions were examined for spatial patterns and seasonal, interannual and decadal variability in nutrient and chlorophyll-a concentrations in the shelf region encompassing the eastern LIS to the southern GoM. Specifically, this analysis was accomplished in two parts. First, the ratios of chlorophyll-a concentrations to a biweekly mean for NB, NS, LIS, RIS and BIS from December 2008 to May 2010 were analyzed. Second, the ratios of nutrients and chlorophyll-a to a monthly regional mean were examined for NB, NS, LIS, RIS, BIS and GoM from 1969-1979 and compared to ratios analyzed from observations made from 1989-1999 for NB, NS, LIS, and GoM. Evaluation and comparison of the two time periods provides a better understanding of spatial and temporal variability in nutrien t and chlorophyll-a concentrations and will be used in conjunction with development of the NPZD model.

SPATIAL AND TEMPORAL DISTRIBUTION OF AMERICAN CONGER EELS, CONGER OCEANICUS, IN RHODE ISLAND AND BLOCK ISLAND SOUNDS

Craig Rockwell, David Taylor, *Department of Marine Biology*, Roger Williams University, Bristol, RI

Bureau of Ocean Energy Management

The American conger eel (Conger oceanicus) is a congrid marine eel with a geographic range that encompasses continental shelf waters from Massachusetts to Florida. Only limited information exists on the general biology and ecology of conger eels, and to date, no studies have examined spatio-temporal abundance patterns of this species in a defined region. In this study, conger eel abundance was quantified in Rhode Island and Block Island Sounds using standard vented and ventless lobster pots (2013: May-Oct; 2014: May-Jun). Spatial and temporal variation in eel catch data were examined relative to site-specific bathymetry and bottom water temperature conditions. Conger eel abundance varied spatially, and was considerably higher at locations in close proximity to Block Island, but the near and far locations all had steady increases in conger abundance over t ime with seasonal variations. Spatio-temporal patterns in eel abundance were significantly related to bottom water temperature, but not depth (Regression: Temp: $R2 = 0.36$, $p < 0.0005$; Depth: $R2 = 0.02$, $p = 0.48$). Future research will examine the biology of conger eels, including the quantification of length-weight relationships, growth rates, gonadal-somatic indices, and length-at-maturity estimates.

MICROBIOLOGY

LOCATED NEAR THE CENTRAL STAIRCASE ON THE 2ND FLOOR OF THE PHARMACY BUILDING

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

COMPARING PLASTID GENOMES OF VERTEBRATA LANOSA AND CHOREOCOLAX POLYSIPHONIAE

Katie Nickles, Eric Salomaki, Chris Lane, *Department of Biological Sciences*, University of Rhode Island, Kingston, RI

RI NSF EPSCoR Summer Undergraduate Research Fellowship Program

Parasites live in or on another organism and exploit the host organism for food. Red algal parasites provide a unique look at how organisms become parasites because the parasite and host usually share a recent common ancestor. This unusual evolutionary phenomenon is called "adelphoparasitism" – adelpho is Greek for "kin". Red algal parasites only exist in the Florideophyceae class due to the triphasic life history characteristic for this group. The parasite mimics the carposporophyte life stage and is able to gain nutrients from the host through secondary pit connections. Parasitic red algae are pigmentless and not known to be able to conduct photosynthesis. Studies in the 1990's demonstrated that the parasites have lost their own plastid as they have transitioned from photosynthetic to parasitic lifestyles. In our study, we examined sequence data from the adelphoparasite Choreocolax polysiphoniae and its host Vertebrata lanosa for differences in gene presence and content. We identified and annotated the V. lanosa plastid, which was smaller than most florideophyte plastids with only 197 genes. Surprisingly, we also identified a ~90,000 base pair DNA fragment from the C. polysiphoniae sequence data that encodes 74 identifiable plastid genes. After annotation of these genes, we are confident this DNA belongs to the plastid of C. polysiphoniae, the first identified parasitic florideophyte plastid.

IMPACT OF ETHANOL AND BENZYL ALCOHOL-CONTAINING HEPARIN FORMULATIONS IN PROMOTING STAPHYLOCOCCUS SPP.BIOFILMS

Sarah Bilida, *Department of Biology*, Rhode Island College, Providence, RI; Megan Luther, Kayla Babcock, Kerry LaPlante, *Department of Pharmacy Practice*, University of Rhode Island, Kingston, RI

RI-INBRE Summer Undergraduate Research Fellowship Program

Background: Heparin &ethanol (EtOH) catheter lock solutions are frequently used for the prevention and treatment of catheter related bloodstream infections. Recent studies demonstrate that heparin products containing alcohol as preservative at concentrations greater than 40% may enhance biofilm (BF) production.

Methods: A modified microtiter assay was used to quantify BF, measured as cell adherence, of S. epidermidis(SE) and S. aureus. After 24 hours of growth, BF was developed and each strain was exposed to either heparin containing preservative (2500 units/mL and 5000 units/mL; benzyl alcohol (BA) 0.45%), preservative-free heparin (5000 units/mL), EtOH, isopropanol (IPA) (40%, 60%, 80%, and 95%) or normal saline control. The activity of bacteriostatic saline (BSS) (0.45% BA) and chemical grade BA (both 0.45%) on BFs were tested separately. Agents were evaluated at either 24 or 72h. Plates were stained and read at OD570 (Ceriet al.).Statistics were conducted using ANOVA with Tukey's post-hoc test.

Results: At 24h, isolates exposed to preservative-free heparin tended to have the lowest BF, while BSS had the highest BF. BSS had significantly higher BF compared to BA and all heparins in three of five strains (0.10-0.89, 95% CI 0.04-1.89,$p<0.03$). There was no significant difference in BF between the two preservative-containing heparins. There was no significant difference between BA and all heparin solutions for 4 out of 5 strains. At 72h, relationships between heparins of different concentrations with and without preservative changed in a strain dependent manner. For bacteria exposed to BA, there was a significantly increased OD compared toBSS (0.3178-.6310, 95% CI 0.0512-0.8754, $p\leq0.017$) in two of the five strains, however in BSSBF was lower than at 24h in 2 strains, possibly due to bacterial detachment.

IPA&EtOH were only tested for 24h and generally increased BF with increased alcohol concentration. Also, the isogenic SA mutant, M7, which lacks the ability to form BFs, appeared to have significantly increased BF after exposure to all concentrations of alcohols tested (0.3577-1.2819,95% CI 0.2243-1.48, $p<0.001$).

Conclusion: BSS demonstrated the most BF at 24h while BA demonstrated the most BF at 72h. Heparin solutions demonstrated less BF, however this was strain dependent. Increased concentrations of EtOH and IPA showed increased BF in all strains except SE. Individual results may be strain dependent and further tests are needed.

IDENTIFICATION AND CLONING OF THE PUTATIVE GANGLIOSIDE BINDING PROTEINS FOR BORRELIA BURGDORFERI FROM MLPD

Ryan Brown, Christopher Reid, *Department of Science and Technology*, Bryant University, Smithfield, RI

RI-INBRE Summer Undergraduate Research Fellowship Program

First recognized in 1975, Lyme disease is now the most commonly reported vector borne illness, despite the fact that it is often asymptomatic. A vast range of symptoms are commonly attributed to Lyme disease including heart, skin, joint and eye. In approximately 15% of patients there are neuropsychiatric manifestations which have now been deemed neuroborreliosis. This project aimed to identify and characterize potential ganglioside binding proteins that could be involved in late stage Lyme disease neuroborreliosis. This would enable us to better understand the molecular mechanisms of Borrelia pathogenesis. We analyzed the Borrelia genomes using a bioinformatic approach where we probed the available Borrelia genomes with botulinum toxin D (a highly characterized ganglioside binding protein) as “bait” to identify potential ganglioside binding proteins from B. burgdorferi sensu lato. We identified MlpD to be the most promising candidate based on it’s ability to bind extr acellularly and possibly to nerve cells, which would allow Borrelia to cause neuroborreliosis. There is also evidence that mlpD is upregulated in animal models. The protein family Mlp is divided into two different groups which differ from each other by approximately 30% (Mlps within the same class possess 60-80% identity). MlpD is classified as an antigenic class II protein, characterized by a shorter C termini with a mass range of 13-15 kDa. The gene mlpD was amplified from B. burgdorferi B31 genomic DNA to include NdeI and XhoI restriction sites. The amplified mlpD was cloned into the pET30a expression vector on NdeI/XhoI fragments to produce a construct with a C-terminal His6 tag. Preliminary expression studies have been carried out and we hope to begin protein expression and purification studies in the near future.

ISOLATION AND CHARACTERIZATION OF NOVEL K2 CLUSTER MYCOBACTERIOPHAGES

Heloise Dubois, *Department of Biology*, Providence College, Providence, RI; Alicia Jancevski, *Department of Chemistry and Biochemistry*, Providence College, Providence, RI

RI-INBRE Summer Undergraduate Research Fellowship Program

Mycobacteriophages are pathogenic viruses that infect and kill mycobacteria, many of which cause diseases including tuberculosis and leprosy. Mycobacteriophage ZoeJ was isolated from a soil sample at Providence College via an enrichment procedure. Plaque morphology and electron microscopy photos suggest that ZoeJ is a Siphoviridae lysogenic phage. DNA sequencing and annotation of the genome indicates ZoeJ contains 57,315 bp and 92 probable genes. Based on homology, ZoeJ has been assigned to the K2 subcluster, a new subcluster consisting of ZoeJ, Mufasa, and TM4. Genomic comparison of the K2 subcluster has revealed a gene sequence containing an integrase which is present in both ZoeJ and Mufasa, yet absent from TM4, a phage commonly used in the study of mycobacteria. Immunity assay results suggest that the integrase of ZoeJ is functional, allowing it to for m lysogens. The putative attP/attB sites of phage integration were identified for both ZoeJ and Mufasa. A PCR assay designed to amplify the regions of phage integration confirmed that the integrase in both ZoeJ and Mufasa is functional, allowing these K2 cluster phages to form lysogens.

USING POMEGRANATE CONSTITUENTS TO SILENCE BACTERIA

Miles Martin, Robert Deering, Jiadong Sun, Fatemeh Akhlaghi, David Rowley, *Department of Biomedical and Pharmaceutical Sciences*, College of Pharmacy, University of Rhode Island Kingston, RI

RI-INBRE Summer Undergraduate Research Fellowship Program

Pomegranates have long been used in folk medicine and are noted for their antioxidant and antibacterial effects. We have shown that punicalagin, a molecule originating from pomegranates, inhibits quorum sensing (chemical bacterial signaling) in Pseudomonas aeruginosa (PAO1) and Chromobacterium violaceum but does not significantly inhibit bacterial growth. Further, we have applied a curve fitting data analysis to determine an average IC50 value of 10.00 µM for punicalagin and 78.40 µM for ellagic acid (a derivative of punicalagin) against PAO1 quorum sensing. These results suggest that punicalagin has the potential to be utilized as a treatment for bacterial infection without killing bacterial cells, which provides a solution to the problem of rapidly increasing resistance to antibiotics.

ANALYSIS OF HFQ FUNCTION IN GROWTH AND OXIDATIVE STRESS ADAPTATION IN THE METAL-REDUCING BACTERIUM *SHEWANELLA ONEIDENSIS*

Nick Mazzucca, Taylor Hunt, Emma Beer, Shelby Scola, Chris Brennan, Meghan Keane, Jess Leonard, Brett Pellock, *Department of Biology*, Providence College, Providence, RI

RI-INBRE Summer Undergraduate Research Fellowship Program

Hfq is an RNA chaperone protein broadly implicated in sRNA function in bacteria. Loss of the RNA chaperone Hfq in the dissimilatory metal reducing bacterium *Shewanella oneidensis* results in slow exponential phase growth, a reduced terminal cell density in stationary phase, a striking loss of colony forming units in extended stationary phase, and an exquisite sensitivity to both hydrogen peroxide and superoxide stress. We have found that the exponential phase growth defect of the hfq mutant in LB is the result of reduced heme levels. Both heme levels and exponential phase growth of the hfq mutant can be completely restored by supplementing LB medium with 5-aminolevulinic acid, the first committed intermediate synthesized during heme synthesis. Increasing hemA expression via an inducible plasmid vector also restores heme levels and exponential phase growth of t he hfq mutant. Our data suggest that reduced heme levels are solely responsible for the exponential growth defect of the *S.oneidensis* hfq mutant in LB medium. We are currently investigating the possible role of reduced heme levels as one potential explanation for the hfq mutant's defect in oxidative stress resistance and stationary phase survival.

BORRELIA BURGDORFERI: VLSE1 AS A POTENTIAL GANGLIOSIDE BINDING PROTEIN

Drew Phelan, Christopher Reid, *Department of Science and Technology*, Bryant University, Smithfield, RI

RI-INBRE Summer Undergraduate Research Fellowship Program

The Center of Disease Control (CDC) estimates that there are around 35,000 cases (including unreported cases) of Lyme disease each year in the United States. Caused by Borellia burgdorferi sensu lato, this disease, if left undiagnosed, can cause a number of neurological affects from myelitis to facial nerve paralysis. The goal of this research is to identify proteins involved in B. burgdorferi recognition of gangliosides. Through the process of bioinformatics and careful analysis of the results, the gene vlsE1 was identified as a potential ganglioside binding protein. VlsE1 is known to be expressed during late stage Lyme disease and is an important virulence factor for establishing infection [1]. The vlsE1 gene was synthesized and sub cloned into the pET28 expression vector to produce an N-terminally His6-tagged construct. After sub-cloning VlsE1 we began est ablishing the expression and purification conditions that produce the best results. Once established we can begin focusing on experiments to identify the function of VlsE1 in B. burgdorferi.

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IDENTIFYING POTENTIAL GANGLIOSIDE BINDING PROTEIN P66 FROM BORRELIA BURGDORFERI

Keyanna Roohani, Christopher Reid, *Department of Science and Technology*, Bryant University, Smithfield, RI

RI-INBRE Summer Undergraduate Research Fellowship Program

Lyme disease is caused by the sensu lato group of species of the bacteria Borrelia burgdorferi (Bb). Transferred from animal to animal via ticks, hosts do not become infected unless they have been exposed for at least 24 hours [1]. Once the host has been infected, Lyme disease is able to avoid the host's immune system and proceed to negatively impact the central nervous system (CNS) [2]. When patients are diagnosed, they are either in the early or late stage of the disease. Antibiotics can effectively be used to treat Lyme disease if a patient is diagnosed within the early stage of the disease [1]. The goal of this project is to identify proteins from Bb that interact with gangliosides. For this project we used bioinformatics to identify membrane associated protein p66. In primate neural tissue, this protein has been shown to have increased expression when in the presence of Bb [3]. P66 was cloned as the full length version (p66FL) and engineered to lack the N-terminal transmembrane domain (p66d27). The p66 gene was cloned into pET30a (+) to generate a construct with a C-terminal His6-tag. We have successfully expressed p66FL in E. coli BL21 (DE3) pLysS and are optimizing conditions for purification.

References:

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2. Gilmore Jr., R.D. et al. (2006) Microbes and Infection. 8 2832-2840
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INHIBITION OF ENTAMOEBA HISTOLYTICA ALCOHOL DEHYDROGENASE 2 (EHADH2) VIA IRON CHELATION AND PYRAZOLINE COMPOUNDS

Kevin Schindelwig Franca, Joseph Tashjian, Avelina Espinosa, *Department of Biology*, Roger Williams University, Bristol, RI; Lauren Rossi, *Department of Chemistry*, Roger Williams University, Bristol, RI

RI-INBRE Summer Undergraduate Research Fellowship Program

Entamoeba histolytica causes 100,000 human deaths per year due to amebiasis worldwide. The current treatment for amebiasis is metronidazole. In the process of growth inhibition of the trophozoite the host may experience severe side effects such as toxic effects to DNA, and treatment is considered a potential carcinogenic by the International Agency for Research on cancer. Alternative treatments for amebiasis are of great interest. The objective of this research was to determine the inhibition effects on the growth of E. histolytica trophozoites using several alternative compounds. Deferasirox, also known as Exjade®, is an FDA approved iron chelator. The tridentate ligands mechanism of action is to remove excess iron from the liver, heart, endocrine tissue by binding iron [Fe 3+] in a 2:1 ratio. Deferasirox may be advantageous in the treatment of amebiasis by removing iron as it acts as a cofactor for the enzyme EhADH2, which is essential to Entamoeba growth. 1,10 phenanthroline was also tested as a control of iron chelation. The following compounds show promising results: series 1 a (1,3-diphenyl-1-carbamoyl-2-pyrazoline) inhibitor 4; series 1 b (3-phenyl-1-propylcarbamoyl-2-pyrazoline) inhibitor 15; and series 2 (1,3,4-triphenyl-1-carbamoyl-2-pyrazoline) inhibitor 27. The inhibitors and iron chelators were tested on their abilities to block growth of E. histolytica at varying concentrations compared to metronidazole. At a concentration of 120 µM, deferasirox, series 1a inhibitor 4, and series 1b inhibitor 15 pyrazolines significantly inhibited trophozoite growth at a rate similar to metronidazole. Based on preliminary data pyrazolines of the 1a and 1b are more efficient at inhibiting amebic growth than series 2 pyrazolines. Series 1a and 1b pyrazolines are less bulky with one less substituent on the pyrazoline ring than serie s 2 pyrazolines, which may allow them to more easily bind and inhibit EhADH2. Future studies will examine more thiocarboxamides and carboxamides pyrazolines to elucidate the mechanism of action and efficacy of these compounds.

GENOME-SCALE METABOLIC MODELING SIMULATES THE GENE-ESSENTIALITY OF CAMPYLOBACTER JEJUNI

Kyle Sexton, Jon Steffensen, Ying Zhang, *Department Cell and Molecular Biology*, University of Rhode Island, Kingston, RI

RI NSF EPSCoR Summer Undergraduate Research Fellowship Program

The species Campylobacter jejuni include a group of highly diverse enteropathogens that are commonly isolated from poultry, cattle, and environmental sources. It is one of the leading causes of food-borne gastroenteritis in humans, and is increasingly shown to resist to antimicrobial drugs. Therefore, it is important to understand the metabolism and evolution of C. jejuni and to identify essential genes that can serve as new drug targets. In this project, we performed comparative analysis between metabolic models of two different C. jejuni strains: RM1221 (Cjr) and 11168 (Cje). Mapping of the complete genomes into metabolic pathways has enabled more accurate analysis of the genotype-phenotype associations, which in turn permitted mapping of evolutionary variations between different strains of the same bacterial species. Additionally, metabolic simulations wer e performed on the Cje model to search for minimal networks, i.e. the minimal set of genes/reactions that are required for the sustainability of an organism. Using 5,000 random simulations, we classified all biochemical reactions in the metabolic network into three categories: 1) core-essential, genes/reactions that are required in all of the random simulations; 2) non-essential, genes/reactions that are always not needed in the simulations under the given condition of a rich medium; and 3) semi-essential, genes/reactions that are essential in some simulations but not in all. Functional analyses of the three categories demonstrated several pathways that are absolutely essential for biomass production, including vitamin/cofactor metabolism and DNA metabolism. Interestingly, the pathways of amino acids metabolism were classified as semi-essential. This reflects the unique life style of C. jejuni, which specializes in the utilization of amino acids. According to a hierarchical clustering analysis, the semi-essential pathways were classified into two independent groups that complement each other to support metabolic versatility in the organism. Overall, this study provided insight into the gene essentiality of C. jejuni and simulated how the inhibition of different genes would affect the mortality of this species.

ISOLATION, CHARACTERIZATION, AND PURIFICATION OF NOVEL PHAGES CERULEAN, MILTON, PENNY, AND SHELDONCOOPER

Ellen Wheeler, Kathleen Cornely, *Department of Chemistry and Biochemistry*, Providence College, Providence, RI; Nicole Cullen, *Department of Biology*, Providence College, Providence, RI

RI-INBRE Summer Undergraduate Research Fellowship Program

Mycobacteriophages are viruses that infect mycobacteria – a species of bacteria that includes M. tuberculosis and M. leprae. Cerulean, Milton, Penny, and SheldonCooper are newly discovered mycobacteriophages that infect Mycobacterium smegmatis which falls under the same genus as Mycobacterium tuberculosis. These new phages were collected from soil at Providence College and enriched. From this enrichment, four plaques were chosen to be purified through multiple rounds of streaking. Once purified, web plates were used to calculate titer and generate a high titer lysate from which DNA was obtained for sequencing. From a PCR gel, it was determined that phages Milton and Penny belong to clusters D and C, respectively. SheldonCooper and Cerulean do not belong in clusters A, B, C, D, or F based on PCR results.

FORMULATION OF PROBIOTIC BACTERIA FOR COMMERCIAL SHELLFISH LARVICULTURE

Meagan Hamblin, Christine Dao, David Worthen, David Rowley, *Department of Biomedical and Pharmaceutical Sciences*, College of Pharmacy, University of Rhode Island, Kingston, RI; Saebom Sohn, Marta Gomez-Chiarri, *Department of Fisheries, Animal and Veterinary Sciences*, University of Rhode Island, Kingston, RI; David Nelson, *Department of Cell and Molecular Biology*, University of Rhode Island, Kingston, RI

University of Rhode Island Coastal Fellows Program

Shellfish larval tank infections create substantial economic losses for the aquaculture industry. Because hatcheries cannot use antibiotics to protect larvae, an effective probiotic product is desired. Previous studies have shown that Bacillus pumilus strain RI06-95 reduces larval mortality by inhibiting the pathogen Vibrio tubiashii RE22. In this experiment, we sought to design a formulation for RI06-95 so that it can be easily processed, stored and used commercially. Two processes, freeze-drying and granulation, were studied and compared. A viability analysis of the formulated products revealed that the lyophilization process maintained a minimum of 48% viability from the original culture, whereas dehydration followed by granulation maintained only 22% or lower viability. A larval bacterial challenge then revealed that the lyophilized product containing suc rose solution reduced larval mortality by nearly 65%, which was significantly more successful than both the non-formulated culture and granulated product. Based on this result, we conclude that lyophilized RI06-95 is a probiotic product that can be used in a commercial shellfish hatchery to inhibit larval tank infection.

MOLECULAR BIOLOGY

LOCATED BEHIND THE CENTRAL STAIRWAY ON THE 1ST FLOOR OF THE PHARMACY BUILDING

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

FUNCTIONAL STUDIES OF THE UNUSUAL N-TERMINUS OF THE ASCIDIAN MYOGENIC REGULATORY FACTOR

Emmanuel Asiedu, Megan Warburton, Lindsay Ratcliffe, Taylor Ferrare, Jacob Mattox, Thomas Meedel, *Department of Biology*, Rhode Island College, Providence, RI

RI-INBRE & RI NSF EPSCoR Summer Undergraduate Research Fellowship Program

Our laboratory studies Myogenic Regulatory Factors (MRFs), which are basic-helix loop helix (b-hlh) transcription factors that regulate metazoan muscle development. An important feature of MRFs that we have taken advantage of in our research is their ability to induce myogenesis in non-muscle cell types. This property allowed us to develop an electroporation-based assay in which we express MRFs in the developing notochord or endoderm of embryos of the ascidian Ciona intestinalis. Because the work of others has demonstrated that MRFs exhibit a high degree of functional conservation, we were surprised that none of the non-ascidian MRFs we tested was able to elicit myogenesis in this assay, whereas the MRF of either Ciona intestinalis or Ciona savignyi could. We hypothesize that this difference is due to the large N-terminal domain of ascidian MRFs that is ab sent from the MRFs of other organisms we have studied. Here we present a series of preliminary studies aimed at elucidating the role of the N-terminus of the Ciona intestinalis MRF (CiMRF) in myogenesis. The approaches we have taken include (1) replacing the CiMRF N-terminus with GFP to determine whether the N-terminus has a specific role in myogenesis or a more generic role, such as in protein stability; (2) determining whether the N-terminus of CiMRF can confer myogenic activity when it is fused to the b-hlh domain and C-terminus of non-ascidian MRFs; (3) using competition assays to determine if the N-terminus functions by interacting with other factors, presumably proteins, to direct myogenesis. Our results indicate that the Ci-MRF N-terminus plays a specific and essential role in directing muscle gene expression; experiments in progress will address the possibility that the N-terminus functions, at least in part, by interacting with another widely expressed protein (or proteins) that is present in the ascidian embryo. [Note: the poster by Ratcliffe, Alashwal, and Meedel describes a bioinformatics-based approach into the properties of the Ci-MRF N-terminus].

EMBRYONIC DEVELOPMENT AND AHR EXPRESSION IN LEUCORAJA ERINACEA (LITTLE SKATE) USING AN EGG CASE MODEL, IN SITU HYBRIDIZATION, AND CLEARED STAINED EMBRYOS

Sara Lazar, Nicholas Andreozzi, Rebeka Rand Merson, *Department of Biology*, Rhode Island College, Providence, RI

RI NSF EPSCoR Summer Undergraduate Research Fellowship Program

Dioxins and polychlorinated biphenyls (PCBs) are toxic substances that occur as waste from industrial processes. The aryl hydrocarbon receptor (AHR), a ligand-activated protein belonging to the bHLH PAS family of transcription factors, binds to these exogenous compounds and mediates the toxic response in most vertebrates. In embryos, exposure leads to cranial-facial deformities, as well as cardiac edema, which result in higher mortality rates. The number of AHR gene copies varies among species, with humans expressing only one AHR. To best study this, we have chosen the little skate, Leucoraja erinacea, the Little Skate, as our model organism because it is possible to follow embryonic development in real time through the egg case. Since elasmobranchs evolve slowly, their AHR represents the form of an ancient AHR. In L. erinacea development, we can observe all embryonic stages from early somite development up through nearly fully developed, pre-hatch skates. These stages were chosen for clearing and staining in order to observe the development of cartilaginous chondrocranium and splanchnocranium. We have discovered that the gill arches develop at stage 3, and the splanchnocranium and placoid scales do not develop until stage 4. This method will allow us to identify developmental abnormalities in future chemical exposure experiments. RNA probes were synthesized using pGEM-T Easy constructs containing target sequences of AHR1, AHR2, and AHR1X. Whole mount in situ hybridization (WMISH) using the AHR2 probe was performed on embryos of each stage. Future experiments are planned in collaboration with EPA. We expect deformities following chemical exposure

ASSESSMENT OF AMINO ACID RESIDUES THAT CONFER LIGAND BINDING OF THE SQUALUS ACANTHIAS ARYL HYDROCARBON RECEPTOR (AHR)

Timothy Bock, Rebeka Rand Merson, *Department of Biology*, Rhode Island College, Providence, RI

RI NSF EPSCoR Summer Undergraduate Research Fellowship Program

Environmental chemicals 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 and chemical makeup of the AHR's ligand binding domain (LBD) determines whether certain ligands will bind and activate this protein. Characterization of the amino acid residues that determine binding may allow identification of at-risk species upon exposure to known AHR agonists. AHR1 in spiny dogfish shark (Squalus acanthia s) does not bind to typical AHR ligands. Homology modeling of the LBDs of AHRs with high binding affinity suggests that specific residues promote ligand binding. Using this approach, we mutated DNA encoding regions of the LBD that are highly conserved in the AHRs with high affinity for known ligands. Three separate constructs were generated with single point mutations and two constructs were generated containing double mutations. Individual constructs were transfected into mammalian C35 (derived from murine hepa 1c1c7) cells, which express a mutant AHR that does not induce expression of target genes, to create multiple variants of the Squalus acanthias AHR1 protein to be tested for binding efficiency. If changes to the LBD restore ligand binding in the mutant proteins, we expect the AHR1 will translocate from the cytoplasm into the nucleus of the cell in the presence of PCB-126, a potent AHR agonist. Experiments designed to assess the transactivation of a luciferase reporter gene regulated by an AHR-dependent murine Cyp1a1 promoter are underway. If specific amino acid residues in the LBD confer binding, this can be used to predict species at risk of the toxic effects mediated by activation of the AHR pathway. Furthermore, this knowledge will contribute to our understanding of the evolution of the AHR gene family.

THE EFFECTS OF GALLIC ACID ON SIGNALING MOLECULES INVOLVED IN THE ATM-CHK2 KINASE SIGNALING PATHWAY

Erika Carragher, JD Swanson, Rhiannon Morrissey, Gwen Beaman, Stephanie Liptak, Kari Clifton, *Department of Biology and Biomedical Sciences*, Salve Regina University, Newport, RI

RI NSF EPSCoR Summer Undergraduate Research Fellowship Program

Gallic acid (GA) belongs to a class of secondary plant metabolites called phenolics which are produced in the head structure of the glandular trichomes of Rubus leaf extracts. Due to the cell proliferative response and nuclear localization of gallic acid in plants, it is hypothesized that gallic acid has therapeutic effects on mammalian cell cancers by activating signaling pathways that are responsible for cell cycle regulation. These signaling pathways when treated with gallic acid have been shown to cause cell cycle arrest, apoptosis, and angiogenesis. The purpose of this study is to identify a mechanism of action for the therapeutic effects of the plant phenolic gallic acid on mammalian stomach cancer cell lines by identifying proteins that are co- or differentially regulated by GA. Studies have shown that gallic acid activates the ATM-Chk2 kinase signalin g pathway in other types of cancer cells. This pathway inactivates the phosphorylation of cdc25C and cdc25A, and as a downstream effect phosphorylated cdc2 does not get dephosphorylated by cdc25 phosphatases, keeping cdc2 in its inactive form where it cannot bind with Cyclin B1 to form mitosis promoting factor, thus halting the cell cycle. In order to examine the signaling molecules involved in the ATM-Chk2 kinase signaling pathway, proteins were harvested from human gastric adenocarcinoma, or AGS, cells that were treated with 0ul , 40ul, and 60ul of GA at 0, 6, 12, and 24 hour time points, and BCA protein assay analysis was done to determine the protein concentrations from the cells. The proteins harvested fell between 0.94 mg/ml and 1.77 mg/ml concentrations. In the future, cell culture conditions will continue to be modified in order to reach the desired 4 mg/ml concentration needed for automated western blot analysis. When the desired protein concentrations are obtaine d they will be used to load equal amounts of protein. Specific phosphorylated and un-phosphorylated primary antibodies will be utilized to identify the presence of any of these specific ATM-Chk2 kinase signaling pathway proteins. Identifying the regulation of these proteins will help to clarify a possible mechanism of action for the therapeutic effects of gallic acid on mammalian cell cancers.

REVISION WITHIN THE GENUS CRYPTONEMIA USING MORPHOLOGY AND MOLECULAR GENETICS

Joseph Guerreiro, *Department of Biology*, Rhode Island College, Providence, RI; Craig Schneider, *Department of Biology*, Trinity College, Hartford, CT; Chris Lane, *Department of Biological Sciences*, University of Rhode Island, Kingston, RI

RI NSF EPSCoR Summer Undergraduate Research Fellowship Program

Cryptonemia is a genus of algae that can be found throughout the world, but its species can be challenging to identify by their appearance. At present, there are 47 species listed in the genus Cryptonemia in the online database AlgaeBase, but little molecular work has been done on the genus. Using gene sequence data from the plastid-encoded large subunit of the RuBisCO operon (rbcL) as a genetic marker, we analyzed Cryptonemia samples from Bermuda, St. Croix, and Key West. Collected over past two decades, the sequence data from these samples were compared, along with morphological data, to historical collections. The results show that four species of Cryptonemia are currently found in the tropical Atlantic flora, including two new species, C. antricola and C. perparva. Some Bermuda samples were also placed in the classification C. crenulata, a species that ha s not been reported in Bermuda since the late 1800s. Our results also allow for a revision of previous identifications made throughout the past two centuries.

AMPLIFIED FRAGMENT LENGTH POLYMORPHISM (AFLP) ANALYSIS FOR DETECTING GENETIC VARIABILITY IN LEISHMANIA SPECIES

Christine Ortiz, Karly Douglas, Alison Shakarian, *Department of Biology and Biomedical Sciences*, Salve Regina University, Newport, RI

RI NSF EPSCoR Summer Undergraduate Research Fellowship Program

Leishmania are parasitic protozoa that cause visceral and cutaneous leishmaniasis in humans. The presentation of the disease is dependent on the specific species of the parasite injected into the human host through the bite of the sand fly vector. For example, L. major and L. mexicana cause cutaneous leishmaniasis, L. donovani causes visceral leishmaniasis and L. tarentolae is non-pathogenic in human host. This project aims to identify genes that are uniquely expressed in each of the different species in order to identify proteins that are potentially associated with each species' specific pathogenicity. Amplified Fragment Length Polymorphism (AFLP), a PCR-based technique that can identify polymorphisms across genomes, was used to compare the genes being expressed in the four different species of Leishmania. To accomplish this, two EcoRI and eight MseI prim ers were combined into 16 primer sets and used to amplify fragments of cDNA from the different Leishmania species. Polyacrylamide gel electrophoresis analysis of the amplified fragments confirmed that unique fragments were present in the expressed genes. For example, the EcoRI-ACA primer sets amplified and detected five unique fragments for L. donovani, four unique fragments for L. mexicana and two unique fragments for L. tarentolae. The EcoRI-ACC primer sets detected two unique fragments in L. donovani, and four unique bands each in L. mexicana, L. major, and L. tarentolae. The percent polymorphism detected for the EcoRI-ACA and EcoRI-ACC primer sets were calculated to be 6.25% and 2.56%, respectively and the percent of monomorphic fragments were 9.4% and 15.34%, respectively. This data indicates that there is genetic variation among Leishmania species with different disease profiles. Future studies include continuing to collect data using other primer combinations and sequ encing the unique gene fragments to determine potential protein production that may lead to the pathogeneses of each species.

MICROSATELLITE ANALYSIS IN ULVA RIGIDA AND ULVA COMPRESSA

Timothy Roosa, Kari Clifton, JD Swanson, *Department of Biology and Biomedical Sciences*, Salve Regina University, Newport, RI; Carol Thornber, *Department of Biology*, University of Rhode Island, Kingston, RI

RI NSF EPSCoR Summer Undergraduate Research Fellowship Program

Green algae plays an important role as a primary producer in almost all marine ecosystems but an overabundance can have negative effects. Aquatic plant species can be out-competed and animal species will be driven out due to lack of nutrient availability due to algal blooms. The Narragansett Bay, RI is home to both Ulva rigida and Ulva compressa, two species of green macroalgae that are known to cause harmful algal blooms which can lead to widespread ecological and economical damage to the area of occurrence. Little is known about the biology of these blooms, and furthermore it is unknown whether or not a bloom is caused by a single individual or multiple individual ecotypes. We want to understand the population makeup of harmful algal blooms in order to determine their origin and how their populations change over time. Microsatellites were elucidated by sear ching for di, tri, tetra, penta, and hexa- nucleotide repeats from NGS transcriptome data using Microsatellite Commander v2. Fifteen microsatellites were chosen based on the size of the repeats and the amount of repeats and primers were designed using Primer3. The Microsatellites were amplifies from two individuals using PCR and then visualized using 2% agarose gel electrophoresis to determine whether or not the microsatellites were designed correctly. These markers will be used to determine whether or not the blooms were caused by a single individual or by multiple individuals. This information can be used to better understand the nature of these blooms to predict and prevent any harmful algal blooms in the future.

ACETYL FENTANYL OVERDOSE & REVERSAL IN A HIGH-FIDELITY PATIENT SIMULATION MODEL

Julia Suits, *Chemical Technology Program*, Community College of Rhode Island, Warwick, RI; Clinton Chichester, *Department of Biomedical and Pharmaceutical Sciences*, College of Pharmacy, University of Rhode Island, Kingston, RI

RI-INBRE Summer Undergraduate Research Fellowship Program

Background: Since March 2013 opiate abuse increased rapidly in Rhode Island. Overdosing killed a total of 75 Rhode Islanders this year alone. Opiates are Central Nervous System (CNS) depressants, which means that the physiological aspects of the body are decreased. Most fatalities caused by drug overdosing are due to severe respiratory depression. Heroin is the most common fatal overdose drug. However, a new street drug called Acetyl Fentanyl is now contributing to the death rate. This drug was appeared in Rhode Island last year, and is manufactured in illicit laboratories in Mexico and smuggled into the United States. The manufacturing process involves adding acetic anhydride to Fentanyl producing Acetyl Fentanyl. Naloxone is a drug used to reverse the effects of opioid agonists. In this study, high-fidelity simulators, were programed to illustrate the effects of a opioid overdose.

Methods: Thorough literature research was done on opiates and overdose before developing the simulation. CAE Healthcare high-fidelity patient simulators with the Müse software were used to demonstrate the effects of overdose on opiates and overdose reversal with Naloxone. Initially, increasing doses of both Fentanyl and Morphine were tested in order to best display an overdose case. We then modeled an Acetyl Fentanyl overdose that resulted in apnea. Naloxone (2mg) was administered twice to successfully reverse the overdose.

Results: Our scenario accurately depicted vital signs of an Acetyl Fentanyl overdose and reversal. Acetyl Fentanyl caused the respiratory rate to drop to zero after a three minute period. Two doses of Naloxone were then given three minutes apart to depict an opiate reversal.
Conclusion: Using high-fidelity patient simulators, we were able to develop and accurately depict what happens during an opiate overdose case and illustrate Naloxone reversal. This scenario will be used to train first responders in RI, along with abusers and their families about Naloxone.

DEVELOPING SIMULATIONS TO ILLUSTRATE THE USE OF SEDATIVE DRUGS IN ICU AND AMBULATORY CARE SETTINGS

Clemens Jung, *Department of Life Sciences – Pharmacy*, Technische Universität Braunschweig, Braunschweig, Lower Saxony, Germany; Clinton Chichester, D*epartment of Biomedical and Pharmaceutical Sciences*, College of Pharmacy, University of Rhode Island, Kingston, RI

Technische Universität Braunschweig and the University of Rhode Island College of Pharmacy Exchange Program

BACKGROUND: Sedation is an important component of the clinical daily routine. Twenty nine percent of all procedures in ambulatory anesthetics in the United States are either topical/local, IV sedation or monitored anesthesia care (Bayman et al. 2011). A module of simulations were created to provide PharmD students an overview about commonly used drugs for sedation and the procedures used for Monitored Anesthesia Care (MAC). MAC is a specific type of anesthesia service typically used for diagnostic or therapeutic procedures.

METHOD: An extensive literature search was conducted to determine which drugs in what settings are commonly employed for sedation. Three cases based on existing clinical cases in the medical literature were created and were programmed into the simulator. CAE Healthcare high-fidelity human patient simulators (HPS, PediaSIM and ECS) with Müse software were used.

RESULTS: The first case involves a 55-year old female patient with severe liver disease who undergoes an endoscopic procedure. This scenario demonstrates the drug combination of midazolam and fentanyl, which is commonly used in the US for this type of diagnostic procedures. The second scenario demonstrates specific advantages of ketamine. In this case, a 9-year old asthmatic child undergoes a burn debridement under ketamine sedation. The final scenario takes place in an emergency department. A 75- year old man suffers a respiratory failure in the ER, which requires a rapid sequence intubation using propofol sedation. The patient is later transferred to the ICU and students observe the patient on a ventilator under sedation by propofol. The simulations were tested with a group of five nursing students in BPS333 (Nursing Pharmacology). A brief introduction to the topic of anesthesia was given to the students prior to the simulations. The total time for the first run was approximately one and a half hour. A debriefing followed student participation in the scenarios. Overall feedback from the participants was positive.

CONCLUSION: As confirmed with the first test run using a human patient simulator is an effective method to demonstrate various drugs and settings used for sedation and MAC. However, for the successful development of a simulation it is important and necessary to consider about the outcome and the methods in an early stage of the development process. For the future it is planned to create an assessment tool to evaluate the effectiveness of the simulations.

TRANSFORMATION IN ARABIDOPSIS THALIANA AND FRAGARIA TISSUE CULTURE

Megan Sylvia, JD Swanson, *Department of Biology and Biomedical Sciences*, Salve Regina University, Newport, RI

RI NSF EPSCoR Summer Undergraduate Research Fellowship Program

Transformation is a common technique used by scientists to study genes of interest by observing effects of inserted genes on the organism's phenotype. Salve Regina University chiefly studies genes and their resulting functions through RNA analysis techniques, such as qPCR. While the data collected from these studies provides valuable correlational evidence between a specific gene and its function, it can only definitively reveal where and when a gene is turned on in an organism. However, transformation can provide both mutational and complementation evidence to support the current correlational evidence provided by RNA experiments such as qPCR; therefore further supporting the relationship between genes and function. Arabidopsis thaliana is a model organism that can be transformed using Agrobacterium-mediated transformation, specifically the floral dip method. Consequently, after the plant begins to develop floral buds, it is inverted and dipped into a solution containing cultured strains of Agrobacterium, sucrose, and DMSO. The Agrobacterium then inserts a segment of its T-Plasmid known as T-DNA into the Arabidopsis plant. Like Arabidopsis, Fragaria, or "allstar" strawberry, can be transformed via Agrobacterium, however instead of using the floral dip method, Fragaria must be degenerated into callus before it can be transformed. Sterile leaf Fragaria explants can be induced to callus by plating them in Murashige and Skoog Basal Media with vitamins, sucrose, TC agar, and various plant hormones/antibiotics. However, while the Fragaria explants have remained alive in several MS media variations for over 2 weeks, callus formation has only been observed in the control organism explants, blackberry. Therefore, the goal of this research project is to 1) effectively create transgenic Arabidopsis thaliana plants via the floral dip method and 2) optimize the protocol to callus Fragaria leaf explants in order to effectively transform the plant in the future.

GENERATING IDH MUTANT TRANSGENIC FRUIT FLIES AND CELL LINES TO STUDY GLIOMA METABOLISM

Michaela Brown, Jenna Buccetti, John Mills, Marla Tipping, *Department of Biology*, Providence College, Providence, RI

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. The goal of this research is to establish an IDH1 mutant model in both Drosophila cell lines using the inducible pMT vector, and in vivo using the Gal4 regulated pattB-UASt vector. Through a series of cloning techniques a GFP-tagged IDH mutant model was created to examine localization of cells. 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. Constructs were also cloned into the pattB-UASt vector to be analyzed in vivo. Through western blot analysis of transfected S3 cells our GFP-IDH mutant and SBP-IDH mutant constructs were validated. Our next step is to inject the pattB-UASt constructs into fly embryos. Fro m these models it will be possible to characterize viability, glial cell proliferation, and metabolic status of the IDH mutated gliomas.

GOLD-COATED BICELLES FOR USE IN PLASMON-INDUCED HYPERTHERMIA

Troy Crawford, Jennifer Daniels, Oleg Andreev, Yana Reshetnyak, *Department of Physics*, University of Rhode Island, Kingston, RI

RI-INBRE Summer Undergraduate Research Fellowship Program

The medicinal application of gold nanoparticles, specifically in regards to cancer treatment, is an area that is currently being researched and shows promising results. Multiple organizations of gold nanoparticles have been studied, such as gold nanospheres, nanoshells, and nanorods. Described in this work are the properties of gold-coated, bi-layered micelles, or bicelles. Gold coated plasmatic liposomes have previously been studied for the release of drugs using near infrared light stimulation. However, disk-like shells have not yet been observed, but are expected to show promising hyperthermia potential. This is due to the structure being similar to that of gold nanorods, which heat up at a much greater rate than nanospheres. In the future, we plan to extend the study to research the effect of these bicelles on tumor treatment via radiation enhancement an d hyperthermia. This study explored bicelles with different q-values (ratio of DMPC to DHPC), varying thicknesses of the layer of gold surrounding the bicelle, and multiple methods used in coating. We then added pH Low Insertion Peptide (pHLIP) to the coated bicelles, so that they would attach themselves to acidic tumors, thereby increasing the radiation absorbed. Additionally, PEG was added to allow the bicelles to avoid detection from the body's immune system. The properties of the bicelles were observed using spectrophotometry, dynamic light scattering (DLS), and transmission electron microscopy (TEM).

RNA OXIDATION IN A D. MELANOGASTER MODEL OF ALS

Danielle Lafond, Geoffrey Stilwell, *Department of Biology*, Rhode Island College, Providence, RI; Asli Sahin, Robert Reenan, *Department of Molecular and Cell Biology*, Brown University, Providence, RI

RI-INBRE Summer Undergraduate Research Fellowship Program

Amyotrophic lateral sclerosis (ALS) is an adult-onset neurodegenerative disease affecting the upper and lower motor neurons. It 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. Any one of over 100 point mutations in the Cu/Zn superoxide dismutase 1 (SOD1) gene are known to cause fALS. SOD1 mutations result in a toxic gain of function and numerous cellular and molecular processes contribute to disease pathogenesis. Oxidative stress is one prominent feature associated with ALS and free radical toxicity is implicated as an early pathological event. While major consequences of oxidative stress include DNA damage, lipid peroxidation and protein misfold ing, the effects of oxidative damage to RNA have not been well characterized. Using a monoclonal antibody (15A3) that recognizes hydroxylated guanine, we will assess oxidative damage to both RNA and DNA by immunohistochemistry in a Drosophila melanogaster model of ALS in which the human H71Y SOD1 mutation was introduced by homologous recombination. Preliminary results will be presented and further investigations will assess the role of oxidative stress in affected cell populations within this Drosophila model using genetic and molecular tools available. These studies, conducted within the well-defined genetic system of Drosophila, will offer new insight into the role of oxidative damage in ALS.

IN VITRO ANALYSIS OF MYOGENESIS IN UBE4B DEFICIENT MICE

Jaclyn Lata, Sarah Spinette, *Department of Biology*, Rhode Island College, Providence, RI

RI-INBRE Summer Undergraduate Research Fellowship Program

Protein turnover in cells is controlled by the ubiquitin conjugation system which marks proteins for degradation by the proteasome and is composed of the three classes of enzymes E1, E2, and E3. Ube4b is an E3 ligase which has a conserved U-box domain that is essential for mediating ubiquitin conjugation. Its ubiquitylation activity has been suggested to be important for cancer but its specific role as a tumor suppressor or an oncogene is unclear. Other experiments have led us to hypothesize that it may function during the growth and differentiation of skeletal muscle. To determine how the lack of Ube4b could affect skeletal muscle in vitro and in vivo a line of transgenic mice was developed in which the U-box domain of Ube4b was removed only in skeletal muscle through expression of iCre-recombinase controlled by the MyoD promoter. These studies where designe d to analyze myogenesis in the Ube4b deficient mice in vitro. To isolate satellite cells (SCs), or muscle specific precursor cells, Fluorescence-activated cell sorting (FACS), and myofiber isolation techniques where used. These SCs were then placed in culture and allowed to divide and differentiate into myoblasts and then myotubes. Myoblast proliferation, apoptosis, and differentiation could thus be examined at different time points during myogenic differentiation in vitro using specialized immunohistological staining. Since proliferation and apoptosis are both critical to cellular transformation in cancer and myogenesis, the analysis of these processes in cells isolated from Ube4b deficient mice may help us better understand the function of Ube4b.

ISOLATION AND PURIFICATION OF ENTAMOEBA HISTOLYTICA ALCOHOL DEHYDROGENASE 2 (EHADH2) ENZYMATIC ACTIVITIES AND INHIBITION BY PYRAZOLINE DERIVATIVES

Joshua Leitao, Harsha Kumar, Avelina Espinosa, *Department of Biology*, Roger Williams University, Bristol, RI; Lauren Rossi, Erica Oduaran, *Department of Chemistry*, Roger Williams University, Bristol, RI

RI-INBRE Summer Undergraduate Research Fellowship Program

The bifunctional alcohol/ aldehyde dehydrogenase enzyme in Entamoeba histolytica (EhADH2) belongs to the ADHE iron dependent family of enzymes and is essential for trophozoite growth and survival. EhADH2 catalyzes the conversion of acetyl Co-A to acetaldehyde and the final reduction of acetaldehyde to ethanol by its separate ALDH and ADH domains respectively. The expression of EhADH2 is necessary for amebic survival, making it an ideal target for therapeutic treatment. Entamoeba histolytica causes amebiasis in humans with 100,000 deaths per year, infecting 50 million people worldwide. The current treatment for amebiasis is metronidazole, which inflicts severe side effects on patients. Previous inhibition research has demonstrated that pyrazoline derivatives display inhibitory effects on the activity of EhADH2 at concentrations non-toxic to humans. The primary focus of research was to determine the effect of alternative treatments for amebiasis through inhibition by pyrazoline inhibitors and iron chelators on the EhADH2 enzyme. A kinetic assay was performed to calculate the Ki value of inhibitors 4 [3-(4-chlorophenyl)-1-(bromophenylcarboxamide)-2-pyrazoline], inhibitor 15 [3-4(-chlorophenyl)-1-N-propylcarboxamide-2-pyrazoline], and inhibitor 28 [3-(4-cholorphenyl)-4-phenyl-1-(4-chlorophenyl carboxamide)-2-pyrazoline] at the rate of enzymatic affinity with the substrate acetaldehyde at NAD+ μM/min as the unit of measurement; then, the Ki value for the inhibitor was calculated using Michaelis-Menten and Lineweaver-Burke equations. Inhibitor 4 [3-(4-chlorophenyl)-1-(bromophenylcarboxamide)-2-pyrazoline] prevented amebic growth due to its structural differences compared to the other synthesized inhibitors, suggesting it is the most efficient inhibitor tested up to date.

ADVANCEMENT OF CONCATEMERIC EPITOPE VACCINES BY CHARACTERIZATION OF COMBINATORIAL EPITOPE ASSEMBLIES FOR OPTIMAL EXPRESSION, SOLUBILITY AND IMMUNOGENICITY

Ariel Lopez, Rui Liu, Lenny Moise, *Institute for Immunology and Informatics*, University of Rhode Island, Providence, RI

RI-INBRE Summer Undergraduate Research Fellowship Program

Background: Reverse vaccinologists face a major obstacle in how to harness the abundant candidate antigens that emerge from T-cell epitope predictors to construct an immunogenic and protective vaccine. The delivery of epitopes as a concatemeric protein is a promising solution to this problem. We hypothesize that different epitope orders will have different levels of protein expression, solubility and immunogenicity. Our goal is to establish an experimental approach that addresses the hurdle of finding viable epitope orders for epitope driven vaccines by: evaluating expression, solubility, and immunogenicity properties of a complete set of concatemeric proteins.

Methods: Four T-cell epitopes fromCMV, EBV, and influenza wereselectedand a library of combinatorial epitope proteins was recombinantly constructed. Expression was evaluated using a SuperFolder GFP. The total protein expressed is directly proportional to the SuperFolder GFP fluorescence. Solubility was evaluated using a Split GFP fragment that is fused with our protein of interest. An in vitro complementation assay was performed by mixing cell lysate and a complementation detection fragment. Lastly, immunoreactivity of successfully expressed concatameric proteins will be evaluated with PBMC from HLA-A2 positive human subjects.

Results: Different levels of expression in E.coli were observed in fluorescence imaging and in quantitative absorbance analyses suggesting that the order of epitopes produce different levels of protein expression. Solubility screening, which is in progress, is to be followed by immunoreactivity evaluation studies.

Conclusions: Narrowing down potential epitope orders will help to identify promising concatameric protein vaccine candidates.

ENZYME KINETICS OF MOUSE SULFOTRANSFERASES

Emily Martell, Adam Auclair, Roberta King, *Department of Biomedical and Pharmaceutical Sciences*, College of Pharmacy, University of Rhode Island, Kingston, RI

RI-INBRE Summer Undergraduate Research Fellowship Program

Bisphenol A (BPA) is a widespread endocrine disrupting chemical that is found in many common everyday items. It is well know that BPA is metabolized by glucuronidation by adults and by sulfonation in newborns. From Wen at el, data demonstrated altered regulation of hepatic phase II metabolism in mice during pregnancy, a down regulation of UDP-glucuronosyl transferases (UGT), and increased sulfotransferase mRNA expression. However, information regarding pregnancy-induced enzyme kinetic changes of phase II-metabolized drugs is limited, and is an integral part of understanding drug risk and exposure. It is hypothesized that BPA is sulfonated at low concentrations by SULT 1A1 and at high concentrations by SULT 2A1. This research should show the enzyme kinetics of BPA sulfonation. The studies were conducted with mouse SULT 1A1 and 2A1 plasmids transformed into E. Coli and isolated by ultra-centrifugation. The collected supernatant was then used in enzyme assays with 3'-phosphoadenosine-5'-phosphosulfate (PAPS) and the substrate for 30 minutes at 37OC, then analyzed using HPLC. The results showed that the mSULT 1A1 is active for p-nitrophenol (PNP) a known substrate, however studies will have to continue with BPA and mSULT 2A1.

BIOINFORMATIC ANALYSIS OF THE UNUSUAL N-TERMINUS OF THE ASCIDIAN MYOGENIC REGULATORY FACTOR

Lindsay Ratcliffe, Thomas Meedel, *Department of Biology*, Rhode Island College, Providence, RI; Hany Alashwal, *Department of Biomedical and Pharmaceutical Sciences*, College of Pharmacy, University of Rhode Island, Kingston, RI

RI-INBRE Summer Undergraduate Research Fellowship Program

Ongoing studies in our lab indicate that the unusually large N-terminus of CiMRF, the Myogenic Regulatory Factor of the ascidian Ciona intestinalis, is essential for the myogenic activity of this protein (see poster by Asiedu et al.). Here we describe the bioinformatics approach we used to investigate the sequence, structure, and potential function of this 364 amino acid domain. The Basic Local Alignment Search Tool (BLAST) was used to analyze the amino acid sequence of both the N-terminus and the entire CiMRF protein (592 amino acids). Conserved sequences were found only in the entire protein and consisted of the cysteine/histidine-rich domain, the basic helix-loop-helix domain, and Domain III near the C-terminus. A Position-Specific Iterated BLAST (PSI Blast) confirmed that CiMRF had a unique N terminal sequence as no conserved domains were found within the 364 amino acids. The structure of the N terminus was explored through ab initio or de novo modeling, which is a low accuracy method used to predict 3D structures of proteins without a known structural homology. The challenge of this prediction method is that sequences with more than 20 amino acids are more difficult to predict from the secondary structure, alone. Two academic servers, Phyre2 and I-TASSER, were used to predict the structure of the CiMRF N-terminus. The results from these servers produced similar secondary structure predictions and multiple disorder regions overlapped between servers. Others have connected disorder, structurally unstable regions, with functional significance. 3D models predicted by the two servers did not align and can be attributed in part to a lack of structural homologies and multiple regions of high disorder. Finally, our BLAST analysis found that the N-terminus of the Phallusia mammillata MRF, another ascidian, had regions of sequence similar to the CiMRF N-terminus. We are currently constructing a plasmid that will express a chimeric MRF in which the P. mammillata N-terminus is swapped for the N-terminus of CiMRF to determine if these regions of similarity have functional significance.

SITE-DIRECTED MUTAGENESIS OF A PUTATIVE CDK PHOSPHORYLATION SITE CLUSTER IN THE FANCD2 PROTEIN

Lauren Shanahan, Rebecca Boisvert, Niall Howlett, *Department of Cell and Molecular Biology*, College of the Environment and Life Sciences, University of Rhode Island, Kingston, RI

RI-INBRE Summer Undergraduate Research Fellowship Program

Fanconi anemia (FA) is a rare genetic disease characterized by bone marrow failure, congenital defects, and increased cancer susceptibility. FA is caused by biallelic mutations in any of sixteen known genes. The protein products of these genes function collectively in the FA-BRCA pathway to repair DNA interstrand-crosslinks (ICLs). Upon exposure to ICL-inducing agents the FA core complex catalyzes the monoubiquitination of the FANCD2 protein on K561, activating this protein for ICL repair. FANCD2 is also posttranslationally modified by phosphorylation following exposure to DNA damaging agents and upon disruption of DNA replication. However, no studies have explored if FANCD2 is phosphorylated independently of DNA damage. We have recently identified a cluster of three putative Cyclin-Dependent Kinase (CDK) phosphorylation sites proximal to K561. Specifically, FANCD2 S525, S624 and S726 were identified via protein molecular modeling and multiple sequence alignment approaches. A sequence alignment from multiple species shows a high degree of evolutionary conservation of these residues. In order to examine the importance of these residues, they were mutated by site-directed mutagenesis to alanine and aspartic acid amino acids separately. FANCD2 single, double, and triple mutants for each residue were generated in the pENTR/D-TOPO entry vector. These cDNAs were subsequently recombined into the pLenti6.2/V5-DEST destination vector, which has recently been sequence verified and tested for expression in both HeLa and Cos7 cells. The next step in this project is to create stable cell lines expressing wild type or mutant FANCD2 in FA-D2 patient cells to determine if these sites have a role in the function of FANCD2. This will provide further insight into the regulation and DNA damage-independent posttranslational modification of this i mportant tumor suppressor protein.

APPLICATION OF DNA SHUFFLING TO UNDERSTAND STRUCTURE AND FUNCTIONAL DIFFERENCES BETWEEN CSK AND SRC

Moira Welch, *Department of Chemistry and Biochemistry*, Providence College, Providence, RI; Yixin Cui, Gongqin Sun, *Department of Cell and Molecular Biology*, University of Rhode Island, Kingston, RI

RI-INBRE Summer Undergraduate Research Fellowship Program

Src and Csk are well known members of the protein tyrosine kinase family, a family of proteins important in the regulation of cellular growth and division. The goal of this research is to figure out the structural basis of the tyrosine kinase for substrate specificity using Src and Csk as a model. DNA shuffling is used to generate hybrids between Csk and Src, and mutants that affected substrate specificity are identified by high-throughput screening. The hybrid kinases are co-expressed with a given protein substrate, and the hybrid able to phosphorylate the given substrate is identified by antibodies recognizing a specific phosphorylation site. The protein substrate used is kdSrc, which can be phosphorylated on tyrosine416 by Src and on tyrosine527 by Csk. When this substrate is co-expressed with a library of Csk-Src hybrids, the hybrids able to phosphorylat e tyrsoine527 on kdSrc are identified by antibody specific for tyorsine527 and the hybrids able to phosphorylate tyrsoine416 on kdSrc are identified by antibody specific for tyorsine416. Once the hybrid kinases for each given site are identified, the genetic sequences of these kinase hybrids are determined. When the genetic sequences of many mutants with similar substrate specificity are compared, the region of the sequence responsible for substrate specificity can be identified. This information can be used to design inhibitors for specific kinases. Such inhibitors can be used to block the growth of cancer cells.

NEW SPECIES OF CARDIOSPORIDIUM PARASITE FOUND ON RHODE ISLAND SHORES

Alexis White, *Department of Biology*, Providence College, Providence, RI; Chris Paight, Chris Lane, *Department of Biological Sciences*, University of Rhode Island, Kingston, RI

RI-INBRE Summer Undergraduate Research Fellowship Program

With the immense biological diversity on our planet, there exist many organisms that have yet to be discovered, even in our own back yard. This research examines the species diversity of Cardiosporidium, a poorly known genus of apicomplexans, which are a group of parasites encompassing such genera as Toxoplasma, Babesia, and Plasmodium. Recently discovered in Korea, there is very little known about Cardiosporidium. Species in this genus parasitize tunicates - marine invertebrates commonly referred to as a “sea squirts”. Similar to its close relative Cardiosporidium cionae, the Rhode Island parasite inhabits the pericardial body, a small clot of cells that oscillates inside the heart cavity as it beats. Using DNA extraction and sequencing, we have found the DNA signature of two new species of Cardiosporidium on the upper east coast of the United States, in sites along Rhode Island and Connecticut shores. Not only has the known geographical range of Cardiosporidium been expanded, but the number of hosts has increased as well.

PROCESS DEVELOPMENT OF TCR SPECTRATYPE ANALYSIS

Jeremiah Alves, Alan Rothman, Barbara Payne, *Department of Cell and Molecular Biology*, University of Rhode Island, Kingston, RI

Biotechnology Certificate Program

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 in a published TCR spectratype method. We analyzed TCR Vβ gene usage using the method of 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 three labeled and one unlabeled 3' primers, and twenty fo ur 5' primers. Replicate experiments were performed using the same input cDNA. Low efficiency of and high variation in amplification was detected for some forward primers when they were matched with the labeled reverse primers. More efficient and consistent amplification was observed for all twenty-four forward primers when paired with the same unlabeled reverse primer. Our data show variability in measured TCR Vβ gene usage following the published method, which is at least in part due to inconsistent amplification using the labeled 3' primers. Since the labeled primers are needed only for fragment analysis, we are exploring the use of deep sequencing to define the TCR diversity. Additional experiments are being performed in order to better quantify assay variability for future use with clinical samples.

GENE EXPRESSION ASSESSMENT OF FOUR LEISHMANIA SPECIES USING AMPLIFIED FRAGMENT LENGTH POLYMORPHISM (AFLP)

Karly Douglas, Christine Ortiz, Alison Shakarian, *Department Biology and Biomedical Sciences*, Salve Regina University, Newport, RI

Independent Research

Leishmania are parasitic organisms that can result in visceral and cutaneous leishmaniasis in humans. Visceral leishmaniasis, an enlargement of internal organs is typically caused by L. donovani and cutaneous leishmaniasis, which results in skin sores and lesions, is caused by L. major and L. mexicana. In this study, we used AFLP to characterize polymorphisms and differences in gene expression among four different species of Leishmania in an effort to characterize protein encoded by unique fragments, to determine if they play a role in the pathogenicity of the species of Leishmania. The four species of Leishmania used were L. mexicana, L. donovani, L. tarentolae, and L. major. Purified cDNA was obtained for each of the four species and subjected to restriction digestion and adapter ligation using Mse1 and EcoR1 specific sites. Individual fragments were ampl ified with multiple successive rounds of PCR using 16 primer combinations and analyzed by gel electrophoresis. We identified several primer combinations that resulted in unique amplified fragments for cDNA. One polymorphic fragment was determined when the EcoRI primer AAC was combined with 8 different MseI primers, and two polymorphic fragments were determined when the EcoRI primer ACC was combined with the same 8 different MseI primers. Taken together, these results indicate that AFLP is a viable technique to assess polymorphic differences in the Leishmania genome and differences in gene expression.

MICROSATELLITES – CONSERVED SEQUENCES BETWEEN LEISHMANIA MEXICANA AND LEISHMANIA DONOVANI

Cilicia Nascimento, Syeda Sultana, *Department of Biology and Biomedical Science*, Salve Regina University, Newport, RI

Independent Research

Leishmania is a parasite predominantly found in subtropical countries and causes cutaneous or visceral leishmaniasis, a disease characterized by the rupture of the host macrophages either in the skin or internal organs; visceral leishmaniasis causes over 20.000 deaths each year. Currently, as with most parasitic infections, microscopy is the gold standard method to diagnose leishmaniasis, biopsy smears are used for cutaneous leishmaniasis and aspirate from lymph nodes, spleen or bone marrow are collected for visceral leishmaniasis. But this technique is time-consuming and depends on the person capacity to distinguish Leishmania from any other protozoan parasite or from an artifact. Immunoassays can also be used as diagnosis for other parasitic infections, but in the case of leishmaniasis it's not an effective diagnosis due to the low production of antibodie s in the cutaneous form and despite the abundant production of IgG in visceral leishmaniasis, these are nonspecific antibodies, therefore useless for this purpose. In this study, the possibility of a molecular diagnosis is studied determining the specificity of microsatellite containing primers designed using msatcommander, software that detects these sequences and generates complementary primers. Microsatellites are short repetitive sequences, used as markers to genome fingerprinting and population studies, they are present in every known sequence, usually in noncoding regions of the genome.

In this work, primers from chromosome seven of L. mexicana containing microsatellites are used to determine which fragments are similar or different between L. mexicana and L. donovani. Primers 91 and 110 showed reproducible results, however they are not significantly different among L. donovani and L. mexicana to be used as diagnosis; and nonspecific primers might cause wrong diagnosis, primer 178 also has reproducible results and is a good parameter for diagnosis, because band sizes found in L. donovani and L. mexicana are significantly different (p

THE IDENTIFICATION OF MICROSATELLITE GENETIC MARKERS IN LEISHMANIA MEXICANA

Syeda Sultana, Alison Shakarian, *Department of Biology and Biomedical Sciences*, Salve Regina University, Newport, RI

Independent Research

Leishmania is a protozoan parasite responsible for the onset of cutaneous, visceral, and mucocutaneous Leishmanasis. The symptoms of the disease depend on the species of Leishmania. The intent of this experiment is to identify differences in the genomic sequences of L. mexicana chromosomes 00, 7, and 14 in comparison with gDNA from Leishmania major, L. donovani, and L .jena via microsatellites generated from the L. mexicana genome. Microsatellites are DNA repeat sequences of varying length that act as DNA markers. The methods used during this experiment included microsatellite identification, designing primers, polymerase chain reaction (PCR), and an agarose gel electrophoresis. Our preliminary results indicated that from chromosome 14 microsatellites 1, 3, and 4 are homozygous and exist at a single allele in the L. mexicana genome. For chromosome 7, micro satellite 91 is heterozygous and is present in two distinct alleles. Chromosome 00 microsatellite 4, and 36 are present in three alleles, microsatellite 28 is heterozygous and present in two alleles, and microsatellites 1, 2, 3, 06, and 49 are all homozygous and present in one allele. The presence of an amplified fragment indicates that the microsatellite is present in the L. mexicana genome. Future experiments will include a comparison of these microsatellite sequences with other Leishmania species, to determine if there are differences in the microsatellite markers between old world and new world Leishmanasis species.

NEUROSCIENCE

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

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

CREATING AMYOTROPHIC LATERAL SCLEROSIS MODELS IN *D. MELANOGASTER*

Victoria St. Amand, Saman Nayyab, Neil Van Noppen, Jose Hurtado, Geoff Stillwell,
Department of Biology, Rhode Island College, Providence, RI

RI-INBRE & RI NSF EPSCoR Summer Undergraduate Research Fellowship Programs

Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disease that results in progressive muscle weakness, paralysis, and motor neuron loss. Over 150 mutations in the highly conserved Cu, Zn superoxide dismutase (SOD1) gene cause familial ALS (fALS) in humans. Although this disease has been intensively studied in vertebrate systems, we used fruit flies (Drosophila melanogaster) as a genetically tractable system with the aim of creating a representative ALS model in a well-defined genetic background. In this study, we introduced precise mutations (A4V, H46R, D101N, S111C) into SOD1 using site directed mutagenesis. Mutant DNA fragments were cloned into Drosophila transformation vector pW25 and successful insertion was confirmed by sequence analysis. In addition, fusion of mutant SOD1 with green fluorescent protein (EGFP) will enable optical tracking of SO D1 aggregation. These studies will enable insertion of ALS-associated SOD1 alleles into D. melanogaster. Future work will include a cellular and molecular analysis of mutant SOD1 in vivo.

THE PROTECTIVE EFFECTS OF SOCIAL ENRICHMENT ON EARLY STRESS AND LATER PSYCHOPATHOLOGY

Kaitlyn Dahlborg, Megan Grammatico, Katherine Sulka, Christopher Bloom, *Department of Psychology*, Providence College, Providence, RI

RI-INBRE Summer Undergraduate Research Fellowship Program

Recent work in our laboratory demonstrated that rats exposed to 2,3,5-trimethyl-3-thiazoline (TMT) in juvenility displayed more anxiety-like behavior in adolescence compared with controls. Differences in anxiety-like behavior were eliminated following subsequent exposure to inescapable shock (IS). In adulthood, TMT-exposed rats demonstrated lowered pain sensitivity when compared to controls, a difference that was not demonstrated in adolescence. The present experiment investigates the effects of environmental enrichment on the previously demonstrated life-long effect of early life exposure to environmental stress on pain sensitivity and anxiety-like behaviors. Utilizing a factorial design, rodents were assigned to four conditions. Groups consisted of animals exposed to TMT in juvenility on PND 23-27 and controls exposed only to water. Animals were furth er divided into animals reared with frequent opportunity to spend time in a socially enriched environment (SEE) and controls. Animals assigned to the SEE condition were provided daily access for 2 hours in a socially enriched environment beginning on PND 28 for the remainder of the experiment. In adolescence, all animals were tested for anxiety-like behaviors and for pain nociception. On PND 43, animals were exposed to IS and tested again for changes in anxiety and pain perception. In adulthood, animals were again tested on EPM and plantar test to assess the effect of experimental procedures across the lifespan. Following adulthood testing, all animals were euthanized and brains were harvested for later analysis. Results are discussed in the context of the role of social support in ameliorating the effects of early life stress across the lifespan.

THE DEVELOPMENT OF A PARENT-ADOLESCENT STRESSOR FOR EYE TRACKING

Kristen Wilkinson, Kelly Pisapia, Nathan Felkel, Eric Vaught, Emily Cook, *Department of Psychology*, Rhode Island College, Providence, RI

RI-INBRE Summer Undergraduate Research Fellowship Program

Adolescence is a time of risky behaviors. Previous research has indicated that these risky behaviors may be related to increases in normative stressful experiences. Two contexts where these stressful transitions may occur are within parent-adolescent relationships and peer relationships. Research relying on animal models has demonstrated that stress causes functional and structural changes in the prefrontal cortex and amygdala that are partially explained by parasympathetic stress response. Few studies, however, have examined the influence of interpersonal stressors on neural indices associated with cognitive control and emotion regulation in humans, particularly adolescents. Exceptions include research that utilizes FMRI and demonstrates increased activation in the amygdala and in the prefrontal cortex to stressors. FMRI studies give us insight but tend to be limited in their sample size given the cost. A more cost-effective method is eye-tracking. Eye tracking can be used to assess pupil dilation and attention as measures of emotional and cognitive processing. To date, no laboratory paradigm exists to assess adolescents' neurobiological response to parent-adolescent conflict using eye-tracking. Such a stimuli needs to be developed in order to understand the primary aim of our grant, which is to examine the neurobiological mechanisms by which interpersonal stressors impact risk behaviors. The current study utilized qualitative methods to help develop the visual stimuli. Qualitative methods included a literature review, interviews with mothers, focus groups with teens and observations of previously recorded parent-adolescent interactions. These data sources will guide the content of the parent-adolescent stimuli (e.g., dialogue, tone of interaction) and establish the feasibility of this approach with adolescents. Results sugg ested that personal responsibilities (e.g., cleaning bedroom) were a common topic of conflict between teenagers and their parents. Results further indicated that more serious conflicts involved hostile as well as critical remarks toward the teen's character and little to no eye contact from both the parent and the teen. Focus groups indicated that the family based stimuli should refrain from a "staged" appearance. The literature suggested that lighting and background stimuli must be controlled for when developing a family based stimuli. The information gathered will aid in the development of a family based stimuli for eye tracking.

MOTOR NEURON POSITIONING DEFECTS IN A TAG-1 KNOCKOUT MOUSE

Kristen D'Elia, *Department of Biology and Department of Psychology*, Providence College, Providence, RI; Heather Anderson-Keightly, Tracey Suter, Alexander Jaworski, *Department of Neuroscience*, Brown University, Providence, RI

RI-INBRE Summer Undergraduate Research Fellowship Program

The mechanisms that regulate the formation of neuronal connections between the central and peripheral nervous system (CNS and PNS, respectively) are not fully understood. Motor neurons have the unique ability among CNS neurons to extend axons towards their muscle targets in the PNS while their cell bodies remain within the spinal cord. Identifying the factors and mechanisms that prevent motor neuron cell bodies from migrating out of the spinal cord along with their axons is central to understanding CNS-PNS boundary formation and function. Preliminary studies have implicated the neural adhesion molecule TAG-1 in this process, as misplaced motor neuron cell bodies were observed outside the spinal cord at the forelimb level of TAG-1-deficient embryonic day 11.5 (E11.5) mice. Here, we created a timeline of motor neuron mispositioning in TAG-1 mutant mice betwe en E10.5 and E13.5, and we find that this phenotype persists at later stages of development. We also investigated the severity of the phenotype in different anterior-posterior regions of the spinal cord and examined the association of Schwann cells with motor axons. Our results confirm and extend the notion that TAG-1 regulates motor neuron positioning and maintenance of the CNS-PNS boundary.

DEVELOPING CONCEPTIONS OF PLAY

Rose DeRienzo, *Department of Psychology*, Providence College, Providence, RI; David Sobel, *Department of Psychology*, Brown University, Providence, RI

RI-INBRE Summer Undergraduate Research Fellowship Program

How do children conceptualize what play is and how do their conceptions of play change throughout development? Previous research has investigated the way children understand their own cognition and how their understanding changes and grows with development. Sobel (in press) studied children's conceptions of learning and found that between the ages of 4-8 children's descriptions of learning change from merely the content of what they learn to explaining the process of how they learn something. Investigating how children of different ages conceptualize the word play allows us to find out if similar cognitive changes take place and how metacognition develops. Study one examined how children spontaneously talk about play via CHILDES language analysis. We followed one child's utterance of the word "play" from age 1 to 7 and analyzed if each utterance referred to: content (what the child was playing), source (who the child was playing with), process (how the child was playing), and/or outcome (what happened if play occurs). This allowed us to analyze how the child thought about the word "play" throughout his development. In study two, children between the ages of 4-10 were asked what play meant. They then engaged in a structured interview about what kinds of things they played and how they played with those things. As children develop, their conceptions of play become much more complex and involved as they explain not only the content of play, but the source, process, and outcome of play. Older children seem to give metacognitive responses that indicate they better understand the process and circumstances where play would occur. The changes in types of responses throughout development shed light onto how metacognition changes and develops as a child grows.

UNDERSTANDING TODDLERS' CONCEPTION OF PRETENSE: ACTION VS. MENTAL STATE BASED

Jennifer Van Reet, Lauren Elia, Ana Leon, Kelly Murner, *Department of Psychology*, Providence College, Providence, RI

RI-INBRE Summer Undergraduate Research Fellowship Program

Previous research has shown that young children are able to pretend and understand the pretense of others by age two but that children under age six incorrectly claim pretending is simply an action, not a mental state. However, previous research has only tested children's understanding of pretending explicitly. The current study is designed to determine whether 18-24 month olds have an action or mental state based implicit understanding of pretense and explore the relationship between executive function and understanding of pretense. To examine toddlers' concept of pretense, looking time at an experimenter pretending correctly with both a familiar and novel object will be measured. For the "acting as if" condition, the experimenter will claim the novel object is unknown to her. In the "knowledge" condition the experimenter will claim to have previous knowledge of the object. It is expected that toddlers in the "acting as if" condition will display longer looking times, indicating that children are able to represent pretense as mental-state-based and appreciate the role of knowledge in pretense. A comprehension of pretense assessment and parent questionnaire will measure children's pretend behavior and their comprehension of others' pretense. Executive function measurements will consist of three tasks that assess inhibitory control, delay of gratification, and working memory. It is hypothesized that children who are able to understand pretense as a mental state based activity will display higher executive functioning. Pilot testing ensured the procedure was developmentally appropriate; data collection is now ongoing. If results follow the hypotheses, findings will suggest that children can represent the experimenter's knowledge as different from their own, inhibiting their knowledge to take that of the experimenter's into consideration. These findings would be beneficial in determining the value of pretense in learning and early childhood education.

THE CIRCADIAN ROLE OF LIGHT AND FOOD PRESENTATION ON BEHAVIORAL AND PHYSIOLOGICAL OUTCOMES IN FISCHER RATS

Meghan Gallo, Pamela Snodgrass-Belt, *Department of Biology*, Providence College, Providence, RI

Independent Research

Circadian rhythms, endogenously generated biological oscillations, permit organisms to synchronize to and predict their environmental cycles, resulting in the animals' ability to thrive. In mammals, central and peripheral circadian oscillators govern these internal rhythms, controlling both physiological and behavioral outputs. Time cues, like sunrise or feeding, provide a cue for these rhythms to synchronize or "entrain" to environmental cycles. Understanding how multiple entraining factors, including both light and food presentation, set and drive multiple rhythms may be of clinical importance. Here we show data collected from rats housed on a normal 12:12 light dark cycle. Animals, separated into three experimental conditions, received food at various time points. Group 1, the control, had access to food 24 hours a day. Group 2 and Group 3 had unlimited access to food for eight hours per day. Group 2 had access to food in the middle of the light cycle, while Group 3 had access to food in the middle of the dark cycle. By decoupling the light and food entrainment signals, we investigated the individual roles of these environmental cues on food consumption, wheel running activity, neuronal activity, and endocrine signals.

ASSESSING EARLY VERB COMPREHENSION WITH DYNAMIC STIMULI

Saadet Alparsian, Emely Bueno, Stephanie Chauvin, Beverly Goldfield, *Department of Psychology*, Rhode Island College Providence, RI

RI-INBRE Summer Undergraduate Research Fellowship Program

The goal of this research is to adapt the Preferential Looking Task (PLT) to reliably assess verb comprehension. The PLT assesses comprehension by comparing visual attention to two images (target/distracter) displayed on a computer monitor before (baseline) and after (test) the target image is labeled. Comprehension is defined as an increase in visual attention to the target image during test compared to baseline. In previous research (sample 1) we used the PLT to measure comprehension of nouns versus verbs. We tested 12 nouns represented by color photos of objects (e.g., juice, cow) ; 12 verbs were tested with video recordings of actors performing actions (e.g., jump, throw). Visual attention during baseline and test trials was monitored by a Tobii T60XL eye-tracker system. Children aged 16 and 18 mos demonstrated comprehension of nouns but not verbs, suggesting late onset of comprehension of words for actions. However, recent work on the development and neurological foundation of visual attention suggests an alternative hypothesis: complex dynamic stimuli elicit longer bouts of visual attention (Richards, 2010; Shaddy & Colombo, 2004) and may be mediated by different neurological processes than attention to static visual displays (Reynolds & Richards, 2009). In sample 1, both nouns (static displays) and verbs (dynamic displays) were presented during a 5 sec baseline trial in order to create equivalent experimental paradigms for the noun and verb groups. Although a 5 sec baseline may have been optimal for meaningful exploration of the static object images, this same 5 sec exposure may have been insufficient for viewing the dynamic scenes of actors and actions used for verbs. The current study tests the hypothesis that children's poor performance on verbs is related to limitations on visual attention created by the 5 sec baseline trial. For sample 2, we tested the same 12 verbs, but lengthened the baseline trial to 10 sec and the test trial to 5 sec. Results indicate verb comprehension across the 12 verb trials for 16, 18, and 20-month olds. These data suggest that reliable assessment of verb comprehension depends crucially on providing an adequate assessment interval for the visual exploration and interpretation of dynamic stimuli.

SOCIAL JUDGMENTS AND VISUAL ATTENTION AS A FUNCTION OF AFROCENTRIC FACIAL PHENOTYPICALITY: A SOCIAL RELATIONS ANALYSIS

Jessica Hunter, Sathiarith Chau, Nick Collette, Travis Dumais, Thomas Malloy, *Department of Psychology*, Rhode Island College, Providence, RI

RI-INBRE Summer Undergraduate Research Fellowship Program

Highly Afrocentric faces are those with the darkest skin tone, broad noses and full lips. Past research documents that people with the strongest Afrocentric features are judged more negatively by Whites than those with weaker Afrocentric features. We applied a new statistical model to analyze Whites' visual attention to, and social judgments of Black faces that vary in Afrocentricity. Social Relations Modeling showed that there are individual differences among perceivers in the extent to which they attend to facial features, the judgments they make and the speed with which they make them. Also, some faces elicited more visual attention than other faces that elicited less visual attention. Social relations modeling is a new approach for understanding how Whites process Black faces varying in Afrocentricity that holds considerable promise.

D2-DOPAMINE RECEPTORS ARE TARGETED TO PLASMA MEMBRANE MICRO-COMPARTMENTS BY THE FOURTH TRANSMEMBRANE DOMAIN

Craig Irving, Christopher Octeau, Joseph Schrader, Meenakshi, Abraham Kovoor, *Department of Biomedical and Pharmaceutical Sciences*, College of Pharmacy, University of Rhode Island, Kingston, RI

RI-INBRE Bridges to Graduate School Program

D2 dopamine receptors (D2R) are the common molecular target of all available antipsychotic drugs. We have previously reported that D2R is localized to two distinct cellular pools, which are either soluble or insoluble in cold solutions of the non-ionic detergent Triton X-100. Both pools of D2R are found in both mouse brain and after transient expression of D2R in HEK-293 cells. We have shown that the detergent-soluble D2R originates from a region of the plasma membrane that allows the receptor to randomly interact with other cellular components while the insoluble D2R originates from plasma membrane micro-compartments that restricts access to many cellular proteins. Thus it is likely that the the two different pools of receptor may initiate or modulate distinct sets of cellular signals. To identify the D2R epitopes that were responsible for mediating the micro-compartmentalization of D2R we designed truncated D2R constructs and examined the detergent-solubility of these constructs after expression in HEK-293 cells. Our findings indicate that the fourth transmembrane region of D2R is likely to be the critical domain responsible for targeting D2R to the detergent-insoluble cellular fraction.

LEARNING CAUSAL REASONING THROUGH PRETEND PLAY

Lauren Elia, Ana Leon, Kelly Murner, Jennifer Van Reet, *Department of Psychology*, Providence College, Providence, RI

RI-INBRE Summer Undergraduate Research Fellowship Program

Pretend play is often viewed as a positive way for children to learn, contributing to improved performance on various types of cognitive tasks, including deductive, syllogistic, and counterfactual reasoning, as well as theory of mind (Walker, Ganea, & Gopnik, 2014). Previous research has also found that understanding causal powers assists in making predictions and taking initiative to bring about desired results (Gopnik and Sobel, 2000). To explore if pretend play supports the development of causal reasoning, the current study investigated whether children would transfer a causal rule demonstrated in pretend play to reality. Preschoolers (N = 54) observed an experimenter pretend that two out of four blocks were "blickets" (either red blocks or square blocks, depending on condition), by pretending that a special "blicket detector" would make a chime sound whenever a "blicket" was placed on it. Next, participants were given a false belief task as a distractor and to measure the participant's theory of mind ability. Children were then shown a real blicket detector and another set of four blocks (red triangle, red cylinder, yellow square, blue square). Children were prompted to "make the detector go". Results showed that children did not transfer the pretend rule to the real phase at a rate significantly greater than chance (all ps > .16): 55.6% of 3 year olds, 66.7% of 4 year olds, and 44.4% of 5 year olds chose a block that was categorized as a "blicket" in the pretend phase as their first choice in the real phase. These results suggest that pretend play, while beneficial, may be an unreliable tool to convey novel causal properties.

NATIVE VOLTAGE-SENSITIVE SODIUM CHANNELS MICROTRANSPLANTED INTO XENOPUS LAEVIS OOCYTES

Heather Conboy, Steven Symington, *Department of Biology and Biomedical Sciences*, Salve Regina University, Newport, RI

Independent Research

There is a great need for more in vitro assay systems to assess the effects of environmental contaminants on mammalians, particularly ion channels. These assay systems should be inexpensive, efficient, high throughput and capable of assessing differences in ages and gender of species. We utilize fluorescent microscopy and immunohistochemistry to assess whether or not voltage-sensitive sodium channels from rat brain were incorporated into the plasma membrane of Xenopus oocytes following intracellular injection. Our results indicate that frequency of incorporation is not affected by different tissue. Furthermore, our results also show that voltage-sensitive sodium channels from rat brain are incorporated into the plasma membrane of oocytes. The purpose of this research is to characterize an in vitro system to see how toxins affect the central nervous syste m. In this system, post natal date (PND) 15 and PND 90 rat brain neurolemma laced with rhodamine is microtransplanted into the plasma membrane of Xenopus laevis oocytes. It could then be concluded that the incorporation between the PND 15 and PND 90 is not significantly different, as well as the alive/death ratio between PND 15, PND 90 and P2 buffer injected oocytes. After immunohistochemistry double staining, native voltage gated ion channels were then identified in the membrane.

FUNCTIONAL CHARACTERIZATION OF VOLTAGE-SENSITIVE CALCIUM CHANNELS MICTROTRANSPLANTED TO XENOPUS LAEVIS OOCYTES

Samantha Neff, Abigail Toltin, Steven Symington, *Department of Biology and Biomedical Sciences*, Salve Regina University, Newport, RI

Independent Research

Voltage-sensitive Cav channels activate upon membrane depolarization and facilitate calcium entry in response to an action potential or subthreshold depolarizing signals. When calcium enters a cell through a voltage-sensitive Cav channel, it serves as a messenger that will initiate various intra-cellular events. The purpose of this study was to confirm the presence of Cav channels in the developing rat brain and assess the function of these native channels when they were microtransplanted into Xenopus laevis oocytes. To do this, western blot and electrophysiological techniques were used. Western blotting confirmed the age-dependent expression of Cav channels in juvenile and adult rat brain tissue. Two-electrode voltage clamp electrophysiology confirms that Cav2.2 channels were functional when microtransplanted into Xenopus oocytes. Thus, microtransplanted rat brain neurolemma into Xenopus oocytes is a practical method to study functional native calcium channels.

ASSESSMENT OF DENDRITIC LENGTH AND COMPLEXITY IN THE STRIATUM AND PREFRONTAL CORTEX FOLLOWING NEONATAL HYPOXIA-ISCHEMIA IN RATS

Zahra Melendez, Keyshia Melendez, *Department of Biology*, Rhode Island College, Providence, RI; Steven Threlkeld, *Department of Psychology*, Rhode Island College, Providence, RI

Independent Research

Neuronal plasticity is the ability for the nervous system, more specifically neurons, and their synapses to modify their function and morphology due to experience, injury and other factors. Historically neuroscientist have utilized and developed various methods in order to measure neuronal plasticity. For example, manually tracing of neurons and their processes using a microscope drawing tube. Technological advances have allowed for the development of more accurate three-dimensional neuronal reconstruction software and encoder systems to measure plasticity. Relatively straightforward histological techniques, such as the golgi-cox method, paired with these technologies allow for a higher degree of efficiency and more accurate analyses compared to previous methods. These contemporary tools are used to measure dendritic arborization, spine density and dendritic length. We explore the application of this technology in two studies where the influence of neonatal hypoxic-ischemic (HI) brain injury and early behavioral experience were assessed on rat medium spiny projection neurons, within the striatum and on pyramidal neurons within the prefrontal cortex. The main objectives of these studies were to further investigate the role of early life behavioral training and brain injury on neuronal plasticity. We hypothesized that HI injury would result in less complex medium spiny and pyramidal neurons and that behavioral experience would induce plasticity, which may underlie recovery of function.