2012 Rhode Island Summer Undergraduate Research Fellowship Conference

Friday, July 27, 2012
8:00 AM

Center for Biotechnology & Life Sciences
University of Rhode Island

Supported by

Rhode Island NSF EPSCoR
Experimental Program to Stimulate Competitive Research

RI-INBRE & RI EPSCOR

SUMMER UNDERGRADUATE RESEARCH FELLOWS (SURF) CONFERENCE

FRIDAY, JULY 27, 2012

CENTER FOR BIOTECHNOLOGY & LIFE SCIENCES

UNIVERSITY OF RHODE ISLAND

KINGSTON, RI

8:00 – 8:45 AM	***CONTINENTAL BREAKFAST & SURF GROUP A POSTER SET-UP***
8:45 – 9:10 AM	***WELCOMING REMARKS***
9:10 – 10:10 AM	***SURF POSTER SESSION*** **- GROUP A**
10:10 -10:20 AM	***INTERMISSION I & SURF GROUP B POSTER SET-UP***
10:20 – 11:20 AM	***SURF POSTER SESSION*** **- GROUP B**
11:20 -11:30 AM	***INTERMISSION II & SURF GROUP C POSTER SET-UP***
11:30 – 12:30 PM	***SURF POSTER SESSION*** **- GROUP C**
12:30 PM	***LUNCH***

LIST OF SUMMER RESEARCH FELLOW POSTERS

***Please note that the poster numbers listed in the following tables also correspond with the page numbers in the abstract book. Students are listed under the institution where their research was conducted.*

University of Rhode Island

Poster #	Summer Fellow	Mentor
1	Soliel Doman	Aftab Ahmed
100	James Stevenson	Clinton Chichester
12	Lauren Boltz	Geoffrey Bothun
123	Farid Topchiev	
92	Annalisa Sharkey	Bongsup Cho
74	Stephen Norris	
132	Jillian Zoglio	Brenton DeBoef
26	Tori Deschenes	Anne DeGroot
18	Jeff Chau	
30	Evan Dunphy	Caroline Gottschalk Druschke
30	Peter Schooling	
45	Allison Holevoet	Graham Forrester
68	Sarah Merolla	
16	Jeremy Carreiro	Art Gold
3	Samantha Nicodemus	Niall Howlett
88	Ryann Rossi	Brita Jessen
25	Alyssa Dantonio	Roberta King
110	Kevin Sun	Abraham Kovoor
109	Elizabeth Sullivan	Christopher Lane
24	Melisa Curran	
85	Alyssa Rogers	
108	Madeleine Suits	Mindy Levine
116	Kevin Szulak	Wei Lu

University of Rhode Island (continued)

Poster #	Summer Fellow	Mentor
91	Eric Shabashevich	David Nelson
75	Kevin Northup	Keykavous Parang
125	Holly Tran	Yana Reshetnyak
60	Zachary Lariviere	David Rowley
56	Kristen Knoph	Navindra Seeram
94	Adam Silva	Angela Slitt
10	Emily Bishop	Carol Thornber
51	Hannah Jones	Hiro Uchida
117	Rachel Thakore	Daniel Udwary

Brown University

Poster #	Summer Fellow	Mentor
90	Christian Selinski	Edward Hawrot
107	Elena Suglia	Gary Wessel
119,120,188	Cynthia Gaudet	Barbara Stonestreet
67	Nicholas DeLeo	John Marshall
11	Eimear Black	Amit Basu

Bryant University

Poster #	Summer Fellow	Mentor
73	Christina Nadolny	Kirsten Hokeness & Christopher Reid
70	Ryan Miller	
58	Jacqueline Kratch	
129	Zoe White	Dan McNally
54	Trevor Kent	Julia Crowley Parmentier
46	Garrett Holmes	Christopher Reid

Providence College

Poster #	Summer Fellow	Mentor
48,86	Matthew Hurton	Nicanor Austriaco
86	Stephen Rogers	
34	Ryan Frazier	
133	Natasha Zupkus	Maia Bailey
29,83	Brenna Peters	Christopher Bloom
29,83	Caroline Doyle	
29,83	Ryan Post	
21	Eliza Conaty	Joseph DeGiorgis
78	Ryan Paranal	
67	Nicholas Marcello	
126	Lauren Trotta	Patrick Ewanchuk
121	Matthew DeBlois	Jeffrey Markert
122	Kelsey Garlick	
80	Meaghan Keane	Brett Pellock
80	Zach Sexton	
80	Matt Goulet	
80	Taylor Hunt	
61	Alexandra Male	Jennifer Van Reet
61	Christina Taylor	
61	Christina Lavigne	

Rhode Island College

Poster #	**Summer Fellow**	**Mentor**
19	Kristen Chauvin	Karen Almeida
35,82	Christopher Funk	
35,82	Cailyn Mather	
35,82	Katelyn Pina	
31,47,50	Masharee Hopkins-Jones	Deborah Britt
31,47,50	Sabrina Elgar	
31,47,50	Kyle Inman	
22	Kristen Wilkinson	Emily Cook
22	Kyle Fernandes	
22	Kayla Flynn	
39	Kirstie Lepore	Beverly Goldfield
39	Kevin Fornari	
39	Christina Gencarella	
89	Kyle Schoolcraft	Brea Govenar
55,66	Lorin Kinney	Thomas Malloy
66,124	Rachel Traghella	
66	Brandon DeSimone	
81	Kaitlyn O'Connor	Thomas Meedel
81	Clifford Picket	

Rhode Island College (continued)

Poster #	**Summer Fellow**	**Mentor**
101	Irina Maglysh	Sarah Spinette
101	Sabiha Rahman	
101	Jacklyn Lata	
118,120	Nicholas Lafond	Steven Threlkeld
118,120	Katrina Feyerherm	
118,120	Molly LaRue	
5	Duane Barnes	John Williams
15	Titalayo Adedji-Campbell	
64	Jose Lora	

Rhode Island School of Design

Poster #	Summer Fellow	Mentor
102	Mengzhou Li	Neal Overstrom
102	Eliza Squibb	

Roger Williams University

Poster #	Summer Fellow	Mentor
65	Kelsey Lucas	Sean Colin
65	Eric Klos	
33	Hanna Sobon	Avelina Espinosa
33	Kevin Schindelwig Franca	
33	Layla Ferland	
71	Andrew Mitchell	
131	Alicia Wilson	Lonnie Guralnick
79	Christopher Pellichero	Dale Leavitt
17	Peter Cavedon	Kathryn Markey
40,52	Joshua Jones	Andrew Rhyne
40,52	Allex Gourlay	
41	Catherine Grimm	Roxonna Smolowitz
43	Allison Hall	David Taylor
59	Nicholas Kutil	
62	Garrett LeBlanc	
77	Michael Pallotta	
105, 106	Laura Stevenson	Kerri Warren
105, 106	Janani Subramaniam	

Salve Regina University

Poster #	Summer Fellow	Mentor
49	Alexandra Igo	Jameson Chace
8	Nicole Bickford	
57	Olivia Kopin	
127	Madison Van Orden	
	James Diamontopoulos	Sarah Materese
32	Caitlyn Farragher	
93	Sarah Showalter	Susan Meschwitz
38	Mallory Goding	
13	Taylor Braun	
99	Brian Somba	Bernard Munge
96	Morgan Smith	
4	Alexander Antonopoulos	
95	Bridget Smith	Alison Shakarian
23	Tia Crowther	
112	Stephanie Beels	
2,95	Erika Albretsen	
23, 44	Lana Hoertz	
114,115	Craig Irving	Steven Symington
115	Karl Varkey	
114	Justin Gay	
114	Wayne Bainter	

Salve Regina University (continued)

9,42, 103	Alexandria Bierce	John-David Swanson
42, 103	Alyssa Guarracino	
9,42, 103	Kelsey Stafstrom	
6,14,36	Amy Battocletti	
6,14,36	Shennel Gelin	
14	Matthew Breseman	

PURIFICATION AND PARTIAL CHARACTERIZATION OF SNAKE LEAF-NOSED VIPER (ERISTICOPHIS MACMAHONII) HEMOGLOBIN

Soliel Doman, *Department of Biotechnology*, Community College of Rhode Island, Warwick, RI; Humera Faraz, Iqbal Chaudhary, *HEJ Research Institute of Chemistry*, University of Karachi, Karachi, Pakistan; Aftab Ahmed, *Department of Biomedical and Pharmaceutical Sciences*, University of Rhode Island, Kingston, RI

RI-INBRE Summer Undergraduate Research Fellowship Program

Hemoglobin is a respiratory protein present in the erythrocytes of all vertebrae. Typically, it consists of two identical pairs of α and β globin chains. Our preliminary investigation on snake Leaf-Nosed Viper hemoglobin is being conducted with the intent to characterize and better understand the interspecies relationships among various snakes at the molecular level. The Leaf-Nosed Viper is a deadly poisonous snake, classified in the family Viperidae, represented in Pakistan by five genera, seven species, and subspecies. The hemoglobin was isolated from the washed RBCs with physiological saline and the globin was further isolated by treatment in cold acidified acetone. The globin chains were separated by reversed-phase HPLC. The corresponding βII globin chain was oxidized and digested with TPCK treated trypsin. Peptides were separated by RP-HPLC, and homogeneity of the peptides was checked by MALDI-TOF mass spectrometry. Partial amino acid sequence of βII chain was deduced by Edman degradation of the intact globin chain and of the purified tryptic peptides in an automated protein sequencer. The primary structure of the βII chain of the LNV was aligned to other reported βII chain sequences, and it was found to be highly conserved.

DETERMINING DIFFERENCES IN PROTEIN EXPRESSION PATTERNS AMONG FOUR DIFFERENT LEISHMANIA SPECIES THROUGH CDNA CLONING AND SEQUENCING

Erika Albretsen, *Department of Biology*, Salve Regina University, Newport, RI; Alison Shakarian, *Department of Biology and Biomedical Sciences*, Salve Regina University, Newport, RI

RI EPSCoR Summer Undergraduate Research Fellowship Program

Leishmania is a protozoan parasite of the Trypanasomatidae family that can cause two different disease manifestations in humans. Cutaneous leishmaniasis causes ulcerating skin lesions and is generally found to be associated with L. mexicana and L. major. Visceral leishmaniasis causes inflammation of the liver, spleen, and bone marrow and is found to be associated with L. donovani. On the other hand, it has been found that L. tarentolae is not pathogenic and does not cause either of the two manifestations. The purpose of this research is to determine if there are different proteins expressed by these organisms that may be a contributing factor in the varying pathogenicities associated with the four Leishmania species. The total RNA of each species was isolated and cDNA was synthesized. Amplified Fragment Length Polymorphisms (AFLP) reactions with 6 selective primer sets were completed to identify polymorphic and unique cDNA fragments among the four Leishmania species.. These unique cDNA-AFLP fragments were cloned into pCR-TOPO plasmid vectors using E. coli. Colonies were isolated and subjected to PCR to determine if the size of cDNA fragments matched the originally cloned AFLP fragments. The plasmids were subsequently isolated, sequenced and subjected to a nucleotide BLAST search. Results showed genes for ribosomal and unknown hypothetical proteins expressed by these parasites in a species specific manner. Interestingly, it was also found that the parasites differentially expressed genes for superoxide dismutase, calreticulin, aminopeptidase and an ATP-binding cassette protein. Additional cDNA-AFLP fragments are currently being isolated and sequenced. Future experiments include verification of differential expression of the genes identified by AFLP analysis using real time qPCR.

CHARACTERIZATION OF A PUTATIVE HISTONE H4 BINDING DOMAIN IN THE FANCONI ANEMIA D2 PROTEIN

Samantha Nicodemus, *Department of Cell and Molecular Biology*, Community College of Rhode Island, Warwick, RI; Karissa Neira, Nial Howlett, *Department of Cell and Molecular Biology*, University of Rhode Island, Kingston, RI

RI-INBRE Summer Undergraduate Research Fellowship Program

Fanconi Anemia (FA) is a rare genetic disorder characterized by bone marrow failure and heightened cancer susceptibility. The FA proteins function in the FA-BRCA pathway to repair damaged DNA and to maintain chromosome stability. A major step in the activation of this pathway is the mono-ubiquitination of the FANCD2 and FANCI proteins. FANCD2/I mono-ubiquitination, catalyzed by the FA core complex, targets these proteins to chromatin. The regulation of FANCD2/I mono-ubiquitination and the mechanism by which these proteins are targeted to chromatin are poorly understood. We have recently identified a putative histone H4 binding domain in the FANCD2 protein based on homology with the known H4 binding domain of Drosophila melanogaster p55, a chromatin remodeling protein. Histone H4 is a core component of the nucleosome, a discrete subunit of chromatin comprised of a short stretch of DNA wrapped around an octamer of histone proteins (H2A)2(H2B)2(H3)2(H4)2. We hypothesize that the FANCD2 H4 domain may facilitate binding and retention of FANCD2 in chromatin. To begin to investigate the function of the putative FANCD2 H4 domain we have used a site-directed mutagenesis approach to mutate two potentially important amino acids within this domain, E956 and E964. Using this approach, we have successfully generated cDNAs for two novel FANCD2 mutants: FANCD2 E956A, E964A and FANCD2 E956K, E964K using Invitrogen Gateway technology, and are in the process of recombining these cDNAs in the pLenti6.2 lentiviral vector. These lentiviral vectors will be used to transduce FA-D2 (FANCD2-/-) patient-derived cells to determine the functional significance of mutation of this putative domain. For example, we will examine the effects of mutation of the H4 binding domain on FANCD2/I mono-ubiquitination, chromatin localization and its ability to rescue the DNA damage hypersensitivity of FA-D2 cells.

LABEL FREE SURFACE PLASMON RESONANCE (SPR) IMMUNOASSAY FOR THE DETECTION OF INTERLEUKIN-8 CANCER BIOMARKER

Alexander Antonopoulos, Bernard Munge, *Department of Chemistry*, Salve Regina University, Newport, RI

RI-INBRE Summer Undergraduate Research Fellowship Program

Methods for measuring protein biomarkers with high sensitivity and ultralow detection limit (DL) promise to provide valuable tools for early diagnosis of diseases such as cancer, and for monitoring therapy and post-surgical recurrence. Surface plasmon resonance (SPR) coupled to nanoparticle-antibody labels for signal amplification in immunoassays is an emerging approach for detecting proteins in biomedical samples. Herein, we report on a label free sensitive SPR-based immunosensor that detects Interleukin-8, a cancer biomarker protein in serum. SPR immunoassays involve attaching capture antibodies (Ab1) to an SPR chip and measuring signals after capture of the protein analyte from the sample. The Ab1 concentration was optimized to lower non-specific binding which often controls the sensitivity and DL. This approach provided a spectrum which can be used as a means for the detection of Interleukin-8, as well as for further amplification of the system through use of nanoparticle-antibody bioconjugates. Work is in progress to enhance the sensitivity and lower the DL using a nanoparticle-antibody bioconjugate signal amplification strategy. These bioconjugates consist of a streptavadin coated magnetic bead bound to a secondary antibody (Ab2), IL8 antigen, and biotinylated polyethylene glycol polymer brushes. These biotinylated polyethylene glycol polymer brushes prevent aggregation and nonspecific binding, all while improving detection limit through increased binding to the Ab1 on the SPR chip.

OPTIMIZED SYNTHESIS OF 1,2,3-TRIAZOLE COMPOUNDS BY MICROWAVE

Duane Barnes, *Department of Physical Science*, Rhode Island College, Providence, RI

RI INBRE Summer Undergraduate Research Fellowship Program

1,2,3-Triazoles exhibit multiple types of biological activity, including anticancer and estrogenic activity. We are synthesizing three scaffolds of 3,4,5-triaryltriazoles, using microwave assisted organic synthesis (MAOS), which can be further modified to produce a large library of compounds. Computations show binding constants to the estrogen receptor for some of these similar to that of tamoxifen. Traditionally, triazole compounds are synthesized by click chemistry using bench top methods that require refluxing for up to two days. Doing these reactions with a microwave reactor reduces reaction time from days to minutes. As a result, a library of triazole compounds can now be prepared in a short time and then modified in a two or three step sequence to produce the compound libraries for anticancer screening.

ULVA SPECIES REPRODUCTIVE STRATEGY DURING BLOOM FORMATION

Amy Battocletti, Carol Thornber, *Department of Biological Sciences*, University of Rhode Island, Kingston, RI; Shennel Gelin, Metthew Breseman, J.D. Swanson, *Department of Biology and Biomedical Sciences*, Salve Regina University, Newport, RI

RI EPSCoR Summer Undergraduate Research Fellowship Program

Climate change may lead to an increase in harmful green macroalgal blooms impacting Narragansett Bay. These blooms are dominated by the species Ulva compressa and Ulva rigida, which have a biphasic, isomorphic lifecycle based on reproduction via the release of zoospores and gametes, but may also reproduce clonally via vegetative reproduction. The environmental conditions associated with vegetative reproduction are not understood, and the predominant reproductive strategy used by Ulva spp. during bloom formation in the bay is still unknown. It is also unknown if environmental cues trigger either of these reproductive strategies in a way that significantly contributes to bloom formation. At three sites within Narragansett Bay, temperature loggers were placed subtidally and Ulva samples collected biweekly. Microsatellite analyses are being developed to determine if individuals are genetically identical or unique. DNA is being extracted from the samples and will be amplified via PCR. The nine previously identified microsatellite markers are currently being checked for polymorphism to ensure adequate heterozygosity across populations before use. Comparisons of genotypes will indicate the number of identical clones versus distinct individuals. These bloom formation dynamics will then be correlated with the continuous temperature data to investigate the possible causal role of temperature in determining the reproductive strategy. This knowledge will inform management decisions regarding the mitigation of economic and ecological bloom impacts.

SPERMATHECA STRUCTURE AND ULTRASTRUCTURE IN THE SOCIAL WASP

Douglas Biancur, Elisabeth Arevalo, *Department of Biology*, Providence College, Providence, RI

RI EPSCoR Summer Undergraduate Research Fellowship Program

Female insects have a specialized structure, known as the spermathecae, for storing sperm after mating occurs. The spermatheca consists of a large reservoir where the sperm is kept viable by hormones produced by elongated glands that are connected to the reservoir by ducts. We examined the spermatheca structures and ultrastructures in the paper wasp, Polistes fuscatus, to characterize the morphology and to observe stored sperm in an inseminated female. To carry this out we collected female wasps early in their life cycle, before worker emergence, when there were only one or few wasps per nest, known as foundresses (egg layers). Three different microscope techniques were implemented for the study. Gross structure and morphology was obtained by using the light microscope with toluidine blue stain. To further characterize the internal morphology, we used both confocal and electron microscopy. Under the confocal microscope we used DAPI staining to detect the presence of sperm nuclei in the lumen of the spermatheca. To validate our claims and to confirm the presence of the stored sperm, we prepared the sample for the electron microscope. Samples were stained with osmium and uranyl acetate that allowed us to identify sperm ultrastructures. The spermathecae, plays an important role in social insects of the order Hymenoptera. Because of their haplodiploid system, foundresses can lay haploid eggs that become males, or use the stored sperm to fertilize their eggs to produce diploid females. This method of characterizing the spermatheca can be used as an assay for determining which one of the sometimes hundreds of females in a colony is inseminated and therefore is the egg layer.

TESTING THE OPTIMAL NUTRIENT RANGE AND NUTRITIONAL CONTENT OF SPINACH

Nicole Bickford, Margaret Kane, Jameson Chace, *Department of Biology and Biomedical Sciences*, Salve Regina University, Newport, RI; Susan Meschwitz, *Department of Chemistry*, Salve Regina University, Newport, RI

Salve Regina University Sustainability Fellowship Program

In a world facing such issues as limited natural resources, global climate change, population increase, food security, and various health concerns, a method for sustainable, healthy, and efficiently grown food production is necessary. Hydroponics meets this criterion by growing plants in a controlled environment without soil resulting in greater crop density and a longer growing season. In order to obtain the maximum benefits offered by hydroponics, growth must be efficient, yield must be maximized, and cost must be lowered. Spinach (Spinacia oleracea) is high in nutritional content with carotenoids, iron, and various vitamins and is a widely consumed good, so it was studied for this purpose. In the first part of the study spinach growth was compared at opposing ends of the specified nutrient range for spinach. Three types of spinach (Corvair, Savoid, and Smooth-Leaf) were grown in two Tower Hydroponic Systems maintained at 1260 ppm and 1610 ppm with 6.5 pH. No significant differences were found in leaf production between the two systems. The second study analyzed the nutritional content to compare these two nutrient-controlled hydroponic systems with conventionally-grown spinach. Total iron and carotenoid concentrations were quantified in Corvair and Savoid spinach using UV-visible spectrophotometry and high-performance liquid chromatography (HPLC) respectively. Data has been gathered for iron content and is in the process of being analyzed. Beta-carotene concentrations among these three growing conditions are being measured. In conclusion, spinach production is possible at lower nutrient levels while maintaining high production and high nutritional content. This study demonstrated the possibility for hydroponics to create methods of indoor farming and to eventually pave the way to a sustainable and healthy future.

GROWING RASPBERRY CALLUS AND STUDYING THE EFFECT OF GALLIC ACID

Alexandria Bierce, Alyssa Guerracino, Kelsey Stafstrom, John-David Swanson,
Department of Biology and Biomedical Sciences, Salve Regina University, Newport, RI

RI-INBRE Summer Undergraduate Research Fellowship Program

Gallic acid is a secondary metabolite found in many species including the Rubus genus. Especially in raspberries, the glandular trichomes have been found to be rich in secondary plant metabolites. Previous research has indicated that when plant callus is exposed to high concentrations of gallic acid (12-20 ug/mL), the cell cycle is halted, but in lower concentrations (1-10uM) it speeds up the cell cycle. Conversely, when high concentrations of the secondary metabolite (12-20 ug/mL) were applied to HeLa cells, the cell cycle was halted and there was no further growth. To investigate the effect of gallic acid on plant cells, raspberry leaves have been sterilized, cut, and placed onto media containing thidiazuron to form callus. Once the callus has formed, the leaves will be transferred to media containing different concentrations of gallic acid. To identify genes that are potentially affected by gallic acid, we extracted DNA from the leaves of a ‘heritage’ raspberry plant. Twelve different genes partially involved in the cell cycle were tested with the DNA. We found that five of the primers (CDKA1, RaspCDKB1, CYCD3, CDKA, and GL2) produced amplification products. Next, we will run qPCR in order to quantify the changes in transcript levels in callus in respect to gallic acid.

DEVELOPING A PROTOCOL FOR ANALYSIS OF ALGAL CELLS USING FLOW CYTOMETRY

Emily Bishop, Carol Thornber, *Department of Biological Sciences*, University of Rhode Island, Kingston, RI

RI EPSCoR Summer Undergraduate Research Fellowship Program

Adult and juvenile algae are often easily distinguishable based on their chemical and physical differences. However, the bloom-forming species Ulva compressa does not demonstrate any morphological differences between ploidy levels. Data on the population structures of this species, in combination with temperature and salinity readings, is key to predicting pre-bloom conditions. By developing a final protocol for analyzing algal cells with flow cytometry, we will examine the ploidy levels of U. compressa populations from three sites in Greenwich Bay, RI. After refining a protocol for preparing tissue samples for flow cytometry analysis, we collected ten individuals from each site to determine a rough estimate of the presence of different life stages in each U. compressa population. Our results showed high quality of sample preparation, but more testing needs to be done to generate concrete results. By further refining our protocol, and continuing to sample individuals from these sites, we will better understand population dynamics in Greenwich Bay and predict pre-bloom conditions in Ulva species.

SYNTHESIS OF GLYCOSYL TRIAZOLE LIBRARY AS INHIBITORS FOR LYTIC TRANSGLYCOSYLASES USING UGI-4CCR AND 'CLICK CHEMISTRY'

Helene Kuhn, Amit Basu, *Department of Chemistry*, Brown University, Providence, RI;
Christopher Reid, Eimear Black, *Department of Biology*, Bryant University, Smithfield, RI

RI-INBRE Summer Undergraduate Research Fellowship Program

Peptidoglycan is a polymer consisting of sugars and amino acids that form a mesh-like layer outside the plasma membrane of bacteria, forming the cell wall. The sugar component of peptidoglycan consists of alternating residues of β-(1,4) linked N-acetylglucosamine (GlcNAc) and N-acetylmuramic acid (MurNAc). Lytic transglycosylases are involved in catalyzing the cleavage of the glycosidic bond between MurNAc and GlcNAc in peptidoglycan by forming a 1,6-anhydro bond in the MurNAc residue. These lytic transglycosylases have been implicated as space makers for the insertion of new peptidoglycan into the cell wall during cell growth.

No synthetic inhibitors of LTs are currently known however, natural inhibitors do exist such as bulgecin A. Therefore the goal of the research is to determine if it is possible to make small molecules which can disturb the biosynthesis of the bacterial cell wall. Such small molecules which could act as inhibitors can be accessed by using the Ugi-4CCR and the 'click' reaction. These molecules are formed using an N-acetylglucosaminazide glycone which will be clicked to an aglycone moiety made by Ugi-reaction. The Ugi 4 component condensation reaction involves the reaction with aldehyde, acid, amine and isocyanide. The aldehydes used in this reaction will vary for each molecule so we can form a library. The main aim is to find out if these small molecules can be an impulsive for new scaffolds for bioactive compounds.

Using 'click chemistry' and Ugi-4CCR allows us to build up a library of small molecules that can be tested as possible inhibitors for LTs. Potentially active molecules enable us to design and study a scaffold for new antibiotics.

EFFECT OF FATTY ACID IMPURITIES WITH VARYING DEGREES OF TAIL SATURATION ON MODEL MEMBRANE PHASE BEHAVIOR

Lauren Boltz, Yogi Kurniawan, Geoffrey Bothun, *Department of Chemical Engineering*, University of Rhode Island, Kingston, RI; Keerthi Venkataramanan, *Biotechnology Doctoral Program, College of Science*, University of Alabama in Huntsville;

Research Experience for Undergraduates, NSF Energy for Sustainability Program, (CBET-0966818)

The synthesis of n-butanol, a known biofuel, occurs during glycerol fermentation by the bacteria Clostridium pasteurianum. A recent study has shown that the only impurity present in crude glycerol that can significantly hinder biofuel production through cellular inhibition is a fatty acid that contains an 18-carbon (C18) polyunsaturated acyl tail. In this case inhibition was due to cellular membrane fluidization caused by the fatty acid. The purpose of this research is to characterize the effect of the degree of unsaturation in fatty acids on membrane structure through phase behavior studies. This was achieved using 1,2-dipalmitoyl-sn-glycero-3-phosphorylcholine (DPPC) liposomes and C18 stearic (unsaturated), oleic (monounsaturated), and linoleic (polyunsaturated) fatty acids. The temperatures and enthalpies of phase nucleation or gel-to-fluid melting of DPPC membranes were analyzed at 1, 5, 10, and 15 mol% fatty acid using differential scanning calorimetry (DSC). Complimentary experiments were conducted using dynamic light scattering and optical microscopy to determine if, in addition to changes in phase behavior, the fatty acids promote micelle formation or the formation of alternate structures. Preliminary results show that higher degrees of fatty acid unsaturation lead to greater membrane disorder; suppressing the membrane pre-transition and reducing melting cooperativity. The results provide further knowledge of how feedstock impurities impact fermentation processes, especially on crude glycerol utilization for biofuel production.

STUDIES DIRECTED TOWARD THE SYNTHESIS OF PHEVALIN AND RELATED CYCLIC DIPEPTIDES AS POTENTIAL QUORUM SENSING MODULATORS

Taylor Braun, Susan Meschwitz, *Department of Chemistry*, Salve Regina University, Newport, RI; David Rowley, *Department of Biomedical and Pharmaceutical Sciences*, University of Rhode Island Kingston, RI

RI-INBRE Summer Undergraduate Research Fellowship Program

Traditional treatment of infectious diseases is based on compounds that kill or inhibit growth of bacteria. A major concern with this approach is the frequent development of resistance to antibiotics. Quorum sensing is the chemical signaling process that allows bacteria to communicate with one another to coordinate their behavior and function like a multi-cellular organism. Small molecules called autoinducers are released by bacteria that bind to and stabilize receptor proteins. Subsequently, the ligand-protein complex initiates transcription of the quorum sensing genes. The discovery of the role of quorum sensing in regulating bacterial virulence has afforded the novel opportunity to control infectious bacteria without interfering with growth. The long-term goal of this project is the design and synthesis of small molecules capable of quorum sensing inhibition. These compounds include the natural product phevalin, a known regulator of virulence factor expression, and other related cyclic dipeptides. The synthesis involves peptide coupling using carbodiimide chemistry, reduction of an ester functional group to an aldehyde, followed by cyclization. Reactions were tracked using TLC, IR, and NMR. Other cyclic dipeptides synthesized will have differences in the acyl groups attached to the ring and in the location and substitution of an aromatic functionality. Optimization of a synthetic procedure will allow for the efficient creation of a library of phevalin derivatives and the investigation of the potential of this class of compounds as quorum sensing modulators.

IDENTIFICATION OF POLYMORPHIC MICROSATELLITE MARKERS IN ULVA RIGIDA AND ULVA COMPRESSA

Matthew Breseman, JD Swanson, Kristen Dostie, Shennel Gelin, *Department of Biology and Biomedical Sciences*, Salve Regina University, Newport, RI; Amy Battocletti, Carol Thornber, *Department of Biological Science*, University of Rhode Island, Kingston, RI.

Rhode Island Science & Technology Advisory Council

The macroalgal genus Ulva is present in shallow coastal systems worldwide including Narragansett Bay, RI. During the summer months, Ulva forms large macroalgal blooms which can have negative ecological and economic impacts on the coastal ecosystem. Of two common Ulva species, U. rigida and U. compressa, only 255 nucleotide sequences have been submitted to GenBank. The aim of our research is to utilize di- and tri-nucleotide repeats found in DNA sequences, known as microsatellites, to create unique fingerprint profiles of U. rigida and U. compressa. Nine microsatellite primer pairs designed for U. intestinalis have been previously found to amplify microsatellite sequences in U. rigida and U. compressa however their polymorphic nature has not yet been determined. We have successfully optimized the annealing temperature for all nine primers by running successive 15℃ range PCR temperature gradients from 50℃ to 65℃. The optimal temperature was found to be between 47.9℃ and 50.5℃. To further elucidate additional Ulva microsatellite markers, 100mg of extracted U. rigida DNA was sent to Washington State University to be sequenced using the PacBio third-gen sequencing method. Once the data is received and assembled the sequence will be scanned for potential microsatellite regions using Msatcomander. We will initially order 100 microsatellite primer sets designed from Primer3 and amplify them initially with 10 test samples collected during summer 2012. The goal is to collect samples biweeekly with the aim to identify many additional polymorphic microsatellite markers to fingerprint U. rigida and U. compressa.

ARYLPHOSPHONIUM SALT CONJUGATES: SYNTHESIS, AND TOXICOLOGICAL ANALYSIS

Titilayo Adedeji Campbell, John C. Williams, *Department of Chemistry*, Rhode Island College, Providence, RI

RI EPSCoR & RI INBRE Summer Undergraduate Research Fellowship Programs

Arylphosphonium salts (APS) are cationic lipophiles that readily cross cell membranes. APS are antimicrobial and have shown anti-cancer activity in a variety of assays. These compounds are highly selective for malignant cells, and for mitochondria in normal cells. They have been used as carrier molecules to transport DNA-alkylating agents into mitochondria. APS can target and inhibit unchecked cell growth. The triphenylmethyl "motif" has been identified as a recurring structure component in potent anticancer agents. Compounds with this structure unit have been found to cause cell cycle arrest, inhibit the polymerization of tubulin, detach mitochondrial hexokinase in cancer cells and to inhibit some calcium channels. APS is an analogue of this motif with phosphorus rather than carbon at the center of the tetrahedral geometry. We are preparing a library of substituted triarylphosphonium-alpha-toluic acids that will be functionalized to make ester and amide conjugates for screening against cancer cell lines and to extend a study on DNA toxicity underway in our collaborator's laboratories.

INTERMITTE STREAMS IN FORESTED WATERSHEDS OF RI CAN SERVE AS SUBSTANTIAL NITROGEN SINKS

Jeremy Carreiro, Kelly Addy, Molly Welsh, Art Gold, *Department of Natural Resources Science*, University of Rhode Island, Kingston, RI

RI EPSCoR Summer Undergraduate Research Fellowship Program

As the world population grows, more nitrogen (N) is deposited into the environment by human activities. One such activity that contributes greatly is the use of N fertilizers. Runoff of excess N results in nutrient-rich coastal water-ways. In this situation, eutrophication occurs, which results in oxygen depletion, harmful algal blooms, fish kills, and eelgrass degradation. We assessed the ability of six intermittent headwater streams in forested watersheds of RI to serve as natural N sinks, areas of the landscape that retains N preventing its flow to coastal waters. Using a series of bromide-nitrate slug tests, we determined the nitrate-N removal rate of each stream reach. We found substantial N removal rates at all sites, with the highest rates in the fall. Higher rates of N removal in fall may be due to the seasonal abundance of dead leaves in stream and in debris dams. Factors correlated with more N removal include shallower stream depths, lower rates of flow and longer residence times. We attributed long retention times within the streams with pools in the stream reach and water interaction with the stream-bed and groundwater. To maintain the N removal potential of intermittent streams, preserving the woody structure of the riparian areas will be critical as these streams are built on woody structure-debris dam formation for pool establishment and root networks that create opportunities for interaction with groundwater.

THE QPX QPCR ASSAY

Peter Cavedon, Roxanna Smolowitz, Kathryn Markey, *Department of Biology*, Roger Williams University, Bristol, RI

RI EPSCoR Summer Undergraduate Research Fellowship Program

The protistan parasite QPX (Quahog Parasite Unknown) has been the cause of mass mortalities in hard clams (Mercenaria mercenaria) on the Atlantic coast of the United States and Canada. To determine the presence and amount of QPX cells in hard clam tissues, a qPCR assay is in development which will use DNA extracted from infected clam tissues to determine the amount of QPX cells present in a sample. The proposed qPCR method will use a standard curve composed from a dilution series of a plasmid containing inserted copies of the QPX genome. The goal is to equate DNA extracted from known numbers of cultured QPX cells with QPX DNA inserted into a plasmid in order to develop a reliable standard curve. To determine the average amount of QPX DNA and cells, several cultures of QPX cells were washed, counted, and the cell number results were averaged. DNA was then extracted from these QPX cultures. To develop the plasmids, PCR was first run on extracted QPX DNA samples. The PCR product in the gel was cut out and purified using a Promega Wizard SV Gel and PCR Clean-Up Kit. The purified product was inserted into a vector using an Invitrogen Purelink Quick Plasmid Miniprep Kit, and the vector was then serially diluted to create a standard curve for the qPCR assay. Our next step is to equate the amount of QPX DNA in each culture with the amount of DNA on our plasmid standard curve. Once we have done this we will known the equivalency between plasmids origin QPX DNA and QPX DNA/average number of cells in the culture. This will form the basis for the diagnostic test using real time qPCR.

KNOWLEDGE, ATTITUDES, AND PRACTICES RELATING TO HPV, CERVICAL CANCER, AND VACCINATION AGAINST HPV IN A LOW-INCOME, UN-INSURED, URBAN POPULATION (OLNEYVILLE, RI, USA)

Jeff Chau, Macayla Landi, *College of Pharmacy*, University of Rhode Island, Kingston, RI; Shahla Yekta, *College of Nursing*, University of Rhode Island, Kingston, RI; Tanie Medieros, MPH, Clinica Esperanza, Providence, RI; Annie de Groot, *Institute of Immunology and Informatics*, University of Rhode Island, Kingston, RI

Institute of Immunology and Informatics

Human Papillomavirus (HPV) is a highly recognized sexually transmitted disease among the medical world. However, awareness within certain populations about this growing virus, its relation to cervical cancer, and methods of prevention is still necessary. A study on the knowledge, attitudes, and practices of patients relating to HPV in a low-income, uninsured, urban population was conducted. The goal was to evaluate these factors regarding HPV and cervical cancer as it is believed that the particular community has negatively associated the vaccine with promiscuity. In addition, this study provided insight into the willingness of the observed individuals to be vaccinated while also educating patients. Ultimately, by understanding patients, vaccine acceptability can then be improved. To conduct this study, an electronic questionnaire, available in Spanish and English, was given—gathering information regarding patient demographics, sexual histor y, use of contraception, knowledge/opinions about HPV, and the series of vaccines to prevent cervical cancer. It was found that English speakers are more likely to become sexually active at a younger age than Spanish speakers and also have more partners. However, they are also more likely to use protection during intercourse. The greater likelihood of not using protection during intercourse for Spanish speakers may be one reason why the HPV vaccine is frowned upon as it allows for safer intercourse, in regards to HPV infection, and could then suggest a reason to withhold the use of protection. As Spanish speakers are a majority within the community, it is important to have a greater understanding to correct the misconceptions and improve patient healthcare. Overall, about 41% of our sample size did not know about HPV prior to the survey. The data suggests that there are still communities highly unaware of HPV or even falsely aware and thus need to be educated to better indiv idual and community healthcare.

VIRTUAL SCREENING OF DRUG LIBRARY TO INDENTIFY NOVEL INHIBITOR OF NAMPT

Kristen Chauvin, *Department of Physical Science*, Rhode Island College, Providence, RI

RI-INBRE Summer Undergraduate Research Fellowship Program

Nicotinamide adenine dinucleotide (NAD+) is commonly known as a cofactor in oxidation/reduction reactions, for example during glycolysis and oxidative phosphorylation. However, in addition to its role in metabolic processes that provide cellular ATP, NAD+ is also consumed by DNA repair proteins during times of stress. Cancer cells exhibit an upregulation of DNA damage repair and cellular survival proteins, and these proteins convert NAD+ to nicotinamide (NAM). The enzyme Nicotinamide phosphoribosyltransferase (NAMPT) is the rate-limiting step in the NAD+ salvage pathway, which is the main biosynthetic pathway to recycle NAM back to NAD+. Disruption of the NAD+ salvage pathway causes a reduction in the amount of cellular NAD+ available, thus threatening cellular survival. Inhibition of NAMPT by NAM analogs such as FK866 result in depletion of NAD+ and consequent apoptosis of malignant cells, providing incentive for further research that may identify novel inhibitors of NAMPT activity.

The goal of my research is to identify novel inhibitors of NAMPT through a virtual docking screen of small molecular libraries. I will be using the freeware program AutoDock Vina, which was developed in the Molecular Graphics Lab at the Scripps Research Institute, and the NCI-Developmental Therapeutics Program Diversity Set III focused drug library. Our hypothesis is that compounds that disrupt the dimerization plane will inhibit NAMPT activity; therefore our virtual docking screen will target the dimerization plane. The target surface will include post-translational modification sites located at or near the dimerization surface. The first area to be screened includes one end of the substrate channel. At this location the channel connects the active site to a surface cavity that spans the dimerization plane and contains a post-transationally modified K389 residue from each monomer. Compounds that bind effectively will be tested in biochemical assays to determine the mechanism and extent of NAMPT activity inhibition.

PURPLE LOOSESTRIFE AND ITS GENETIC DIVERSITY

Richard Cimini, Matt Vumback, Maia Bailey, *Department of Biology*, Providence College, Providence, RI

RI EPSCoR Summer Undergraduate Research Fellowship Program

Purple Loosestrife is an extremely invasive species that outcompetes other organisms native to that environment. Evolutionary theory suggests that invasive species should reproduce hermaphroditically resulting in a swifter expansion into the environment (Baker 1955). Additionally, populations resulting from several serial colonization events are expected to have low genetic diversity causing a lessened ability to adapt. In the case of Purple Loosestrife, however, mating can only occur between individuals with different genetically determined flower types. Although this seems to be a limiting factor to the plant's reproductive success, we still observe invasive properties such as a high rate of seed production (unpublished data) and a speedy ability to adapt to new environments (Colautti et al. 2010). Our goal is to explain this somewhat contradicting phenomenon, that an invasive species has such a specific mating pattern yet still appears to thrive. To further understand Purple loosestrife we are examining the correlation between diversity of genetic markers and flower diversity in small Rhode Island populations. Populations currently scored show that even very small populations (< 50 individuals) are genetically variable for neutral markers indicating that dispersal and gene flow help maintain sufficient genetic variation in populations for this mating system to function. Further work will be needed to estimate gene flow in these populations.

THE AMYLOID PRECURSOR PROTEIN OF ALZHEIMER'S DISEASE CLUSTERS ON THE SURFACES OF AXOPLASMIC ORGANELLES AND LOCALIZES TO THE ORGANELLE/MICROTUBULE INTERFACE

Eliza Conaty, Rylie Walsh, Paul Poidomani, Joseph DeGiorgis, *Department of Biology*, Providence College, Providence, RI

RI INBRE Summer Undergraduate Research Fellowship Program

Alzheimer's Disease (AD) is the leading cause of dementia in the United States and currently afflicts approximately 26 million people worldwide. The incidence of AD is projected to climb to 100 million by the year 2050, and it is predicted to cost 20 trillion dollars over the next 40 years to the US alone. The massive neuronal death characteristic of AD is associated with neuritic plaques made of a protein fragment called amyloid beta (Abeta). Mutations in Abeta's full-length parent protein Amyloid Precursor Protein (APP) are linked to familial forms of the disease. Very little is known, however, about APP's wild-type function or how mutations in APP lead to the pathogenesis of AD. Here we use a combination of confocal light and transmission electron microscopy (TEM) to determine the subcellular location of APP in the squid axon, which serves as a major model for understanding neuronal function. By immunofluorescent antibody labeling we detect APP in punctate structures that are in association with microtubules or are free along the coverslip surface, findings which are consistent with putative vesicle labeling. At the EM level we find APP to be associated with isolated axoplasmic vesicles and localized in clusters at the vesicle surface. In addition, when vesicles are added to exogenous bovine microtubules a subset of vesicles bind to the microtubule surface and label for APP at the vesicle/microtubule interface. Previous work has shown that Kinesin-3 also localizes to the surfaces of axoplasmic vesicles. Currently we are determining the distribution of APP and Kinesin-3 through double labeling experiments.

THE EFFECT OF PARENT-ADOLESCENT RELATIONSHIPS AND STRESS RESPONSE ON ADOLESCENT RISK BEHAVIOR

Kyle Fernandes, Kayla Flynn, Kristen Wilkinson, Amanda Welch, Emily Cook,
Department of Psychology, Rhode Island College, Providence, RI

RI INBRE Summer Undergraduate Research Fellowship Program

Despite prevention and intervention efforts, adolescent risk behavior (substance abuse, delinquent, and aggressive behavior) is still a major health concern and economic cost. Thus, understanding individual differences in risk behavior remains a priority. An important factor that may account for individual differences in risk behavior is adolescents' stress response brought on or exaggerated by negative parent-adolescent relationships. Few studies, however, have examined how experiences within the parent-adolescent relationship and a dysregulated stress response may work in concert to increase adolescent risk behavior. Potentially, a dysregulated stress response could be an explanatory mechanism (i.e., mediator) that accounts for why problems within parent-adolescent relationships may be associated with risk behavior. Specifically, negative parent-adolescent relationships may increase the cumulative effect of stress on underlying biological systems resulting in a dysregulated stress response and assaults to the prefrontal cortex that may impact engagement in risk behavior. Alternatively, a dysregulated stress response may act as a moderator of the association between parent-adolescent relationships and risk behavior such that youth who evidence a dysregulated stress response will be more vulnerable to increases in risk behavior associated with negative parent-adolescent relationships.

The proposed study will examine these two ways in which adolescents' stress response may affect the association between parent-adolescent relationships and risk behavior in 100 mothers and adolescents. Data collection will include a home-visit where we will collect observational data of mother-adolescent interactions, survey data from moms and youth, and physiological measures to assess stress response in youth (heart rate, blood pressure, and cortisol). Results from this study will shed light on the relationship among parent-adolescent relationships, stress response, and risk behavior during the critical period of adolescence. This information can then be used to inform prevention efforts by identifying the potential mechanisms for intervention and for which youth intervention is needed.

VERIFICATION OF EXPERIMENTALLY DERIVED OPTIMAL REACTION CONDITIONS ON THE ENZYMATIC ACTIVITY OF A SECRETED LIPASE EXPRESSED BY LEISHMANIA DONOVANI

Tia Crowther, Lana Hoertz, *Department of Biology*, Salve Regina University, Newport, RI; Alison Shakarian, *Department of Biology & Biomedical Sciences*, Salve Regina University, Newport, RI.

RI INBRE Summer Undergraduate Research Fellowship Program

This research was conducted to verify the reproducibility of the effects of various metal ions on the enzymatic activity of LdLip3, a secreted lipase from Leishmania donovani. It is hypothesized that LdLip3 may play a role in the parasites' survival, development and/or its pathogenesis within the human host. Enzymatic activity of purified HA-tagged - LdLip3 was tested under varying conditions that consisted of a range of pH's (4-8) over a thirty minute incubation period at three different temperatures, 26˚, 37˚, and 42˚C. 4MU-palmitate was used as the substrate. The fluorescence of 4MU (4-methylumbelliferone) is directly proportional to its cleavage by theLdLip3 enzyme. In addition, it is known that metal ions are often cofactors for many enzymes. A total of nine metal ions were tested including Fe+2, Ca+2, Cu+2, Na, Mg+2, K, Zn+2, Co+2, and Mn+2, to determine if they facilitate an enhanced LdLip3 reaction. Results of this study show the optimal enzymatic activity of purified LdLip3 is obtained at pH 8 with the addition of Zn+2 at 42˚C. When compared to previous data collected under the same specifications but carried out on several independently derived LdLIP3 samples by three different students, distinct, reproducible trends in the pattern of specific activity were obtained for the various reaction conditions tested. Thus, we have been able to verify reproducible results under varying enzyme reaction conditions. Importantly, we have established reproducible optimal reaction conditions for temperature, pH and metal ion cofactors for LdLIP3 when using 4MU- palmitate as substrate. This enzyme reaction condition (pH 8 with the addition of Zn+2 at 42˚C) will be used in future studies to identify potential inhibitors of the LdLIP3 secretory lipase from this important group of human pathogens.

BRIDGING THE GAP BETWEEN FREE-LIVING TAXA AND PARASITES USING COMPARATIVE EVOLUTIONARY GENOMICS

Melissa Curran, *Department of Biology*, Providence College, Providence, RI; Ian Misner, Christopher Lane, *Department of Biological Sciences*, University of Rhode Island, Kingston, RI

RI EPSCoR Summer Undergraduate Research Fellowship Program

Oomycetes have been the cause of extensive disease, from the devastating potato and tomato pathogen Phytopthora infestans, to numerous animal pathogens. Thus far, research has been concentrated among the virulent species within the Peronosporalean clade. This project investigates a facultative pathogen of fish and a free-living saprobe, within the Saprolegniales - a clade notably containing closely related free-living and parasitic species. With the nuclear genome sequences of facultative parasite Achlya hypogyna and free-living saprobe Thraustotheca clavata, we performed comparative genomic analyses in the context of six available oomycete genomes and twenty-three genomes from related lineages. Through the utilization of Evolutionary Gene Networks (EGNs), the OrthoMCL database, and PFAM domain assignments, protein families were identified that contain greater numbers of proteins in pathogenic species than free-living taxa. Of particular interest are proteins that are secreted as part of the oomycete infection cycle (secretome) because they play a direct role in nutrient uptake and host aversion. Phylogenetic analysis of the 54 shared secretome proteins revealed 13 horizontal gene transfer events from either the bacterial or fungal lineages into the oomycetes. This transfer of fungal genes could be a key factor contributing to the development of a parasitic lifestyle. In addition, elicitin-like (EL) genes were identified within the shared secretome of Achlya hypogyna and Thraustotheca clavata. The understanding of these genes' expression levels through the application of quantitative PCR has increased available information on these traditionally Peronosporalean, host-infiltrating proteins and how they are repressed in Achlya hypogyna and Thraustotheca clavata. Evidence of EL proteins outside of the Peronosporalean clades implies an earlier acquisition of this gene than previously accepted. This finding could suggest a possible mechanism for parasitic evolution through the mutation of existing genes present in facultative and free-living oomycetes to serve novel, parasitic functions.

ARE PREGNANT WOMEN AND THEIR EMBRYO/FETUS AT A HIGHER RISK OF BPA EXPOSURE AS A RESULT OF THEIR ALTERED SULFOTRANSFERASE ACTIVITY?

Alyssa Dantonio, Roberta King, Emine Yalcin, *Department of Biomedical and Pharmaceutical Sciences*, University of Rhode Island, Kingston, RI

RI INBRE Summer Undergraduate Research Fellowship Program

Bisphenol A (BPA), one of the most widespread endocrine disrupting chemicals, is found in everyday items such as in the lining of food and beverage cans, water bottles, baby bottles, IVs, metal equipment, piping, and even dental sealants. Increased exposure to BPA has been correlated with increased incidence of type II diabetes mellitus, effects on brain, behavior, and prostate, slowing of whole body metabolism and insulin disruption in peripheral tissues. At relatively high exposure concentrations with laboratory models, BPA has been proven to cause these adverse physiological changes. However, human risk is dependent not only on the amount of BPA ingested, but also on how efficiently individuals metabolize BPA to non-toxic and excreted metabolites such as BPA-sulfates and BPA-glucuronides. During pregnancy, it is known that many physiological changes occur. These changes must be better understood so that teratogenicity and maternal toxicity can be avoided. This study measured BPA-sulfonation, a mechanism of BPA clearance and detoxification, in a mouse model of pregnancy, and compared pregnant mice to control virgin mice. Specifically, pregnant and virgin S9 liver samples were incubated with the substrate BPA (0.5–75μM) and the donor PAP-35S (2.8–60.57μM) for 30–60 minutes at 37ºC, and then tested by HPLC and a radio detector to quantify the ratio of PAP-35S to conjugated BPA-S. These results indicate that one sulfotransferase is effective at higher BPA concentrations (10-100μM) and the other sulfotransferase is effective at lower BPA concentrations (0.5-5μM). In addition, the pregnant mice catalyzed BPA sulfation at lower rates than the control virgin mice. This indicates that during pregnancy, BPA clearance may be substantially slower than under normal conditions. If this result applies to humans, pregnant women and their fetus may be more susceptible to BPA exposure.

EPITOPE VALIDATION OF TICK SALIVOME DERIVED PEPTIDES

Tori Deschenes, Benjamin Javery, Wendy Coy, Anne De Groot, *Department of Cell and Molecular Biology*, iCubed, University of Rhode Island, Providence, RI; Tom Mather, *Department of Plant Sciences and Entomology*, Center for Vector Born Disease, University of Rhode Island, Kingston, RI

TRIAD/University of Rhode Island Biotechnology

Background: Tick-borne diseases (TBDs) affect most parts of the world and constitute serious public health problems in vital need of solutions. A vaccine against ticks, or the pathogen transmission process, would prevent the spread of multiple vector-borne diseases and its discovery would represent a major milestone for improvement of public health. It is well established that ticks and other arthropod vectors manipulate host responses by secreting proteins from their salivary glands during feeding. Our epitopes for testing we're mined using immuninformatics tools from these tick salivome proteins.

Methods: The MHC Class II binding assay we used at I'Cubed yields an indirect measure of peptide-MHC affinity. In this assay, soluble HLA molecules, unlabeled experimental peptides and a europium-labeled control peptideare allowed to reach steady equilibrium, the HLA molecule complexes are captured on an ELISA plate coated with anti-human DR antibody. Time-resolved fluorescence measuring bound HLA-labeled control peptide is assessed at 615 nm by a Walllac Victor3 unit. Binding of experimental peptides is expressed as the percent inhibition of the labeled control peptide (experimental fluorescence / control fluorescence multiplied by 100).

Results: 32 Peptides we're tested against 4 alleles (of 8) that make up the predictive "supertype allele" in the EpiMatrix algorithm. Each peptide was tested at 3 concentrations; 100 uM, 10 uM and 1 uM. These 3 concentrations mark cutoff points for assessed binding affinity. Of the tested peptides for each allele, the following were shown to be at least moderate binders. DRB1*0101, 26 of the 32 (81.2%). DRB1*0401, 17 of the 32 (53.2%). DRB1*0701, 18 of the 32 (56%). DRB1*1501, 28 of the 32 (87.5%) Of the false positives, 4 peptides did not bind against all 4 alleles. These 4 will be retested to confirm.

FACTORS INFLUENCING HOMARUS AMERICANUS ABUNDANCE AND DISTRIBUTIONS ALONG NEWPORT NECK

James Diamantopoulos, *Department of Biology*, Salve Regina University, Newport, RI

RI EPSCoR Summer Undergraduate Research Fellowship Program

American lobster (Homarus americanus) are an important commercial fishery in Rhode Island. Long-term decline of annual landings is concerning, and the causes may be due, in part, to the North Cape oil spill of 1996, increased predation, pollution, and overfishing. Here we explore current population status in the undersampled near-shore rocky intertidal zone of Newport Neck and use habitat selection to model future lobster distribution and abundance with climate change. We tested the hypotheses that there would be an equal ratio of male to female lobsters and that lobster abundance would be evenly distributed. Using sets of vent-less traps we monitored once every four days in shallow (<15 m) near-shore habitat between Easton's Beach and Gooseneck Cove. There is a 3:1 male sex-biased ratio, suggesting that females either have low survival in the near-shore environment or migrate to greater depths. Lobsters were found in all locations but more abundantly in rocky headwall areas, a trend especially strong among females. This distribution is probably related to larger substrate size and higher substrate heterogeneity. There was no significant difference in lobster captures per day between shallow (<5 m) and deep (5-15 m) traps. Lobster abundance positively correlated to species richness, perhaps an indirect measure of local productivity. Based on preliminary results, near-shore environments of Rhode Island are a principle habitat for undersized lobster males, who prefer higher habitat heterogeneity and productivity. Gravid females inhabit near-shore habitats - providing refugiae from trapping pressure. Future climate change sea level rise will impact available substrate of Newport Neck and affect lobsters. Based on present measures of lobster habitat selection, predictions of local lobster distribution abundance with climate change are possible.

CASPOFUNGIN INDUCES AIF1-DEPENDENT PROGRAM CELL DEATH IN SACCHAROMYCES CEREVISIAE

Faith Donaghey, Christopher Chin, Katherine Helming, Nicanor Austriaco, *Department of Biology*, Providence College, Providence, RI

RI INBRE Summer Undergraduate Research Fellowship Program

Caspofungin was the first member of a new class of antifungals called echinocandins to be approved by a drug regulatory authority. Like the other echinocandins, caspofungin blocks the synthesis of β(1,3)-D-glucan of the fungal cell wall by inhibiting the enzyme, β(1,3)-D-glucan synthase. Loss of β(1,3)-D-glucan leads to osmotic instability and cell death. In recent years, several laboratories have shown that a wide range of antifungal drugs leads to programmed cell death (PCD) in yeast that is reminiscent of apoptosis in mammalian cells. We now provide evidence that Saccharomyces cerevisiae cells cultured in media containing caspofungin manifest the classical hallmarks of PCD in yeast, including the generation of reactive oxygen species (ROS), and the fragmentation of mitochondria. Our data also suggests that this cell death phenomenon requires the pro-apoptotic gene, AIF1, but not the yeast metacaspase, YCA1.

NON-SUICIDAL SELF-INJURY: THE INTERACTIVE STRESSOR MODEL

Caroline Doyle, Brenna Peters, Ryan Post, Christopher Bloom, *Department of Psychology*, Providence College, Providence, RI; Josh Mazick, *Department of Psychology and Mathematics*, Providence College, Providence, RI

RI INBRE Summer Undergraduate Research Fellowship Program

Non-suicidal self-injury (NSSI) is the intentional destruction of one's own body tissue in absence of intent to die. In the past, stimulant models using such drugs as caffeine and pemoline have been used to elicit self-injury in animals; although the topography of this induced self-injury resembles the behavior of NSSI, we contend that it ignores important environmental antecedents. To investigate the role of one such environmental factor, stress, our lab has developed an animal model of NSSI that includes environmental stressors preceding a nociceptive/physical stressor that we believe more accurately represents human cases of NSSI. Previous research in our lab indicates that an environmental stressor paired with a nociceptive stressor affects pain tolerance differently than exposure to only one of the two stressors alone, suggesting that both the order and combination of the stressors are relevant. The proposed study will use water avoidance and inescapable shock as environmental and nociceptive stressors, respectively. Sham versions of each stressor will serve as a control. To determine the effect of the stressor tasks, we will use an open field test and a forced swim test to quantify anxiety and depression, respectively. To measure the analgesic effect of the stressors, both the Hargreaves test and formalin test will be used. We hypothesize that animals exposed to the environmental stressor followed by the nociceptive stressor will have a marked analgesic effect when compared to the sham controls.

IDENTIFYING THE CHANNELS AND BARRIERS TO COMMUNICATION BETWEEN MARINE SCIENTISTS, FISHERIES MANAGERS AND FISHERMEN IN THE STATE OF RHODE ISLAND

Evan Dunphy, *Department of Communications*, University of Rhode Island, Kingston, RI;
Peter Schooling, *Department of Marine Affairs*, University of Rhode Island, Kingston, RI

RI EPSCoR Summer Undergraduate Research Fellowship Program

In Rhode Island, like many coastal states, fisheries managers, marine scientists and fishermen are forced to interact in order to set regulations for the state's commercial fishing industry. Frequently, discussion of these contentious issues leads to frustration, anger and a break down in constructive communication. This communication study involves identifying the channels and barriers to communication between marine scientists, fisheries managers and fishermen in the state of Rhode Island. The goal of our work is to find the problematic areas, and make recommendations on how to improve the communication between these parties. Over six weeks, we interviewed 17 individuals. The results of our rhetorical analysis suggest that there are many common arguments, and these 3 key issues emerged: the accuracy and timeliness of stock assessment data, the politically complex management system that is detrimental to expedient decision-making, the disagreement between scientists and fishermen on the importance of the impacts of climate change. Taking these key issues into account, our list of recommendations improves upon the status quo. These changes would help to make the entire system, including scientists, managers and commercial fishermen, a more streamlined and efficient process. Our conclusion proves that further research and more recommendations should be implemented in order to remedy these communication barriers. This will ultimately build a more socially and economically viable commercial industry in Rhode Island.

INVESTIGATING THE ROLE OF BCP1 THROUGH THE EFFECTS OF DOXORUBICIN ON A MUTANT STRAIN OF S. CERVISIAE

Sabrina Elgar, Kyle Inman, Masharee Hopkins-Jones, Deborah Britt, *Department of Biology*, Rhode Island College, Providence, RI

RI INBRE Summer Undergraduate Research Fellowship Program

Responding to DNA damage is one of the most important functions in the survival of all living organisms. DNA repair is a very specific and complex series of events that includes many components. In human cells, one such component is BCCIP, a protein that interacts with the tumor suppressor BRCA2 and the cell cycle regulator CDKN1A. In response to DNA damage, BCCIP assists in halting the cell cycle and participates in homologous recombination repair with BRCA2. In order to better understand the role of BCCIP in DNA repair, we have chosen to study the protein's fungal homolog, Bcp1, in S. cerevisiae. To evaluate the hypothesis that Bcp1 plays a role in the DNA damage response, two strains of yeast were tested: SEY is the control strain and AAY is a strain with a temperature sensitive mutation in Bcp1. Both strains were treated with the DNA damaging drug doxorubicin. Doxorubicin is commonly used as a treatment for many cancers such as those of the breast and lung. The drug works by preventing the DNA double helix from separating after the enzyme topoisomerase II has broken the chain for replication, thus causing DNA double strand breaks. When cultured in the presence of a doxorubicin concentration that inhibited the growth of SEY, AAY continued to grow as well as the untreated cells. These results suggest that Bcp1 may participate in DNA repair, but be detrimental in the presence of doxorubicin. The contribution Bcp1 makes to DNA repair still needs to be investigated.

THE EFFECTS OF SUBSTRATE HETEROGENEITY ON AMERICAN SPIDER CRAB ABUNDANCE AND DISTRIBUTION ALONG NEWPORT NECK, RI

Caitlyn Farragher, Sarah Matarese, Jameson Chace, *Department of Biology*, Salve Regina University, Newport, RI

RI EPSCoR Summer Undergraduate Research Fellowship Program

Substrate heterogeneity is a key habitat component for benthic marine mircoinvertebrates. American spider crabs (Libinia) are an important detritivore and food resource for many organisms in the near-shore marine ecosystem. Determining the factors that influence spider crab abundance and distribution along Newport Neck, RI is important for understanding the dynamics of this ecosystem today, as well as providing a predictive tool for community-level response to sea level rise with climate change. Since summer of 2011 modified lobster traps where placed at 10 sites divided into 3 sub-sites along the neck of Newport, RI. The traps are checked and rebaited at least once every four days. The substrate size and diversity was determined by using 0.5 m2 quadrats in the sub-tidal zone near the traps. 388 males and 105 females were captured and released during the summer of 2012. We tested two key habitat selection predictions: spider crabs are more abundant in areas with (1) higher habitat heterogeneity and/or (2) higher species richness and diversity. Preliminary results suggest that spider crabs are most abundant sites 8 and 9, which are in the most protective coves and are likely to have the greatest coverage by fine sediments. Species richness, but not diversity, was positively correlated to abundance. Spider crabs respond numerically to substrate type, especially those of protected coves, and to high species richness, which is probably a function of localized productivity. An understanding of spider crab habitat selection can be used to predict populations' change with climate change. As a food source for many organisms, including game fish and migratory sea ducks and sea birds, the distribution and abundance of spider crabs can create a trophic cascade in the near-shore ecosystem of Rhode Island.

DISCRIMINATION AND AGGREGATIVE PATTERNS AMONG AND BETWEEN E. INVADENS IP-1 AND E. INVADENS VK-1:NS

Layla Ferland, Kevin Schindelwig Franca, Kate Higgins, Hanna Sobon, Avelina Espinosa, *Department of Biology*, Roger Williams University, Bristol, RI

RI INBRE Summer Undergraduate Research Fellowship Program

The purpose of this study was to determine the mechanisms of aggregative discrimination in two strains of Entamoeba, the relevance of signaling is to determine phylogenetic relatedness, and analyze if aggregation is essential in pathogenesis. The Entamoeba lineage constitutes an ideal model to determine the behavioral and signaling cues needed for aggregation. Chemical cues have been reported for E. histolytica but not E. dispar, suggesting cell-communication between con-specifics and behavioral differences with relevance for disease. Entamoeba varieties have been reported as 'morphologically undistinguishable'. Two strains of E. invadens have been isolated from different hosts (VK-1: NS - komodo dragons and salamanders and IP1- snakes) but were classified within the same 'species'. Trophozoites of each strain aggregate only with members of their own variety, suggesting they are able to associate based on behavioral and chemical communication. Adaptations to different environments and horizontal gene exchange could have influenced diversification of each lineage. Measurable aggregation and behavioral cues in fluorescence micrographs of Entamoeba varieties suggest that these characteristics should be included in phylogenetic studies. The next step is to determine if aggregative signals are used in pathogenic processes.

GENETIC CHARACTERIZATION AND COMPLEMENTATION STUDIES OF BXI1 IN THE REGULATION OF THE UNFOLDED PROTEIN RESPONSE AND CELL DEATH IN SACCHAROMYCES CEREVISIAE

Ryan Frazier, Alfredo Gonzalez, Nicanor Austriaco, OP, *Department of Biology*, Providence College, Providence, RI

RI-INBRE Summer Undergraduate Research Fellowship Program

Bax inhibitor-1 (BI-1) is an anti-apoptotic gene whose expression is upregulated in a wide range of human cancers. Studies in both mammalian and plant cells suggest that the BI-1 protein resides in the endoplasmic reticulum and is involved in the unfolded protein response (UPR) that is triggered by ER stress. We have been investigating the function of the yeast gene, *BXI1*, which appears to be a homolog for mammalian BI-1. To further characterize the function of *BXI1*, we have created double mutants between our gene and *IRE1*, which encodes the ER-localized endonuclease known to regulate the unfolded protein response (UPR). Our oeBXI1 *Δire1*, and oeIRE1 *Δbxi1* double mutants have allowed us to characterize the genetic interactions between these two genes in the regulation of the UPR and cell death in yeast. Furthermore, we have investigated the functional properties of *BXI1* through a series of complementation experiments with its putative human an plant homologs. Finally, we have also undertaken a series of experiments to reconcile our studies with the published work of the Madeo Laboratory, which had characterized *BXI1* as the *YBH3* gene.

ACTIVITY STUDIES OF NICOTINAMIDE PHOSPHORIBOSYLTRANSFERASE (NAMPT)

Karen Almeida, Chris Funk, Cailyn Mather, Katelyn Pina, *Department of Physical Sciences*, Rhode Island College, Providence, RI

RI-INBRE & RI EPSCoR Summer Undergraduate Research Fellowship Programs

Nicotinamide adenine dinucleotide (NAD) is the second most important cellular metabolite, behind ATP, and is a cofactor in more than 200 oxidation reduction reactions. NAD is also consumed in the upregulation of poly (ADP ribose) polymerases (PARP) and Sirtuin proteins . While normal oxidation reduction reactions involve a steady state NAD cyclic value, DNA damage repair causes a stress that increases the activity of PARP and Sirtuin proteins and the consumption of NAD. The resulting nicotinamide (NAM) must then be recycled back to NAD using a two step NAD salvage pathway. The first and rate-limiting step of this pathway is the conversion of NAM to nicotinamide mononucleotide (NMN) by Nicotinamide phosphoribosyltransferase (NAMPT). The consumption of NAD and the ability to alter its recycling rate through NAMPT activity has possible cancer treatment applications. NAMPT is a 55-kDA enzyme that forms a homodimer to create two active sites.

Nampt expression was induced with IPTG, verified by immunoblot analysis and purified on Nickel NTA resin. Protein purity was determined by SDS-PAGE gel electrophoresis followed by Coomassie staining, supplemented by concentration determination by A280 analysis. Nampt activity was monitored via conversion of its enzymatic product, Nicotinamide mononucleoside NMN, to a fluorescence derivative. A linear relationship between fluorescence intensity and NMN concentration will be presented along with experiments investigating enzyme kinetics (including optimization of time course and preliminary Michaelis–Menten kinetics). These analyses will be instrumental in evaluating Nampt kinetics, by matching calculated Nampt Km and Kcat to literature values and comparing generated mutants and potential inhibitors with this framework.

DNA EXTRACTION METHODS FOR MACROALGAL ULVA SPECIES

Shennel Gelin, Kristen Dostie, Matthew Bresemen, JD Swanson, *Department of Biology and Biomedical Science*, Salve Regina University, Newport, RI; Amy Battocletti, Carol Thornber, *Department of Biological Science*, University of Rhode Island, Kingston, RI.

Rhode Island Science & Technology Advisory Council

Macroalgal Ulva is a bloom-forming algae that can create negative environmental and economical impacts in the Narragansett Bay, RI. In order to better understand these harmful environmental impacts, we intend to use microsatellite markers to DNA fingerprint different Ulva clones and determine the genetic structure of Ulva populations. To determine the best method to extract DNA from Ulva cells we investigated different methods of disrupting cell walls. One method involved grinding the sample with a mortar and pestle using liquid nitrogen and the other method involved grinding the sample using a PCT shredder. Using both disruption methods, two different extraction processes were tested and compared to determine which process yielded a higher concentration and purer quality of DNA. The first method tested was a QIAGEN DNeasy Plant Mini Kit in which a step-by-step protocol was followed to isolate DNA from the samples. The second method that was used was a phenol/chloroform extraction which uses CTAB buffer. After testing both methods of disruption and both methods of extraction, we checked the samples on a 1% agarose gel in 1X SB buffer in order to compare the bands and see which methods were more efficient. In addition the DNA samples from both extraction methods were quantified using a spectrophotometer. It was found that both disruption methods produced similar yields and the phenol/chloroform method yielded higher concentrations of DNA. We expect to extract DNA from about 480 samples by the end of summer and use the DNA to identify microsatellite regions within the genome.

3D ANIMATION OF IRESSA (GEFITINIB) DRUG RESISTANCE MECHANISM IN ADDITION TO 3D PRINTING OF CYTOSOLIC CELLULAR SIGNALING PROTEINS, INCLUDING RAS PROTEIN

Sean Gilman, Pharmacy, *3D Center for Biomedical Sciences*, University of Rhode Island, Kingston, RI; Stephan Norris, *Computer Engineering, 3D Center for Biomedical Sciences*, University of Rhode Island, Kingston, RI; Roy Bergstrom, *Instructional Technology and Media Services*, University of Rhode Island, Kingston, RI; Bongsup Cho, *College of Pharmacy*, University of Rhode Island, Kingston, RI

Champlain Foundations

URI's 3D Center for Biomedical Sciences produces high definition animations and models that serve as highly effective teaching tools which can enhance student understanding of complex cellular biological processes. An animation has been produced describing how a certain protein kinase becomes resistant to a cancer drug known as Iressa (Gefitinib). Protein kinases are a group of enzymes that transfer a molecule such as the cofactor ATP to one or more amino acid residues in a signaling protein. Iressa is a kinase inhibitor that blocks the binding of ATP and therefore, no phosphorylation can occur. However, the kinase domain can become resistance to Iressa by mutation of one amino acid via Threonine to Methionine mutation. This allows the endogenous ATP molecule to bind back to the protein. To understand how signaling is initiated, a series of cytosolic cellular signaling proteins, such as the Ras protein, have been modeled, colored, and printed to show the downstream pathway related to the animation. The kinase protein has also been printed to allow students to manipulate amino acids as well as bind both the ATP and Iressa molecules in the active site of the protein. Other molecular models printed include Fullerene Buckyball with and without a water molecule and Fullerene bonded to an antibody to showcase a drug delivery method.

ISOLAATION AND STRUCTURAL IDENTIFICATION OF COMPOUNDS FROM BOXELDER MAPLE (ACER NEGUNDO) LEAVES

Mallory Goding, Susan Meschwitz, *Department of Chemistry*, Salve Regina University, Newport, RI; Navindra Seeram, *Department of Biomedical and Pharmaceutical Sciences*, University of Rhode Island, Kingston, RI

RI-INBRE Summer Undergraduate Research Fellowship Program

Plants from the maple genus (Acer) have been widely used in traditional systems of medicine. The Boxelder maple (A. negundo) species is native to North America and, although not as well known as the sugar maple (A. saccharum), still widely regarded for its sap, which is used for the production of maple syrup. There have been previous phytochemical investigations into the constituents of maple syrup but limited data is available about the constituents of the various plant parts of the Boxelder maple species. In the current study, a methanolic extract of A. negundo leaves underwent a liquid-liquid partitioning with ethyl acetate (EtOAc) and butanol. The EtOAc-soluble fraction was subjected to a series of chromatographic isolation procedures including silica, and Sephadex LH-20 column chromatography, as well as semi-preparative and analytical high performance liquid chromatography (HPLC). Chemical structures of isolates are to be established via their nuclear magnetic resonance (1H-NMR and 13C-NMR) and mass spectroscopy (MS) data. Further work will be done to assess the bioactivity of these compounds.

ASSESSING WORD COMPREHENSION IN YOUNG CHILDREN

Kevin Fornari, Christina Gencarella, Kirstie Lepore, Beverly Goldfield, *Department of Psychology*, Rhode Island College, Providence, RI

RI-INBRE Summer Undergraduate Research Fellowship Program

Language skill is an important index of developmental status. For children 3 years and older, word knowledge can be reliably assessed by standardized tests that require pointing to pictures or acting out commands. However, such tests are inadequate for children with physical impairment (e.g., cerebral palsy) or poor social engagement (e.g., autism spectrum disorder).

The Preferential Looking Task (PLT) was devised to assess the developmental course of word comprehension in infants and toddlers who have a limited behavioral repertoire. The PLT measures word comprehension by comparing visual attention to two images (target and distracter) on a computer screen before (baseline) and after (test) the target image is labeled. Comprehension is defined as an increase in attention to the target during test compared to baseline. Thus, the PLT is ideally suited for testing children with physical or behavioral limitations at any age.

The specific aim of this one-year pilot study is to develop a test of word comprehension using the PLT to assess nouns, adjectives, and verbs in a sample of normally developing 4 and 5-year-olds; a standardized assessment is also administered and we test the hypothesis that the two insruments are correlated. The PLT will then serve as baseline data for a subsequent investigation of word comprehension in children with physical and social limitations.

We selected 120 words from frequency word lists. Color photos representing nouns and adjectives were selected from archives; we created video clips of actors performing actions to represent verbs. All items are being pilot tested to control for item saliency and level of difficulty. A final set of 35 target words will be administered. Visual attention is measured using a Tobii T60 XL eye tracker. Data analyses will examine the correlation between PLT and standardized test scores as well as age and sex differences.

METHODS FOR MONITORING THE EFFECT OF OCEAN ACIDIFICATION ON OTOLITH DEVELOPMENT, SURVIVAL, AND GROWTH IN CLARK CLOWNFISH AMPHIPRION CLARKIA

Allex Gourlay, Andrew Rhyne, *Department of Biology and Marine Biology*, Roger Williams University, Bristol, RI; Joshua Jones, *Department of Science and Technology*, Bryant College, Smithfield, RI; Eric Wilcox Freeburg, *Department of Environmental, Earth, and Ocean Sciences*, University of Massachusetts Boston

RI EPSCoR Summer Undergraduate Research Fellowship Program

Ocean Acidification is an escalating environmental issue that is being widely studied globally. It is unknown how this process effects the growth and development of fish larvae. When carbon dioxide is converted to carbonic acid from bicarbonate, the water becomes more acidic, and limits the available carbonate essential for calcifying organisms. Otoliths, which are calcium carbonate based structures in the inner ear of fish, are responsible for the detection of general movement, balance and orientation. It is unknown how they are affected by ocean acidification. The premise of this experiment is to examine the effects of pH on otolith development.

Twelve ten gallon tanks were equipped with a system with the ability to pump carbon dioxide into the water to mimic this natural process. These tanks were controlled and monitored using logger systems to record the pH variance over the eight to ten day larval development period of Clark clownfish (Amphiprion clarkii). Once the larvae hatched the fish were counted and stocked into the tanks at a density of 1 per liter with an Isochrysis sp. algae density of 200,000 cells per mL and 2 Pseudodiaptomus sp. copepod nauplii per mL. Algae and copepod counts were made twice daily and added when needed to maintain target density. All tanks were siphoned daily with a 25 percent water change, and water quality tests were performed to observe the conductivity, alkalinity, ammonia, and pH of the water. Once the fish completed metamorphosis, the fish in each tank were counted and measured to evaluate their survival rate and extent of growth. Each individual fish was preserved for otolith analysis. The resulting stages of the otoliths will be examined to determine how ocean acidification affects their development.

A MORE SENSITIVE AND PRECISE METHOD FOR THE QUANTIFICATION OF P. MARINUS IN THE EASTERN OYSTER

Catherine Grimm, Rozanna Smolowitz, Kathryn Markey, *Department of Biology*, Roger Williams University, Bristol, RI

RI EPSCoR Summer Undergraduate Research Fellowship Program

Perkinsus marinus, also called Dermo, is a protozoan that causes extensive disease in the eastern oyster, Crassotrea virginica. Significant mortality from Dermo continues to occur in oyster populations all the way from the Gulf of Mexico, along the Eastern coast, to as far north as Maine. The current diagnostic method consists of culturing infected minced oyster tissues and allowing the P. marinus trophozoites to form hypnospores that are easily visible when stained with iodine and examined microscopically. The occurence and severity of infection can be calculated from the stained tissue using the Mackin index. This method is subjective, because results are dependent on the ability of the person analyzing the tissue. The results are further complicated by other Perkinsus species, which will give false positive results. New diagnostic methods, such as real-time quantitative PCR, seek to correlate the amount of measured P. marinus DNA to a rating on the Mackin index. This will increase the specificity and reliability of rating Dermo infections. Due to its reproductive method, more than one copy of DNA may be found in each organism; therefore, controls for the qPCR using Dermo culture extracts are variable. This study removed this variance by creating a plasmid using the TOPO cloning kit to develop standards for Dermo qPCR. DNA extracted from mature Dermo cultures of known cell numbers will be equated with DNA copies in the plasmid controls to produce a control curve for use in diagnostic test methods.

THE EFFECTIVENESS OF THE PLANT POLYPHENOL GALLIC ACID ON CELL CYCLE ARREST IN GASTRIC CANCER CELLS

Alyssa Guarracino, Kelsey Stafstrom, Alexandria Bierce, JD Swanson, *Department of Biology and Biomedical Sciences*, Salve Regina University, Newport, RI; Songhua Zhang, Steve Moss, *Division of Gastroenterology*, Brown University, Providence, RI

RI-INBRE Summer Undergraduate Research Fellowship Program

Naturopathic medicines have been widely used in the medical field to treat diseases and illnesses such as cancer, but little is known of the molecular mechanisms underlying these naturopathic treatments. Exposure to gallic acid, a secondary metabolite found in certain plants, has been shown to cause cell cycle arrest and subsequent apoptosis in cancerous cell cultures. Previous studies have shown arrest in the G2/M phase of the cell cycle following exposure of 40-50 µM of gallic acid. To date, no studies have focused on gastrointestinal related cancers making this study especially relative because we are looking at the effect of nutraceuticals. Gastric cancer cells (Human gastric adenocarcinoma cells (AGS) bought from ATCC) were grown in F-12K medium supplemented with 10% FBS, 100 Units/ml Penicillin and 100 µg/ml Streptomycin. Cell culture incubations were performed at 37 C in a humidified incubator with 5% CO2. When cells reached 75-80 % confluence, they were exposed to 0, 10, 20, 30, 40 or 50 µg of gallic acid for 0, 12, 24, or 48 hours. Flow cytometery was used to measure the number of cells in each phase of the cell cycle. RNA will be extracted from the cells and qPCR will be run to measure the amount that specific genes are being expressed in these cells using primers designed for genes that are associated with the G2/M phase. The data from this research can be used to validate the effectiveness of gallic acid on cancer cells and may potentially lead to new treatments for patients with stomach cancer.

MERCURY IN THE SEDIMENTS OF THE NARRAGANSETT BAY ESTUARY (RHODE ISLAND, USA): CONTAMINATION FROM A HISTORICAL AND SPATIAL PERSPECTIVE

Allison Hall, David Taylor, *Department of Marine Biology*, Roger Williams University, Bristol, RI; David Murray, Warren Prell, *Department of Environmental and Geological Sciences*, Brown University, Providence, RI

RI-INBRE Summer Undergraduate Research Fellowship Program

Estuarine and marine sediments are repositories for heavy metal contaminants, including mercury (Hg), thus providing a historical record of contaminant inputs into aquatic ecosystems. This study presents stratigraphic profiles of total Hg concentrations measured in sediment cores collected from the Narragansett Bay. Sediments were collected at 12 sites across an anthropogenic gradient using a gravity corer. Sub-samples were taken continuously at 2-cm increments and analyzed for total Hg content (ppm dry weight) using automated combustion atomic absorption spectroscopy. The same sub-samples were analyzed for total organic carbon (TOC; %) with combustion gas chromatography. A positive relationship was found between total Hg and TOC (Linear regression: $R2 = 0.444$, $df = 1,34$, $p < 0.0001$), reflecting Hg's affinity for sediments within the fine fraction dictated by in situ biogeochemical conditions. Accordingly, all Hg values were normalized by TOC. Five representative cores were dated using Lead (Pb)-210 , a naturally occurring radioactive element (half-life = 22.3 yr). Irrespective of spatial location, sediment total Hg concentrations were low in the deeper portions of the cores (mean Hg = 0.009 ± 0.02 ppm), coinciding with the pre-industrial time period. Cores demonstrated a mid-depth maximum in Hg content (mean Hg = 0.10 ± 0.09 ppm) that presumably resulted from industrial revolution contaminant inputs that began in the mid-1800s and persisted into the mid-1900s. Finally, the Clean Water Act of 1972 was reflected as total Hg concentrations decreased in the surface samples (mean Hg = 0.04 ± 0.05 ppm). Sediment total Hg concentrations varied spatially throughout the Bay, with concentrations greatest in the north and decreasing southward (range Hg = 0.26-6.59 ppm). Sediment Hg concentrations were particularly elevated in the developed regions of the Bay, hence reflecting these areas as the initial centers of industrial growth and the dominant sources of historical and recent contaminant inputs.

CHARACTERIZATION OF THE SECRETORY LIPASE LDLIP3 FROM THE HUMAN PATHOGEN LEISHMANIA DONOVANI UNDER VARIOUS ENZYME REACTION CONDITIONS

Lana Hoertz, Alison Shakarian, *Department of Biology and Biomedical Sciences*, Salve Regina University, Newport, RI

RI EPSCoR Summer Undergraduate Research Fellowship Program

Leishmania donovani, a protozoan parasite, is the causative agent of the often fatal disease visceral leishmanisis. It has been shown that these organisms exhibit lipolytic activity during their growth in vitro. Lipases are enzymes that are known to aid in the development and virulence of several pathogenic organisms such as Candida albicans and Staphylococcus warneri. Little information is known, however, about the role of lipases in Leishmania species. We hypothesize that lipase may play a part in Leishmania's ability to survive within the human host as well as its pathogenesis. Previously in our lab, the L. donovani secretory lipase gene (LdLip3) was episomally expressed with an HA tag by transfected promastigotes. The purified protein was tested for enzyme activity by performing assays with McIlvaine's buffer pH 4-8 at 26°C, 37°C, and 42°C using 4MU-palmitate as a substrate. The results showed that there was an average thirty fold increase in specific activity when comparing the purified protein to the unpurified supernatant samples. Metal ions are known to be cofactors for a variety of enzymes, greatly affecting their activity, therefore, in the current study a panel of metal ions were tested to determine their effect on the activity of purified LdLIP3. Optimal conditions for this enzyme were established at pH 8, 42°C, with the addition of Zn+2, whereas the addition of Mn+2 consistently produced a strong inhibitory effect. We are currently in the process of validating this previously collected data via the same experimentation protocol. Chelating agent studies with EDTA are also in progress to verify the effect of metal ions on the enzyme activity. Once established, optimal reaction conditions will be used in future enzyme studies to identify potential specific LdLIP3 inhibitors.

CHANGING USE OF CORALS AS HABITAT BY A CARIBBEAN DAMSELFISH IN RESPONSE TO THE DECLINE OF PREFERRED CORALS.

Allison Holevoet, *Department of Biological Sciences*, University of Rhode Island, Kingston, RI; Graham Forrester, *Department of Natural Resources Science*, University of Rhode Island, Kingston, RI

RI EPSCoR Summer Undergraduate Research Fellowship Program

A decline in the health of tropical reef ecosystems has been well documented over the last few decades. We wanted to know whether declines in the abundance of the three spot damselfish (Stegastes planifrons) are related to shifts in habitat use by juveniles so we tested whether progressive loss of the damselfishes' preferred habitat of Montastraea annularis corals caused increasing use of secondary habitats (which contained either other species of branching corals, or dead coral). Beginning in 1992, eight reefs around Guana Island have been monitored annually to see how the habitat preference of S. planifrons has changed over time. At each reef, the habitat features within individual damselfish home ranges (roughly 30 x 30 cm in area) were compared to the habitat features in a set of randomly selected locations of similar size. Randomly selected sites were compared to the home ranges to determine what types of habitats the fish are choosing relative to what is available. Comparisons of habitat selection in 1993, 2001, and 2010 show that the fraction of damselfish in preferred (M. annularis dominated) home ranges has declined. From this, we suggest that the loss M. annularis has led to increasing use of secondary habitats by S. planifrons, where their survival is reduced and that the loss of preferred habitat is a major cause of the decline in small reef fishes like S. planifrons.

A COMPARISON OF GC-MS DERIVATIZATION METHODS FOR ANALYZING MURAMIC ACID CONTENT IN SOIL MICROBIAL COMMUNITIES

Garrett Holmes, Julia Parmentier, Dan McNally, Christopher Reid, *Department of Science and Technology*, Bryant University, Smithfield, RI

RI EPSCoR Summer Undergraduate Research Fellowship Program

Muramic acid is a structural molecule that is essential to the peptidoglycan layer of the cell wall of bacteria. Because the molecule is limited to bacterial cell walls, muramic acid is an ideal biomarker in the laboratory for the measuring bacterial content. Correlating muramic acid content to a specific cell number is an important tool for understanding soil health and possibly detecting alterations to microbial communities due to site contamination. This project examined a variety of GC-MS derivatization procedures for analyzing levels of muramic acid in soil to determine how these levels correlate to specific cell counts. Pseudomonas aeruginosa and Bacillus subtilis were grown and used as test organisms, representing both Gram-positive and Gram-negative bacteria. These bacteria were quantified using serial dilutions and plating methods to determine the specific amount of bacterial cells in each sample that were correlated to the GC-MS data. Previous studies measure only the mass of bacteria, as opposed to specific cell number. In addition, this study compares three different techniques: alditol acetate acetic anhydride derivatization, aldononitrile derivatization, and methyl ester O-methyl (MMA) derivatization for their ability to be used to accurately measure amounts of bacteria in contaminated soil. Using GC-MS in both scan and SIM mode, specific molecules were detected that indicated the presence of muramic acid. Our results suggest that the MMA methods exceed the accuracy of the classic alditol acetate method. These results could improve the accuracy of soil health testing in the ongoing research for diesel contaminated sites at Prudence Island in Rhode Island.

EFFECT OF PHLEOMYCIN ON SACCHAROMYCES CEREVISIAE WITH A TEMPERATURE SENSITIVE MUTANT FORM OF BCP1 PROTEIN

Masharee Hopkins-Jones, Sabrina Elgar, Kyle Inman, Deborah Britt, *Department of Biology*, Rhode Island College, Providence, RI

RI-INBRE Summer Undergraduate Research Fellowship Program

Saccharomyces cerevisiae is a species of budding yeast that carries the gene Bcp1, which is one of the many genes responsible for yeast growth. Bcp1 is the fungal homolog to the mammalian gene BCCIP, a tumor suppressor. The overall purpose of this research is to investigate the hypothesis that Bcp1 plays a role in DNA damage response pathways. The specific objective of this project was to investigate how the drug phleomycin affects growth of a yeast strain with a Bcp1 temperature sensitive mutation (AAY) relative to its parental strain (SEY). Phleomycin is an antibiotic of the Bleomycin family that binds and intercalates DNA, which destroys the double helix and leads to double and single strand breaks. Both strains were cultured in the presence of phleomycin for 48-72 hours. The exposure showed that Phleomycin inhibited growth of the SEY and AAY, but AAY had a greater sensitivity to the drug. The results suggest that Bcp1 is needed to repair DNA damage induced by phleomycin.
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GENOME REDUCTION IN YEAST INVOLVES PROGRAMMED CELL DEATH

Matthew Hurton, Emily Roblee, Nicanor Austriaco, Richard Bennett, *Department of Biology*, Providence College, Providence, RI

RI-INBRE Summer Undergraduate Research Fellowship Program

Genetic reduction is of great significance in many biological pathways, a primary example being production of gametes. After discovering that a tetraploid strain of Candida albicans undergoes significant cell death on reduction to a diploid state, we set out to determine the means of death and if it was a general, genome-reduction-related process. To measure this cell death cells were grown on either pre-sporulation or sporulation media for tetraploid and diploid cells, respectively, in both Candida and Saccharomyces backgrounds. Measurements of viability were made with methylene blue or propidium iodide staining, with tetraploid cells on average showing 23% survival by day two compared to 89% for a diploid control. To test the mechanism of death, assays were done to measure ROS levels and caspase activity. Cells were stained with dihydrorhodamine and then viewed under a confocal fluorescence microscope, with on average 52% of the tetraploid showing fluorescence whereas only 2% fluorescence was visible in the diploid control. A second assay was done using FLICA protocol for caspase activity, with tetraploids showing around 53% fluorescent cells while only 2% of diploids showed fluorescence. These results indicate high levels of both ROS and caspase activity in the tetraploid, suggesting programmed cell death via apoptosis. Similar experiments in diploid strains of Candida albicans, Candida lusitania, and Saccharomyces cerevisiae are being carried out to show that programmed cell death is a generally retained feature of genome reduction in yeast. Further research will be done with knockout strains of apoptotic genes and sporulation-specific strains to further understand the mechanism of death.

DOES SUB-TIDAL SUBSTRATE HETEROGENEITY PREDICT NEAR SHORE MARINE SPECIES DIVERSITY?

Alexandra Igo, Sarah Matarese, Jameson Chace, *Department of Biology*, Salve Regina University, Newport, RI

RI EPCSCoR Summer Undergraduate Research Fellowship Program

The richness and abundance of marine species, specifically macro invertebrates, is greatly dependent on the type of habitat available. Substrate heterogeneity was predicted to positively influence the diversity of marine organisms in the shallow water (< 5 m) of Newport Neck. Ten sites along Newport Neck were chosen and each divided into three sub-sites, creating a total of thirty sampling locations. Modified lobster traps and small fish traps were placed at each site and species richness and abundance were recorded every three days. Divers estimated substrate size and diversity from 0.5 m^2 quadrats placed in sub-tidal zones at each trap location. A total of six species, including rock crabs (Cancer irroratus) and lobsters (Homarus americanus), were identified along Newport Neck by our trapping efforts. Richness ranged from 1 to 6 species among all sites, with the highest richness at sites 6, 9, and 10. The most common species captured in the modified lobster traps were lobsters, spider crabs (Libinia sp.), and rock crabs. The most common species captured in small fish traps were perch (Perca sp.), Asian shore crabs (Hemigrapsus sanguineus) and small lobsters. Abundance ranged -8-15 individuals/trap day, with highest total abundance at sites 6, 9, and 10 and lowest at sites 2, 5 and 8. Based on these preliminary results, sites 10 and 6 had the overall highest species diversity (Simpson's Index = 1.44). We predict that sub-tidal substrate heterogeneity will be positively correlated with species richness, abundance and diversity. Understanding habitat selection of benthic near shore marine organisms today allows for modeling future distribution and abundance with sea level rise that is concomitant with climate change. From this, we can predict that future areas of high species richness and abundance will be in locations with the most heterogeneous rocky sub-tidal zone.

EFFECTS OF BLEOMYCIN ON SACCHAROMYCES CEREVISIAE WITH A TEMPERATURE-SENSITIVE FORM OF BCP1

Kyle Inman, Sabrina Elgar, Masharee Hopkins-Jones, Deborah Britt, *Department of Biology*, Rhode Island College, Providence, RI

RI-INBRE Summer Undergraduate Research Fellowship Program

Knowledge about the cellular response to DNA damage is essential for the understanding of multiple diseases. The protein BCCIP is known to play a part in the human DNA damage repair pathway, but its exact function is unclear. Identifying BCCIP's role in DNA repair could increase our understanding of this pathway, which is important in the prevention of cancer. We studied an analogous protein in yeast, known as Bcp1, since yeast cells are easier to work with and have shorter generation times than human cells. Complete inactivation of Bcp1 arrests cells during mitosis, so we used a mutant strain called AAY with a temperature-sensitive form of the protein. This mutation decreased the functionality of Bcp1 enough for an effect to be seen, but not enough to cause cell cycle arrest. To test the effects of the loss of Bcp1 on AAY's response to genetic damage, we grew cultures of AAY in the presence of bleomycin, a drug that damages DNA via oxidative stress and causes double strand breaks. The parent strain (SEY) served as a control. Serial dilutions and cell counts were used to determine the extent of growth in the presence of the drug. We found that AAY was more susceptible to bleomycin than the parental strain. These results may have been due to the decrease in functional Bcp1 in the temperature-sensitive mutant. However, another study suggested that AAY has a weaker cell wall than SEY. Since bleomycin causes oxidative stress, this weaker cell wall could be responsible for the differing responses to the drug. Preliminary data seemed to support this conclusion, as AAY grown in the presence of NaCl, which provided osmotic support, was no more susceptible to bleomycin than SEY. Further research is necessary to confirm this result.

ECONOMIC IMPACT OF PROPOSED CLOSINGS IN STELLWAGEN BANK NATIONAL MARINE SANCTUARY TO THE TOWN OF GLOUCESTER

Hannah Jones, *Department of Environmental and Natural Resource Economics*, University of Rhode Island, Kingston, RI

RI EPSCoR Summer Undergraduate Research Fellowship Program

Stellwagen Bank National Marine Sanctuary is valued as a rich ecosystem that is valued by many for tourism, recreational and commercial fishing. The sanctuary is valued by fishermen throughout Massachusetts from as far east as Provincetown and north up to Gloucester. For a town dependent on fishing like Gloucester, Stellwagen Bank has untold importance. Stellwagen Banks natural geography provides rich fishing grounds that serve as an important ecosystem for many ocean dwellers, including the American Lobster. The rise and recent fall of the American Lobster are tantamount to that of Gloucester. With lobster fetching some of the lowest prices in years, lobstermen rely on a steady catch from Stellwagen Bank. Unfortunately, there is a proposed closing the area to lobster fishing, which will have a profound impact on the lobstermen who rely on the area. The purpose of this study was to address the economic impact of such a closure on the town of Gloucester. The study hopes to address the ripple effect that such a closing would have on the town of Gloucester in monetary terms. This means that the survey will try to extract impacts felt beyond the harbor. Additionally, the survey also hopes to address the impact of anomalous weather patterns on lobstermen. This year, due to a temperate winter, the lobster have molted early, leaving behind a soft shell which is too delicate to be shipped. Variations in weather, regulations and price of the lobster create an unstable future for the quintessential fishing village of Gloucester. This study hopes to quantify the importance of lobster to Gloucester before regulations alter Gloucester's heritage for future generations.

SYSTEM STABILITY OF AN EXPERIMENTAL SETUP MONITORING THE EFFECT OF OCEAN ACIDIFICATION ON MARINE ORGANISMS

Joshua Jones, *Department of Science and Technology*, Bryant College, Smithfield, RI; Allex Gourlay, *Department of Biology and Marine Biology*, Roger Williams University, Bristol, RI; Eric Wilcox Freeburg, *Department of Environmental Earth and Oceans Sciences*, University of Massachusetts Boston

RI EPSCoR Summer Undergraduate Research Fellowship Program

Ocean acidification, an effect of global climate change, is becoming an increasingly important topic aiming to narrow the diverse predictions of its effect on marine species, populations, and ecosystems. The rising demand for fossil fuels leads to a larger abundance of carbon dioxide emissions into the atmosphere, much of which is absorbed by the ocean. This greater uptake of carbon dioxide by the ocean reduces the availability of carbonate ions many marine species need to form calcium carbonate. The area of interest for this research is the otolith (ear bone) growth through calcification. The species Amphiprion clarkii is the preferred species for the experiment due to its short larvae stage. Modeling climate change and more specifically ocean acidification for experimentation involves recreating a complex system. To conduct research on the effects of the changing pH in the ocean, we built a system comprised of 12 tanks with corresponding log boxes which monitor pH of each experimental tank. Solenoid valves are connected to each tank and are triggered to release carbon dioxide into the tank when the log systems record a pH above the targeted level. The consistency and steadiness of the pH in each tank reflects directly on the rate and abundance of carbon dioxide being pumped into the tank. Prior to experimentation on fish larvae, we monitored how stable the pH levels were in each tank. Logs were recorded and downloaded to analyze. By monitoring the fluctuation of pH levels in a tank, the system can be adjusted to minimize the pH variation to maintain the most consistent environment for the larvae. This system, once perfected, could also be utilized for studying the effect of ocean acidification on other marine organisms.

THE EFFECTS OF SUBSTRATE COMPOSITION ON SPECIES DIVERSITY ALONG NEWPORT NECK, RHODE ISLAND IN THE CONTEXT OF CLIMATE CHANGE

William Kelly, Jameson Chace, Sarah Matarese, *Department of Biology and Biological Sciences*, Salve Regina University, Newport, RI

Salve Regina University Biology Workstudy

Climate change is becoming an increased global concern, especially along coastal areas where 44% of the world's population lives and where marine productivity is the highest. Predicted sea-level rise concomitant with climate change will impact both human societies and ecosystem function. A better understanding of organismal habitat selection in the near-shore coastal zone is needed to predict how sea-level rise will influence future distribution and abundance of marine organisms at the base of these dynamic and productive ecosystems. Substrate heterogeneity may be key to the habitat selection of barnacles (Chthamalus), mussels (Mytilus edulis), snails (Littorina littorea), and crabs (Callinectes sapidus), (Hemigrapsus sanguineus), and (Carcinus maenas) along the temperate, western Atlantic rocky intertidal zone. Along Newport Neck, Rhode Island in 2012, we tested the hypothesis that increased substrate heterogeneity will positively affect species diversity. Using 0.5m2 quadrating in the intertidal zone the presence of marine invertebrates and substrate composition were surveyed at 30 sites along Newport Neck, Rhode Island. Current intertidal substrate compositions and future supertidal substrate compositions 1 m above the high tide mark were compared. We used species richness and Simpson's Diversity Index to analyze the relationship between substrate composition and species diversity. An increase in physical habitat heterogeneity increases species diversity. Future sea-level rise in the area will decrease habitat heterogeneity and therefore, we predict, will decrease intertidal zone species diversity.

ANALYSIS OF HEAVY METALS CONCENTRATIONS FROM A WORLD WAR TWO ERA PETROLEUM HYDROCARBON SPILL.

Trevor Kent, *Department of Biotechnology*, Community College of Rhode Island, Warwick, RI; Julia Crowley Parmentier, *Department of Science and Technology*, Bryant University, Smithfield, RI

RI EPSCoR Summer Undergraduate Research Fellowship Program

As part of an ongoing study of long-term natural attenuation of gasoline and diesel fuel contamination in beach sediments at the Prudence Island T-Dock, in Narragansett Bay, RI, we determined the concentrations of acid-extractable heavy metals within and outside of the contaminant plume. We are concerned with the toxic effects of heavy metals from the petroleum hydrocarbons, including copper, lead, zinc, chromium, barium, vanadium, nickel, and cadmium on marine life and humans. . We also monitored other metals including iron, magnesium, manganese, silver, arsenic, selenium and strontium. Samples were collected from the north side of the T-dock at the high tide mark and the low tide mark within the contaminant plume and north of the plume. Additional samples were collected offshore of the plume and from the south side of the T- dock. Samples were collected at multiple depths at each site allowing for comparison between contaminated sites and clean site. The samples were all acid digested (3:1 HCl:HNO3) and then diluted to a specific concentration for analysis using an ICP-MS.

The levels detected are similar to concentrations reported in a 1996 single source sample. The total range of concentration varied no more than a factor of three for all metals except for strontium. High Sr concentrations correlate with high shell content. Metals concentrations within the contaminant plume were generally higher than background samples. Maximum Pb concentrations of 11 and 27 mg/kg were detected at depths of 0.5 m below the surface in Test Pits 1 and 6 located approximately 10 - 15 m north of the T-dock, within the contaminant plume. These samples also had concentration of V, Cr, Ni, Zn, Fe and Mg approximately 2 times background concentrations. None of the samples exceeded NOAA ERL-ERM guidelines for heavy metals.

THE CROSS-RACE EFFECT AT ENCODING AND RECOGNITION

Lorin Kinney, Thomas Malloy, *Department of Psychology*, Rhode Island College, Providence, RI

RI-INBRE Summer Undergraduate Research Fellowship Program

The cross-race effect (CRE) is a phenomenon where individuals of one race have better facial memory accuracy for members of their own race over other races. Despite being well documented, debate surrounds the factors responsible for the effect. According to the Categorization-Individuation Model (CIM), perceptual expertise, social categorization, and motivation are considered the main variables in explaining the CRE. During exposure to faces, motivation to store same race (SR) faces over other race (OR) faces in memory would lead to the CRE. At recognition, faces that are poorly encoded will require more attention, take longer to identify, and are more likely to be misidentified. Forty Caucasian participants, 20 male and 20 female, will view black male, black female, white male, and white female faces in a yes-no recognition task to determine the magnitude of the CRE. Participants will view 32 faces at encoding, then a distracter task, followed by a yes-no recognition task involving 64 faces, where 32 are new. Using eye-tracking software during both the encoding and recognition tasks, gaze behavior, differences in area of interest fixation, and pupil dilation will be measured for SR and OR faces in order to determine differences in visual behavior that. Based on the CIM, it is expected that more attention will be paid to SR faces than OR faces at encoding, and those who pay more attention to SR faces at encoding are expected to have a larger CRE. At recognition, more visual search of OR faces, as well as a more delayed yes-no response will be associated with a larger CRE.

ISOLATION AND STRUCTURAL IDENTIFICATION OF BIOACTIVE COMPOUNDS FROM BLACK CUMIN (NIGELLA SATIVA) SEEDS

Kristen Knoph, Hang Ma, Tao Yaun, Navindra Seeram, *Department of Biomedical and Pharmaceutical Sciences*, University of Rhode Island, Kingston, RI

RI-INBRE Summer Undergraduate Research Fellowship Program

Ranunculaceae (Nigella sativa L.) is an annual herbaceous plant and widely cultivated in Mediterranean countries. Its seeds, commonly named black cumin, have been used as a food spice in Arabian cuisine as well as in Middle Eastern folk medicine for the treatment of hypertension, abdominal pain, asthma and diabetes. Due to these ethnomedicinal uses, we investigated the phytochemical components of black cumin seeds and evaluated the biological activities of the isolates. In the current study, black cumin seed oil was obtained by hexane extraction and the crude extract was subjected to a series of chromatographic isolation approaches including silica gel chromatography, C-18 medium pressure liquid chromatography (MPLC) and LH-20 column chromatography, as well as semi-preparative and analytical high performance liquid chromatography (HPLC). Chemical structures of the isolated compounds were elucidated by spectroscopic techniques including nuclear magnetic resonance (1H-NMR and 13C-NMR) and confirmed by HPLC chromatography as well as mass spectroscopy (MS) data. Two isolated compounds have been identified as thymoquinone (1) and hydro-thymoquinone (2) and were evaluated for their anti-diabetes activity. Further work is being conducted to isolate other compounds from black cumin.

COMPARISON OF NUTRITIONAL CONTENT IN HYDROPONICALLY GROWN AND CONVENTIONALLY GROWN MICROGREENS

Olivia Kopin, Jameson Chace, *Department of Biology and Biomedical Sciences*, Salve Regina University, Newport, RI; Susan Meschwitz, *Department of Chemistry*, Salve Regina University, Newport, RI

Salve Regina University Sustainability Fellowship Program

Fifty percent of the world's population lives in areas that lack access to arable lands and high quality soil, leading to societal caloric and nutritional imbalances. Hydroponic agriculture is an alternate method for healthy and resourceful food production without soil and therefore has wide applicability to urban areas. The hydroponic method includes growing plants without soil, eliminates the use of pesticides and provides greater crop density and a year-round growing season. Microgreens are an ideal crop for such areas, and especially for hydroponics because they grow quickly (14 days between germination and harvest), easily (under traditional T-12 florescent lights), and produce a high density of greens that have the highest nutritional content that is often lacking among the urban poor. The purpose of this study was to quantify the nutritional content of microgreens grown in a hydroponic system with conventionally grown microgreens. The seeds were placed directly into an elevated growing system (EGS) and allowed to germinate. The EGS is a typical flood-and-drain hydroponic system with automated watering (30 minutes in each hour) and T-12 florescent lighting (12L:12D), where pH (6.1) and nutrient levels (910 ppm) were monitored and adjusted daily. After a growth period of two weeks, the microgreens were harvested. Total of 30.1-g/sq. m was obtained. Microgreens were also purchased from Stop and Shop in Newport, RI. Immediately the microgreens were processed for beta-carotene and iron content and then analyzed using High Performance Liquid Chromatography and Microscale UV-Visible Spectrophotometric measurement by standard addition. For the typical consumer there are questions about the nutritional content of microgreens grown hydroponically or conventionally. Furthermore, as hydroponics expands into urban areas, there needs to be an evaluation of practices for growing the healthiest crops under the most affordable conditions. The Hydroponic Research Lab at Salve Regina University provides this type of information.

PCR TOXINOTYPING OF A CLOSTRIDIUM DIFFICILE

Jackie Kratch, Christopher Reid, Kirsten Hokeness, *Department of Science and Technology*, Bryant University, Smithfield, RI

RI-INBRE Summer Undergraduate Research Fellowship Program

Clostridium difficile is a Gram-positive pathogen that spreads easily through hospitals and can infect anyone. A suspected toxin A-/B- strain of C. difficile was acquired by the laboratory. Prior to further use, the C. difficile isolate was toxinotyped via polymerase chain reaction (PCR). The toxinotyping protocol developed by the National Microbiology Laboratory (NML) in Winnipeg was utilized. C. difficile infections are mediated by toxins A and B and cause damage to the intestinal epithelia. Primers specific for binary toxin subunit B (cdtB), toxin A (tcdA),toxin B (tcdB), and the negative regulator tcdC (binary toxin) were used. To confirm that a potential toxin negative strain was indeed C. difficile primers specific for the species specific region of triose phosphate isomerase (tpi) were used as a control. Results from PCR analysis and culturing on C. difficile selective media indicated the organism was not C. difficile.

MERCURY ACCUMULATION IN CARTILAGINOUS FISH FROM RHODE ISLAND'S COASTAL WATERS

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

RI-INBRE Summer Undergraduate Research Fellowship Program

Mercury (Hg) is a toxic environmental contaminant that bioaccumulates in fish tissues, including numerous marine species. Cartilaginous fish of the subclass Elasmobranchii are important ecological constituents of marine ecosystems, yet the fate of Hg contaminants in their body tissues is largely unknown. In this study, four species of elasmobranchs: little skate (Leucoraja erinacea), winter skate (L. ocellata), smooth dogfish (Mustelus canis), and spiny dogfish (Squalus acanthias), were collected from Rhode Island/Block Island Sound, and the Hg content (ppm wet wt) of their white muscle tissue was analyzed using automated combustion atomic absorption spectrometry. Diet and feeding habits for each species were also assessed by stomach content and stable nitrogen (d15N) and carbon (d13C) isotope analyses. Mean Hg concentrations differed significantly among species, with highest levels measured in smooth dogfish, followed by spiny dogfish, and little and winter skates, respectively. There was a significant relationship between muscle Hg concentrations and body weight for all species, although bioaccumulation rates were greatly accelerated in dogfish relative to skates. Moreover, smooth dogfish had a higher Hg content relative to spiny dogfish at a given weight, which is further explained by this species higher trophic level status, as determined from d15N signatures. The enriched d13C values of skates and smooth dogfish indicated benthic foraging, which was further confirmed by the dominance of decapods and crustaceans in the stomach contents. Conversely, squid and butterfish were the principal prey of spiny dogfish, and the contribution of these pelagic prey was reflected in the depleted d13C signature. Future work includes researching the effect of habitat use, analyzing prey Hg, and analyzing Hg concentrations of the liver to better understand bioaccumulation patterns in these species.

EXPLORATION OF DEEP SEA SEDIMENT FUNGI FOR NOVEL ANTIBIOTICS

Zachary Leriviere, Robert Deering, Stephanie Forschner-Dancause, David Rowley, *Department of Biomedical and Pharmaceutical Sciences*, University of Rhode Island, Kingston, RI; David Smith, *Graduate School of Oceanography*, University of Rhode Island, Narragansett, RI

RI-INBRE Summer Undergraduate Research Fellowship Program

The World Health Organization has identified antimicrobial resistance as one of the top three greatest threats to human health. In order to keep pace with rising drug resistance, new sources of novel antibiotics must be identified. Due to obvious technical challenges, sediments from ocean depths below 2000 meters remain one of the least explored environments for microbiology and thus represent an untapped resource for biomedical discovery. In 2010, IODP Expedition 329 to the South Pacific Gyre (SPG) cored the sediment stack underlying average ocean depths of 5,057 m. Sediments for microbial cultivation were sampled at each of six sites within the gyre and a seventh control site just south of the gyre's edge. In total, 105 samples were collected from sediment cores within the gyre ranging in depth from 1.3 to 75.3 meters below the seaflooor (mbsf) and an additional 27 subcores ranging in depth from 1.4 to 126.9 mbsf were collected from the control site. In total, 120 pure fungal strains were isolated from the deep ocean sediments. In this study, four of the fungal strains were intensively studied for their production of bioactive molecules. The fungi were cultivated in multi-liter scale using marine media and extracted with organic solvent. Pure compounds were isolated from the extracts using a combination of chromatographic techniques, especially high-performance liquid chromatography (HPLC). The molecular structures of several compounds were deduced using nuclear magnetic resonance (NMR) and mass spectrometry. To date, the known fungal metabolites cyclopiazonic acid, cyclopenin, cyclopenol, viridicatol and rugulovasin have been identified from the four strains. Further pure compounds are currently under investigation.

THE EFFECT OF EGO DEPLETION ON ADULTS' AND PRESCHOOLERS' MENTAL REPRESENTATION OF PRETENSE

Christina Lavigne, Cristina Taylor, Katherine McNulty, Alexandra Male, Jennifer Van Reet, *Department of Psychology*, Providence College, Providence, RI

RI-INBRE Summer Undergraduate Research Fellowship Program

Everyone has a limited amount of self-control, a resource which can be 'depleted' temporarily after it is used. Previous research has shown that children, but not adults, use self-control when representing pretense: after reading a story about pretending, children are slower to respond to a word associated with a real aspect of the story (a real associate), than to both a word associated with a pretend aspect of the story (a pretend associate) or a word unassociated with the story (control). This study was designed to examine how adults and children process pretending after being depleted, as well as the effect of engaging in pretense on inhibitory control (self-control). Experimenters hypothesize that overall, after being depleted the adults will have significantly varying reaction times between the real, pretend, and control associates. This study was presented on a computer. First participants were depleted using a standard Stroop task, in which they were instructed to pay attention to the appearance of a word, not its meaning. Second, participants read stories about pretending. Directly after reading each story, participants saw a picture of either a pretend, real, or control associate and named it out loud into a microphone; reaction time (ms) to speak was measured. Participants then completed a second Stroop test to check if activating pretense increases participants' self control. Participants were both adults (undergraduate students) and children ages 4 to 5. Child participants experienced a non-verbal version of the same procedure. Initial results show that adults reacted significantly quicker to the real associate than to any other associate. Therefore, after being depleted, adults reacted the quickest to real associates. Further results and implications of this research will be discussed.

HABITAT EFFECTS ON MERCURY BIOACCUMULATION IN BLACK SEA BASS (CENTROPRISTIS STRIATA)

Garrett LeBlanc, Carissa Gervasi, David Taylor, *Department of Marine Biology*, Roger Williams University, Bristol, RI

RI-INBRE Summer Undergraduate Research Program

Mercury (Hg) is a widespread environmental contaminant that bioaccumulates in fish muscle tissue, and therefore poses a risk to human consumers. Understanding the human risk from Hg exposure requires insight into: (1) species-specific Hg concentrations and (2) variability in fish Hg content as a function of life history (e.g., habitat use and diet). In this study, an important recreational fish, the black sea bass (Centropristis striata) was collected from inshore (Narragansett Bay) and offshore (Rhode Island/Block Island Sound) habitats using trawls and hook & line. For black sea bass (n=191), white muscle tissue was analyzed for total Hg and results were evaluated relative to fish age and habitat use (inshore vs. offshore). The otoliths of the inshore population were analyzed for strontium (Sr) concentrations for the detection of a salinity signature, and thus, a verification of site fidelity in the Bay. Visual analysis (frequency of occurrence) of stomach contents was also performed to assess variation in diet across habitats. Irrespective of habitat-type, the Hg content of black sea bass muscle tissue was positively correlated with fish age, indicating the bioaccumulation of Hg. Black sea bass collected from inshore habitats, however, had higher Hg levels at a given age than conspecifics from offshore locations. Further, within the inshore habitat, individuals with a lower Sr concentration (lower salinity signature) had higher Hg levels. The cumulative results indicate that Hg levels in black sea bass vary significantly over relatively small spatial scales (5 km). Finally, the diet of the inshore population was dominated by crabs (59%), while the offshore conspecifics fed on crabs (30%), shrimp (16%), and algae (12%). This information can be helpful to guide the consumer on what fish to eat. Future work will include the analysis of black sea bass stable isotope signatures and otolith microchemistry (for offshore fish).

HIGH SCHOOL RESEARCHERS AT THE UNIVERSITY OF RHODE ISLAND

Sage Cohen, Julian Natale, Ella DeMarco, Mindy Levine, *Department of Chemistry*, University of Rhode Island, Kingston, RI

RI-INBRE Summer Undergraduate Research Fellowship Program

High school students have conducted high level research in the chemistry department at the University of Rhode Island. In particular, they have worked on two projects: the detection of small-molecule explosives using fluorescent organic nanoparticles (S. Cohen), and mechanisms for the eco-friendly production of biofuels (J. Natale and E. DeMarco).

DNA BINDING STUDIES OF TRIARYLCINNAMYL PHOSPHONIUM SALTS

Jose Lora, John Williams, Karen Almeida, *Department of Physical Sciences*, Rhode Island College, Providence, RI

RI INBRE Summer Undergraduate Research Fellowship Program

Arylphosphonium Salts (APS) are organic compounds that have both cationic and lipophilic character, which facilitates their transport through plasma membranes and cell walls to accumulate in the cytoplasm or the mitochondria. These cationic lipophiles have been intensely investigated and categorized as potential chemotherapeutic agents as they exhibit a high selectivity for carcinoma cells over normal cells. This is due to the greater need for energy in cancerous cells for their high proliferation, which increases the amount of mitochondrial byproducts relative to normal cells. This, in turn, increases the negativity of the mitochondrial membrane's potential difference, and thus its affinity for cations. Therefore, the presence of APS in the mitochondria of cancerous cells is more prevalent than in that of normal cells. The high selectivity of arylphosphonium salts and their ability to cross plasma membranes is the main target of our interest in studying these molecules. In a previous study, a systematic set of structurally related APS was selected to assess their toxicity and interactions with DNA. Cinnamyl triphenylphosphonium chloride (CTP-Cl), showed the greatest interaction with DNA as determined by DNA gel shift assays and qPCR analyses.

This work examines a set of triarylcinnamylphosphonium salts (TCP's) for DNA binding by varying the substituents on the three phenyl rings of the starting reagent triphenylphosphine while maintaining the cinnamyl group as the fourth substituent. Antibacterial toxicity on other sets of arylphosphonium salts vary as a function of substituents on three of the aryl groups: CH3O>CH3>F~H. A computational model for the interaction of the major groove of DNA and the TCP's gave slightly different results. These studies will define the association of these compounds with DNA and explore their mechanism of action in an effort to understand their effects and selectivity on cancerous cells versus normal cells.

FLUID INTERACTIONS THAT ENABLE STEALTH PREDATION BY THE UPSTREAM FORAGING HYDROMEDUSAE CRASPEDACUSTA SOWERBYI

Kelsey Lucas, Sean Colin, John Costello, *Department of Biology*, Roger Williams University, Bristol, RI; Kakani Katija, *Department of Applied Ocean Physics and Engineering*, Woods Hole Oceanographic Institution, Woods Hole, MA; Eric Klos, *Graduate School of Oceanography*, University of Rhode Island, Narragansett, RI.

RI EPSCoR Summer Undergraduate Research Fellowship Program

Unlike most medusae which forage with tentacles trailing behind their bells, several species forage upstream of their bells using aborally located tentacles. It has been hypothesized that these medusae forage as stealth predators by placing their tentacles in more quiescent regions of flow around their bells. Consequently, they are able to capture highly mobile, sensitive prey. In this study, we used digital particle image velocimetry (DPIV) to quantitatively characterize the flow field around Craspedacusta sowerbyi, a freshwater upstream foraging hydromedusa, to evaluate the mechanics of its stealth predation. We found that fluid velocities were minimal in front and along the sides of the bell where the tentacles are located. As a result, the deformation rates in the regions where the tentacles are located were low, below the threshold rates required to elicit an escape response in several species of copepods. Estimates of their encounter vol ume rates were examined based on flow past the tentacles and trade-offs associated with tentacle characteristics were evaluated.

INTERPERSONAL ATTRACTION WITHIN AND BETWEEN SOCIAL GROUPS

Lorin Kinney, Rachel Traghella, Brandon DeSimone, Thomas Malloy, *Department of Psychology*, Rhode Island College, Providence, RI

RI-INBRE Summer Undergraduate Research Fellowship Program

The social context hypothesis states that interpersonal behavior is moderated to meet the norms of different social groups. Consequently, social identity is group specific. Research on trait perception has demonstrated that within social groups a person's traits are judged similarly by others, whereas across groups there is much less agreement about those traits. The present research used the key person design with a sample of 250 participants from three social groups (family, friends, co-workers) to study interpersonal attraction and affect within and between social groups. Agreement of liking-disliking for a person was expected with groups, whereas across group interpersonal affect was expected to be inconsistent. Results supported this hypothesis. Accumulating evidence suggests that social identity is group specific because people adjust behavior to meet the specific norms within different social groups.

THE DISTRIBUTION OF THE AMYLOID PRECURSOR PROTEIN OF ALZHEIMER'S DISEASE AT THE NEURONAL SYNAPSE

Nicholas DeLeo, Nicholas Marcello, Joseph DeGiorgis, *Department of Biology*, Providence College, Providence, RI

RI-INBRE Summer Undergraduate Research Fellowship Program

Abeta, the predominant peptide that builds up in the lesions of Alzheimer's patients is a fragment of a larger amino acid sequence termed the amyloid precursor protein (APP). Mutations in the gene that encodes APP lead to early onset forms that are heritable. Furthermore, the APP gene lies on chromosome 21 and individuals with Trisomy 21 (Down's syndrome) have an additional APP gene and often develop AD due to a dose effect. Although, APP is clearly a causal component of this devastating neurodegenerative disorder, little is known about its wild-type function or how mutations in the gene manifest into the debilitating disease. Recently, a few studies reported that APP resides in the presynaptic terminal and is associated with synaptic vesicles, a finding that is intriguing. However, here in our early investigations using confocal microscopy we found that APP occupies a distinct and non-overlapping distribution that lies adjacent to the presynaptic vesicle pool. At present we are carrying out EM immuno-localization experiments to determine the specific distribution of APP as it relates to the synaptic terminal.

RESTORED CORALS AS FISH HABITAT: USE OF ELKHORN CORALS BY JUVENILE 3-SPOT DAMSELFISH

Sarah Merolla, *Department of Biological Sciences*, University of Rhode Island, Kingston, RI; Graham Forrester, *Department of Natural Resources Sciences*, University of Rhode Island, Kingston, RI

RI EPSCoR Summer Undergraduate Research Fellowship Program

In response to the recent decline in coral cover, various restoration methods have been used to rebuild coral populations. Coral reefs provide essential habitat for many reef fish, and thus it would be expected that coral restoration could recreate habitat for multiple species. In this study we monitored three-spot damselfish juveniles at a restored elkhorn reef in the British Virgin Islands. The purpose of this study was to determine whether three-spot damselfish would use transplanted Acropora palmata as a habitat. From when we first monitored the study site in 1992 until 2005, the reef had always lacked both live elkhorn corals and three-spot damselfish juveniles. Starting in 2005, we began transplanting elkhorn coral fragments to the study site. In 2010 we observed several three-spot damselfish juveniles on the reefs associating with the elkhorn transplants, and began to systemically monitor the juveniles on the reef each year. After three years of monitoring, it became apparent that most individual juveniles are associated with transplanted Acropora palmata. Therefore, we deduced that the three-spot damselfish are using the elkhorn transplants as habitat. This study demonstrates that coral restoration not only rebuilds coral populations, but also aids in the creation of habitat for other species.

ISOLATION AND STRUCTURAL IDENTIFICATION OF COMPOUNDS FROM BOXELDER MAPLE (ACER NEGUNDO) SAPWOOD

Michael Millen, Susan Meschwitz, *Department of Chemistry*, Salve Regina University, Newport, RI; Navindra Seeram, *Department of Biomedical and Pharmaceutical Sciences*, University of Rhode Island, Kingston, RI

Salve Regina University Department Work Study

Phytochemicals are non-nutritive plant chemicals that have protective or disease preventative properties. Polyphenols are phytochemicals that are found to have antioxidant properties and are potentially beneficial to the medical community. The Boxelder Maple (Acer negundo) is a species that is indigenous throughout eastern North America in which the chemical constituents have not been fully investigated. In order to isolate the polyphenols from Acer negundo, a series of methanol extractions were taken from the sapwood of the tree. After liquid-liquid partitioning, the ethyl acetate soluble fraction was subjected to column chromatography with silica and Sephadex LH-20, followed by preparative thin layer chromatography and semi-preparative high performance liquid chromatography (HPLC) in order to continue the purification of these polyphenols. Nuclear magnetic resonance (NMR) and mass spectroscopy (MS) will later be used on the purified compounds in order to propose structures, and assays will be run in order to determine the antioxidant properties of these compounds.

THE CHARACTERIZATION OF CD1034 FROM CLOSTRIDIUM DIFFICILE, A MEMBER OF THE POORLY CHARACTERIZED GLYCOSYL HYDROLASE FAMILY 73

Ryan D. Miller, Zachary Suter, Danielle Gutelius, Kirsten Hokeness, Christopher Reid, *Department of Science and Technology*, Bryant University, Smithfield, RI

RI-INBRE Summer Undergraduate Research Fellowship Program

Peptidoglycan (PG) is composed of repeating, alternating units of β-1,4N-acetylglucosamine (GlcNAc) and N-acetylmuramic acid (MurNAc), which are cross linked by the peptide tetramer extending off MurNAc, making it a meshed, sheath-like structure. PG is a major component of the bacterial cell wall. This project seeks to characterize the protein encoded for by the CD1034 gene in Clostridium difficile strain 630, which is a highly conserved cell wall-acting enzyme. It is believed that the protein in question is an endo-β-N-acetylglucosaminidase (GlcNAcase), meaning that it is an enzyme which cleaves peptidoglycan between GlcNAc and MurNAc. This enzyme's function classifies it under the glycosyl hydrolase 73 CAZy family, which hydrolyzes the glycosidic bonds which hold PG subunits together. Little is known about this class of enzymes, despite being involved in many essential functions of bacterial life. In characterizing GlcNAcases, there is potential for exploiting their function for the purposes of antimicrobial development. By potentially inhibiting GlcNAcases, the specific jobs the proteins are tasked with (septum degradation, exporting of toxins, recycling of cell wall, assembly of flagella, etc.) will be impossible. The inhibition would severely weaken the bacteria, and thereby help patients fight off infection. Discovering expression conditions, purification methods, and characterization of this protein's binding affinity for PG were the main goals of this project. Researching this protein's binding affinity may lead to development and the discovery of substrate inhibitors for future projects. A variety of expression conditions were explored: temperatures, media, and induction agent concentrations. It was found that the most soluble protein was produced at 18˚C within terrific broth with an isopropyl thiogalactopyranocide (IPTG) concentration of 0.025mM. Due to the weak binding affinity for the His-tag on the N-terminus, we are attempting to create a His-tag fusion protein of CD1034 for the C-terminus.

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

Andrew Mitchell, Avelina Espinosa, *Department of Biology*, Roger Williams University, Bristol, RI; Lauren Salerno, *Department of Biology and Chemistry*, Roger Williams University, Bristol, RI; Lauren Rossi, *Department of Chemistry*, Roger Williams University, Bristol, RI

ASM Undergraduate Research Fellowship Program

Entamoeba histolytica is the leading cause of amebic dysentery worldwide and whose infection plagues developing nations. A bifunctional iron(II) and NAD+ dependent alcohol dehydrogenase enzyme, E. histolytica alcohol dehydrogenase 2(EhADH2), catalyzes the last two step in the parasite's fermentative pathway. EhADH2 catalyzes the conversion of acetyl-CoA 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. Past pyrazoline carbinol compounds have been shown to competitively inhibit the activity of EhADH2 at concentrations non-toxic to humans. We synthesized three series of pyrazoline derivatives. Preliminary testing of these compounds exhibited successful inhibition of EhADH2 by two of these series. Recombinant E. coli cells expressing EhADH2 were cultivated and stored as pellets. These pellets were lysed via sonication and EhADH2 was isolated by dialysis followed by FPLC. The isolated EhADH2 was used to measure enzymatic activity in the presence of the inhibitor. To further characterize these inhibitors Ki values will be determined.

THE ISOTHIOCYANATE, SULFORAPHANE, ALTERS THE ACIDIFICATION OF THE VACUOLE TO TRIGGER YEAST CELL DEATH

Michael Murphy, Douglass Tucker, Stacy Thomas, Alexander Wilcox, Nicanor Austriaco, *Department of Biology*, Providence College, Providence, RI; Stephan Patrick Joly, *Department of Biology*, Catholic University, Washington, D.C.

RI-INBRE Summer Undergraduate Research Fellowship Program

The isothiocyanate, sulforaphane (SFN), isolated from broccoli and other cruciferous vegetables is a potential chemotherapeutic agent. Recent studies in mammalian cells have suggested that SFN works by causing cell cycle arrest and/or initiating programmed cell death (PCD). However, the precise mechanism by which SFN kills cells remains elusive at this time. In order to identify genes that may be involved in the cell's response to SFN, we initiated a genetic screen using the yeast knockout (YKO) library of the budding yeast, Saccharomyces cerevisiae, to identify loss of function mutants that are sensitive to SFN. The data from the screen in unison with that of a parallel microarray analysis narrowed the focus of our study to genes involved in vacuolar acidification and alkalization. We have identified 311 SFN sensitive and 11 SFN resistant mutants in yeast ORFs that had been previously linked to vacuolar acidification. The identity of these genes suggested that SFN kills cells by making the yeast vacuole more basic. This hypothesis was confirmed with confocal microscopy that compared the vacuole acidification levels of these cells before and after they were cultured in SFN.

SCREENING OF GLYCOSYL TRIAZOL BASED INHIBITORS FOR ANTIMICROBIAL ACTIVITY

Christina Nadolny, Kirsten Hokeness, Christopher Reid, *Department of Science and Technology*, Bryant University, Smithfield, RI; Amit Basu, *Department of Chemistry*, Brown University, Providence, RI

RI-INBRE Summer Undergraduate Research Fellowship Program

The need for development of new antibiotic treatment to antibiotic-resistant pathogens has been identified by the Infectious Disease Society of America (IDS) as an issue of critical importance to America. Multi-drug resistance in bacteria has increased over the past several decades and has proved to be a tremendous problem.

In an attempt to identify novel compounds with antimicrobial activity, microbial whole cell assays were run on a variety of Gram positive and negative organisms. Assays were performed on a series of glycosyl triazole based inhibitors prepared in the laboratory of Dr. Basu (Brown University). The advantage of whole cell screening allows us to identify active compounds that are capable of being taken up by the bacterial cell. We are investigating both spore germination (gram positives) and vegetative cell assays (gram positive and negative). The inhibitors are intended to exploit unique protein-ligand contacts in lytic transglycosylases (LTs) and N-acetylglucosaminidases (GlcNAcases), which are key building blocks of bacteria cell wall.

The initial inhibitor screening identified compounds that seem to be showed a minimum inhibition of 20%. Preliminary results show that GNAz11 and GNAz15 have inhibition of over 20% for Branhamella catarrhalis, GNAz11, GNAz13, GNAz14, GNAz15, GNAz16, GNAz17, and GNAz18 for Escherichia coli DH5-alpha, minimal inhibition for Bacillus cereus and Pseudomonas aeruginosa, and no inhibition for Staphylococcus aureus or Candida albicans. The aim is to identify new lead compounds that may represent a new class of antibacterial agents.

3D CENTER FOR BIOMEDICAL SCIENCES: ANIMATION, VISUALIZATION, AND PRINTING

Stephen Norris, Jeffrey Ferrucci, *3D Center for Biomedical Sciences: Animation, Visualization, and Printing*, University of Rhode Island, Kingston, RI

3D Animation at URI

Scientific visualization can offer additional instruction for students outside of the classroom. Animations and interactive games allow students to review material outside of class. Difficult material can be presented in multiple ways to help reinforce the concepts discussed in the lectures. 3D animation is an effective way of showing complex chemical reactions or any process that textbooks may not be able to express as easily in words and pictures. A visual representation in collaboration with a voiced over explanation of a process can assist students in comprehending confusing concepts.

An interactive game discussing the material can further reinforce the material. It is a simple extension of a 3D animation utilizing something interactive to reinforce the concepts of the animation. Like an animation, students are provided with a visual representation of the process. However, students can be asked questions testing their comprehension of the concept, breaking animations down into an multiple events and responses from the students.

The Secretory Pathway project's goal is to take the student step by step through the Secretory Pathway and to test the student's complete continuous understanding of the process. Questions are accompanied by corresponding animations to illustrate the process they are trying to understand.

CYCLIC POLYARGININE PEPTIDE-FATTY ACID CONJUGATES: SYNTHESIS AND COMPARATIVE CELLULAR UPTAKE STUDIES

Kevin Northup, Donghoon Oh, Keykavous Parang, *Department of Biomedical and Pharmaceutical Sciences*, University of Rhode Island, Kingston, RI

RI-INBRE Summer Undergraduate Research Fellowship Program

University of Rhode Island College of Pharmacy Dean's Fellow

Positively-charged linear cell-penetrating peptides (CPPs) have been previously shown to enhance the cellular uptake of cell-impermeable compounds across cellular phospholipid bilayer. The cellular delivery of negatively-charged phosphopeptides by linear CPPs still remains a major challenge. More stable cyclic CPPs have not been explored extensively as molecular transporters. Herein, we report design and evaluation of cyclic polyarginine peptides attached to a fatty acyl chain for cellular uptake studies of phosphopeptides. Cyclic pentaarginine [R5] peptide conjugated with 12-dodecanoyl (10 μM) increased cellular uptake of GpYEEI (5 μM) in human ovarian carcinoma (SK-OV-3) cancer cell line by 2.6 fold versus that of cyclic pentaargine [R5] without a dodecanoyl chain (10 μM). A number of cyclic [R5] peptides containing shorter (C8) and longer (C16) fatty acyl chains were synthesized to determine the effect of chain length on cellular delivery of phosphopeptides. Cyclic [R5] peptide was synthesized using Fmoc solid-phase peptide synthesis from H-Arg(Pbf)-2-chloro-trityl resin through coupling of four arginine residues and one lysine. The peptide was conjugated with fatty acids of varying lengths (C8, C12, and C16) via a single lysine (K) residue. Cyclic peptide-fatty acid conjugates were purified by reversed-phase preparative HPLC. The structure of the peptide-fatty acid conjugates was confirmed by MALDI-TOF mass spectrometry. Herein, we report comparative cytotoxicity and molecular transporter ability of peptides for a negatively-charged phosphopeptide in SK-OV-3 cell line.

DOES THE ENDOPLASMIC RETICULUM UNDERGO FRAGMENTATION DURING YEAST CELL DEATH?

James O'Brien, *Department of Biology*, Providence College, Providence, RI

RI-INBRE Summer Undergraduate Research Fellowship Program

The budding yeast, Saccharomyces cerevisiae, is known to undergo programmed cell death when cultured in ethanol, a phenomenon accompanied by fragmentation of the mitochondria. Unexpectedly, by visualizing an ER-localized Sec63p-RFP, we have observed what appears to be fragmentation of the endoplasmic reticulum. Using a genetic approach, we have also identified three mutants in BXI1, VPS1, and FZO1, which appear to have fragmentated ER. Bxi1p is an ER localized protein, Vps1 is a vacuole-localized protein implicated in vacuole structure and fusion, and Fzo1p is a mitochondrial-localized protein linked to mitochondrial fusion and fission. We have begun characterizing this phenotype using fluorescence loss in photobleaching (FLIP) to determine if the observed punctate pattern is indicative of ER fragmentation. This would be the first demonstration of ER fragmentation in yeast.

BLUE CRAB ABUNDANCE AND SIZE DISTRIBUTION IN THE NARRAGANSETT BAY AND TIDAL RIVERS (RI/MA, USA)

Michael Pallotta, David Taylor, *Department of Marine Biology*, Roger Williams University, Bristol, RI

RI EPSCoR Summer Undergraduate Research Fellowship Program

The blue crab, Callinectes sapidus, is a temperate species that occupies Mid-Atlantic estuaries during several life history stages. Recent empirical data, however, indicate that the abundance of blue crabs has significantly increased in southern New England estuaries. The objectives of this study were to analyze spatial and temporal patterns in blue crab abundance and size-frequency distribution in the Narragansett Bay and its associated tidal rivers (RI/MA, USA). Stomach content analysis will also reveal the prey items that the crabs are feeding on. Data were collected from the Juvenile finfish seine survey by the Rhode Island DEM, who recorded the abundance and size of the blue crabs captured at 18 stations throughout Narragansett Bay. This abundance data from 2009-2011 suggests that the greatest number of crabs populate the area in July, with the highest amount in the year 2010. Blue crab specimens were also collected in the RWU survey from three sites at different locations in each the Seekonk and Taunton rivers May through July. The stomach contents of these specimens were extracted from the crabs and preserved. After the contents were preserved, preliminary visual analysis of the stomach contents revealed that the crabs feed primarily on bivalves and crustaceans, as well as some small bony fish. Future work on this project will include a genetic analysis of the stomach contents using environmental PCR to specifically identify prey items and stable isotope analysis of claw tissue to verify stomach content data.

MYOSIN VI ASSOCIATES WITH MOTILE PIGMENT GRANULES IN THE SQUID PHOTORECEPTOR

Ryan Paranal, Joseph DeGiorgis, *Department of Biology*, Providence College, Providence, RI

RI-INBRE Summer Undergraduate Research Fellowship Program

Within the squid photoreceptor, pigment granules migrate within a central shaft in response to light and act as molecular sunglasses to shade the photosensitive regions of the cell. Here, by electron microscopy, we find that these granules are 1-μm teardrop-shaped organelles that are in contact with multiple microtubules. This suggests that granule migration is a microtubule-based process. At the contact points between the granule and microtubule, we find electron-dense specializations that appear as patches within the granule membrane and extend to the exterior cytoplasm, which may link the organelle to microtubule-based motors. In addition, by confocal microscopy, pigment granules contain myosin VI on their surfaces and are bound to the cortical actin network. Currently, we are working on cloning and expressing the myosin VI tail to serve as a positive control for biochemical investigations.

DEVELOPING A TOOL FOR COLLABORATIVE STOCK ASSESSMENT OF QUAHOGS IN NARRAGANSETT BAY

C.R. Pellichero, *Department of Biology*, Providence College, Providence, RI; D.F. Leavitt, *Department of Biology and Marine Biology*, Roger Williams University, Bristol, RI

RI EPSCoR Summer Undergraduate Research Fellowship Program

Accurate stock assessment is important for keeping commercially fished resources at a sustainable yield. In Rhode Island, the bay quahog (Mercenaria mercenaria) is one of the most important commercial fisheries in Narragansett Bay. The state fisheries management agency assumes the job of stock assessment of these shellfish as part of their management process. However, due to increased responsibilities coupled with decreased staff, the RI Department of Environmental Management is hard pressed to undertake large-scale quahog stock assessments. The goal of this research is to improve the annual quahog stock assessment by establishing groundwork for cooperation between commercial bull rake fisherman and the RI DEM Marine Fisheries Division. We will work to determine a method to assess quahog standing stock through calibrating and standardizing the catch efficiency of a bull rake.

Using an Ashtech Mobile Mapper 100 Differential GPS (dGPS) with antenna, we will record the linear distance of one tow of the bull rake. The dGPS antenna will be mounted on the bull rake's stale with distance measured to sub-meter accuracy through post-processing of the transect. After recording tow length, we can use the width of the different bull rakes to calculate the area of each sample plot to measure the quahog catch per unit area. There will be divers under water to collect the quahogs that were missed by the bull rake to allow us to estimate catch efficiency of the bull rake compared to the known catch efficiency of the standard hydraulic dredge used in the RI-DEM Marine Fisheries' surveys. The size distribution of the catch will be observed and recorded. Once standardized, the fisherman will be recruited to provide expanded site assessments of quahog standing stock using the protocol developed through this research, in collaboration with the routine annual dredge survey of the RI-DEM.

CONSTRUCTION AND CHARACTERIZATION OF A NULL ALLELE OF THE HFQ GENE OF THE DISSIMILATORY METAL REDUCING BACTERIUM SHEWANELLA ONEIDENSIS

Christopher Brennan, Matthew Goulet, Taylor Hunt, Meaghan Keane, Nicholas Mazzucca, Zachary Sexton, Brett Pellock, *Department of Biology*, Providence College, Providence, RI

RI-INBRE Summer Undergraduate Research Fellowship Program

Shewanella oneidensis is a dissimilatory metal reducing bacterium that can utilize a wide variety of terminal electron acceptors under anaerobic conditions, including a variety of soluble and insoluble heavy metals. Our goal is to identify and characterize genes that encode small regulatory non-coding RNAs (sRNAs) in S. oneidensis. sRNA genes are regulated by changes in environmental conditions and can mediate both positive and negative regulatory outcomes by inexact base pairing to their mRNA targets.
The well-conserved RNA chaperone protein Hfq has been widely implicated in bacterial sRNA function. To better understand sRNA function, we have constructed a null allele of the S. oneidensis hfq gene. The S. oneidensis hfq mutant has a slow growth phenotype in exponential phase and saturates at a lower density than wild type cultures under both aerobic and anaerobic conditions. The hfq mutant also exhibits a decreased rate of anaerobic Cr(VI) reduction relative to wild type cells and is highly sensitive to oxidative stress. We are currently further evaluating the growth and survival of the Shewanella hfq null mutant. In addition, we are investigating the mutant's sensitivity to a variety of physical stresses, evaluating the capacity of the hfq mutant to reduce metals other than Cr(VI), and determining the role of Shewanella hfq in sRNA function.

FUNCTIONAL EVOLUTION OF THE MYOGENIC REGULATORY FACTOR FAMILY

C.J. Picket, Katie O'Connor, Thomas Meedel, *Department of Biology*, University of Rhode Island, Kingston, RI

RI-INBRE Summer Undergraduate Research Fellowship Program

Myogenic Regulatory Factors (MRFs) are a class of basic helix-loop-helix (b-hlh) transcription factors involved in regulating muscle development in all animals. Our project was designed to test two questions about MRFs: 1. Are MRFs functionally conserved? 2. Does the alanine/threonine (A/T) dipeptide of the basic domain constitute a myogenic code in non-chordate invertebrates as it does in the chordates? In order to address these questions we used a simple in vivo assay based on misexpressing MRFs in the notochord of Ciona intestinalis embryos. Previous studies in our lab using this assay demonstrated that the Ciona MRF (Ci-MRF) could elicit the expression of a variety of muscle-specific genes in this assay, thereby demonstrating its utility and the myogenic activity of Ci-MRF. Initial experiments using MRFs from different organisms failed to elicit myogenesis in this assay. Sequence comparisons revealed that CiMRF possessed an extensive amino terminal region upstream of the b-hlh domain that was absent from the other MRFs, and we reasoned that this region might contain elements necessary for MRFs to function in Ciona. We tested this idea by generating chimeric constructs in which the b-hlh domain of other organisms was substituted for the corresponding domain of Ci-MRF sequence. Using these chimeric plasmids we show that MRFs from all animals we tested are myogenic and that the A/T dipeptide is indeed required for their complete activity. Our results support the idea that MRFs show extensive functional conservation and that the A/T dipeptide is a critical component of their activity. In anticipation of future experiments designed to identify the crucial functional elements of the large amino terminal domain of CiMRF, we will also present the results of experiments intended to determine if a nuclear localization signal exists in the basic domain of CiMRF.

NICOTINAMIDE PHOSPHORIBOSYLTRANSFERASE (NAMPT) POINT MUTATION GENERATION

Karen Almeida, Chris Funk, Cailyn Mather, Katelyn Pina, *Department of Physical Sciences*, Rhode Island College, Providence, RI

RI-INBRE & RI EPSCoR Summer Undergraduate Research Fellowship Programs

NAD+/NADH are customarily known for their role in oxidation/reduction reactions in biological systems. Recently it has been established that NAD+ is consumed during times of stress. In such situations, increased activity of poly (ADP-ribose) polymerase has been noted resulting in an increase in DNA damage repair. Stressful situations also cause heightened activity of Sirtuin proteins and cellular survival. The increased activity of these protein families results in the conversion of NAD+ to nicotinamide (NAM). As NAD+ levels decrease, the NAD+ salvage pathway is activated in an attempt to replenish the stock. In the two-step process, NAM is recycled back to NAD+. In the first step, nicotinamide phosphoribosyltransferase (NAMPT) converts NAM to nicotinamide mononuculeotide (NMN). This step is the rate-determining step and has been linked to diseases such as cancer, diabetes, and Alzheimer's disease. NAD+ is then regenerated by the adenylation of NMN.

NAMPT is a 55-kDa enzyme existing as a homodimer with two active sites. Currently there are 19 known post-translational modification (PTM) sites reported for NAMPT. The modifications can be the phosphorylation of serine or tyrosine or the ubiquitination of lysine. Our hypothesis states that the PTMs of NAMPT regulate the overall activity of the protein. The PTM will be modified in a series of systematic mutations. Each PTM involving serine will be changed to alanine or glutamate to represent the unphosphorylated or phosphorylated states respectively. Sites involving tyrosine will be mutated to phenylalanine or glutamate to represent the unphosphorylated or phosphorylated states respectively. The ubiquitination sites will be changed to alanine. The first mutations studied will be S199D, S200D, H247A, and K389A. It is the aim of this experiment to gain a more comprehensive understanding of the regeneration of NAD+ while furthering our knowledge on the role of NAMPT in the restoration of NAD+.

A NOVEL MODEL OF HUMAN PHOBIA: THE DISCRIMINATED CONDITIONED PUNISHMENT MODEL

Christopher Bloom, Caroline Doyle, Brenna Peters, Ryan Post, *Department of Psychology*, Providence College, Providence, RI; Josh Mazick, *Department of Psychology and Mathematics*, Providence College, Providence, RI

RI-INBRE Summer Undergraduate Research Fellowship Program

A specific phobia is a marked and persistent fear that is excessive or unreasonable, cued by the presence or anticipation of a specific object or situation. Traditionally, the signaled avoidance (SA) paradigm has been used in an attempt to better understand human phobia. In recent years, animal models of this type have been criticized for ineffectively mimicking phobia. The SA model characterizes phobia as an avoidance behavior by presenting environmental cues which act as warning signals to an aversive stimulus (i.e., shock). Discriminated conditioned punishment (DCP) is an alternative paradigm that characterizes human phobia as a choice behavior using the same warning signals as in SA, but focuses on the punishment of an otherwise adaptive behavior. The present study quantifies the differences between the paradigms and suggests that DCP offers a better paradigm for human phobia. Rats were assigned at random to each of the two conditions (N=16). Data on point of response in the series of warning signals and percentage of trials resulting in shock were collected. Results indicate that rats in the DCP paradigm respond significantly earlier than rats in the SA paradigm (M=1.23; M=2.73), [$F(1,14)=2762.55$, $p<0.001$]. Results indicate that rats in the DCP paradigm were shocked a significantly lower percent of the time than rats in the SA paradigm (M=3.65; M=37.65), [$F(1,14)=62.46$, $p<0.001$]. These results suggest that DCP may be a better model of human phobia than SA.

MACROALGAE & BLUE MUSSELS AS BIOINDICATORS OF HUMAN INFLUENCE IN THE NARRAGANSETT BAY

Margaret Resnick, *Department of Biological Sciences*, University Of Rhode Island, Kingston, RI; Autumn Oczkowski, *Atlantic Ecology Division*, US Envrionmental Protection Agency, Washington, D.C.

RI EPSCoR Summer Undergraduate Research Fellowship Program

Recent concern over nitrogen (N) inputs to Narragansett Bay has led many adjacent sewage treatment plants to upgrade their facilities to tertiary treatment. While these upgrades have cost over $50 million in East Providence alone, the impacts of the N reductions are unknown. While it is generally agreed upon that we should limit the amount of N put into an ecosystem, we do not necessarily have a clear understanding of how N moves through them. In order to observe these types of effects (e.g. where and how far does sewage flow), scientists can use the stable isotopes of N (∂15N) to infer something about the distribution and transport of sewage N in an ecosystem. We developed a new research method to monitor ∂15N in the Narragansett Bay ecosystem. By using mussels as biological autosamplers, we measured nitrogen flow in three dimensions. Five lines were deployed at five sites on June 19, 2012 in the west passage of the Narragansett Bay. Bags of six individual blue mussels (Mytilus edulis) were placed at depths of five, ten, and fifteen feet vertically from the bottom of the bay and retrieved on July 17, 2012. By placing samples at varying depths and locations, we hoped to see variations of ∂15N not only spatially but vertically in the bay. In addition to blue mussels, macroalgae (Ulva sp.) was placed on two separate occasions (June 19 – 26 ,2012 and June 26 – July 3, 2012) on these lines at depths of five and ten feet to serve as a potential secondary source by which we might be able to interpret the flow of dissolved bio-available N (e.g. nitrate and ammonium) through the Bay. Preliminary results suggest that the lines may be an effective way to monitor the distribution of sewage N in Narragansett Bay.

A MOLECULAR ASSESSMENT OF THE DIVERSITY OF BROWN ALGAE IN BERMUDA

Alyssa Rogers, Tom Shamp, Thea Popolizio, Christopher Lane, *Department of Biological Sciences*, University of Rhode Island, Kingston, RI

RI EPSCoR Summer Undergraduate Research Fellowship Program

For nearly 60 years there has been very little investigation of the marine algal flora in Bermuda, which is an ideal location for a biodiversity assessment due to its size and location. Residing approximately 1000km off the coast of North Carolina, Bermuda is influenced by the cool water from the north during winter and by the warm water eddies that spin off the Gulf Stream for the much of the other seasons. The Bermuda Seaweed Project aims to extensively survey the marine algal flora in Bermuda and generate morphological and molecular data for the 450+ red, green and brown Bermudian seaweeds in order to better understand and add to the currently recognized flora. Whereas the focus of the project has thus far been on red algae, data presented here demonstrate similar trends for the brown algae of Bermuda. DNA barcode data were produced for the brown algal samples from the cox1 and rbcL genes of the mitochondrion and plastid, respectively. Phylogenetic trees were produced from these data in order to assess the algal diversity. Based on these analyses, it appears that species diversity in several Bermuda brown algal genera is currently underestimated. Morphological examination of preserved samples will be undertaken to determine whether the newly detected Bermudian species are known from other parts of the world or represent novel species. The Bermuda Seaweed Project will result in Bermuda being the first country to have is its entire algal flora catalogued morphologically and molecularly, resulting in the most comprehensively described marine flora in the world.

A GENETIC SCREEN FOR HIGH COPY SUPPRESSORS OF BXI1 IN THE BUDDING YEAST, SACCHAROMYCES CEREVISIAE

Stephen Rogers, Matthew Hurton, Nicanor Austriaco, *Department of Biology*, Providence College, Providence, RI

RI-INBRE Summer Undergraduate Research Fellowship Program

Bax inhibitor-1 (BI-1) is an anti-apoptotic gene whose expression is upregulated in a wide range of human cancers. Our laboratory has published data suggesting that the yeast gene, BXI1, is a homolog of BI-1, which links the unfolded protein response and programmed cell death. We have initiated a high copy suppressor screen using a yeast genomic library to find other genes that will help us better understand the function of BXI1. These include genes that function in a manner similar to that of BXI1 or genes that operate within the same pathway as BXI1. We are both selecting for and screening for high copy suppressors that allow Δbxi1 cells to survive culture conditions known to induced programmed cell death in yeast, including high temperature, addition of β-mercaptoethanol, a drug known to induced the unfolded protein response, and hydrogen peroxide, a reactive oxygen species. The screen and selection are currently underway, and several potential candidate mutants have been identified.

IDENTIFICATION OF ANTIGENIC TARGETS OF CANDIDA ALBICANS SPECIFIC ANTIBODY FRAGMENTS

Keyana Roohani, Kristie Aicardi, Christopher Reid, *Department of Science and Technology*, Bryant College, Smithfield, RI; Sonia Nesbitt, Joseph Bliss, *Department of Pediatrics*, Women and Infants Hospital, Providence, RI

Rhode Island Science & Technology Advisory Council

Candida albicans is an oppurtunistic pathogen that inhabits the mouth, throat, intestines, and genitourinary tract. These human fungal pathogens are guides for studies of other pathogenic fungi. The hyphal form of C. albicans is the pathogenic form of the organism. In immune compromised patients such as neonates, this organism can cause systemic infection with high mortality rates. In associaton with Women and Infants hospital, we have been endeavering to identify the antigenic target of the specific antibody fragment scFv12, which has been shown to recognize the hyphal cell surface. The purpose for this project is to develop an extraction process for identifying teh antigen recognized by the scFv fragment. Affinity chromatography cyanogen bromide agarose-scFv12 resin, was employed to purify the antigen from a lyticase solubilized fraction of C. albicans hyphae. The protein antigen was then subject to proteomic analysis by mass spectrometry.

LEAF LITTER DECOMPOSITION AND PROCESSING IN A CARIBBEAN MANGROVE FOREST AND ITS IMPLICATIONS FOR CARBON SEQUESTRATION

Ryan Rossi, *Department of Marine Biology*, University of Rhode Island, Kingston, RI; Jessen Brita, *Department of Oceanography*, Graduate School of Oceanography, University of Rhode Island, Narragansett, RI; Johnson David, *The Ecosystems Center*, Marine Biological Laboratory, Woods Hole, MA

RI EPSCoR Summer Undergraduate Research Fellowship Program

Mangrove ecosystems are highly productive coastal wetlands that provide ecological services on local and global scales. Mangroves are potential sites of carbon sequestration due to this high productivity and efficient carbon storage within tree and leaf biomass. An essential process for carbon sequestration is organic matter processing, which includes macro-invertebrate shredding and microbial decomposition activities. This study investigates the effect of macro-invertebrate (e.g., crabs) shredder activity on the rate of leaf litter decomposition and processing. Fallen leaves of Rhizophora mangle were collected from three sections within the Jobos Bay National Estuarine Research Reserve (Aguirre, Puerto Rico) in a fringing mangrove forest. Collected litter was deployed in mesh bags that excluded macro-invertebrates or bundled by monofilament to allow for macro-invertebrate shredder activity. Litterbags and leaf bundles were incubated on the mangrove platform surface and buried in crab burrows. Replicates were removed at intervals up to 24 days. The rate of litter processing was determined by mass loss of leaves and shredder activity was measured by loss of leaf area. While no significant loss of leaf material due to microbial decomposition was observed within the 24-day incubation, a significant effect of macro-invertebrate shredding on the mangrove platform and within the crab burrows was observed. These results illustrate the major role of macro-invertebrate activity for organic matter cycling in a coastal mangrove forest.

COMPARISON OF RIBBED MUSSEL POPULATIONS IN COASTAL MARSHES ALONG A NITROGEN-LOADING GRADIENT IN NARRAGANSETT BAY

K. Schoolcraft, J. Hall, B. Govenar *Department of Biology*, Rhode Island College, Providence, RI; S.M. Moseman-Valtierra, *Department of Biological Sciences*, University of Rhode Island, Kingston, RI

RI EPSCoR Summer Undergraduate Research Fellowship Program

Coastal marshes are critically important ecosystems that serve as buffers from storms, filters for runoff water entering the ocean, and nurseries for migratory fish and bird species. With increasing residential development and human activities along the coastline, marshes are subjected to greater inputs of nutrients and pollutants. As part of a larger project to investigate the flux of greenhouse gasses from marshes resulting from elevated surface temperatures, along the nitrogen-loading gradient in Narragansett Bay, we have characterized the variation in ribbed mussel populations at three sites during the summer of 2012. Similar to previous work conducted at the same sites in 1998 and 1999, there was a positive correlation between mussel density and nitrogen-loading, but an inverse relationship with mussel size. These results will be compared to growth rates, age, and fecundity, as well as gut content and tissue stable isotope analyses to identify the underlying mechanisms of how nitrogen-loading effects mussel populations and their contribution to greenhouse gas emissions in coastal marshes.

CONSTRUCTION OF A TARGETING VECTOR TO OPTIMIZE SENSITIVITY OF THE ALPHA3 SUBUNIT OF THE NICOTINIC ACETYLCHOLINE RECEPTOR

Christian Selinski, Tanya Sanders, Edward Hawrot, *Department of Molecular Pharmacology, Physiology and Biotechnology*, Brown University, Providence, RI

RI EPSCoR Summer Undergraduate Research Fellowship Program

RI EPSCoR Summer Undergraduate Research Fellowship Nicotinic acetylcholine receptors (nAChRs) are cholinergic receptors that form ligand-gated ion channels in the plasma membranes of certain neurons and on the postsynaptic side of the neuromuscular junction. The α1 subunits of skeletal muscle nAChRs have a high affinity to a competitive antagonist, alpha-Bungarotoxin (α-Bgtx), a component of venom from snakes in the cobra-family (Elapid), whereas nAChRs in neurons are, in general, composed of subunits which are insensitive to α-Bgtx and encoded by ancestrally related genes. We have induced sensitivity to this toxin by substituting two amino acids in the loop C region of the normally Bgtx insensitive α3 subunit with the corresponding residues from the α1 subunit. We made a target vector for incorporation of this mutation into the genome of a mouse. A point mutation that created a frame shift was removed from our initial construct. The desired DNA was identified by diagnostic restriction enzyme cleavage analysis. The new targeting vector will be transfected into a murine ES cell line that will subsequently be used to create a knock in mouse in which the desired toxin-sensitive alteration in the nicotinic acetylcholine receptor will replace the native sequence in the genome.

THE ROLE OF THE RPOE GENE OF PHAEOBACTER SP. STRAIN S4 ON GROWTH, TROPODITHIETIC ACID PRODUCTION AND BIOFILM PRODUCTION

Eric Shabashevich, Wenhig Zhao, David Nelson, *Department of Cell and Molecular Biology*, University of Rhode Island, Kingston, RI

RI-INBRE Summer Undergraduate Research Fellowship Program

Background: Recent studies suggest that bacterial strains belonging to the genus Phaeobacter have strong inhibitory activity against pathogenic marine bacteria. Phaeobacter sp. strain S4 was isolated from the inner shell surface of an oyster and found to have probiotic activity, protecting oyster larvae from infection by two different bacterial pathogens of oyster larvae, Vibrio tubiashii and Roseovarius crassostreae. Probiotic activity of Phaeobacter species may be affected by growth rate, tropodithietic acid (TDA) production, biofilm formation, the ability to attach to the oyster, and survival under stressful environments. The purpose of this study was to investigate the effects of mutation to the gene encoding the alternative sigma factor, rpoE, upon Phaeobacter S4.

Methods: Phaeobacter S4 wild type and rpoE mutant strains were obtained and subjected to several different tests. Growth rate was determined by optical density at 600nm and by colony forming units (CFU) using serial dilution and plating. TDA secretion was determined by a bioassay using Phaeobacter cells to inhibit Vibrio anguillarum growth. Biofilm formation was determined by crystal violet staining. Survival under stress conditions was examined using heat shock and starvation.

Results: The rpoE mutant exhibited slower growth rate, TDA production, and biofilm formation compared to the wild-type. Heat shock and starvation stress tests did not show any significant difference between the mutant and wild type.

Conclusions: Our data suggest that rpoE is involved with the regulation of growth, TDA production, and biofilm formation of Phaeobacter S4. Growth rate, TDA production, and biofilm formation are all important for the probiotic activity of Phaobacter S4 and may require expression of rpoE for full activity.

INVESTIGATION OF STRUCTURAL AND THERMODYNAMIC RATIONALE BEHIND THE SEQUENCE DEPENDENT NUCLEOTIDE EXCISION REPAIR OF BULKY CLUSTER DNA LESIONS

Annalisa Sharkey, Vipin Jain, Bongsup Cho, *Department of Biomedical and Pharmaceutical Sciences*, University of Rhode Island, Kingston, RI

RI-INBRE Summer Undergraduate Research Fellowship Program

University of Rhode Island College of Pharmacy Dean's Fellow

DNA is under constant assault, resulting in different types of DNA damage. When a polymerase encounters a lesion, it can bypass by replicative polymerase, either inserting the correct base (error-free) or incorrect base (error-prone). Arylamine carcinogens are implicated in the etiology of various sporadic human cancers and may form C8-substituted dG adducts in vivo. These DNA adducts are known to exist in a mixture of three distinct conformations; major groove (B), stacked (S), wedged (W), and their population ratio depends on the type of lesion and its neighboring bases. 2-Acetylaminofluorene (AAF) is a commonly studied arylamine carcinogen and an excellent substrate for nucleotide excision repair. We reported previously that the E. coli UvrABC system removes the dG-AAF adduct in the NarI sequence (5'--CG1G2CG3CC--3') in a sequence dependent manner. The results showed that dG-AAF at G1 and G3 exhibit higher ratios of syn S- and W-conformers, resulting in greater repair efficiencies. The work was extended to cluster adducts, i.e. dG-AAF at G1G2, G2G3, and G1G3. Dramatic sequence effects were observed in their repair efficiencies with the order of reparability as G2G3 > G1G3 > G1G2. The circular dichroism (CD) and UV-melting results revealed that dG-AAF at G2G3 destabilizes and destacks the duplex more than G1G3 and G1G2 making it a better repair substrate. We hypothesize that the severity of base destacking and duplex destabilization largely depends on the distance of bases between the two adducts in a di-adduct duplex. In this study, we designed duplexes in which the two adducted guanines were separated by growing numbers of cytosine starting from 0 to 3. Our results confirm that the di-adduct duplex with one base gap is destacked and destabilized the most whereas increases in the gap assists the duplex to slightly regain stability, thus making the distortion less recognizable.

STUDIES DIRECTED TOWARD THE SYNTHESIS OF N-ACYL CYCLOSERINE DERIVATIVES AS POTENTIAL QUORUM SENSING INHIBITORS

Sarah Showalter, Susan Meschwitz, *Department of Chemistry*, Salve Regina University, Newport, RI; David Rowley, *Department of Biomedical and Pharmaceutical Sciences*, University of Rhode Island, Kingston, RI

RI-INBRE Summer Undergraduate Research Fellowship Program

Bacterial infections are a leading cause of death in children and the elderly in the United States. Unfortunately the overuse of antibiotics to treat bacterial infections has contributed to a rise in drug resistance, resulting in many strains of bacteria being increasingly more difficult to treat. Many pathogenic bacteria communicate with each other through quorum sensing, a phenomenon which uses small molecules called autoinducers to control virulence factors necessary for infection. These autoinducers offer the opportunity to investigate and inhibit quorum sensing systems at the molecular level and provide a potential route to novel antibacterial therapeutics. The long-term goal of this project is to synthesize molecules with the potential to mitigate the toxic effect of bacteria by down regulation of quorum sensing. We have chosen as our target molecules N-acylated derivatives of cycloserine, the FDA approved antibiotic for the treatment of tuberculosis. These derivatives will be structurally similar to the N-acyl homoserine lactones (AHLs), the most studied and understood autoinducers of quorum sensing. A one-step synthesis to prepare a library of N-acyl cycloserine derivatives is underway. In order to identify key structural features that affect quorum sensing modulation, the acyl groups will differ extensively in terms of chain length and the type and placement of functional groups. For acyl groups that include a phenyl ring, we will examine the inhibitory effects of the nature and position of substituents on the ring. All derivatives will be tested for their inhibition of quorum sensing in sensor strains, specifically, bioluminescence inhibition in Vibrio harveyi and E. coli JB525, and pigment production in Chromobacterium violaceum. The molecules 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.

FASTING ALTERS DRUG UPTAKE TRANSPORTER GENE EXPRESSION IN LIVERS OF FASTED C57BL/6 AND LEPOB/OB (OB/OB) MICE

Adam Silva, Jialin Xu, Angela Slitt, *Department of Biomedical and Pharmaceutical Sciences*, University of Rhode Island, Kingston, RI

RI-INBRE Summer Undergraduate Research Fellowship Program

The liver relies upon a network of membrane associated transport proteins, which bring chemicals from the blood into hepatocytes, and then out of hepatocytes into bile. Organic anion transporting polypeptide (Oatp) and Organic cation transporter (Oct) family mediate uptake of chemicals from blood into hepatocytes. Sodium taurocholate co-transporting polypeptide (Ntcp) is also critical for liver clearance, as it participates in enterohepatic bile acid recirculation. As the incidence of obesity has increased over the past two decades, understanding how diet or nutritional status intersects with regulation of transporter expression is important for better understanding of liver clearance. Fasting is a common practice used in dieting and before surgery. How obesity impacts fasting regulation of liver clearance mechanisms is of importance given the number of obese individuals who may partake in the latter practices and may also use medications or herbal remedies. The purpose of this study was to determine whether Oatp1, Oct, and Ntcp is altered in liver after fasting in lean and obese mice. Lean (C57BL/6) and Obese (Lepob/ob, ob/ob) were fed Ad libitum (fed) or food-deprived (fasted) for 24 hours. Livers were collected, total RNA was isolated, cDNA was then synthesized, and quantitative real-time PCR was performed. Target gene expression was normalized to B2M expression. In fed mice, Oatp1a1, 1a4, 1b2, 2b1, and Oct1 mRNA expression was decreased, whereas Oct2 and 3, and Ntcp were increased in livers of ob/ob mice compared to lean mice. In lean mice, fasting decreased Oatp1a1, 1a4, 1b2, 2b1, Oct1, and Ntcp, and increased Oct2 and 3 mRNA expression in liver. In ob/ob mice fasting decreased Oct2 and Ntcp, and increased Oatp2b1, Oct1 and 2 mRNA expression in liver. In summary, gene expression differences were observed in livers from lean and obese mice, as well as after fasting.

CLONING AND SEQUENCING OF CDNA-AFLP FRAGMENTS OF FOUR DIFFERENT LEISHMANIA SPECIES

Bridgett Smith, Erika Albretsen, *Department of Biology*, Salve Regina University, Newport, RI; Alison Shakarian, *Department of Biology and Biomedical Sciences*, Salve Regina University, Newport, RI

RI-INBRE Summer Undergraduate Research Fellowship Program

Leishmania, a protozoan parsite, is known to be morphologically indistinguishable among various species. However, when examining the pathogenicity of each species, some are known to be pathogenic, causing cutaneous or visceral disease in humans, while others are non-pathogenic. Cutaneous leismaniasis produces an ulcerated skin leison, while visceral leismaniasis causes a distended abdomen, due to infection and inflammation of the liver and spleen. We hypothesize that uniquely expressed proteins may contribute to the differences seen in the pathogenicity among species. Thus, in the current study, cDNA-AFLP (amplified length fragment polymorphism) technique was used to identify uniquely expressed molecules among four species: L. donovani, L. major, L. tarantole, and L. mexicana. As such, the primer set ACC-CTC was used to amplify the cDNA of the four species. The AFLP reactions were run on agarose gels, to allow for the identification of unique and polymorphic fragments among the species. Results showed a unique amplified fragment of approximately 550 base pairs that was produced with L. donovani cDNA. The cDNA-AFLP fragments were cloned using a TOPO-TA cloning kit and verified by colony PCR. Sequencing results of positive clones showed a unique fragment for L.donovani of 566 base pairs which contained both the ACC and CTC primers. Further analysis indicated that the AFLP fragment from L. donovani coded for an ATP-binding casette (ABC) protein. The role of ABC proteins in the biology of Leishmnaia are currently being investigated to identify a possible link to species specific pathogenicity among this important group of human parasites.

NOVEL MULTI-LABELED MAGNETIC BEADS WITH POLYMER BRUSHES FOR ULTRA-SENSITIVITY ELECTROCHEMICAL DETECTION OF PROTEIN CANCER BIOMARKERS

Morgan Smith, Kara Lombardo, Gregory Keras, Bernard Munge, *Department of Chemistry*, Salve Regina University, Newport, RI

RI EPSCoR Summer Undergraduate Research Fellowship Program

Background and Objectives: Rapid, extremely sensitive and accurate biosensor arrays for clinical measurements of biomarker proteins for early detection and monitoring of cancer are critically important and will lead to inexpensive devices for reliable on-the-spot cancer diagnosis, improved therapeutic outcomes at lower costs, decreased patient stress, and new targeted therapies. Methods: Herein we report on ultrasensitive GSH-AuNP immunosensor based on novel multi-labeled magnetic beads, (HRP/MB/Ab2)-PEG with specially designed polyethylene glycol polymer brushes to minimize non-specific binding (NSB) and particle aggregation. The GSH-AuNPs were bioconjugated to the primary antibodies (Ab1) and used to capture a cancer biomarker, human interleukin-6 (IL-6) in a sandwich electrochemical immunoassay coupled to horseradish peroxidase enzyme labels. Results: The “stealth” (HRP/MB/Ab2)-PEG bioconjugate gave extremely low NSB resulting in a remarkable long linear dynamic range of 105 and ultralow DL of 10 fg/mL (500 aM) for electrochemical detection of IL-6 in 10 μL serum. Accuracy of the immuonsensor was determined by measuring IL-6 in head and neck squamous cell carcinoma (HNSCC) cell lines with excellent correlation to the standard ELISA method. Conclusions: These (HRP/MB/Ab2)-PEG based immuonsensors show great promise for the fabrication of ultrasensitive biosensor microarrays for point-of-care cancer diagnosis.

AGGREGATIVE BEHAVIOR, PHYLOGENETIC RELATEDNESS AND PATHOGENESIS IN ENTAMOEBA

Hanna Sobon, Kevin Schindelwig Franca, Layla Ferland, Avelina Espinosa, *Department of Biology*, Roger Williams University, Bristol, RI

RI-INBRE Summer Undergraduate Research Fellowship Program

The purpose of this study was to determine the mechanisms of aggregative discrimination in three strains of Entamoeba and analyze if aggregation is essential in pathogenesis. Positive control experiments and paired experiments with fluorescent dye were conducted to observe the interactions between three different strains: E. moshkovskii snake, E. invadens IP-1, and E. terrapinae. One paired experiment was performed observing the aggregation interactions between E. moshkovskii snake and E. terrapinae, and a second between E. invadens IP-1and E. terrapinae. Over a span of 36 hours there was little aggregation between E. terrapinae and E. invadens IP-1, and higher levels of aggregative behavior between E. moshkovskii snake and E. terrapinae. The highest levels of interaction were observed between members of the same population (e.g. E. terrapinae with E. terrapinae). This study suggests that E. terrapinae and E. moshkovskii might be more closely related phylogenetically than E. terrapinae and E. invadens IP-1. The next step is to determine if aggregative signals are used in pathogenic processes.

SELECTIVE UPTAKE OF FLUORESCEIN-LABELED ARYLPHOSPHONIUM SALTS BY NORMAL VS. TUMORIGENIC CELLS

Jose Solares, Alex Amer, Sarah Spinette, *Department of Biology*, Rhode Island College, Providence, RI; John Williams, Jr., *Department of Physical Sciences*, Rhode Island College, Providence, RI

RI-INBRE Summer Undergraduate Research Fellowship Program

Aryl phosphonium salts (APS) are cationic lipophiles that cross cell membranes. Derivatives with functional groups and fluorescent molecules attached have been synthesized using microwave accelerated organic synthesis (MAOS) and bench top methods. All reactions show good yields and dramatically decreased reaction times using MAOS. In order to determine whether APS can be used in targeting cancer cells, murine mammary cells and metastatic murine mammary cells were cultured in these compounds in order to determine the compound's specificity. The fluorescein attached to the APS made it possible to image the cells with the compound under fluorescent microscopy. The uptake dynamics also show an SAR common to toxicology of these compounds.

MULTIPLEX ELECTROCHEMICAL IMMUNOSENSOR FOR PROTEIN CANCER BIOMARKERS USING NANOSTRUCTURED SWNTS-SPE ARRAYS

Brian Somba, Bernard Munge, *Department of Chemistry*, Salve Regina University, Newport, RI

RI-INBRE Summer Undergraduate Research Fellowship Program

Background and Objectives: Rapid, extremely sensitive and accurate biosensor arrays for clinical measurements of biomarker proteins for early detection and monitoring of cancer are critically important and will lead to inexpensive devices for reliable on-the-spot cancer diagnosis, improved therapeutic outcomes at lower costs, decreased patient stress, and new targeted therapies. Protein arrays for measuring multiple protein cancer biomarkers in clinical samples hold great promise for reliable early cancer detection. Methods: Herein, we report a prototype 2- sensor electrochemical immunoarray based on dual single-wall carbon nanotube screen printed electrodes arrays decorated with gold nanoparticles (SWNT-AuNP) for the simultaneous detection of multiple protein biomarkers for oral cancer in serum. The prototype sensor design utilizes an electrochemical flow cell coupled to a dual sensor electrode array. Results: Immunoarray procedures were designed to measure Interleukin-8 (IL-8) and interleukin-6 (IL-6) simultaneously in a single serum sample. All of these proteins are elevated in serum of patients with oral cancer with relatively similar levels of serum concentration. Horseradish peroxidase (HRP) was used as label on detection (secondary) antibodies in a sandwich immunoassay format. Secondary antibodies (Ab2) that are bound to biotinylated PEGs conjugates to HRP multi-labeled Magnetic beads providing the necessary higher sensitivity required for IL-8 and IL-6 detection at physiological levels. Conclusions: These results show great promise for real time multiplexed cancer biomarker detection for point-of-care diagnostics.

APS RGD CONJUGATES

Adrian Soto, John Williams, *Department of Physical Sciences*, Rhode Island College, Providence, RI

RI-INBRE Summer Undergraduate Research Fellowship Program

Arylphosphonium salts (APS) are cationic cytotoxic lipophiles that can cross cell membranes. APS are selectively taken up by malignant cells and mitochondria in healthy cells and have been used as transport molecules to deliver DNA-alkylating molecules into mitochondria. This makes APS and their conjugates potential anti-cancer agents. Some possible targets are cell cycle arrest, tubulin polymerization, mitochondrial hexokinase disassociation, and calcium channel inhibition. Conjugates to the APS can make small or major changes in structure that may alter the mechanism and potency of their cytotoxic action. Capping a polypeptide synthesized on a polystyrene resin with APS can make a conjugate molecule designed to target specific cell types and regions of cells. Polypeptides, usually pentamers or less, can be efficiently made by microwave assisted organic synthesis to any specified amino acid sequence. Wang-resin based solid-state synthesis has been used to synthesize RGD trimers that were then conjugated to arylphos- phonium salts to make compounds that may be preferentially taken up by cancer cells. By combining RGD and APS the accumulation of APS in non-malignant cells may be prevented. All steps in SSPS were done using a microwave in about 1/40 of the overall reaction time with high yields.

CONDITIONAL SKELETAL MUSCLE-SPECIFIC DEPLETION OF UBE4B ACTIVITY

Jacklyn Lata, Irina Maglysh, Sabiha Rahman, Megan Radka, *Department of Biology*, Rhode Island College, Providence, RI

RI-INBRE Summer Undergraduate Research Fellowship Program

Ube4b is an E3 ligase that ubiquitylates substrates for their subsequent degradation by the proteasome. Recently, we demonstrated that Ube4b is alternatively spliced specifically during striated muscle differentiation and regeneration. In vitro, Ube4b ubiquitylated UNC45B, a chaperonin essential for myofibril assembly. In addition, expression of a Ube4b mutant lacking the catalytic U-box region generates a lethal phenotype where embryos die by E13 from cardiomyocyte apoptosis. Together these data imply that Ube4b has an important role in myogenesis. To test this hypothesis in vivo, we have developed a line of transgenic skeletal muscle-specific conditional knockout mice. To create this line, mice that were positive for MyoDiCre were bred with mice harboring a conditional Ube4b allele in which the exon coding for the catalytic U-box domain is flanked with LoxP sites. Cre-mediated recombination thus removes the coding sequence for the U-box, causing the inactivation of the Ube4b enzyme. Since MyoD expression occurs solely in cells of the skeletal muscle lineage, when the iCre gene is inserted downstream of its promoter Cre expression is only observed in skeletal muscle tissue. This way, recombination of the U-box allele and inactivation of Ube4b is localized in skeletal muscle, allowing for the cardiac muscle to develop normally. Mice lacking active Ube4b show significantly reduced postnatal growth after P6 and die between P2-21 (Ave=P10). Additional preliminary western blot data showed no difference in the expression levels of certain muscle proteins including skeletal muscle myosin heavy chain and sarcomeric alpha-actin. Currently, gross overall necropsy and histological analysis of various skeletal muscles are being performed on mutant and wild type littermates.

PLANKTON STUDIO: VISUAL INQUIRY AND IMAGING AS TOOLS FOR FACILITATING THE INTEGRATION OF ART AND SCIENCE

Mengzhuo Li, Eliza Squibb, Beal Overstrom, *The Nature Lab*, Rhode Island School of Design, Providence, RI

RI EPSCoR Summer Undergraduate Research Fellowship Program

Visual inquiry and imaging play an important role in scientific creativity and communication. Training in artistic skills has been shown to enhance mental imaging capacity yet the development of visual-spatial thinking skills is often overlooked in the classroom. At the Rhode Island School of Design, the Nature Lab offers living and preserved natural science specimens as a resource for exploring functional morphology and the significance of form and pattern through deep observation and hands-on access in a studio setting. In this study we used living marine plankton from Rhode Island coastal waters as the focus of a studio-based learning exercise involving careful observation and representation with the goal of investigating multiple modes of inquiry as an educational tool. A curriculum module was developed and tested with educators from the arts, sciences, and humanities. Participants were asked to observe select live marine specimens under stereo and compound microscopes, and to create sketches and representations of significant features. Teams then categorized the drawings and diagrams based on collaboratively-generated definitions of art and science. This type of exercise can foster fresh dialogue on the relationship of art and science and encourage the use of multiple modes of inquiry in formal and informal learning environments.

INVESTIGATION OF HUMAN CYCLIN-DEPENDENT KINASES AND THEIR EXPRESSION POST-EXPOSURE TO GALLIC ACID

Kelsey Stafstrom, Alyssa Guarracino, Alexandria Bierce, JD Swanson, *Department of Biology and Biomedical Sciences*, Salve Regina University, Newport, RI

RI-INBRE Summer Undergraduate Research Fellowship Program

Many natural substances, especially phenolic acids, have been shown to have significant medicinal qualities. Gallic acid (GA) is a phenolic found in glandular trichomes of many prickled plants such as raspberries and blackberries. Data suggests that GA causes significant cell proliferation when exposed to plant cells, though conversely it can stop cell division entirely in animal cells. This experiment aims to apply concentrations of GA to gastric cancer cells to test its effectiveness of preventing cancer cells from dividing. We will use several human cyclin-dependent kinases (CDKs) to test for gene expression of the exposed cancer cells. CDKs are cell cycle regulating genes that have known expression at specific points in the cell cycle. From surveying known Human CDKs, we selected CDK1, CDKN3, CDK4, CDK6, CDK7 and CDK14 for further study. To this end we designed primers for the specific genes using Primer3 and tested them using Human DNA. The project will continue with extracting RNA from AGS gastric cancer cell lines treated with GA and running qPCR with the primers to highlight the cells' activity, or nonactivity, in the cell cycle.

DEVELOPING INTER-PROFESSIONAL COMMUNICATION SKILLS USING A HIGH-FIDELITY HUMAN PATIENT SIMULATOR

James Stevenson, *Department of Biology*, Providence College, Providence, RI; Clinton Chichester, Amanda DeAngelis-Chichester, *College of Pharmacy*, University of Rhode Island, Kingston, RI

RI-INBRE Summer Undergraduate Research Fellowship Program

Miscommunication in healthcare settings can become a hindrance to providing safe and effective patient care and is a common cause of morbidity and mortality. Clinicians operate in an environment where a safe and effective outcome is directly related to the communication skills of people from multiple professions with differing responsibilities and degrees of training. We have developed an educational scenario using a high-fidelity human patient simulator that focuses on inter-professional communication and teamwork and is adaptable for students with varying levels of education. The scenario is modeled from a case of “serotonin syndrome”, a condition of serotonergic hyperactivity due to antidepressant drug interactions. Students or current healthcare professionals develop their skills in communication by conducting this role-playing simulation exercise. Along with improving communication skills, each discipline is also required to complete objectives specific to their area of expertise. Nursing must conduct an assessment of a patient presenting with altered mental status, neuromuscular hyperactivity and autonomic instability. Psychiatry is responsible for diagnosing serotonin syndrome as a result of serotonergic drug interactions while pharmacy must develop a treatment plan to attenuate the symptoms and treat the underlying pathophysiology. The inter-professional team must work together in a simulated hospital environment with a high-fidelity human patient simulator modeling the condition in real time. To complete the scenario, the inter-professional team is required to develop a report using a standardized communication strategy called SBAR and deliver their report to a physician actor. Preliminary trials indicate utilizing nursing and pharmacy students in a joint exercise facilitates communication skills better than working in groups of students from the same discipline. By working together on a challenging case in a realistic clinical environment, either students or practicing healthcare professionals can develop inter-professional communication and teamwork skills using this novel high-fidelity human patient simulation.

TEMPERATURE-SENSITIVE COMPONENTS OF DEVELOPMENTAL CADMIUM TOXICITY

Laura Stevenson, *Department of Environmetal Sciences*, Roger Williams University, Bristol, RI; Janani Subramaniam, Kerri Warren, *Department of Biology*, Roger Williams University, Bristol, RI

RI EPSCoR Summer Undergraduate Research Fellowship Program

Cadmium is well-studied environmental pollutant linked in humans to renal and bone injuries, immune deficiencies and cancer. However, the mechanisms of toxicity are poorly understood, especially at sub-lethal concentrations. Temperature is reported to increase heavy metal sensitivity in many animals, and many of cadmium's toxic mechanisms appear to be temperature-dependent. In the face of global climate change, temperature-dependent toxicity is of special relevance to the fate of aquatic ectotherms. The zebrafish, Danio rerio, is used in the present study to examine the effects of small temperature shifts on embryo-larval responses to cadmium exposure. Zebrafish embryos are exposed to low cadmium chloride doses (0, 0.5 and 5uM CdCl2) and temperature shift that otherwise supports normal development, and examined for the first 5 days of development. Temperature-specific, cadmium-specific and combinatorial effects on morphology and physiology are apparent. This study aims to characterize changes in gene expression that correlate to temperature sensitive components of developmental cadmium toxicity.

THE COMBINED EFFECTS OF CADMIUM AND TEMPERATURE ON ZEBRAFISH LARVAE DEVELOPMENT

Janani Subramaniam, Laura Stevenson, Kerri Warren, *Department of Biology, Marine Biology and Environmental Science*, Roger Williams University, Bristol, RI

RI EPSCoR Summer Undergraduate Research Fellowship Program

Climate change and chemically-contaminated water increasingly impact coastal aquatic ecosystems and the effects of small temperature change fluctuations on developmental metal sensitivity are not well understood. This project utilized a cadmium toxicity bioassay in zebrafish embryo-larvae to examine the combined effect of low-level cadmium exposure with otherwise-tolerable temperature shifts. Zebrafish embryos were exposed to 0, 0.5 and 5um of cadmium chloride (CdCl2) at 25 and 32C and examined from day 2- day 5 of development.

General morphology and developmental progress was assessed as were specifics of cardiovascular development. Vasculogenesis and angiogenesis were characterized using transgenic endothelial cell green fluorescent protein (GFP) zebrafish, vessel maturation and patterning with alkaline phosphatase assays, and vessel integrity and patency with microscopic observation. Temperature-specific outputs included increased rate of developmental progression and elevated heart rate and metabolism. Cadmium-specific, temperature insensitive parameters included cadmium-induced cardiac arrhythmia and cranial hemorrhage. The combined effect of cadmium and temperature, however, reduced cadmium-tolerance more universally, with increased rates of edema, necrosis and disaggregation. These results suggest very low levels of metal contamination of coastal waters pose a significant future environmental threat.

EXPRESSION OF MEIOTIC GENES AND PROTEINS DURING ECHINODERM EMBRYOGENESIS AND GAMETOGENESIS

Elena Suglia, Mamiko Yajima, Eric Gustafson, Gary Wessel, *Department of Molecular Biology, Cell Biology, and Biochemistry*, Brown University, Providence, RI

RI EPSCoR Summer Undergraduate Research Fellowship Program

Meiosis is the production of sex cells, or gametes, in eukaryotes. All cells divide and multiply in a process called mitosis, whereas meiosis, a cell division of spermatocytes and oocytes, is significantly different in that the daughter cells lose three of four chromosomes to become haploid and genetically not identical to the parent cells. Meiosis, a key process of sexual reproduction, is thus consequently critical for production of genetic diversity. Meiosis is a fundamental process shared by all sexually producing organisms and malfunctions of meiotic genes in humans are known to cause infertility and genetic diseases such as Down syndrome. Understanding fundamental mechanisms of meiosis will, therefore, have a significant impact on both the fields of basic life science and medical science. We have used echinoderms, a sister group of chordates, as experimental animals and investigated a series of meiotic gene/protein expression in their ovaries, embryos, and larvae. Meiotic genes that are highly conserved among metazoans such as Rad21, Rad51, Dmc1, and Scm1β, were cloned by polymerase chain reaction (PCR) from ovary cDNA libraries of Sea urchins (Strongylocentrotus purpuratus) or of Starfish (Patiria miniata). Amplified PCR products were then sub-cloned into plasmid vectors and subsequent sequencing revealed that the cloned inserts were indeed the sequences chosen from each of the meiotic genes. To identify the expression of these meiotic genes in vivo, anti-sense RNA probes were made from these cloned sequences of S. purpuratus or P. miniata meiotic genes. In situ hybridization was then performed in P. miniata oocytes and embyros and S. purpuratus larvae by immunofluorescence with 11 different commercial antibodies.

PROGRESS TOWARD THE SYNTHESIS OF A CHIRAL FLUORESCENT POLYAMINE

Madeleine Suits, *Department of Pharmacy*, University of Rhode Island, Kingston, RI; Kayla Flynn, Mindy Levine, *Department of Chemistry*, University of Rhode Island, Kingston, RI

RI INBRE Summer Undergraduate Research Fellowship Program

University of Rhode Island College of Pharmacy Dean's Fellow

We report herein the synthesis of a fluorescent non-natural amino acid intended to monitor the complexation, localization, and delivery of siRNA to live cells. This amino acid will be incorporated into the side chain of a chiral oxazoline, and polymerized to yield chiral fluorescent polymers. Furthermore, due to the fact that the fluorescent monomer exhibits solvatochromism, it will be utilized to observe the intracellular environment surrounding the siRNA-polymer complex. The fluorescent probe itself is synthesized from the corresponding non-natural amino acid, 4-n,n-dimethylamino-1,8-napthalimidoalanine. After removal of the Boc. protecting group, the carboxylic acid is reduced, yielding the corresponding amino alcohol, which is then cyclized to the oxazoline. The next step in our research will be to determine whether the fluorescent polymer binds to siRNA through the use of fluorescence spectroscopy.

THE PLASTID GENOME SEQUENCE OF THE RED MACRO ALGA GRACILARIOPSIS ANDERSONII

Elizabeth Sullivan, *Department of Biological Sciences*, University of Rhode Island, Kingston, RI

RI-INBRE Summer Undergraduate Research Fellowship Program

The red algal plastid is a chlorophyll and phycobilin-containing organelle that performs the vital functions, like photosynthesis, that are necessary for survival in algae and other plants. In addition to photosynthesis, plastids can also biosynthesize amino acids, lipids, and starch. The plastid genome contains a portion of the genes necessary for proper function, with the majority of the genes required having been transferred to the nucleus as part of endosymbiosis. To date, very few red algal plastid genomes have been sequenced, so there is a small reference library available to those who wish to make evolutionary comparison. The data from my current study will expand the understanding of which genes are conserved or under evolutionary selection within the red algae.

The objective of this project is to sequence the plastid genome of Gracilariopsis andersonii. This genome is a critical piece of a larger project examining the role of plastid genes in red algal host/parasite interactions. Next-generation sequencing data from G. andersonii were mined for pieces of the plastid genome. The published genome Gracilaria tenuistipitata was used as a reference for initial assembly, resulting in thirty one assembled pieces (contigs) of the genome. A BLAST database of the remaining sequence reads was constructed to identify fragments that were not shared by the reference genome. After assembly, the genomic contigs were compared to the reference, allowing for a visual comparison with genes and relative placement and orientation. To close the gaps between contigs, PCR primers were designed to ‘walk’ the gaps to closure by sequencing missing data. To date, I have completed ~80% of the G. andersonii genome and have begun annotating the genes and tRNAs encoded in the plastid.

THE 3RD CYTOPLASMIC LOOP OF THE D2 DOPAMINE RECEPTOR IS RESPONSIBLE FOR MICRO-COMPARTMENTALIZATION AT THE PLASMA MEMBRANE

Kevin Sun, *Department of Cellular and Molecular Biology*, University of Rhode Island, Kingston, RI; Jeremy Celver, Abraham Kovoor, Meenakshi Sharma, Chris Octeau, *College of Pharmacy*, University of Rhode Island, Kingston, RI

RI-INBRE Summer Undergraduate Research Fellowship Program

Rhode Island Science & Technology Advisory Council's Governor Carcieri Fellow

Understanding how cells distinguish signals from different receptors that can activate common signaling pathways is a fundamental question in cell biology. One likely mechanism involves segregation of subsets of receptors and signaling molecules into different compartments establishing receptor specific signaling environments within the plasma membrane. The D2 dopamine receptor (D2R) is a clinically important brain molecule because it is the major target of drugs used to treat schizophrenia and Parkinson's disease. A majority of D2R expressed in the plasma membrane of cells, partitions into a biochemical fraction that is resistant to solubilization in non-ionic detergents. Interestingly the pool of receptor which segregates into the detergent resistant fraction was readily internalized in an agonist dependent manner whereas internalization of soluble D2R was not detectable. This data suggests that D2R populations segregating into the detergent-insoluble fraction is functional and that the two D2R populations originate from distinct biochemical environments in the membrane. In this study, we performed experiments to determine the structural basis of D2R compartmentalization by constructing chimeric receptors between D2R and related opioid receptors which unlike D2R are largely localized to detergent soluble membrane fractions. Substituting the third cytoplasmic loop of delta opioid receptor (DOR) with the 3rd cytoplasmic loop of D2R was sufficient to significantly redirect DOR to detergent-resistant membranes. Additional constructs in which portions of the D2R 3rd loop were deleted have allowed us to narrow in on the region of D2R which is responsible for targeting to detergent resistant membranes. Preliminary experiments have identified a protein, filamin, which interacts with the D2R 3rd intracellular loop to likely play a role in fencing D2R into detergent-resistant membrane. These results provide an explanation for how D2R may be compartmentalized in the membrane and thus allow D2R expressing cells to differentiate D2R signaling from signals meditated by closely related receptors.

PYRETHROID MODULATION OF T-TYPE VOLTAGE-SENSITIVE CALCIUM CHANNEL ISOFORMS

Wayne Bainter, Justin Gay, Steven Symington, *Department of Biology and Biomedical Sciences*, Salve Regina University, Newport, RI

RI-INBRE Summer Undergraduate Research Fellowship Program

Pyrethroids are synthetic derivatives of the naturally occurring pyrethrins that are widely used in agriculture and residential pest control programs. Human exposure is virtually assured because of their widespread use, but little is known about how these compounds affect human receptors. Previous studies have shown that T-type calcium channels from a variety of organisms are modified by pyrethroids, however, effects vary depending on the pyrethroid used and the channel examined. To better understand this phenomena, we investigated the structural activity relationship of three different but related pyrethroids (deltamethrin, permethrin, and fenpropathrin) on T-type voltage-sensitive calcium channel isoforms expressed in Xenopus oocytes. To do this, cRNA was transcribed from linearized cDNA encoding Cav3.2 and Cav3.3 using the mMessage mMachine T7 ultra in vitro transcription kit. Newly transcribed cRNA was injected into Xenopus oocytes (0.1 μg/μl and 0.3 μg/μl) and the effect of three pyrethroids (deltamethrin, fenpropathrin, and permethrin) assessed on the voltage-gated characteristics of the expressed channels. Results indicate that only deltamethrin inhibited Cav3.2 in a concentration-dependent manner while permethrin and fenpropathrin had no effect. In contrast, all pyrethroids examined inhibited Cav3.3 in a concentration-dependent manner. These results suggest a stereospecific interaction of pyrethroids on T-type voltage-sensitive calcium channels that is unique to specific calcium channel isoforms.

USING A COMPUTATIONAL APPROACH TO IDENTIFY POTENTIAL INHIBITORS OF THE LEISHMANIA DONOVANI LIPASE, LDLIP3

Stephanie Beels, Steven Symington, Alison Shakarian, *Department of Biology and Biomedical Sciences*, Salve Regina University, Newport, RI

RI-INBRE Summer Undergraduate Research Fellowship Program

Leishmaniasis is a disease caused by a parasite transmitted by the sand fly in areas such as Africa, the Middle East, and Latin America. Leishmania donovani is one species of a single-celled parasite that causes Leishmaniasis, and can often result in a fatal visceral disease. LdLIP3 is a specific secreted lipase found in the supernatant of Leismania donovani that is involved with energy utilization. The long-term goal of this project is to identify efficient natural substrates of LdLIP3 which will facilitate the discovery of possible inhibitors for the enzyme that can be utilized for Leishmaniasis treatment. To that end, we utilized open source computational tools to model the substrate binding site of LdLIP3 and identify the potential inhibitors of lipase activity. A previously crystallized control lipase from Rhizomucor meihei (3TGL), was modeled and docked with two natural substrates (palmitate and stearate) and potential inhibitors. Autodock was utilized for the molecular docking experiments to quantify the various hydrogen bonds, electrostatic interactions, binding affinities and energy minimizations associated with enzyme-substrate relationships. It was found that palmitate is a more efficient natural substrate than stearate for lipase from Rhizomucor meihei. We also evaluated the binding characteristics of three potential competitive inhibitors from the terpenoid class of drugs (citral, menthol, and THC) on the Rhizomucor meihei lipase. Each of the competitive inhibitors were analyzed for binding affinities and energy minimization. The computational data indicate that THC is a stronger inhibitor than citral and menthol. Collectively, the results from the competitive inhibitor study using the control lipase, strongly suggest that THC could be a potential inhibitor for the Leishmania donovani lipase, LdLIP3. Future studies will evaluate the results obtained from the computational approach by determining the effect of the terpenoid drugs of LdLIP3 purified from Leishmania donovani.

A COMPUTATIONAL APPROACH TO DETERMINE THE BINDING SITE OF PYRETHROIDS TO THE BΓ SUBUNIT OF HETEROTRIMERIC G-PROTEINS

Paul Diss, Steven Symington, *Department of Biology and Biomedical Science*, Salve Regina University, Newport, RI

RI-INBRE Summer Undergraduate Research Fellowship Program

Previous reports indicate that a photoactivatable arylazide derivative of fenvalerate ([3H]decyanoazidofenvalerate or [3H]DeCAF) specifically labels the βγ subunit from heterotrimeric G-proteins (Gβγ) in bovine ROS cells and rat brain membrane fragments. The goal of this study was to determine the binding site of fenvalerate and evaluate the structure activity relationship of pyrethroids using a molecular docking computational approach. In these experiments, autodock was utilized to quantify hydrogen bonding, electrostatic interactions, binding affinities and energy minimizations associated with ligand-receptor complex. In control experiments, autodock correctly identified the binding site of M119, a drug that has previously been shown to interact with Gβγ. In computational binding studies using the pyrethroid, fenvalerate, we validated the original labeling experiment and showed that the most energetically favorable binding site for this pyrethroid on Gβγ is located in the fifth blade of the β-subunit. Furthermore, fenvalerate binding at this location was dependent upon hydrophobic interactions between Gβγ and fenvalerate and hydrogen bonds between the ester carbonyl and the protein. In structural activity relationship studies using three different structural analogs of pyrethroids, which possess either a halogenated vinyl group (permethrin), an α-cyano (fenpropathrin) or both (deltamethrin) illustrate that all three bind to the fifth blade on the Gβγ protein. Permethrin, which posses a halogenated vinyl group but not an α-cyano moiety, had the most favorable binding characteristics to Gβγ. Future studies will evaluate the results obtained from the computational approach by determining the binding characteristics of the pyrethroids from purified Gβγ.

CHARACTERIZATION OF ENDOGENOUS VOLTAGE AND LIGAND-GATED CHANNELS IN MICROTRANSPLANTED RAT BRAIN NEUROLEMMA INJECTED INTO XENOPUS OOCYTES

Craig Irving, Wayne Bainter, Justin Gay, Steven Symington, *Department of Biology and Biomedical Sciences*, Salve Regina University, Newport, RI

RI-INBRE Summer Undergraduate Research Fellowship Program

Microtransplantation of mammalian neurolemma is an excellent tool to study the structure and function of transmitter receptors and ion channels associated with the central nervous system. Microtransplanted neurolemma can originate from a variety of sources, posses ion channels and receptors in their native configuration and lipid matrix, and are applicable to examine diseases associated with different channelpathies. This functional system make neurolemma-injected Xenopus oocytes a powerful method for detailed investigations of ion channel function and regulation in the central nervous system. Our preliminary results indicate that Xenopus oocytes, injected with neurolemma fractions prepared from rat brain are capable of reconstituting native ion currents into their plasma membrane. Expressed ion currents were sensitive to tetrodotoxin, ω-conotoxin MVIIC, and TEA; indicating the presence multiple voltage-gated ion channels (voltage-gated sodium channel, calcium channel and potassium channel, respectively). Furthermore, neurolemma-injected oocytes also illustrated a concentration-dependent GABA-sensitive current. Once completely characterized, this procedure will also be amenable to the study of the developmental and reproductive toxicity of various drugs and other environmental contaminants and for the study of additional agents causing acute, chronic and developmental neurotoxicity in mammalian neuronal tissues, including those from knockout mice models and humans.

EXPRESSION OF THE HUMAN N-TYPE CALCIUM CHANNEL IN XENOPUS LAEVIS OOCYTES

Karl Varkey, Craig Irving, Steven Symington, *Department of Biology and Biomedical Science*, Salve Regina University, Newport, RI

RI-INBRE Summer Undergraduate Research Fellowship Program

Voltage-sensitive calcium channels are expressed throughout the central nervous system and regulate many y complex functions. The human N-type calcium channel (Cav2.2), is highly expressed in presynaptic nerve terminals, and is intimately involved with the regulation of Ca2+-dependent neurotransmitter release. Expression of this channel in heterologous expression systems, like Xenopus laevis oocytes, allow for an investigation of the relationship that exists between biophysical features of voltage-sensitive calcium channels and their physiological functions. To further investigate the action of pyrethroids and other environmental contaminants on the voltage- and kinetic properties of expressed Cav2.2, human α1 and β3 subunits in individual plasmids were transformed into E. coli cells, and then purified using a Qiagen Midi Kit. Individual clones were validated by enzyme restriction digests on the purified plasmids. Linearized α1 and β3 cDNA were used as a template to synthesize cRNA with the mMESSAGE mMACHINE T7 Ultra Kit in vitro transcription kit. The resulting cRNA was then injected into defollicated Xenopus laevis oocytes and incubated for 3-5 days. Two electrode voltage clamp electrophysiology were used to characterize expression of Cav2.2 α1 and β3 subunits in the oocytes. Preliminary tests have shown that this channel is blocked by the neurotoxic peptide ω-conotoxin. Successful expression of the human Cav2.2 in Xenopus laevis oocytes will establish the grounds for experimental research to determine the effects of pyrethroids on Cav2.2.

RENAL EXCRETION OF NANOPARTICLES IN MICE

Wei Lu, Aftab Ahmed, Daisy Yan, Liangrain Guo, Kevin Szulak, *Department of Biomedical and Pharmaceutical Sciences*, University of Rhode Island, Kingston, RI

RI-INBRE Summer Undergraduate Research Fellowship Program

Nanoparticles made of gold and CuS possess versatility in cancer treatment. To be considered a viable target for drug delivery, these particles must be readily excreted from the body following systemic administration. The purpose of this study was to determine if these nanoparticles can be metabolized and excreted from the body. Two types of core-shell structured nanoparticles, i.e. hollow gold nanoparticles and hollow CuS nanoparticles, with similar particle size were prepared for the evaluation. Both nanoparticles received surface modification with polyethylene glycol in order to evade the cells of the mononuclear phagocyte system. Following intravenous injection, urine was collected at determined time points. During the first 8 h following administration, significant change of urine color was observed in mice injected with CuS nanoparticles but not gold nanoparticles, indicating renal excretion of CuS nanoparticles. Inductively coupled plasma mass spectrometry (ICP-MS) analysis was performed to test element concentration of copper or gold in urine for each corresponding time point. Over a course of 7 d, our results showed that more than 8% of injected dose of copper was excreted from kidney, while the cumulative renal excretion of gold was only about 0.04% of injected dose. These findings confirmed that hollow CuS nanoparticles but not hollow gold nanoparticles can be considered as biodegradable drug delivery system.

A SYNTHETIC BIOLOGY APPROACH TO NATURAL PRODUCT CLUSTER-BASED MOLECULAR DISCOVERY

Rachel Thakore, *Department of Biology and Community Health*, University of Rhode Island, Kingston, RI ; Caroline Killian, Daniel Udwary, *Department of Biomedical and Pharmaceutical Sciences*, University of Rhode Island, Kingston, RI

RI-INBRE Summer Undergraduate Research Fellowship Program

The Polyketide synthases (PKS) are an enzyme family found in many bacteria and fungi that produce secondary metabolites, and are often used for chemotherapy. We have observed several similar Type III PKS-containing gene clusters with conserved components across numerous bacteria, though the products remain unknown. Our goal is to determine the products of four PKS clusters from Mycobacterium tuberculosis H37Rv, Deinococcus radiodurans, Frankia EAN1pec, and Streptomyces coelicor A3(2). We utilized a gene synthesis approach to create Escherichia coli-adapted PKS genes derived from the genome sequences of M. tuberculosis and D. radiodurans, and began creation of an adaptable system to allow the expression of complete operons, including accessory genes. By mixing and matching pieces of operons from the various organisms, we intend to create a novel system for combinatorial biosynthesis.

EFFECTS OF INTER-ALPHA INHIBITOR PROTEINS ON RADIAL ARM MAZE LEARNING IN A RODENT MODEL OF NEONATAL HYPOXIA-ISCHEMIA

Katrina Feyerherm, Nick Lafond, Molly La Rue, Steven Threlkeld, *Department of Psychology*, Rhode Island College, Providence, RI; Cynthia Gaudet, *Department of Biology*, Rhode Island College, Providence, RI; Courtney Hill, Barbara Stonestreet, *Department of Pediatrics*, Women and Infants Hospital of Rhode Island

RI-INBRE Summer Undergraduate Research Fellowship Program

Neonatal cerebral oxygen deprivation and reduced blood flow (hypoxia/ischemia respectively) can result from umbilical cord occlusion, prolonged labor or preterm birth producing an inflammatory response and neuronal cell death contributing to poor cognitive outcome and learning disabilities later in life. The goal of our investigation was to determine if using naturally derived immunomodulator Inter-Alpha Inhibitor Proteins (IAIPs) could remediate working memory deficits known to be associated with HI injury in a rodent model. Evidence suggests that IAIPs have potential to prevent inflammatory mediated neonatal ischemic brain injury and subsequent behavioral deficits. Complete cauterization of the right common carotid artery and 120 minutes of hypoxia at postnatal day (P) 7 was induced in two treatment groups (HI (n=13) and HI+IAIPs (n=9)). Sham (n=12) surgery was performed on control animals. Treatments of 30 mg/kg of IAIP or saline (vehicle) were administered prior to the hypoxia and 24 hours post-HI insult. As adults (~P130) subjects were tested on a water escape version of the radial arm-maze designed to measure working and reference memory. HI-IAIP and Sham groups exhibited superior working memory and reference memory during the asymptotic portion of testing. Results suggest that administration of IAIPs at the time of neonatal HI injury may reduce the severity of long-term cognitive impairments.

EFFECTS OF HYPOXIA- ISCHEMIA AND INTER-ALPHA-INHIBITOR TREATMENT ON BRAIN WEIGHT AND NEURONAL CELL DEATH

Cynthia Gaudet, *Department of Biology*, Rhode Island College, Providence, RI; Courtney Hill, Barbara Stonestreet, *Department of Pediatrics*, Women and Infants Hospital of Rhode Island; Steven Threlkeld, *Department of Psychology*, Rhode Island College, Providence, RI

RI-INBRE Summer Undergraduate Research Fellowship Program

Reduced cerebral oxygenation (hypoxia) and blood circulation (ischemia) puts premature and very low weight babies at a high risk for poor neurological and behavioral outcomes (Hossain, 2008; Huang & Castillo, 2008). Hypoxia ischemia (HI) is associated with rupturing blood vessels leading to an elaborate inflammatory response and a cascade of prolonged cell death extending days or even weeks. Inter-alpha-inhibitor-proteins (IAIPs) have been shown to reduce inflammation. Due to the limited treatment options available at this time, this study sought a novel treatment using IAIPs as a neuroprotectant. Complete cauterization of the right common carotid artery and 90 minutes of hypoxia was induced in two groups. One group received HI+IAIPs (n=9) and the other received saline vehicle (HI, n=11), while a sham (n=10) group served as the control. Treatments of 30 mg/kg of IAIPs or saline (vehicle) were administered prior to the hypoxia and 24 hours post-HI insult. Subjects were perfused and fixed followed by brain extraction 72 hours post insult. A one-way ANOVA revealed an overall treatment effect ($p=.039$) on brain weight. Post-Hoc analysis of brain weight revealed a significant difference between Sham and HI subjects ($p<.05$). Brain weight analysis is used as an early indicator for severity of injury (Peiffer, Fitch, Thomas, Yurkovic, & Rosen, 2003). An assessment of the number of dying neurons, quantified using Fluoro-Jade b staining was performed on paraffin embedded coronal brain sections. A one-way ANOVA revealed an overall treatment effect on the number of FJB labeled cells between treatment groups ($p=.018$). Post-Hoc analysis revealed that HI subjects had significantly more FJB labeled cells than HI+IAIPs and Sham subjects ($p<.05$). Results suggest that IAIPs have neuroprotectant capabilities. Future studies will seek further support for IAIPs as a therapeutic option for perinatal brain injury.

EFFECTS OF INTER ALPHA INHIBITOR PROTEINS ON AUDITORY PROCESSING FOLLOWING NEONATAL HYPOXIC-ISCHEMIC INJURY

Molly La Rue, Nick Lafond, Katrina Feyerherm, Steven Threlkeld, *Department of Psychology*, Rhode Island College, Providence, RI; Cynthia Gaudet, *Department of Biology*, Rhode Island College, Providence, RI; Courtney Hill, Barbara Stonestreet, *Department of Pediatrics*,Woman and Infants Hospital of Rhode Island

RI-INBRE Summer Undergraduate Research Fellowship Program

Neonatal hypoxia and ischemia occur when brain oxygen and blood flow levels are reduced respectively, leading to significant long-term learning and processing impairments. Inter-alpha inhibitor proteins (IAIPs) are serine protease inhibitors found in human plasma and are known to help reduce acute inflammation (Singh et al., 2010). We hypothesized that IAIPs would improve auditory discrimination following neonatal hypoxia-ischemia in rats. We used a series of modified pre-pulse inhibition paradigms to measure auditory processing in Wistar rats (~150 days postnatal) with either HI + NaCl vehicle, HI + two doses of 30 mg/kg IAIPs, or sham + NaCl vehicle on postnatal day (P) 7. The acoustic startle reflex (ASR) is a rapid contraction of muscles. A reduction or attenuation of this ASR can be induced through pre-pulse inhibition (PPI, Fitch et al., 2008). Tasks used to assess auditory processing included simple pre-pulse (startle reduction), long duration pre-stimulus (0-100ms) silent gap detection, and a short duration pre-stimulus (0-10ms) silent gap detection task. Finally, rats were tested for complex (oddball) tone order discrimination to assess temporal and spectral processing demand. Attenuated response scores were calculated from the peak ASR. No differences were found between treatment groups on the simple pre-pulse inhibition (reflex modification) task. We found no differences on the long duration (0-100ms) silent gap detection task or the short duration (0-10ms) silent gap detection task. These findings suggest that basic pre-pulse inhibition and auditory temporal processing is intact in rats with neonatal HI injury. However, results revealed significant deficits in adult rats with neonatal HI on the complex two-tone discrimination tasks as compared to HI rats treated with IAIPs and shams. We conclude that treatment with IAIPs following neonatal HI protects against complex auditory processing deficit seen in untreated neonatal HI rats.

LOW LEVELS OF GENETIC DIFFERENTIATION AMONG SAMPLES OF SALTON SEA TILAPIA (CICHIDAE: OREOCHROMIS MOSSAMBIQUES X OREOCHROMIS UROLEPIS HORNORUM)

Matthew DeBlois, Kelsey Garlick and Jeffrey Markert, *Department of Biology*, Providence College, Providence, RI

RI EPSCoR Summer Undergraduate Research Fellowship Program

The Salton Sea is a large lake in southern California created in the early 1900s by the accidental release of flood waters from the Colorado River. Currently, the salinity of the Salton Sea is approximately 1.5 times as concentrated as ocean water due to a lack of drainage, constant input of agricultural runoff and high rates of evaporation. African Tilapia were introduced into the Salton Sea in the mid-20th century as a part of an illegal fish farm. Their descendants now thrive in the lake despite the high salinity. The fish family Cichlidae is well known for its rapid rate of adaptation and speciation. Distinct morphological groups have been noted in recent collections of tilapia. The first subtype has green scales and a distinctly flat “beak”, the second has much darker scales and a notably more pronounced beak. In our study, three different samples of fish from the Salton Sea were analyzed: the two adult morphotypes and a group of fry whose adult phenotypes were unknown. By analyzing small repeating DNA sequences known as microsatellites, it was determined that the group with green scales and the group with darker scales likely belonged to the same gene pool, despite their phenotypic differences. The tilapia fry were a genetically distinct unit when compared to the other two groups, although this difference was small. Additional work will be needed to determine whether this low level of genetic differentiation represents the vestiges of multiple introductions, geographic sub-structuring, differential mortality, or some other evolutionary force.

ASSESMENT OF CURRENT GENETIC DIVERSITY OF TILAPIA FISH HYBRIDS OF THE SALTON SEA REVEALS RECENT BOTTLENECK AND OVERALL LOWER GENETIC DIVERSITY COMPARED TO ESTIMATES FROM THE 1990S

Kelsey Garlick, Matthew Deblois, Jeffrey Markert, *Department of Biology*, Providence College, Providence, RI

RI EPSCoR Summer Undergraduate Research Fellowship Program

Levels of genetic diversity in the tilapia fish (putative Oreochromis mossambiques x Oreochromis urolepis hornorum hybrids) in the Salton Sea are crucial to their survival and persistence in an accidental lake with a continually increasing salinity. The sea is currently more salty than seawater and salt levels are rapidly increasing. Many factors including the desert location of the lake and regional water policies put this abundant species at risk and make them a priority to study because they are essential to the survival of the migrating birds who feed on the tilapia. We examined the current level of genetic diversity using 10 DNA fingerprinting markers (microsatellite loci) to measure genetic diversity and to estimate the genetic effective population size and recent demographic changes. Analysis using linkage disequilibrium suggests that the effective population size is approximately 100 fish, which is small when compared to the actual population size (in the millions). However this estimate has wide confidence intervals. Other analyses strongly suggest that there has been a recent bottleneck in the population. All 10 microsatellite loci had excess heterozygosity when compared to the expected heterozygosity, indicating that a bottleneck has occurred and allelic diversity has dropped substantially in recent years. Heterozygosity estimates further suggest a decline in genetic diversity. Our current data set estimates H = 0.59 +/- 0.05 (s.e). Historical estimates from the 1990's trend higher, with H = 0.72 +/- 0.07, though they are not statistically distinct. These results are an important to contribution to the ongoing effort to conserve the Salton Sea and its inhabitants and will be vital to future genetic management policies for the tilapia in the lake.

IMPACT OF NANOPARTICLE BINDING ON MODEL MEMBRANE FUSION AND LIPID MIXING

Farid Topchiev, Aihong Xi, Geoffrey Bothun, *Department of Chemical Engineering*, University of Rhode Island, Kingston, RI

Nanotechnology is at the forefront of scientific innovation. Engineered nanoparticles (NPs) have become heavily utilized for tasks such as drug delivery, water treatment, disinfection, and antifouling surface development. Although the use of NPs yields many benefits, the NPs employed contain complex surface chemistries and exhibit high surface reactivity, which can alter cellular function or cause cytotoxicity through cellular membrane binding and subsequent changes in membrane structure and function. This research seeks to understand the effects NP binding on membrane-related cellular fusion and lipid mixing processes at the biophysical level using model liposomal membrane systems. Fusion allows cells to exchange material, and is critical during cellular differentiation and tissue development and repair. A fluorometric assay has been used with fluorophore labeled and unlabeled zwitterionic, anionic or cationic model cell membranes to examine how fusion or lipid mixing changes in the presence of bound NPs as a function of NP surface chemistry, ionic strength, and temperature (corresponding to different membrane phases). Preliminary results have shown that the binding of iron oxide NPs with anionic polymer coatings hinders lipid mixing between zwitterionic membranes. Phosphate buffered saline (PBS), as opposed to deionized water, promotes lipid mixing with and without the addition of iron oxide NPs by reducing electrostatic repulsion and forming cation bridges between membranes. Understanding the effects that varying NPs have on intermixing of liposomes will enable the quantification of physical nanoparticle-membrane interactions and the identification of “design rules” for reducing or controlling NP-induced fusion activity.

PATHOGEN AVOIDANCE THEORY

Rachel Traghella, *Department of Psychology*, Rhode Island College, Providence, RI

RI-INBRE Summer Undergraduate Research Fellowship Program

The purpose of this study is to test Pathogen Avoidance Theory of weight bias. This theory states that bias against individuals of extreme weights is due to an evolved mechanism that causes humans to avoid stimuli that differ from the average because they may carry pathogens. This theory contrasts with theories stating that weight bias is due to socialization. Pathogen Avoidance Theory suggests that there will be bias towards those who are overweight and those who are thinner than the average weight. To test this theory, 54 participants were recruited (27 male, 27 female) and asked to view a series of 9 images. Each individual saw 3 weight photos (1 extremely thin female, 1 average weight female, and 1 morbidly obese female) and 6 pathogen photos (3 photos of ringworm and 3 photos of leprosy). Participants were asked to rate the targets in the photos on a set of trait dimensions. At the end of viewing all photos, participants responded to statements that measured their attitudes towards individuals of extreme weights and their perceived susceptibility to pathogens. Unknown to participants during the experiment, non-verbal facial reactions were recorded in order to see if there were reactions of disgust for the weight photos that matched the reactions to the pathogen photos. It was hypothesized that ratings of targets in the weight photos would result in a curvilinear function. That is, the targets that strayed from an average weight were rating more negatively then the target of average weight. For pathogen photos, it was predicted that judgments would become more negative as the condition worsens. It was also hypothesized that facial reactions to weight photos and pathogen photos would display similar expressions of disgust, supporting the idea that humans are turned off to individuals of extreme weights because they stray from average.

POSITIVELY-CHARGED MEMBRANE PEPTIDE AS A NOVEL TOXIC PH-SENSITIVE AGENT FOR CANCER TREATMENT

Holly Tran, *Department of Biological Sciences*, University of Rhode Island, Kingston, RI; Anna Moshnikova, Oleg Andreev, Yana Reshetnyak, *Department of Physics*, University of Rhode Island, Kingston, RI

RI-INBRE Summer Undergraduate Research Fellowship Program

University of Rhode Island Provost's Fellow

Cancer is an uncontrolled proliferation of abnormal cells. Conventional treatment is invasive and risks damage to healthy normal body cells. Universally, cancer cells exhibit an acidic extracellular environment. We have discovered positively-charged membrane peptides, which display toxic effects on cancer cells in low pH and high concentration. The peptides interact with cellular membrane at low pH and induce destabilization of plasma membrane and immediate cell death. We assumed that the action of peptides has cooperative nature and linked four peptides together with 4-arm PEG polymer. The compounds were dissolved in dimethylforamide solvent (DMF), kept overnight, and purified using high-pressure liquid chromatography (HPLC). The purified solution was then lyophilized and dissolved in dimethyl sulfoxide (DMSO). Cervical carcinoma cells (HeLa) and adenocarcinoma cells (A549) were treated with increasing concentration (1 -8 μM) of monomeric and tetrameric peptides for 2 hours. An MTS assay was performed to measure cell death. We observed pH-dependent cell death. The cytotoxic effect at low pH was monitored for lower concentration of tetrameric peptide in comparison to that of the monomer. To record changes of cellular morphology in a process of treatment, HeLa GFP (HeLa cell with stable expression of green fluorescent protein) was treated with tetrameric peptide at both normal and low pH (pH 7.4 and pH 6.1). We observed membrane blebbing and loss of GFP signal within a couple of minutes after treatment at low pH. The effect might be explained by significant destabilization of membrane. We believe that optimization of constructs might lead to novel formulation of pH-selective agent for treatment of acidic tumors.

POSITIVE AND NEGATIVE EFFECTS OF TEMPERATURE CHANGE ON TWO SOUTHERN NEW ENGLAND INTERTIDAL CONSUMERS.

Lauren Trotta, Patrick Ewanchuk, *Department of Biology*, Providence College, Providence, RI

RI EPSCoR Summer Undergraduate Research Fellowship Program

Climate change has wide ranging effects on temperature and weather patterns, species distributions and ecological interactions. Marine intertidal species may provide a unique model to address the effects of these changes as they are situated at the border of marine and terrestrial environments. In addition, species at the extremes of their thermal limit may be positively or negatively affected by temperature as shown through changes in an organism's feeding rate, growth rate, and survivorship. We conducted laboratory experiments using two important consumers in the southern New England shoreline, Nucella lapillus and Urosalpinx cinerea, both of which play an important role in maintaining the abundance and diversity of intertidal species. We examined the response of N. lapillus and U. cinerea to potential changes in average summer water temperature as well as changes to their competitive interactions. Our results indicate that elevated temperature negatively impacted feeding rate and survivorship of N. lapillus, where as it positively impacted U. cinerea. The potential alterations in water temperature could impact the species distribution, abundance and community composition.

THE EFFECT OF LIGHT INTENSITY ON BETA-CAROTENE LEVELS IN HYDROPONIC AND AGRICULTURALLY GROWN BASIL

Madison Van Orden, Jameson Chace, *Department of Biology and Biomedical Sciences*, Salve Regina University, Newport, RI; Susan Meschwitz, *Department of Chemistry*, Salve Regina University, Newport, RI

Salve Regina Sustainability Fellowship Program

Beta-carotene is a building block for vitamin A that is important in overall eye health and is found in many vegetables and leafy greens. Previous studies have shown that beta-carotene concentration varies among plant varieties and growing conditions. Indoor hydroponic agriculture systems may lack the necessary conditions for beta-carotene formation compared to traditional outdoor, soil-based agriculture; however, this has not been tested. At Salve Regina University Hydroponic Research Lab, plants are grown hydroponically in a soilless, climate controlled environment where light sources, nutrient concentration and watering can be controlled. In this study, we tested the hypotheses that(1) light intensity (watts/m2) affects the beta carotene content and that (2) this beta-carotene content in hydroponic plants is equal to that of plants grown in soil. Basil (Ocimum basilicum) (n=14) were placed in a hydroponic Elevated Grow System (EGS) with a monitored pH level of 6.1 and an average nutrient concentration of 910 ppm. Watering was automatic at 30 min/hour and light regime cycled 12L:12D. One set of plants (n= 9) was exposed to LED light, while the others (n= 5) to T-5 flourescent grow lights. Additionally, plants (n=3) grown under outdoor soil-based agriculture and (n=12) collected from local supermarkets were used in comparative analysis. All basil was analyzed for beta-carotene using High Preformance Liquid Chromotography technique. Harvested volumes were approximately equal per plant. In the EGS, light intensity was significantly greater under T-5 than LED lights. Beta-carotene content will be compared among these four growing conditions (hydroponic: T-5 and LED, soil: organic and conventional). Commercial basil is grown under a variety of methods that result in variation of beta-carotene content, which is unknown to both the retailer and the consumer. Growing basil hydroponically could promote increased commercial availability of basil with high concentrations of beta-carotene.

DISTRIBUTION AND RELATIVE ABUNDANCE OF MNEMIOPSIS LEIDYI ALONG A DOWN-BAY WATER QUALITY GRADIENT IN NARRAGANSETT BAY

Lena Weiss, Caroline Karp, *Center for Environmental Studies*, Brown University, Providence, RI; Barbara Sullivan-Watts, *Graduate School of Oceanography*, University of Rhode Island, Narragansett, RI

Center for Environmental Studies Summer Research Fellowship

Jellyfish have the ability to negatively impact marine environments through voracious predation, high levels of recruitment, and their ability to tolerate areas of poor water quality. When conditions are favorable, these organisms also have the ability to "bloom," aggregating in high densities over a small area. These aggregations quickly decimate prey populations, as well as cause nuisances for humans. Examining jellyfish at a local scale is extremely relevant to Narragansett Bay since the region is susceptible to hypoxia during the summer months and has a vulnerable fishery. This makes the bay potentially vulnerable to an increase in jellyfish populations. There has been extensive documentation of changes in the timing, abundance, and species composition of jellyfish blooms in Rhode Island waters between the 1970s and 2000. Therefore, it is important to understand how trends in local biophysical factors correlate with the timing, abundance and distribution of these blooms. The abundances of gelatinous plankton in Narragansett Bay, as well as zooplankton abundance, water temperature, salinity, and dissolved oxygen were studied at three sites forming a down-bay water quality gradient. These trends were compared to past observations to determine how blooms may be changing due to physical and biological shifts in the bay. Although data collection is still ongoing, bloom patterns this year appear to be unrelated to surface water temperature, which has been determined as the major driver for blooms in previous years. Blooms were not observed until the first week in July, approximately 30 days later than in 2011. Thus, another factor has displaced water temperature as the strongest driver of jellyfish abundance in the bay. Continued data collection will help to determine what this factor is and how this significant biological shift will alter the ecology of the bay.

PAH DEGRADATION CAPABILITY OF A HISTORICAL DIESEL-CONTAMINATED SITE AT PRUDENCE ISLAND, RI

Zoe White, Dan McNally, Julia Parmentier, Christopher Reid, Jamie Pierce, *Department of Science and Technology*, Bryant College, Smithfield, RI

RI EPSCoR Summer Undergraduate Research Fellowship Program

Previous studies have shown that the presence of a toxic chemical will generally decrease the number of bacteria biotypes and cell density. Bacteria that remain at the site adapted to the contamination. In the case of a diesel spill, some bacteria developed the capability to use aliphatic and aromatic compounds found in diesel fuel as carbon and energy sources. The microbial population of diesel contaminated sediment at the intertidal zone is the focus of this study. The diesel spill occurred during WWII era at a refueling depot on the southern tip of Prudence Island. Samples were taken from contaminated and pristine locations along the shoreline about 10 inches below the surface. Their contamination, bacterial growth conditions, and bacterial communities were compared. The organic content of the sediment was negligible, the dissolved oxygen was measured at about 15%, and the salinity was considered brackish. The total petroleum hydrocarbon (TPH) was determined to be around 0.4 mg/gdw sediment. Viable counts using LB medium were approximately 1.87 x 107 cells and 1.21 x 107 cells, while total counts using a Hausser-Petroff counting chamber was 8.26 x 108 for the contaminated site and pristine site has yet to be determined, respectively. The different biotypes for the contaminated and pristine sites were 3 and 5, respectively. The number of biotypes for anaerobic bacteria at the pristine site was 3. A naphthalene screening test revealed 10 biotypes had the capability to utilize naphthalene as a source of carbon and energy. One bacteria species, most likely a P. putida, which grows on glycerol at a doubling time of 1.5 hours, was examined for naphthalene degradation. Preliminary results show a degradation rate of 3mg/L and reached levels below detection in approximately 6 hours. This study indicates the potential for further degradation of residual diesel fuel under specific conditions.

BIODIESEL FROM WASTE BIOMASS: LIGNIN TO LIPID CONVERSION BY DEEP-SEA *RHODOCOCCUS* BACTERIA

Dorian A. Whyte, *Department of Chemistry and Chemical Technology*, Bronx Community College, City University of New York, Bronx NY; David Rowley, *Department of Biomedical and Applied Pharmaceutical Science*, College of Pharmacy, University of Rhode Island, Kingston RI; Aaron M. Socha, *Department of Chemistry and Chemical Technology*, Bronx Community College, City University of New York, Bronx NY, Department of Biomass Science and Conversion Technology, Sandia National Laboratory, Livermore CA

RI-INBRE Summer Undergraduate Research Fellowship Program

Actinobacteria within the genus *Rhodococcus* are illustrious for their ability to oxidatively catabolize alkylbenzenes, aromatic ethers, fused ring systems, phenols and benzoic acid derivatives to TCA cycle intermediates. Due to structural similarities shared between these aromatic compounds and monomers of the highly abundant biopolymer lignin, we hypothesized that *Rhodococcus* isolates derived from deep-sea environments could contain novel oxidative enzymes, possibly with enhanced stability to high pressure processes typically associated with biomass pretreatment. The TCA cycle intermediate acetyl co-A is the bio-synthetic precursor to fatty acids and triglycerides, the raw materials for biodiesel production. Because certain Rhodococci are known to accumulate up to 80% of their dry cell mass as lipids, we envisioned a biorefinery platform for biodiesel production using monolignol feedstocks. Thirty deep-sea bacterial isolates were identified as belonging to the genus *Rhodococcus* using 16S rRNA sequencing techniques. These strains were then screened by analytical HPLC for the catabolism of catechol, *p*-hydroxybenzoate and gentistate. Due to their promising catabolic profiles, isolates SPG 11-39, SPG 11-68 and SPG 11-73 were selected for further study. Growth curves were generated to determine the optimum feeding time for the selected monolignols. Quantitative HPLC analysis was used to determine the extent of monolignol degradation at various times and concentrations. Additional studies to characterize, quantify and optimize lipid production by these organisms are underway.

THE INDUCTION OF CRASSULACEAN ACID METABOLISM AND THE ROLE OF PHOTORESPIRATION IN PORTULACARIA AFRA

Alicia Wilson, Lonnie Guralnick, *Department of Biology*, Roger Williams University, Bristol, RI

RI EPSCoR Summer Undergraduate Research Fellowship Program

Portulacaria afra is endemic to South Africa and is a dominant species in parts of the Eastern Cape. Research is being done on the potential of Portulacaria afra for carbon sequestration in response to global climate change. P. afra is a facultative CAM plant that uses both C_3 and Crassulacean acid metabolism pathways. It closes its stomata during the day to conserve water and use stored malic acid as a CO_2 source when temperatures reach over 25°C, preventing photodamage. Photorespiration may be a factor in the plant's ability to withstand restricted water conditions. Plants were grown in a glasshouse under full sunlight (30°C/17°C) and water was withheld for up to 21 days. The level of carbon fixation enzymes and photosynthetic activity of P. afra leaves were examined under well watered and limited water conditions. Research showed an increase in PEP Carboxylase activity under limited water, indicating the induction of CAM. Noon time showed an increase of 30 fold, 6 PM had an increase of 16 fold, and Midnight had an increase of 7 fold between the control and 21 days water stress. Under 7 day water stress conditions, a diurnal fluctuation of acidity in P. afra leaves between the morning and night was 170 µeq/gfw and the 21 day water stress showed a 197 µeq/gfw fluctuation compared to the control with a fluctuation of 69 µeq/gfw. Glycine Decarboxylase (GDC) protein level, which is an indicator of photorespiration was determined through Western Blots. An increase in protein levels for all times of day was observed for water stressed samples when compared to controls. Further research is being done to study the role of photorespiration and the induction of the CAM pathway.

CONTROLLED REGIOSELECTIVITY IN THE ARYLATION OF INDOLES BY HPMV

Jillian Zoglio, *Department of Chemistry*, University of Rhode Island, Kingston, RI

RI-INBRE Summer Undergraduate Research Fellowship Program

The catalyst HPMV has been known to improve the rate of oxidative coupling and produce selectivity in the arylation of indoles. Protecting groups were placed on the nitrogen atom of the indoles to ensure that it would not be arylated in the reaction. N-pivaloyl and N-tosyl protecting groups were compared to determine which protecting group allowed for better separation and therefore isolation of the resulting product.

Electron withdrawing groups and electron donating groups were used to manipulate the position in which the indole was arylated. A general trend was observed in which electron donating groups did not produce selective arylation while electron withdrawing groups yielded a single product. However, weak electron withdrawing groups also did not allow for selective arylation. Consequently, indoles with electron rich substrates yielded better percent conversion of starting material.

CLIMATE AFFECT ON SEX EXPRESSION IN LOBELIA SIPHILITICA

Natasha Zupkus, Maia Bailey, *Department of Biology*, Providence College, Providence, RI

RI EPSCoR Summer Undergraduate Research Fellowship Program

Among flowering plants, around seven percent are gynodioecious (Richards 1986) meaning that populations consist of hermaphrodite individuals who produce both pollen and seeds and female individuals who only produce seeds. In many of these species, there is a correlation between the proportion of females in a population and its latitude (unpublished data). We are growing a gynodioecious wildflower, Lobelia siphilitica, whose populations span from Indiana up into Ontario, west to Iowa, and east to Massachusetts. In L. siphilitica populations, female frequencies vary from 0 and 100% with females being more common in populations located in the central southern range. Theoretical models suggest that these frequency differences can be attributed to differences in male function among hermaphrodites within populations. To determine whether the female frequency pattern seen in L. siphilitica is the result of hermaphrodite genotypes responding differently to different climates, we carried out a garden experiment in two incubators mimicking climate in different latitudes, Ontario and Indiana. We grew individuals from northern and southern populations in the two incubators and measured pollen viability counts. If variation between hermaphrodites is greater in hot climates, we will have found the likely cause of sex ratio variation. Our results will help us predict how other plants will be affected by climate change.

Author List

Last Name	First Name	Page Number(s)
Addy	Kelly	1,6
Adedeji Campbell	Titilayo	15
Ahmed	Aftab	1,116
Aicardi	Kristie	87
Albretsen	Erika	2,95
Almeida	Karen	35,64,82
Amer	Alex	98
Andreev	Oleg	125
Antonopoulos	Alexandra	4
Arevalo	Elisabeth	7
Austriaco	Nicanor	24,28,48,72,86
Barnes	Duane	5
Bailey	Maia	20,133
Bainter	Wayne	111,114
Basu	Amit	11,73
Battocletti	Amy	6,14,36
Beels	Stepahnie	112
Bennett	Richard	48
Bergstrom	Roy	37
Biancur	Douglas	7
Bickford	Nicole	8
Bierce	Alexandria	9,42
Bishop	Emily	10
Black	Eimear	11
Bliss	Joseph	87
Bloom	Christopher	83,29
Boltz	Lauren	12
Bothun	Geoffrey	12,123
Braun	Taylor	13
Brennan	Christopher	80
Breseman	Matthew	6,14
Brita	Jessen	88
Britt	Deborah	32,47,50
Carreiro	Jeremy	16
Cavedon	Peter	17
Celver	Jeremy	110
Chace	Jameson	8,32,49,53,57,127
Chau	Jeff	18
Chaudhary	Iqbal	1

Author List (continued)

Chauvin	Kristen	19
Chichester	Clinton	104
Chin	Christopher	28
Cho	Bongsup	37,92
Cimini	Richard	20
Cohen	Sage	63
Colin	Sean	65
Conaty	Eliza	21
Cook	Emily	22
Costello	John	65
Coy	Wendy	26
Crowther	Tia	23
Curran	Melissa	24
Dantonio	Alyssa	25
David	Johnson	88
De Groot	Anne	18,26
DeAngelis-Chichester	Amanda	104
DeBlois	Matthew	121,122
Deering	Robert	60
Degiorgis	Joseph	21,67,78
DeLeo	Nicholas	67
DeMarco	Ella	63
Deschenes	Tori	23
DeSimone	Brandon	66
Diamantopoulos	James	27
Diss	Paul	113
Doman	Soliel	1
Donaghey	Faith	28
Dostie	Kristen	14,36
Doyle	Caroline	29,83
Dunphy	Evan	30
Elgar	Sabrina	31,47,50
Espinosa	Avelina	33,71,90
Ewanchuk	Patrick	126
Faraz	Humera	1
Farragher	Caitlyn	32
Ferland	Layla	33,97
Fernandes	Kyle	22
Ferrucci	Jeffery	74
Feyerherm	Katrina	12,118
Flynn	Kayla	22,108

Author List (continued)

Fornari	Kevin	39
Forrester	Graham	45,68
Forschner-Dancause	Stephanie	60
Frazier	Ryan	34
Funk	Chris	35,82
Garlick	Kelsey	121,122
Gaudet	Cynthia	119,120,188
Gay	Justin	111,114
Gelin	Shennel	6,14,36
Gencarella	Christina	39
Gervasi	Carissa	62
Gilman	Sean	37
Goding	Mallory	38
Gold	Art	16
Goldfield	Beverly	39
Gonzalez	Alfredo	34
Goulet	Matthew	80
Gourlay	Allex	40,52
Govenar	B.	89
Grimm	Catherine	41
Guarracino	Alyssa	42,103
Guo	Liangrain	116
Guralnick	Lonnie	131
Gustafson	Eric	107
Gutelius	Danielle	70
Hall	Allison	43
Hall	J.	89
Hawrot	Edward	90
Helming	Katherine	28
Higgins	Kate	33
Hill	Courtney	118,119,120
Hoertz	Lana	23,44
Hokeness	Kirsten	58,70,73
Holevoet	Allison	45
Holmes	Garrett	46
Hopkins Jones	Masharee	31,47,50
Howlett	Nial	3
Hunt	Taylor	80
Hurton	Matthew	48,86
Igo	Alexandra	49
Inman	Kyle	47,50,31

Author List (continued)

Irving	Craig	114,115
Jain	Vipin	92
Javery	Benjamin	26
Joly	Stephen Patrick	72
Jones	Hannah	51
Jones	Joshua	52,4
Kane	Margaret	8
Karp	Caroline	128
Katija	Kakani	65
Keane	Meaghan	80
Kelly	William	53
Kent	Trevor	54
Keras	Gregory	96
Killian	Caroline	117
King	Roberta	25
Kinney	Lorin	55,66
Klos	Eric	65
Knoph	Kristen	56
Kopin	Olivia	57
Kovoor	Abraham	110
Kratch	Jackie	58
Kuhn	Helene	11
Kurniawan	Yogi	12
Kutil	Nicholas	59
La Rue	Molly	118,12
Lafond	Nick	118,12
Landi	Macayla	18
Lane	Christopher	24,85
Lariviere	Zachary	60
Lata	Jaci	101
Lavigne	Christina	61
Leavitt	DF	69
LeBlanc	Garrett	62
Lepore	Kirstie	39
Levine	Mindy	63,108
Li	Mengzhuo (Jenny)	102
Lombardo	Kara	96
Lora	José	64
Lu	Wei	116
Lucas	Kelsey	65
Ma	Hang	56

Author List (continued)

Maglysh	Irina	101
Male	Alexandra	61
Malloy	Thomas	55,66
Marcello	Nicholas	67
Markert	Jeffrey	121,122
Markey	Kathryn	17,41
Matarese	Sarah	39,42,53
Mather	Cailyn	35,82
Mather	Tom	26
Mazick	Josh	29,83
Mazzucca	Nicholas	80
McNally	Dan	46,129
McNulty	Katherine	61
Medeiros	Tanie	18
Meedel	Thomas	81
Merolla	Sarah	68
Meschwitz	Susan	8,13,38,57,69,93,127
Millen	Michael	69
Miller	Ryan D.	70
Misner	Ian	24
Mitchell	Andrew	71
Moseman-Valtierra	SM	89
Moshnikova	Anna	125
Moss	Steve	42
Munge	Bernard	4,96,99
Murphy	Michael	72
Murray	David	43
Nadolny	Christina	73
Natale	Julian	63
Neira	Karissa	3
Nelson	David	91
Nesbitt	Sonia	87
Nicodemus	Samantha	3
Norris	Stephen	37,74
Northup	Kevin	75
O' Connor	Katie	81
O'Brien	James	76
Octeau	Chris	110
Oczkowski	Autumn	84
Oh	Donghoon	75
Overstrom	Neal	102

Author List (continued)

Pallotta	Michael	77
Paranal	Ryan	78
Parang	Keykavous	75
Parmentier	Julia	46,54,129
Pellichero	C.R.	79
Pellock	Brett	80
Peters	Brenna	29,83
Pickett	C.J.	81
Pierce	Jamie	129
Pina	Katelyn	35,82
Poidomani	Paul	21
Popolizio	Thea	85
Post	Ryan	29,83
Prell	Warren	43
Radka	Megan	101
Rahman	Sabiha	101
Reid	Christopher	11,46,58,70,73,87,129
Reshetnyak	Yana	125
Resnick	Margaret	84
Rhyne	Andrew	40
Roblee	Emily	48
Rogers	Alyssa	85
Rogers	Stephen	86
Roohani	Keyana	87
Rossi	Ryann	88
Rossi	Lauren	71
Rowley	David C.	13,54,60,93,130
Salerno	Lauren	71
Sanders	Tanya	90
Schindelwig Franca	Kevin	33,97
Scholz	Carmen	12
Schoolcraft	K.	89
Schooling	Peter	30
Seeram	Navindra	38,56,69
Selinski	Christian	90
Sexton	Zachary	80
Shabashevich	Eric	91
Shakarian	Alison	2,23,44,95,112
Shamp	Tom	85
Sharkey	Annalisa	92
Sharma	Meenakshi	110

Author List (continued)

Showalter	Sarah	93
Silva	Adam	94
Slitt	Angela	94
Smith	Morgan	96
Smith	Bridgett	95
Smith	David	60
Smith	Amanda	129
Smolowitz	Roxanna	17,41
Sobon	Hanna	33,97
Socha	Aaron	130
Solares	Jose	98
Somba	Brian	99
Soto	Adrian	100
Squibb	Eliza	102
Stafstrom	Kelsey	9,42,103
Stevenson	Laura	105,106
Stevenson	James	104
Stonestreet	Barbara	118,119,120
Subramaniam	J.	105,106
Suglia	Elena	107
Suits	Madeleine	108
Sullivan	Elizabeth	109
Sullivan-Watts	Barbara	128
Sun	Kevin	110
Suter	Zachary	70
Swanson	J.D.	6,9,14,36,42,103
Symington	Steven	111,112,113,114,115
Szulak	Kevin	116
Taylor	David	43,59,62,77
Taylor	Cristina	61
Thakore	Rachel	117
Thomas	Stacy	72
Thornber	Carol	6,10,14,36
Threlkeld	Steven	118,119,120
Topchiev	Farid	123
Traghella	Rachel	66,124
Tran	Holly	125
Trotta	Lauren	126
Tucker	Douglass	72
Udwary	Daniel	117
Van Orden	Madison	127

Author List (continued)

Van Reet	Jennifer	61
Varkey	Karl	115
Venkataramanan	Keerthi	12
Vumback	Matt	20
Walsh	Rylie	21
Warren	K.S.	105,106
Weiss	Lena	128
Welch	Amanda	22
Welsh	Molly	16
Wessel	Gary	107
White	Zoe	129
Whyte	Dorian	130
Wilcox	Alexander	72
Wilcox Freeburg	Eric	40,52
Wilkinson	Kristen	22
Williams	John	15
Wilson	Alicia	131
Xi	Aihong	123
Xu	Jialin	94
Yajima	Mamiko	25,107
Yalcin	Emine	116
Yan	Daisy	18
Yekta	Shahla	56
Yuan	Tao	42
Zhang	Songhua	91
Zhao	Wenjing	132
Zoglio	Jillian	133
Zupkus	Natasha	