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Spring Research Symposium



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The role of Non-canonical(NC) NF-kB in canine DLBCL

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NF-kB is a highly conserved family of transcription factors, that control innate and adaptive immune responses. NF-kB activation is highly regulated through either the canonical or the non-canonical pathway. The canonical pathway is activated by hundreds of immune and inflammatory stimuli and regulates over three hundred genes involved in innate and adaptive immune responses. The non-canonical (NC) NF-kB pathway is activated by a very restricted set of stimuli and regulates the expression of cytokines and chemokines that lymphoid organogenesis, B-cell maturation, proliferation and B cell survival. In health, the NC NF-kB pathway is highly regulated only being activated by B cell growth factors and inflammatory stimuli. However, this pathway has been shown to be constitutively active in certain cancers of humans including multiple myeloma, Chronic Lymphocytic Leukaemia (CLL) and B and T cell lymphomas. This constitutively active pathway is believed to promote survival and proliferation of neoplastic plasma cells and lymphomas and inhibiting this pathway could have beneficial effects in the treatment of lymphoma. Cells from the lymph nodes of dogs with Diffuse Large B-cell Lymphoma (DLBCL) were tested for the presence of constitutively active NC NF-kB activity by qRT-PCR and Western Blot analysis (WB). The cell lines GI-1 a B-cell leukaemia cell line that has been around for about 10 years, CLBL-1 a recently characterized cell line that came from a canine malignant lymph node and the 17-71 cell line that was derived from the peripheral blood of a dog with multicentric lymphoma were also interrogated by WB for NC NF-kB pathway activity in order to examine the significance of the pathway in lymphoma development and survival. We found that canine DLBCL cells had evidence of constitutively active non-canonical NF-kB pathway activity based on the over-expression of non-canonical regulated genes and the presence of increased p52:p100 ratio. Our results suggest that this pathway may be involved in lymphomagenesis in canine patients and that the dog may serve as an important spontaneous model for human DLBCL subtypes exhibiting constitutive non canonical NF-kB activity.

This project was sponsored by NIH MARC Grant # 5T34GM008422.

Comparing the Microbial Communities of Black Band and White Plague Disease

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Coral disease is now considered one of the leading threats to coral reefs around the world and may have contributed to the greatest loss of coral cover in the Caribbean, where many of the known diseases can be found. Some of the better characterized diseases include yellow band, white pox, white plague, and black band disease. The latter two have had shown devastating power in the Caribbean where they affect a large population of reef building corals. Without these species of corals, the composition of the reefs changes from coral- to algal-based and mankind loses the services these corals provide. This study is novel in that it compares the communities of black band and white plague disease. Also, there will be an attempt to trace the origin of these microbes by analyzing the microbial communities in the surrounding water column and sediments. Black band and white plague disease samples were collected from reefs around the island of St. Thomas. Samples were also taken from the surrounding water column and sediment near infected colonies and control colonies. DNA was extracted from the samples using established protocol. The universal bacterial 16S rDNA primers were used in polymerase chain reaction (PCR) analyses to amplify bacterial DNA within the samples. Specific bacterial primer used in previous studies were then used in PCR reactions to isolate certain bacteria that have been observed in both BBD and WPD samples from previous studies. Thus far, the preliminary results show that there is indeed bacteria within all samples collected and that DNA has been extracted. The specific primers were not successful in amplifying any DNA from the samples. It could be that the protocol has not been optimized or that these species of bacteria are cannot be found in the samples taken so far. Optimization of extraction and PCR protocol is still being conducted. This study will be the first of its kind in the Virgin Islands region as well as in the literature. Identification of specific bacterial species found in association with the coral diseases in this study will enhance research in other regions of the globe, by suggesting certain species that should be screened for. This study will add to the knowledge of the variability of coral diseases around the world. It can be used as reference for future research on black band and white plague disease. This study aims to isolate the environmental factors that may initiate coral diseases, another first.

This research supported by the Lana Vento Charitable Trust and VI EPSCoR.

**Threats to Our Reef
Lionfish-The Eating Machine**

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The invasion of the Atlantic by the Indo-Pacific Lionfish (*Pterois Volitans/Miles Complex*) began in the early 1990s off the coast of Florida. By 2008 the invasion made its way to the United States Virgin Islands. On November 25, 2008 the first Indo-Pacific Lionfish was removed from Frederiksted Pier on St. Croix. Because there are no predators to the lionfish in the Caribbean the invasion continues. This project aims to identify the prey of the invasive lionfish recovered off the westend of St. Croix as a means to possibly predict what aquatic species will face extinction as the invasion continues. The fish removed were characterized by sex, length, and weight. Through dissection the stomach contents were analyzed and when possible the species were identified. The male to female ratio of the lionfish was also observed. Our stomach content analysis showed the juvenile yellowhead wrasse, shrimp and the bicolor damselfish were the most frequent prey. Trends in the weight, length, and male to female ratio of the lionfish were also observed. Female lionfish were generally larger and bigger than male lionfish.

This research was funded by NSF HBCU-UP (Grant Number HRD – 0506096) and by the NIH MBRS-RISE Grant Award No. GM061325.

Coral Community Structure Change of Brewers Bay, St. Thomas USVI After a 30-Year Period

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Coral reefs, while they cover less than .1% of the ocean surface area, harbor up to 25% of all marine fish species and have been estimated to be associated with 1-9 million species of organisms. This ecosystem provides humans services valued at approximately \$US375 billion worldwide. Global warming, ocean acidification, over fishing, eutrophication, and coastal development threaten the health and prosperity of coral reefs. Synergism of these coral hazards has resulted in a significant decline in coral cover on many shallow Caribbean reefs in the last three decades. Not all reefs have been affected similarly, however, and some shallow reefs that are protected from local sources of stress have proven more resilient to large-scale regional stressors like thermal anomalies that result in mass bleaching. Here we investigate changes in the health and structure of a shallow Caribbean reef today over the same time period.

In 1978-1982 an assessment of coral diversity and abundance was conducted at 3 sites in Brewers Bay, St. Thomas (USVI) by the Division of Natural Resources Management prior to and after an extension of the St. Thomas airport runway. Since then the area has been subjected to multiple hurricanes, at least one mass coral bleaching event, and at least one large disease outbreak and it is periodically subjected to sedimentation and nutrient inputs from a densely populated watershed. In the spring of 2012, we conducted an identical abundance survey to that of 1978-1982 assessment under the assumption that coral community structure has changed. A total of 3 sites and 12 transects were surveyed for coral diversity, coral cover, and rugosity as well as lesion presence and mortality (not included in original assessment). Changes in coral cover, species composition and rugosity in relationship to disease prevalence are investigated using uni- and multi-variate statistical tools.

A change in coral benthic composition or community structure can mean a decrease in rugosity or habitat space necessary for other species' survival. Studies such as these are therefore important for identifying these changes, which may ultimately result in a decrease in coral reef ecosystem productivity.

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Genetic Variants in CYP4F2 Gene Affects Maximal Diastolic Blood Pressure and BMI in Response to Exercise Training

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High blood pressure is a major risk factor for heart disease, stroke and kidney disease. Cardiovascular diseases (CVD) and its risk factors are highly heritable and prospective research has suggested that they have a strong genetic basis. In addition, there is evidence that suggests that CVD and its response to exercise training is also heritable. Our goal was to investigate the association of genetic variants of the cytochrome P450 (CYP4F2) gene with hypertensive characteristics and other CVD risk factors in response to exercise training. We focused our analysis on a 1347 G/A polymorphism (rs2108622) of the CYP4F2 gene, that was previously associated with hypertension, with carriers of the A allele being at higher risk relative to non-carriers. One hundred and eighty four sedentary, normotensive and dietary stabilized; older Caucasian men and women were involved in the study. DNA was extracted before and after a six month endurance exercise training program and the genotypes were determined by PCR-RFLP technique. The PCR products were subjected to sequencing to confirm genotypes. Data were analyzed using SPSS software to test for statistical significance for the hypertensive phenotypes before and after training. The A allele associated significantly with body mass index and maximal diastolic blood pressure ($p=0.036$ and 0.044 respectively) in response to exercise training. Overall, our finding suggests that CYP4F2 variation explains the inter-individual differences in the response of hypertensive phenotypes to exercise training. Further work is still being done to genotype more individuals. In conclusion, these data contribute to the growing understanding of CYP4F2 as a candidate gene for hypertension and other CVD.

This work was supported by NIH AR059913, UMD Kinesiology GRIF (KCJ) and NIH MBRS RISE Research Program Grant #GM061325.

Caribbean hybrid *Acropora prolifera* viability restricted to shallow reef zones

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The critically endangered Atlantic scleractinians *Acropora palmata* and *Acropora cervicornis* (Lamarck 1816) produce a fecund hybrid, *Acropora prolifera*, providing a genetic store for the genera as well as limited functional ecological redundancy. This study hypothesized that environmental and genetic factors limit the typical spatial distribution of *A. prolifera* to extreme shallow water reef zones, where *A. palmata* typically occurs as opposed to deeper reefs (>5m) where *A. cervicornis* is primarily found. At Flay Cay (US Virgin Islands), four visibly healthy *A. prolifera* colonies growing at least 5m apart were fragmented, weighed (buoyant weight), and placed at 2-3m (control), 10m, and 22m depths in different light and current regimes for seven months to assess viability and growth characteristics relative to environmental characters via change in buoyant weight and a photographic time course. Colony maternal inheritance and genotype were also determined. Depth (df=23, $p=0.0001$) and genet ($p=0.0069$) both affected fragment growth rates (with no interaction). Control fragments grew faster than both 10m and 22m fragments (48%, 65% respectively) and three of four genets exhibited different growth rates. Frequency analysis showed depth significantly increased mortality ($p=.004$), disease incidence ($p=.0026$), and macroalgal interaction prevalence ($p=0.0001$). Scanning electron microscopy determined neither depth nor genet caused a difference in axial corallite outer diameter (df=11, $p=0.1526$, $p=.3447$ with no interaction) or a variation in number of axial concentric rings (all-3). The hybrid *A. prolifera* may be restricted to its shallow depth range by environmentally driven post-settlement factors that limit viability at deeper depths.

This project was supported by the Lana Vento Charitable Trust and VI EPSCoR.

Comparison of Angle Resolved Photoemission and Scanning Tunneling Microscopy on High T_c superconductors using the YRZ Model

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The goal of this study is to use the Yang-Rice-Zhang (YRZ) to model the behavior of electrons in a sample of $\text{Bi}_2\text{Sr}_2\text{CaCu}_2\text{O}_8$ for the pseudo gapped, and superconducting state. Two experimental techniques are known to perform such a task and they are in some disagreement. Angle resolved photoemission spectroscopy (ARPES), a direct measurement, and Scanning tunneling Microscopy (STM), which provides similar information, and determines electron behavior after some considerable data processing; appear to show somewhat different behaviors for high temperature cuprate superconductors. While the YRZ model appears to work for photoemission, it must now be tested against the STM data. The main thrust of this project is to determine the energy range the model agrees with the STM data, if at all. In order to accomplish this, constant energy contour images were first created. The Gwidyon software was then used to create autocorrelation images to determine the quasiparticle interference (QPI) patterns. The QPI image and crystal symmetry were then used to determine the q-vectors of the octet model. This calculation was scripted in MATLAB and ran to calculate the dimensions of the q-vectors for Binding energies between -105 meV and 105 meV. The q vectors connected points of high density of states in the constant energy surfaces, reflected by bright spots in the autocorrelation images. One of the major findings includes the fact that the graphs are not symmetric in binding energy, as was previously believed.

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Content not available.

The Evasive Nature of the HIV Virus

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HIV, the virus that causes AIDS, is one of the most studied diseases in the scientific world today primarily because of the commonality of the disease, and the inability to develop an efficient cure. People with HIV-1 often times are treated with Highly Active Anti-Retroviral therapy (HAART). Maraviroc is a type of HAART drug that blocks HIV-1 from interacting with the transmembrane protein CCR5 receptor on human cells (macrophages, microglial cells, t-lymphocytes etc.) (Lavi, et al., 1997). Characteristics of HIV-1 that has facilitated its evasive nature is its coined term "quasispecies" where individual viruses of one generation can differ up to 10% from each other. In addition to this naturally high mutation rate, the reverse-transcription cycle is prone to error thus making HIV-1 even harder to target. These properties may allow HIV-1 to switch to receptors, and utilize the CXCR4 receptor instead of its preferred CCR5 (Mosier, 2009). When this switch occurs, HAART is less effective and patients experience worsened symptoms and develop AIDS at a faster rate. Labs all over the world are working to eventually develop a way to prevent HIV from interacting with the CXCR4 receptor, and in an effort to assist in this enormous project, our lab made clones of the viruses' envelope gene acquired from 12 patients with HIV-1. We did this by ligating the gene into a TOPO-TA vector and transforming into Top 10 *E. coli* cells. Each patient had two samples for two time points, where the first is before treatment with Maraviroc and the second is after two weeks of treatment on Maraviroc. Clones from first time point is expected to be CCR5 utilized, while the second time point is expected to be CXCR4 utilized. I transformed these DNA samples into *E. coli* bacteria and screened for envelope by PCR. Developing clones that represent the viruses' quasispecies population, transfecting, then placing the viruses produced in a human cell environment will help us to detect which viruses utilize CXCR4, or both receptors. The next steps are sequencing the envelopes we harvested to determine what mutations, due to spontaneous recombinations in the life cycle, are prone to utilizing a particular type of receptor. This will eventually lead to much more effective binding inhibitory medicine.

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Investigation of Doping in Graphene

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Graphene is comprised of a single layer of carbon atoms; it can be created by mechanical exfoliation of a single or a few layers of the material from bulk graphite, or grown by chemical vapor deposition through catalysis of methane on a metal substrate. This material has remarkable properties for both fundamental studies as well as potential applications in advanced electronic devices. Its mobility at room temperature, for example, is extraordinarily high (more than 10 times that of silicon) and is essentially unchanged by chemical doping. In a manner similar to that in semiconductors, the possibility to dope graphene either substitutionally or by using an organic route means is of particular interest for applications in electronic devices and chemical sensors. Little is known about how to controllably dope graphene at this stage. Since many semiconductor devices depend on the ability to dope the material, it is important to understand how to carry out this process in graphene so that its potential for electronic devices can be fully explored. This will be the focus of the proposed research.

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Successfully Classifying Promoter Regions of *Escherichia coli* into Different Functional Types Using Machine Learning

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Promoters are regions of DNA found at the beginning of DNA segments called genes. Promoters are essential in cells because they are the binding sites for the protein RNA polymerase. Once bound to the promoter, given the right conditions, RNA polymerase will initiate the transcription of that gene, creating messenger RNA that is later translated to make a protein encoded by that gene. In our research project we studied different types of *E. coli* promoters. Each promoter is specifically recognized in the cell by one of six different sigma (σ) subunits of RNA polymerase, $\sigma70$, $\sigma54$, $\sigma38$, $\sigma32$, $\sigma28$, and $\sigma24$. Different σ 's are active under different environmental conditions and are critical in regulating gene expression in *E. coli*. There is structural similarity shared by $\sigma70$, $\sigma38$, $\sigma32$, $\sigma28$, and $\sigma24$, so these proteins are called the $\sigma70$ family. Sigma 54 is outside of the $\sigma70$ family. The purpose of this project is to see how accurately machine learning algorithms can classify previously identified promoter sequences into different functional categories using tetra-nucleotide frequencies as distinguishing features. We wrote a PERL script to create a program that would calculate the frequencies of tetra-nucleotides in each sequence. Those frequencies were then used as input for classification algorithms in the WEKA data mining platform. We used two algorithms, the C4.5 tree algorithm and the Naïve Bayes algorithm, to classify promoter regions into different classes, for example $\sigma70$ vs. $\sigma54$. Our first null hypothesis is that there is no difference between the distribution of correct and incorrect sequence instances classified using the C4.5 algorithm and the expected for random guessing, 50% correct. In fact, the accuracy for the C4.5 classification is 68%, and using the X^2 (Chi-squared) test, we reject this null hypothesis (p-value <0.0001). Our second null hypothesis is that there will not be a significant difference in the accuracy of classification of promoters between classifications into one class that is recognized by a $\sigma70$ family member and another class recognized by $\sigma54$. Using the C4.5 tree algorithm, pairs of classes that included $\sigma54$ had an average correct classification of 73%, whilst pairs of classes that only included members of the $\sigma70$ family had an average correct classification of 62%. Using a X^2 test, we reject this second null hypothesis (p-value \approx 0.0002). The result is that there is a significantly greater accuracy of classifications of promoters when one of the promoters is outside the $\sigma70$ family than when both are in the $\sigma70$ family. In addition, we will present results using the Naïve Bayes algorithm and discuss efforts to increase the accuracy of classification and identify those features that are most useful in these classifications.

This research was supported by NSF HBCU-UP grant number HRD- 0506096. The promoters used in this research were retrieved from the RegulonDB Database. <http://regulondb.ccg.unam.mx/>

Comparison of Parents with F₁ and F₂ Generations of Sorrel (*Hibiscus sabdariffa*)

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Hibiscus sabdariffa or sorrel is grown throughout the world for its fresh juice made from the calyxes of the plant. Plant height can reach up to 7 feet and branches between varieties vary. The objective of this study was to compare the height, branches and floral induction of two parent Caribbean sorrel varieties with F₁ and F₂ generations. The parental varieties were St Kitts dark (SKD) and Trinidad black (TTB). Data was collected on height, branching and floral induction at two week intervals. Parent SKD had the greatest amount of fruit-bearing branches than TTB, F₁ or F₂ populations. The TTB x SKD F₁ resulted in taller plants than either parent or the SKD x TTB F₁ plants. However, all F₂ generation plants were shorter than either parent. All F₁ plants initiated flowers when SKD did which was two weeks before TTB, however all the F₂ initiated flowers with TTB. Sorrel is selfpollinating causing inbred varieties but hybrid vigor can be obtained by crossing varieties which can improve production.

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Investigating the role of an actin-binding protein, palladin, in human fibrotic disease

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Fibrosis is a condition in which the interstitial spaces become filled with excess extracellular matrix fibers, which causes hardening of the organ that can lead to organ failure. Fibrosis can occur in any organ, and it is currently believed that approximately 45% of all disease-related deaths in the United States are caused by some form of fibrosis. Pancreatic fibrosis is caused by binge drinking, and it leads to a progressive, irreversible loss of pancreas function. In the pancreas, in response to alcohol metabolites, pancreatic stellate cells (PSCs) become activated and differentiate into highly proliferative myofibroblasts that display enhanced motility, contractility and adhesive abilities. These activated PSCs are believed to be the primary cellular mediators of pancreatic fibrosis. Recent studies have shown that the actin-binding protein, palladin, is highly expressed in activated PSCs. In the current study, we tested the hypothesis that palladin plays a key role in the activation of stellate cells to become myofibroblasts. The myofibroblast phenotype arises due to changes in gene expression, which are regulated by transcription factors such as myocardin-related transcription factor (MRTF) and serum response factor. It has been shown previously that palladin can translocate between the cytoplasm and the nucleus, and further, that palladin binds to MRTF and may regulate its transcriptional activity in the nucleus. CDC42 is a small GTPase that plays a critical role in the nuclear translocation of MRTF. Interestingly, our preliminary results show that palladin regulates the activity of CDC42 in PSCs: when palladin is knocked down, CDC42 activity decreases **dramatically**. This leads us to hypothesize that palladin plays a key role in regulating the activity of CDC42, and thus that palladin expression is required for the nuclear import of MRTF. We utilized cytoplasmic/nuclear extraction to separate the cytosol and nucleus in the PSCs, and immunoblot analysis and immunofluorescence microscopy to detect MRTF localization in three treatments (normal PSCs, palladin-knockdown (KD) PSCs, and CDC42-knockdown (KD) PSCs). Our findings show that in normal PSCs, MRTF was detected predominantly in the nucleus. In contrast, MRTF was detected at higher levels in the cytosol of both palladin KD and CDC42 KD cells. Together, these results highlight the role of palladin in MRTF localization and suggest that palladin may play an important role in regulating MRTF-mediated gene transcription. Understanding the pathway and roles that these three molecules play in pancreatic fibrosis will aid in our future goals to create drug therapies that are effective in controlling not only the progression of pancreatic fibrosis, but many fibrotic diseases.

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Damsel fish territorial behaviors on the spread of disease in coral reefs

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Damsel fish are territorial species and have territories of corals that they feed on and prevent other predators from feeding on. The damselfish could be a factor in the spread of disease on coral reefs and may be a vector in the spread of disease.

If disease is spread by corallivores that have fed on infected corals, the presence of territorial damselfish would prevent the spread of disease by preventing a disease carrying corallivore from infecting an uninfected coral. However, if a coral within the damselfish's territory is infected with a disease (that can be transmitted by feeding) the corals in the damselfish's territory would become infected and would continuously be re-infected until the epidemic has passed or the corals die.

The hypotheses tested are: the presence of damselfish in coral reefs is responsible for the spread of disease in reefs.

Data collected by the U.S. Virgin Islands Territorial Coral Reef Monitoring Program was used to develop and analyze models of damselfish territories and the prevalence of disease in reefs that are colonized by different species of damselfish. The results of the model were compared to actual disease data taken from field observations of coral communities that have been colonized by damselfish.

This project was sponsored by NIH MARC Grant # 5T34GM008422.

Characteristics of Sorrel Parents, F1 and F2 Generations

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Sorrel, *Hibiscus sabdariffa* also known as, Jamaican sorrel or Roselle, develops a bright calyx of petals with an acidic flavor. Sorrel is used to make juice, punch, wines or a variety of delicacies such as jams and chutney. It is harvested mostly during the Christmas season and is part of most of the islands' traditions. The purpose of this experiment is to evaluate characteristics of parental, F1 and F2 generations of sorrel from selected crosses. The developmental characteristics of F1 and F2 generations of sorrel were compared to their parents in regards to plant height, number of branches and floral induction on a bi-monthly basis. Three sorrel varieties were used in this experiment included a Day Neutral variety from St Kitts (KDN), 268100 from Nigeria (100) and a local white variety from St Croix (W). Three hybrids were used in this experiment were KDN x 100, 100 x KDN and W x 100. A 100 x KDN also had an F2 generation. This experiment has far reaching agricultural implications because it was found that all hybrids with the parental 100 and KDN grew faster. It was also found that the white variety took the longest to initiate floral buds. The F1 generations kept the characteristics of the parents with regard to height, leaf shape and flowering time. However, the F2 generation had characteristics of both parents.

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Is there a social preference of the rock boring sea urchin, *Echinometra lucunter*?

Tricia Greaux

Dr. Teresa Turner (mentor)
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The rock boring sea urchin, *Echinometra lucunter*, has not been extensively studied and little is known about its social preferences. The survival of this intertidal herbivore is crucial in order to create a better environment for the survival of coral reefs. *Echinometra lucunter* eats algae which harms corals. Using a transect (3.5 m) and 6 quadrats (25 cm x 25 cm), a density analysis was conducted in the field to determine the natural occurrence of the *Echinometra lucunter*. The coefficient of variance was used to reveal aggregation. I observed these sea urchins push each other as a form of intra-specific competition. An experimental study was designed to determine the social preference by using a Y-maze choice experiment conducted at the William P. MacLean Marine Center, St. Thomas campus, University of the Virgin Islands. In the choice experiment, there was a group of juveniles in one arm of the Y-maze, with a single adult in the other arm (n=22). This was done to determine if, like other species of sea urchins, the juvenile *Echinometra lucunter* would aggregate with other juveniles. The other possibility is that adults protect the juveniles under their spine canopy. Therefore, the juvenile would travel towards the adult. A chi-squared test (independence) revealed there was no strong difference in the choice between both arms (p-value =0.088). This means the juvenile sea urchins did not respond differently to the cues from the groups; juveniles vs. single adult.

This research was supported by the Emerging Caribbean Scientists (ECS) Honors Fund.

Sediment Based Pollutant Regulation and Mitigation

Nathan Gubser

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The USVI has been mandated by Environmental Protection Agency (EPA) via the Clean Water Act to develop standards for the maximum quantities of pollutants that specific territorial bodies of water are to receive. The primary issue seen in St. Croix's associated waters is turbidity. Turbidity is the measure of water's clarity in regards to cloudiness from suspended solids via sources such as sedimentation and erosion. Boiler Bay is a site where significant terrestrial erosion is occurring from an old dirt road that directly discharges into the bay and causes turbidity. Boiler Bay is located within the St. Croix East End Marine Park (STXEEMP) and contains a myriad of protected marine environments and endangered species, such as coral and sea turtles. Previous studies at Boiler Bay estimate the annual delivery of 2.5 tons of sediment into this body of water. Sedimentation such as that from the Boiler Bay watershed has been considered one of the primary stressors to coastal aquatic habitats in the Virgin Islands.¹

This study utilized an EPA approved YSI 6920 sonde to gather high resolution measurements of turbidity, conductivity, ph, and dissolved oxygen parameters. A tipping bucket rain gauge was concurrently monitoring precipitation to accompany these data sets. This combined data provided a detailed understanding of turbidity in Boiler Bay and also examined the links between these parameters and the previous study's sediment quantifications. "The type of high-resolution water quality data intended for this study is commonly discussed as an important, but lacking source of information in Local Action Strategy meetings".² Furthermore, this research will introduce potential schematic design options that could mitigate Boiler Bay's erosion rates and improve this aquatic environment's overall health.

¹ Rogers, C. S., Miller, J., Muller, E. M., Edmunds, P., Nemeth, R. S., Beets, J. P., et al. (2008). Ecology of Coral Reefs in the US Virgin Islands. In B. M. Riegl, *Coral Reefs of the USA* (pp. 303-373). Springer Science+Business Media B.V.

² Reale-Munroe, K.(2011). Terrestrial Sediment Delivery and Nearshore Water Turbidity – A Case Study From the East End of St.Croix, USVI. *Unpublished Manuscript*. University of the Virgin Islands, VI.

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Coral Reef Ecosystems in the Virgin Islands

Jemica Gumbs

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Coral reefs deliver ecosystem services to tourism, fisheries and shoreline protection. However, coral reefs are fragile ecosystems, partly because they are very sensitive to water temperature. They are under threat from climate change, ocean acidification, blast fishing, cyanide fishing for aquarium fish, overuse of reef resources, and harmful land-use practices, including urban and agricultural runoff and water pollution.

Using a variety of data analysis programs, including Mathematica, the project collected numeric data on how Virgin Islanders visually perceive coral reef ecosystems, their knowledge of the reefs and ways in which they contribute or harm the continuity of healthy reefs within the waters surrounding their islands. An image analysis was also executed to compare the ratings versus the image structure for each coral reef ecosystem used throughout the project.

The information collected from this project will be beneficial to any coral reef outreach project within the Virgin Islands, and aiding understanding of factors influencing visual and aesthetic perceptions of coral reef conservation in the region.

This research project was made possible through NSF HBCU-UP Research Fellowship, funded through a grant from the National Science Foundation (NSF) grant number HRD – 0506096. Additional funding for the data collection and analysis of the research undertaken is provided by the NSF VI-EPSCoR incubator project “The role of experiential social learning in achieving semantic transformation in community attitudes, beliefs and behaviors towards coral reef resilience” (K. Alexandridis, K. Engerman and T. Turner), grant number 203058.

Molecular detection of *Rickettsia* sp. in *Rhipicephalus sanguineus* (the brown dog tick) from dogs in the United States Virgin Islands

Akacia Halliday

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Rhipicephalus sanguineus is a species of tick common throughout the world. In addition to its commonality, *R. sanguineus* is known to carry bacterial pathogens such as *Bartonella* sp., *Anaplasma* sp., and *Rickettsia* sp. (just to name a few). When *R. sanguineus* bites a person or animal it may transmit these pathogens. Spotted fever group rickettsiae are known to cause severe illness in humans. SFG rickettsiae largely affect travelers and people who visit high incidence areas. To evaluate the risk of human or animal infection with vector-borne pathogens in the U.S.V.I., a local veterinary clinic provided us with *R. sanguineus* collected from dogs. We tested the specific hypothesis that SFG rickettsiae are present in *R. sanguineus* in the U.S.V.I. Molecular assays, specifically PCR analyses, using *Rickettsia*-specific primers were employed to test our hypothesis. One set of PCR primers target the citrate synthase (*gltA*) gene specific to the *Rickettsia* sp. A second set of primers target a fragment of the outer membrane protein A (*rompA*) gene that is only present in SFG rickettsiae. Total DNA was extracted from 22 individual adult ticks using the Qiagen DNeasy Blood and Tissue Kit, and the PCR assays were employed to screen the DNA extracts for the presence of rickettsial DNA. Preliminary results indicate the presence of *Rickettsia* sp. within 22/22 (100%) of the tick DNA extracts by DNA agarose gel-based visualization of an amplified DNA fragment of the expected *gltA* fragment size (381 bp). Interestingly, when the PCR products were separated by gel electrophoresis, additional amplified DNA bands were present. These bands were both larger and smaller than the expected 381bp fragment targeted by the *gltA* PCR primers. However, our *rompA* PCR results demonstrated an absence of amplified DNA fragments from *R. sanguineus* DNA extracts when PCR reactions were visualized by DNA gel electrophoresis even though controls indicated PCR was working properly, suggesting that the *gltA* band may not originate from *Rickettsia* DNA. We plan to further investigate the nature of the ~381 bp fragments generated in the *gltA* PCR by DNA agarose gel purification and DNA sequencing. Sequences of the gel-purified fragments and results from PCR screening of additional *R. sanguineus* DNA extracts will be presented.

This work was sponsored by NSF HBCU-UP grant number HRD-1137472.

Determination of Desirable Traits in F₁ Generation of Sorrel

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Hibiscus subdariffa is a plant that is native to the subtropical and tropical regions of the world. More specifically it is grown in Jamaica and other Caribbean islands, such as St. Croix. The aim of this study was to select for characteristics, such as height, fruiting and number of branches via hybridization of Caribbean and African lines. Three parents were used, being Trinidad black (TTB), Zambian varieties 500726 (26) and 500721 (21). Three hybrids were created, being TTB x 26, TTB x 21, and 26 x TTB. Every two weeks data was collected on specific parameters, such as plant height, number of branches and the time of flower bud initiation over 98 days. In comparing 26, TTB, 26 x TTB and TTB x 26 among each other, there were no large differences between plant heights. However, when number of branches were compared, the hybrids were between the two parents. Additionally, the pattern of flower bud initiation between the parents and the hybrids followed the TTB variety. In comparing TTB, 21 and TTB x 21, the height of the hybrid was greater than both of the parents. Conversely, the hybrid was right between the parentals with regard to the number of branches. When it came to flower bud initiation, the hybrid was similar to 21 and not TTB. Thus, there was no set of F₁ hybrid combinations that followed an all-inclusive pattern of heredity.

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Mitofusin-2 Protects Renal Epithelial Cells Under Stress

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The proximal tubule of the kidney is rich in mitochondria, double-membrane organelles that generate ATP and integrate cell signaling events that result in apoptosis. Mitochondria are dynamic and constantly undergo fusion and fission events that alter their morphology and may perhaps, regulate apoptosis and organ function. We hypothesized that Mitofusin-2 (MFN2), a mitochondrial fusion protein, is important in the renal epithelial cell stress response. To test this hypothesis, the MFN2 floxed gene was conditionally knocked out in the murine kidney. Despite marked mitochondrial fragmentation in the kidneys of juvenile MFN2-cKO mice, renal histology, development and organ function did not differ from wild type. In contrast, primary cultures of MFN2-cKO kidney epithelial cells subjected to chemical ischemia were significantly more susceptible to mitochondrial membrane injury, caspase 3 activation and apoptosis than wild type ($P < 0.05$). Bax, a major cause of apoptosis in these cells was equally activated, but far greater mitochondrial Bax accumulation was detected in MFN2 KO cells. We conclude that MFN2 protects renal epithelial cells by preventing Bax accumulation on the mitochondrial membrane.

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**DNA Extractions of White Mangroves:
The enzymes RNase & Proteinase K produced the purest DNA**

Gemel Joseph & Lavidia Brooks

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Mangroves are known for their high salt tolerance and ability to provide shelter to many aquatic and non-aquatic organisms. They protect coastal lands from erosion by trapping sediment runoff from flowing into oceans and other bodies of water. Molecular data are currently not available for the white mangrove species, *Laguncularia racemosa*, which is one of four species of mangroves found in the Virgin Islands. This is because the DNA extracted in the past by scientists of the VI often was not clean enough to allow for further examination of its genetic variation. In this study, we investigated the question "What method of DNA extraction produces the highest quality DNA sample for *L. racemosa* for use in genetic testing?" There were four DNA extraction methods: the CTAB protocol, the double chloroform (CTAB) protocol and two DNA extraction kits, Epicentre and Ultraclean. After extracting the DNA, we measured the purity and quantity of the sample using UV spectrometry. We cleaned the least pure samples using 3 enzymes: proteinase K, phosphatase and RNase. After which we amplified the DNA samples and analyzed the DNA bands (under UV light) after they were separated in an agarose gel. The use of enzymes proteinase K and RNase produced the best samples with $AAA_{260}: A_{280}$ ratios of 1.85 and 1.86 respectively. The outcome from amplifying and analyzing the DNA bands is inconclusive. The results indicate that we are well on our way to finding the ideal extraction method which produces high quality DNA for further genetic testing.

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Calcareous Soil and Photoperiod Influence on Caribbean and African Sorrel

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Sorrel, (*Hibiscus sabdariffa*) commonly known as Roselle, is an annual plant that is in the Malvaceae family. Sorrel is used to make a health drink filled with high contents of vitamin C and anthocyanins better than cranberry juice. The objective of this study was to evaluate growth of 17 sorrel varieties, from the Caribbean and Africa, for tolerance on high pH caliche soils and their sensitivity to photoperiod. Seedlings of the sorrel were established at one foot spacing in calcareous soils during late March. Plant height, flowering and caliche tolerance recorded over time. For caliche tolerance, plants were ranked from 1-5, with (5) being the best and (1) being severely chlorotic and necrotic. Generally, the varieties from Africa had better tolerance to the high pH soil, ranked above 2.5, than the Caribbean varieties. Five varieties appeared to be photoperiodically day neutral by producing flower buds and flowered in May. From these results, a breeding plan will be developed to combine tolerance to calcareous soils with a day neutral photoperiod to enhance year round production.

This research was funded through VI Dept. of Agriculture Specialty Crops Block Grant and USDA-NIFA-Resident Instruction in Insular Areas (Grant # #2008-34816-20016) and supported by NSF HBCU-UP grant number HRD-1137472.

Key Words: Caliche, Day Neutral, High pH Tolerance, *Hibiscus sabdariffa*, Roselle and recorded.

Protocols and future endeavors for investigating the synergistic benefits of local herbs on the lifespan of normal, aged and diabetic *C. elegans*

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Virgin islanders believe that the ethno-medical preparations enhance therapeutic value, reduce side-effects and give an inexpensive alternative to conventional medicine. Our goal is to investigate the effects of select botanical remedies on the island of St. Thomas USVI with respect to the life span expectancy of normal, diabetic, and aged *Caenorhabditis elegans* animal models. We hypothesize that alternative botanical medicines used in the U.S. Virgin Islands contain a mixture of “active” ingredients that interact synergistically to yield enhanced therapeutic efficacies and reduce toxicities. We have successfully developed a protocol for growing, transferring and observing the lifespan of *C. elegans*. For preservation purposes we have now developed a freezing protocol to store our *C. elegans* for future use. The keys to a successful freeze are using animals at the correct stage of development, the addition of glycerol to the freezing media, and a gradual cooling to -80°C . Freshly starved young larvae (L1-L2 stage) survive freezing best. Well-fed animals, adults, eggs and dauers do not survive well. It is best to use several plates of worms that have just exhausted the *E. coli* OP50 lawn and that contain lots of L1-L2 animals. This data will increase our ability to ascertain the effects that each botanical preparation has on the life-span expectancy of normal, diabetic, and aged *C. elegans* models. High performance liquid chromatography (HPLC) will be used to build profiles for each botanical preparation and their fractions. The fractions are prepared based on polarity using dichloromethane, ethyl acetate and 80:20 ethyl acetate:methanol. These fractions will then be added to cultures of *C. elegans* and the effects will be monitored and recorded.

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Studying the Interactions between Coral Reefs and Participants of Marine Conservation Activities

Kyvonn A. Morton & Antonio Farchette

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The reefs provide an aquatic habitat that promotes enhanced species biodiversity, protection to our coastal shorelines, and promotion of sustainable marine and natural resource-based livelihoods. Coral reefs are being diminished daily due to anthropogenic and natural threats. In this process the role of information and education of the society regarding coral reef health and conservation is critical to prevent coral loss. In order to determine how people's general knowledge affects how they perceive and interact with coral reefs and how participants interact with each other and the environment during marine conservation activities, we administered a pre-survey to determine their attitudes, beliefs, and behaviors about the coral reefs. The participants undertook a boat trip to three different spatial locations, varying in their characteristics (e.g., difference between inshore and offshore reefs). We interviewed focus groups and used their responses in a content analysis of key perceptions and cognitive characteristics in three key temporal stages: (a) before the trip, (b) during, and (c) after the boat trip. The participant behavior in the water was observed in order to study their interests and the complexity of their interactions, and their encounter patterns with the natural system. The data was used to study the relationship between human interactions and the reefs. The participants realized how their actions affect the coral reefs and they noted the differences between inshore and offshore spatial locations. The study experience enhanced their understanding of how human interactions degrade the reef ecosystem and cause detrimental effects. Our study shows that the participants found the research to be vital, educational and have important impacts to the community perceptions regarding coral reef conservation. Finally, our results present a new approach to studying how social learning from experience can benefit and improve perceptions, beliefs, and attitudes toward environmental conservation.

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Do the bacterial communities in corals have an impact on coral disease?

Tobias Ortega-Knight & Danny Lynch

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Coral diseases have significantly contributed to the decline of coral reef ecosystems worldwide. White Plague and Black Band disease emergence in the Caribbean has been especially harmful. Bacteria are largely thought to be the causative agents leading to these diseases, although this has not been conclusively shown. There is uncertainty as to where these pathogenic bacteria originate. Some postulate that these bacteria are always present within the coral holobiont and because of differing environmental stressors an imbalance occurs between these microbes living in association with coral communities. Other scientists propose these bacteria may originate from the soil, water column, or other anthropogenic sources. Since the causative agents have not been precisely identified, we are investigating whether the bacterial communities found in healthy coral can lead to diseased states in the community. To examine this question we are characterizing and quantifying the diversity of the bacterial community of healthy corals. To quantify the bacterial community we are extracting the bacterial DNA from the coral and then using primers that are specific to the 16s ribosomal genes for marine bacteria to PCR amplify a portion of that gene. PCR products will be cloned to separate the different genotypes of bacteria present. DNA sequences from the clones will be compared to existing DNA databases to identify the bacteria present and to determine if any match sequences of known disease causing agents. In addition, real-time PCR will be used to determine the different proportions of differing bacteria present in the samples. By better understanding the diversity and composition of bacterial communities in healthy corals, this information will contribute to our understanding of the causative agents in coral disease. In future studies this information could be useful when looking into how these diseases are transmitted.

This research is funded by the National Institute of Health MBRS-RISE grant #GM061325.

**Distribution and Abundance of Hawksbill (*Eretmochelys imbricata*)
and Green (*Chelonia mydas*) Sea Turtles
within Buck Island Reef National Monument, St. Croix, USVI**

Clayton Pollock

Paul Jobsis, PhD (mentor)
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Buck Island Reef National Monument (BIRNM) provides important habitat for "Critically Endangered" Hawksbill and "Threatened" Green sea turtles. This study assesses the distribution and abundance of sea turtles within a marine protected area. It also investigates potential relationships between the observed distribution and abundance of sea turtles and the benthic composition of certain areas within the park. As part of a renewed effort to collect in-water data from sea turtles at BIRNM this study will be the first in almost a decade to document sea turtle distribution and abundance.

In this study the park was divided into 18 approximately 1.5 km² survey blocks and timed snorkel surveys were conducted along transects within each section to record the abundance, disposition and species of the sea turtles encountered. During approximately 32 survey effort hours we observed 36 turtles; 22 greens and 14 hawksbills. To test for relationships between the observed sea turtle distribution and abundance within the park and the benthic characteristics we calculated a variety of benthic habitat characteristics per section using ArcGIS and JMP software and used MDS to investigate whether benthic communities were consistent between sections. Preliminary analysis using ANOVA, linear regressions and likelihood ratios suggests that sea turtle distribution within the park is patchy and influenced by habitat. This study is part of the critical sea turtle monitoring at BIRNM, identifies salient sea turtle areas within the park and aids in assessing the recovery of these species.

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Engineering Rain Measurement Systems for Caribbean Microclimatology Studies

Dwayne Richardson
David Morris, PhD (mentor)
University of the Virgin Islands

Meteorologists use satellite images to map out the positions of large air masses around the earth within which diverse weather tend to form in areas throughout the world. These systems are used to predict weather on the island of St. Thomas as well. The interaction of these air masses with the varied topography of Caribbean islands like St Thomas, however, produce different rainfall outcomes depending on the precise local geography. We are building a rainfall sensor network that will monitor the precipitation patterns across St Thomas at high frequency (~1 Hz) with ~1km spatial resolution. This sensor network will be the first of its kind deployed in the Caribbean though similar experiments have been previously performed in the US Great Plains region. The data collected will be valuable to a range of scientific and engineering projects of local interest in the Caribbean and broader interest to the international atmospheric science community. Data like this could tell us about the accuracy of more general weather detection systems and if there are any new trends in the weather that are specific to the Island. As a side benefit of this program, the sensor network will provide real-time precipitation data to the Virgin Islands Robotic Telescope at Etelman Observatory which will be used to trigger automatic closure of the observatory in advance of rainfall events to protect the telescope hardware against water damage.

This paper describes the calibration of our prototype rain sensor. We deposited a controlled and measurable volume of water evenly across the sensor. This process was repeated ten times at various sensitivity settings. The water level was measured and compared to the sensor-recorded amount.

Tests demonstrate that the sensor yields approximately 40% accuracy under multiple settings. These results appear consistent. The relatively low level of accuracy may be due to errors inherit to our experiment technique. Field tests will be conducted to further characterize the sensors true level of precision. Work supported by NSF HBCU-UP # HRD-0506096

Larval settlement of the long-spined sea urchin, *Diadema antillarum*, corroborates seasonality and importance of post-settlement processes in the US Virgin Islands

Jamie Spray

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Rapid phase shifts from coral reefs to algal-dominated reefs have occurred throughout much of the Caribbean following a mass mortality event claiming 95-99% of long-spined sea urchins. Such a strong correlation with this event suggests the herbivorous *Diadema* fulfill an important niche in maintaining coral-dominated reefs. Hence, their reestablishment could enable coral reef recovery. Yet, recovery has been variable and patchy across and within the region. Possible reasons for this could be a difference in larval supply between localities, preferential settlement, and post-settlement processes acting strongly on certain populations. To test the larval supply between two sites with contrasting adult population densities, we deployed settlement collectors monthly in Brewer's Bay, St. Thomas. Annual data over two years show a late spring/early summer seasonal peak in settlement, though variation occurs within months possibly due to oceanographic conditions or nutrient flux. Additionally, we predicted that if larval supply accounts for the differences in adult populations, the locality with higher adult density (Runway) would show significantly higher *Diadema* settlement. The results were that Black Point, the site with low adult density, had the higher amount of settlement, whereas Runway had low settlement. Because the site with higher adult density had the lower number of settlers, the larval supply hypothesis is falsified. Therefore, post-settlement processes must affect juvenile recruitment success, and further study on the behavior and nursery requirements of recently-settled juveniles could lend insight into survival mechanisms to ensure healthy adult populations. My current research focuses on feeding habits of juvenile *Diadema antillarum* conducted in the lab on specimens less than 10mm test diameter. Juveniles are presented overnight with a controlled mass of varied macroalgae found in the habitat, and the percent mass consumed is quantified. The research is ongoing. Knowledge of such factors required for survival is information that has not yet been well documented for juvenile *Diadema* recruits.

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