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Student Research Projects
ABSTRACTS

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Laser Capture Microdissection for gene expression profiling of the songbird brain

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The brain interprets its environment and generates behavior through complex interactions of molecular and neuronal activities. This study investigated these mechanisms using songbird vocal communication as a model behavior. The zebra finch, Taeniopygia guttata, songbird has two neural pathways that are involved in learned vocalization. The Posterior Vocal Pathway (PVP) is responsible for vocal motor output, while the Anterior Vocal Pathway (AVP) is necessary to modify the vocal-motor output. In both of these pathways singing behavior induces gene expression, suggesting that a gene cascade is associated with vocal behavior. The potential application of laser-capture microscopy for profiling behavior-induced gene expression in the songbird brain was examined. The two experimental groups were quiet birds and birds that sang ~30 bouts in 30 minutes. Two PVP vocal nuclei, the high vocal center (HVC) and robust nucleus of the arcopallium (RA), and two AVP vocal nuclei, the lateral magnocellular nucleus of the anterior nidopallium (LMAN) and the striatal nucleus (Area X) were analyzed. These brain regions were microdissected from adult zebra finches, >120 days old, using a laser capture microscopy (Arcturus PixCell IIe). RNA (4-10 micrograms) was successfully extracted from captured regions, as confirmed using the Bioanalyzer 2100. This innovation will now allow us to amplify the mRNA and assay for vocalizing gene expression using microarrays.

MARC GM08422 and Duke University Summer Research Opportunity Program

Cloning, Expression, Purification, and Crystallization of Rv1738: An Essential Gene during *Mycobacterium tuberculosis* persistence

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ABSTRACT

Tuberculosis is caused by the bacteria called *Mycobacterium tuberculosis*. The disease was first described in the seventeenth century. Mycobacterium tuberculosis has once again become a devastating human pathogen. Recent research has begun to uncover how *M. tuberculosis* can survive in the face of chemotherapy for many months, called persistence. Rv1738 is a hypothetical protein which has the highest expression under hypoxic, nitric oxide, and environmental stress; all models of persistence. This indicates that Rv1738 has great significance to the survival of *M. tuberculosis* within the host. Since this protein has not been extensively studied, its structure and functions are not known. Therefore, the objective of this research is to successfully purify and crystallize Rv1738 to determine its protein structure. To accomplish this objective the gene that encodes Rv1738 has been amplified using a polymerase chain reaction (PCR) using H37Rv genomic DNA as the template for gene amplification. Rv1738 was cloned and the cDNA was inserted into a bacterial expression vector. The protein was expressed using the E. coli strain BL21(DE3). A 6X-histidine tag was used to purify the protein using metal affinity chromatography. The protein was judged to be 95% pure by visualization on a SDS polyacrylamide gel. Rv1738 was further purified by dialysis and concentrated to prepare for crystallization. Further work is needed as the formation of the Rv1738 protein crystals are still in process. This research was funded by NSF grant number DBI - 0139246 and MARC grant number GM08422.

Silane - PEG Matrix can be used for Fluorescein Adsorption on a Titanium Surface

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Drug eluting stents are a great tool for coronary artery disease; however, there is a problem with an accumulation of blood cells on the stent due to the polymer coating. My specific approach to this problem is to create a coating on the stent that would allow fluorescein (drug substitute) to become entangled within a matrix and diffuse consistently. The matrix consists of silane self assembling monolayers (SAMs) and the attached poly(ethylene glycol) methyl ether (PEG). To test the formation of the SAMs, contact angles for each type of silane were taken. ((chloromethyl)phenylethyl)trichlorosilane SAMs gave the highest contact angles of >100°, where as, the n-dodecyltriethoxysilane gave 70- 80° angles. Also, based on the x-ray photoelectron spectroscopy (XPS) results, the PEG was successfully attached for each type of SAMs. Finally, the fluorimetry results showed that the amount eluted from the matrix was highly variable in the replicates and over time. In conclusion, there was success in forming the intended matrix, however, failure in achieving consistent amounts of eluted fluorescein.

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Towards Understanding Coral Bleaching Sequence Patterns using SPADE

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To understand and monitor better the epidemic of coral "bleaching" more knowledge about this phenomena is needed. It is believed that a series and a combination of different stress factors such as atmospheric and oceanographic condition changes may cause coral reefs to become irritated and expel their vital symbiotic zooxanthellare algae. The study proposes to use SPADE (*S*equential *PA*ttern *D*iscovery using *E*quivalence classes) to better understand coral bleaching phenomena. SPADE looks for frequent event sequences and can be easily molded to find patterns in the change of factors such as sea temperature, air temperature, and water salinity. This work will show that SPADE is an efficient data-mining algorithm for detecting patterns in coral reef bleaching. SPADE can be used both as a verification or discovery drive n mining tool. As a verification driven data mining tool, SPADE will look for facts to support the hypothesis that sea temperature is not the only factor inducing coral reef bleaching. Using SPADE as a discovery driven mining tool new patterns impacting coral reef bleaching may be derived.

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An Assessment of the Diet and Nutrition of the Green Sea Turtle (*Chelonia mydas*) in Captivity

Kate Cassim University of the Virgin Islands Research Advisor: Dr. Donna Nemeth

The nutrition of captive Green Sea Turtles (Chelonia mydas) affects many aspects of their health including growth, development, and physical space requirements. These turtles undergo habitat shifts throughout the first few years of life which represent changes in diet and nutritional requirements which need to be addressed when designing a captive feeding regimen. Many times natural food sources are not available for feeding to captive individuals and therefore an alternative diet must be created that contains the necessary nutrition for the turtles to grow and develop properly. This is true for the Green Sea Turtle in the Virgin Islands because the sea grass beds that provide a natural food source have been damaged, and destroying habitat to feed captive animals is not a viable solution when alternative food sources are available. Many factors contribute to the determination of the diet for captive individuals including nutritional content, price, and availability. To assess the dietary needs of captive Green Sea Turtles the nutritional content of available food sources will be compared to the natural diet to assess the quality of nutrition in each option as well as the nutritional value and price per pound. The growth rates and dietary history of the turtles at Coral World Ocean Park on St. Thomas in the US Virgin Islands which have been raised in captivity since hatching will be compared to the growth rates of captive turtles at other aquarium facilities as well as those of wild populations in the Virgin Islands. The results of this analysis will provide insight into the best dietary source for captive Chelonia mydas in the Virgin Islands and help to better understand any additional needs in captivity to provide them with the healthiest environment possible. This research is supported by Coral World Ocean Park.

Edge Detection and Feature Extraction of Retinal Images

Alexander David, Computer Science, University of the Virgin Islands Mentor: Baris Sumengen, Lab Head: B.S. Manjunath, Computer Science, UCSB Research supported by NSF ITR research grant 0331697

Analyzing retinal images plays an important role as scientists try to understand and treat retinal detachment. This condition can occur due to injury or disease and almost always leads to impairment or loss of vision. The purpose of our research this summer was to enhance some of the ways that scientists can study these images. Our goals were to extract a set of features from these retinal images that can be used for a similarity search and to test a state of the art edge detection algorithm on our retinal images. Feature extraction is the process of extracting numerical values from images that can later be used for image analysis. For our research, we extracted features for the purpose of using them in a similarity search among the images. The features we extracted were: center of gravity, spread, component mean and component area. Once we had these features we implemented a Graphical User Interface (GUI) so that biologist could use our program and control the parameters of the similarity search. For the other part of our project we implemented a state of the art edge detection algorithm called the Compass Edge Operator (CEO) on our retinal images. Edge detection is the process of finding the edges within an image in an attempt to pull out significant objects in that image. It looks for maximums among the differences in pixel values throughout the image and it returns those maximums. The CEO overlays a circle onto an image, computes signatures for each half and uses the Earth Movers Distance to compute the differences between the signatures. The results from our research show that our features served as a good measure of similarity between image and the CEO was able to detect good edges from our images. We would like to further develop our GUI and make it web accessible and also extract features from the edge images and use them in our similarity search.

The Efficacy of Medicinal Plants on the Inhibition of Two Fungal Strains

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The use of complementary and alternative medicine (CAM) has been increasingly accepted throughout the United States. An alternative system of medicine, curanderismo, is a traditional Mexican healing technique originating in the sixteenth century which fused Aztec and Spanish ideologies. This project aimed to prove or disprove that herbal extracts are effective against fungal strains. The inhibition of two fungal strains *Aspergillus niger* and *Saccharomyces cerevisiae*, by plant species *Zea mays* (Corn silk), *Lavendula vera* (Lavender), and *Hydrastis canadensis* (Goldenseal) was analyze. Ethanolic tinctures were prepared for all plant species and applied to Kirby-Bauer disk diffusion testing. Results for fungal analysis gave inconclusive results. Due to the fact that the results were inconclusive hypotheses could not be rejected and conclusions could not be made. Supported by NSF Grant #0453329

Is a Multi Conformer Protein better than a Single Conformer?

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Crystallography is the science of crystal structure and protein crystallography depends on the use of X-rays and diffraction patterns. To solve the protein structure by X-ray crystallography requires several steps. The final step, the refinement process, utilizes several computer-programs that improve the protein models against its electron density map. The refinement was done in order to minimize errors and structural inconsistencies between the experimental data and calculated data. In the past the objective of refinement focused on elucidating a single protein structure per electron density map. However, the use of multiple models or multi conformers per electron density map dictates a more complete interpretation of the dynamic protein crystal and gives better insight into the connectivity between structure and biochemical function of the protein. From 40 diffraction data sets evaluated by the CESG (Center for Eukaryotic Structural Genomics), 40 sets of electron density maps of proteins structures were already determined by the use of the Fourier Transform technique. One of the 40 protein structures, a 2.51 Å low resolution structure of Arabidopsis thaliana annexin (1YCN) At 1g 35720.1 was analyzed to determine whether 1YCN could be best described utilizing , one , two , four or eight conformers. The process to determine the best conformer model required the use of a statistical tool named R_{free} and a simulating annealing computerized process that better facilitated the elucidation of the electron density map of 1YCN. The interpretation of the diffraction data as multi conformers for this particular protein was validated by the lesser R_{free} obtained via the refinement process and comparison with a single refined conformer model.

The Relation Between Size and Playtime in Spotted Seatrout (*Cynoscion nebulus*) as a Result of Catch and Release Angling

Deia Francis (University of the Virgin Islands) Advisor: Dr. Rebekah Thomas (Texas A & M University – Corpus Christi)

The Spotted Seatrout, *Cynoscion nebulus*, is an important recreational fishery in Texas which represents a large portion of the fishery economic output in Texas. There is an ongoing concern about the physiological stress of catch and release angling of *C. nebulus*. This is a pilot study that looks at the levels of lactate in the blood as a possibility of *C. nebulus* mortality. Blood samples were taken from a total of 14 experimental trout in Aransas Bay and later analyzed for its lactate concentrations according to size and playtime (handling time). Control trout were kept in a pen for later sampling and analysis. There was no correlation found in the lactate concentrations between playtime or between size. However, the results do show that *C. nebulus* are experiencing some type of lactate concentration differences individually, but not in relation to size or playtime. This baseline data will be used for future studies.

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Do localized populations of *Dictyota humifusa* influence color morphs of *Elysia crispate* in St. Thomas' subtidal community?

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Vibrant coloration exhibited by marine organisms has long been the focus of scientific interest and intrigue. As such, numerous studies investigating the coloration of marine organisms have been conducted with respect to lifecycle. sociality, and symbioses. In the coastal waters along the north shore of St. Thomas, USVI, the seemingly localized distributions of a vibrant blue alga, Dictyota humifusa co-occur with the sparse distribution of blue individuals of the herbivorous sea slug *Elysia crispata*. These two brilliantly colored organisms appear to share overlapping distributions, being restricted to a consecutive string of three shallow (<5m), isolated rocky bays on the north shore of the island, occurring solely in areas regularly subjected to high turbulence throughout much of the year. Two laboratory experiments using *E. crispata* from the south shore of St. Thomas (previously unexposed to large aggregations of *D. humifusa*) were conducted by introducing the blue alga into the treatments, with the possibility of noting an acquisition of blue pigmentation; a third experiment in which blue slugs were isolated from *D. humifusa* was run, to monitor any potential color loss upon seperation on the two organisms. Throughout the duration of all three experiments, the slugs universally exhibited drastic color loss. In the experiment 3 (isolation of blue slugs from D. humifusa), subjects lost significant amounts of blue coloration; slugs in experiments 1 and 2 failed to acquire any new pigmentation upon introduction of D. humifusa. This study was crippled by massive mortality rates experienced ubiquitously throughout the three experiments. Poor aquarium conditions and possible temperature shock to the specimens of live rock during transport to the lab are two significant factors contributing to the demise of this study, leaving the possibility of a correlation between these two co-occurring organisms wide open.

Investigating the sociality and olfactory cues in the banded shrimp *Stenopus hispidus*

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Abstract. Sociality plays a key role in the life cycle of all sexually reproducing organisms, and a wide variety of metazoan species occur as mated pairs throughout various stages of adulthood. In preliminary field assessments, we have noted Stenopus hispidus showing a trend toward heterosexual aggregation. Preliminary in situ trials have shown individuals separated from one mated pair to avoid territory occupied by non-mate conspecifics. This study is designed to investigate the sociality of *S. hispidus*, with respect to olfactory cues emitted by both mates and non-mate conspecifics, as well as visual behavioral cues displayed by either mated pairs or randomly paired non-mates. We plan to mark and monitor the home territories of mated pairs of S. hispidus in situ, in hopes of assessing possible territorial boundaries or occupancy patterns displayed in the wild. Laboratory experiments will employ a v-maze as the primary tool for investigating olfaction between the shrimp, while a 36 gallon bow front aguarium will be used for noting and recording visual cues displayed by individual shrimp when in different scenarios. By isolating olfactory cues from individuals and noting the behavioral responses they induce, we hope to learn more about sociality and the role it plays in the life cycle of S. hispidus in St. Thomas' subtidal community.

Quantitative Analysis of Various Organic Samples by Fourier Transform Infrared Spectroscopy

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Fourier Transform Infrared Spectroscopy (FTIR) is widely used in the qualitative and quantitative analyses of both organic and inorganic samples. For example, many state and national crime laboratories tend to use FTIR spectroscopy when identifying and characterizing substances retrieved at different crime scenes. The first main objective was to establish a diverse library of spectra using various sampling techniques. The second objective was to create standard (calibration) curves for compounds of known concentrations, making it easier to determine the percent of a drug in a sample. These will in turn decrease the amount of time and money that is invested into the Forensic analysis of various compound. In the present work, solid, liquid, and powdered samples were prepared using both transmission and reflectance techniques, such as the potassium bromide (KBr) pellet method and attenuated total reflectance (ATR). The samples were analyzed using least squares analysis and the data was plotted in a graph of absorbance versus area under the curve (AUC). Quantitative results showed that the potassium bromide pellet provided the best spectra when compared to the other techniques. Ultraviolet-Visible Spectroscopy confirmed a strong correlation (0.99) between the absorbance and concentration of the diluted caffeine samples; however, the FTIR data of the same samples showed a smaller correlation (0.60) after plotting its calibration curve. Therefore, further experiments are needed to gain a better understanding of this significant difference.

This work was supported by a grant that was awarded to the Forensic Chemistry Program at the University of Mississippi, as well as the National Science Foundation (NSF).

Determining the Genetic Diversity of Solanum conocarpum, A Rare Species Indigenous to the Virgin Islands

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Very little is known about the ecology of the Virgin Islands' rare plant populations. One rare plant, *Solanum conocarpum*, a member of the Solanaceae family, was thought to be extinct until recently. Previously, S. conocarpum was only found on St. John in the U.S. Virgin Islands. In fact just a few years ago, only one single plant was found in St. John. Over 100 individuals have been found since then and some have been cultivated in gardens across the US Virgin Islands. Testing the genetic diversity of this plant by running random amplified polymorphic DNA polymerase chain reactions (RAPD PCR) will help us to determine the species' susceptibility to disasters and ability to reproduce successfully. We hypothesize that because of the rarity of S. conocarpum, the genetic variation will be significantly low compared to non-rare congener such as S. polyganum. Data were collected to test the hypothesis that small population size has resulted in decreased genetic diversity and a low polymorphism level. Six to ten plants from five different populations were sampled. DNA extracted from these samples was isolated and amplified using RAPD PCR. Preliminary data indicate that the heterozygosity of the plant populations is 0.126, which falls at the low end of genetic diversity for plants. The lack of heterozygosity suggests inbreeding and genetic drift. The five populations tested of *S. conocarpum* had a polymorphism level of 0.525. Compared to other common plants with polymorphism levels of 0.87 and greater, we determined that genetic diversity is low. The purpose of analyzing the genetic diversity is that it will provide data for conservation. Since the species is so rare, a conservation plan is needed to prevent it from extinction and to preserve its genetic variation. Future studies include developing a conservation plan for S. conocarpum and submitting the species for the endangered species list.

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Using Data Mining to Evaluate Coral Bleaching Occurrences

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Coral bleaching is the whitening of corals due to stresses in the environment. In recent years coral bleaching has become an epidemic claiming the lives of many of our coral reefs around the world. There are various environmental and atmospheric conditions that can cause this phenomenon, such as sea and air temperatures. However, most researchers theorize that mass coral bleaching is caused by episodes of elevated sea temperatures. This research considers using data mining, which is a process of developing patterns from data to analyze what events contribute to coral bleaching. To measure how the data is separated into clusters the, K-means clustering algorithms, along with two mathematical distance formulas, were used on the coral reef data obtained from NOAA for effective coral bleaching data analysis. This result in data being partitioned into groups and clustered based on the selected atmospheric categories. For instance, sea temperature, air temperature, salinity, wind speed, etc. are just some those categories The results suggest that clustering algorithms can partition coral reef data into groups based on similarities linking data placed in certain clusters. In this way, trends and relationship are observed, aiding in the accurate prediction and warning of future coral bleaching events. Research is still ongoing. While many researchers point the finger to sea temperatures as the major cause of coral of bleaching compared to other atmospheric categories, this research will provide insight into that theory.

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Alcohol's Effect on Neuron Firing

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Neurons are responsible for transmitting messages throughout the body via long distance electrical signals known as action potentials. These depend on the active transport of sodium and potassium ions across the cell membrane. The effect of various drugs on the process of neuron firing is a current research interest. The Hodgkin-Huxley equations, a system of four nonlinear ordinary differential equations, mathematically model the influx and efflux of these ions across the cell membrane. In the presence of alcohol, the release of potassium ions is accelerated. We propose a modified version of these equations, which incorporates the effect of alcohol, and examine its implications through mathematical analysis in dynamical systems. We investigate the qualitative behavior and interpret the results of the steady-state solutions in the fast and fast-slow phase planes.

Thanks to the National Science Foundation (NSF), the National Security Agency (NSA), Loyola Marymount University, and Cal Poly Pomona University for making this research possible.

Is there a Relationship Between Habitat and Parasite Infestation among Caribbean Reef Fishes?

University of the Virgin Islands Dr. Paul Sikkel, Dr. Donna Nemeth, Amber McCammon VI-EPSCoR

The focus of this project is on marine resource management and biocomplexity. The most common measures of fish habitat quality include food availability, shelter, risk of predation, and access to mates and resources necessary for reproduction. One aspect of habitat quality that has been largely ignored is risk of parasitism. Our goal is to assess the relationship between habitat and the per capita risk of infestation of fishes by monogenean flatworms. We quantify the relationship between habitat and Neobenedenia sp. monogenean parasite loads of blue tang (Acanthurus coeruleus) and ocean surgeon (Acanthurus bahianus) by collecting fish, removing parasites with a freshwater dip, and counting the parasites that infest each fish. We hypothesize that habitat quality affects parasite abundance and therefore parasite loads on fish. We compare offshore cay, near shore reef, and near shore trashed reef by collecting a minimum of 15 fish of each species, from each site, in summer, winter and spring. A secondary goal is to examine the effectiveness of cleaner shrimps in removing parasitic monogeneans from fish. We have found that banded coral shrimp (Stenopus hispidus) and Peterson cleaner shrimp (Periclimenes pedersoni) will eat monogeneans provided to them in the lab. However, it is unknown whether any cleaner shrimp remove monogeneans from fish. To determine whether fish interact with cleaner shrimps under captive conditions tanks will be established to house parasitized fish with and without cleaner shrimp. Fish collected from the field will be randomly assigned to tanks and trials will be run by leaving fish in tanks with and without cleaners at 1, 3, and 5-day intervals. Parasite loads will be quantified as described above. This work will improve understanding of factors that affect the commercially important Virgin Islands fishery. The health of our marine reef ecosystem may be at risk if habitat changes have a negative effect on the abundance of cleaner organisms and/or fish populations resulting in fewer hosts and cleaners and therefore an increased risk of infestation per host. This investigation will give insight into the importance of habitat quality on the health of reef fishes and increase understanding of the biocomplexity of marine reef ecosystems.

Imaging and Analysis of Randomly CD-Tagged Cells

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CD-tagging is the use of a retroviral vector to insert a specifically designed DNA sequence into genomic DNA. In this study, a green fluorescent protein-encoding exon was inserted into a 3T3 mouse cell. This led to the specific tagging of the Central Dogma molecules. However, the natural expression of the genome and its protein products was expected to be maintained. Cloned cells were randomly tagged using the CD-tagging procedure. GFP tags were inserted randomly into the genome of the cell. This resulted in the tagging of a wide range of proteins within a single cell type. Tagged cells now express the fluorescent qualities of the protein and can be identified using fluorescence microscopy. Cells were imaged using the Kinetic Scan HCS Reader. After image acquisition, cells that were correctly segmented were chosen using data from a histogram depicting the nuclear intensity of all the imaged cells. Image features were then calculated for these selected cells. The features were used to conduct data clustering of the images. This resulting data were used to construct a consensus tree, which reflected the relationship between the randomly tagged proteins as observed in the feature space. Six distinct cell lines were then identified. Vertical line length of the tree inferred that there are inherent similarities and differences between the tagged clones. Clones 1, 2, and 5 were characterized by long line length. Thus we can predict that the proteins are dissimilar in their function and sub cellular location within the cell. The same prediction can be made about Clones 3 and 6. The tree of unknown clones was then combined with an existing tree of proteins whose sub cellular location patterns have already been identified. Clones 5 and 6 were characterized by short line length; this indicated that they are more similar to the proteins that are located in the nucleus and ER than those found in the DNA and other locations within the cell. Theoretically, these clones should be similar in function and sub cellular localization to these known proteins. This data can be used along with protein sequencing in order to study and identify the roles and sub cellular location patterns of tagged clones within the cells where they are found. Correct application of this knowledge may lead to an array of treatments and possible cures for diseases. Currently, research is still being conducted and will contribute to the enlargement of the database on the sub cellular locations of members of the proteome within a cell.

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Surface Studies of Cesium Absorbed on Aluminum and Paving Brick.

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Ever since September 11, 2001, terrorist attacks using Radioactive Dispersive Devices (RDD) has been a major concern. RDDs are weapons that combine conventional explosives with radioactive materials. Cesium-137 is one possible radioisotope that might be encountered in an RDD. Understanding how cesium (and other radionuclides) interacts with urban surfaces is needed to provide the scientific basis for developing strategies for responding to and recovering from RDD attacks. In this work, cesium hydroxide (CsOH) was used as the source of cesium contaminant. The two urban surfaces that were investigated were aluminum and paving brick. CsOH was placed on samples of both materials. The samples were dried in an oven and then were placed inside the high vacuum chamber of a combined X-Ray Photoelectron/Auger Spectrometer. Control samples of aluminum and paving brick without added Cs were also placed inside the vacuum chamber in order to obtain reference spectra. The samples were then analyzed to determine surface composition. After comparison with reference spectra, Cs peaks were detected on the aluminum surface and one of the paving brick samples (# 2). On the aluminum spectra, peaks of Cs were detected at 75 eV and 724 eV. Aluminum and oxygen peaks were also detected. On the paving brick sample # 2. Cs peaks were observed at 75 eV and 724 eV. Other peaks that were observed are calcium, silicon, sodium, and oxygen.

Coral Bleaching prediction using neural networks data mining algorithm

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Predictive data mining algorithms are studied to assist with the prediction of coral bleaching. The algorithms use historical data to potentially find hidden patterns. Current prediction methods are limited in that they only use near-real time data to determine if conditions are conductive to bleaching, thus they cannot deduce ahead of the data whether conditions might spawn bleaching. This poster presents a neural network architecture to determine to learn how to predict coral bleaching from the historical data and past bleaching events. The information used is from data collected from the SEAKEYS Sombrero Reef station for the month of August 1998 and the reefdase dataset for 1998. Current results shows that the neural network has an accuracy rate of 86 % prediction but more experiments are needed to validate these results.

References: http://www.coral.noaa.gov/crews/index.shtml http://www.cs.waikato.ac.nz/ml/weka/ http://www.inns.org/nnjournal.asp

Determining the Bioactivity of a Marine Sponge Extract Against Leishmania

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Leishmaniasis is a complex of diseases caused by protozoa of the genus Leishmania. The disease is transmitted to humans by sandflies and affects over 2 million people every year all around the world. The treatments currently available for leishmaniasis are slow, expensive, susceptible to drug resistance, and often toxic to patients. Hence, it is vital to identify new sources for more effective drugs. Marine sponges produce a diverse array of biologically active secondary metabolites. Many of these compounds have antimicrobial properties. A crude chemical extract of a green encrusting sponge, Amphimedon viridis, collected from Brewer's Bay, St. Thomas, United States Virgin Islands, exhibited antileishmanial activity. The purpose of this project is to isolate the compound responsible for the observed bioactivity. The crude extract was separated into chemical fractions using vacuum liquid chromatography. Leishmanicidal bioactivity of these fractions was determined in vitro by an Alamar Blue assay utilizing a culture of Leishmania donvani promastigotes maintained at the National Center for Natural Products Research. Bioactive fractions were submitted to thin-layer chromatography and nuclear magnetic resonance spectroscopy for structure characterization; the NMR spectra indicated that the fractions were not pure. In conclusion, three fractions, JP-D, JP-E, and JP-F, displayed promising bioactivities against Leishmania with IC50 values of 8.5, 7.0, and 9.2 µg/ml, respectively. Further research must be done in order to isolate and identify the pure biologically active compound.

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Habitat selection of Red Hind (*Epinephelus guttatus*) on near shore reefs of St. Thomas, U.S. Virgin Islands

Alkin Paul, University of the Virgin Islands

Many marine biologists are interested in where fish settle. Knowledge of habitat selections at all life stages is key information in designing marine protected areas. The knowledge of where fish spawn is vital in sustaining fish populations. Additionally, the survival of these organisms to the age of sexual maturity should also be important. Red Hind (Epinephelus guttatus) are commercially important Caribbean fish in the seabass family, Serranidea. New laws protect aggregation sites but the problem of fish being taken before they get an opportunity to spawn still exists. Little is known about the life cycle of these groupers, especially during their transition from the juvenile to the adult stages. It would be necessary to also protect particular habitats that would allow the juveniles to reach maturity so that the spawning aggregations may have a greater success. A study by Elizabeth Whitman (2003) found that juvenile Red Hinds are usually found around the coral species Porites porites and usually found on the north side of the St. Thomas. The purpose of this study was to expand on Dr. Whiteman's preliminary study to determine whether the results hold other years and other locations. Data were collected on the habitats for all groupers observed in the field from nine sites around the island of St, Thomas in 2005. Each site was surveyed and all groupers were visually recorded. A .5 m x .5 m guadrant was used to record and analyze the habitat composition of all areas where the groupers were observed. Red Hinds were found in a number of habitats. Further studies at different depths are needed to test possible oceanographic influences on fish distribution.

Cloning of Type IV Collagen α5 Chain into an Expression Vector

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The kidney capillary wall comprises three key components: podocytes (glomerular epithelial cells), endothelial cells and the Glomerular Basement Membrane (GBM). The glomerular basement membrane (GBM) is the most important component of the kidney filtration barrier and is abnormal in most renal diseases, such as Alport Syndrome (Hudson 2004). The GBM provides support and some of its components serve as ligands in cell behavior (Hudson 2004). The GBM also functions in the filtration of blood by preventing large proteins and other cellular components from entering the urinary system. The GBM like any other basement membrane is formed by a net-like structure of Type IV collagen as well as other proteins such as laminin. Type IV collagen is encoded by six alpha chains (X1-X6 (IV)). All of the six alpha chains of collagen IV have the same helical organization. Each chain has three domains: a short 7S at the Nterminal, a long collagenous domain in the midsection of the chain, and a noncollagenous domain (NC1) at the C-terminal. These chains come together to form only 3 protomers. The X1-X1-X2 (IV) network is expressed in the immature GBM but is replaced by the X3-X4-X5 (IV) network in the mature GBM. When this important switch fails to occur due to mutation in the X3, X4, or X5 chain, it leads to Alport syndrome. The long term goal is to understand how Collagen IV in the GBM influences behavior of podocytes. This will address many gaps in the knowledge of the interaction of the $\alpha 3$, $\alpha 4$, $\alpha 5$ network and podocytes. We hypothesize that type IV collagen provides signals to podocytes through integrins or other cellular receptors, to maintain the appropriate podocyte function. The purpose of this experiment was to express and characterize the X3, X4, X5 collagen network in vitro. The X3 chain has already been expressed so my goal was to clone $\alpha 5$ chain into an expression vector transfect cloned DNA into a mammalian cell. Two strategies were used to clone the insert (α 5) into expression vector (PIR: α 3). PIR: α 3 was used as the expression vector because it is known to show simultaneous expression of $\alpha 5$. The first strategy included cloning the insert directly into the vector. However, after numerous attempts an intermediate step was used to clone $\alpha 5$ cDNA into PIR: $\alpha 3$ using pBluescript (pBS). The α 5 chain was cloned into pBS (intermediate step) and efforts are underway to get it cloned into PIR: α 3.

This research was conducted at Vanderbilt University, Department of Medicine (2005). Short Term Training Program in Vascular Pathology.

Marine Bio-Products: Examining the Chemistry of Virgin Islands Marine Invertebrates

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The search for medicines and insecticides has crossed from the plains of terrestrial surfaces into the depths of the sea. Marine organisms contain many chemicals they harvest to use for protection, digestion, or other daily behaviors. In these experiments, highly active chemicals will be extracted out of marine invertebrates and tested for medicinal or insecticide uses. Organisms will be collected by SCUBA and extracted with an ethanol/water mixture. The extracts will then be tested for antibiotic, anticancer, and insecticidal activity by examining inhibition of growth of *Escherichia coli* bacteria, *Daphnia* sp., and *Drosophila* sp. respectively. Highly active extracts will be further fractionated by liquid chromatography and examined by nuclear magnetic resonance spectroscopy.

A Wavelet Subband Enhancement to Classification

Keridon Williams, Thomas Merryman, Jelena Kovacevic

Cell protein classification is important. Correctly identifying the type of protein in question reveals information about the cell's structure and function. In this research we explore an algorithm that can enhance typical classification systems by examining the 2D images' fully decomposed wavelet tree. Success, or classification accuracy improvement, will also serve as proof that the subbands in a decomposed data set hold information that is pertinent to classification.

We apply a generic classifier to a data set. This gives us the starting accuracy that we wish to improve upon. We repeat the application of the classifier on subbands to yield a group of decisions. Our algorithm consolidates these choices into one decision through the use of weights that characterize the importance of each individual subband. Once we maximize the classification accuracy we apply these weights to the testing data. We achieve significant classification accuracy improvement indicating that subbands hold useful information.

Influence of Solid and Liquid Media on Pineapple Micropropagation

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Pineapple is indigenous to American tropics and has been distributed and grown throughout the world's equatorial area. The object of this study was to evaluate the micropropagation of different varieties found in the Caribbean as well as those grown in other parts of the world. Eleven pineapple varieties were obtained from the USDA Tropical Germplasm Repository in Hilo, Hawaii. Shoots from each variety were grown on gelled or liquid Murashige and Skoog medium with either Benzyladenine (BA) at a concentration of 2 mg/L or a combination of BA and Kinetin (Kin) at a concentration of 2 mg/L. After 35 days the new shoots were separated and transferred to MS medium without plant growth regulators. Placing the plants in media free of plant growth regulators allowed them to grow and develop. Only one variety, 'Black Antigua', exhibited a significant difference in proliferation between BA and the combination of BA and Kin. There was however, a significant difference in proliferation between plants grown on solid media versus plants grown on liquid media within all varieties. Plants on the liquid media proliferated significantly better, in most varieties up to six times as many plants, than those grown on solid media. Pineapple varieties respond better to liquid than solid media for shoot proliferation in vitro.

This research was funded through a USDA Southern Regional Hatch project.

FosB Expressions in the Pedunculopontine Nucleus of L -dopa-induced Dyskinesia Mice.

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Long- term L-dopa treatment for Parkinson's disease patients usually causes abnormal involuntary movement. Well-established dvskinesia. Hemi-Parkinsonian rat model lesioned with 6-hydroxydopamine (6-OHDA) are widely used to the research causes of Parkinson's disease. As the mechanism of Ldopa-induced dyskinesia is unclear, which might be related to other gene expression, the use of transgenic/knock out mice would be a good opportunity to investigate why dyskinesia materializes with chronic L-dopa treatment in Parkinson's disease patients and how to better medicate patients. By studying the Hemi-Parkinson's mice model, Dr. Kang's lab observed L-dopa induced dyskinesia in Parkinsonian disease mice similar to human patients. These mice over expressed FosB, a product of one oncogene, in Pedunculopontine nucleus (PPN) a small nucleus of the brain stem. The level of FosB expression in PPN highly correlated with the dyskinesia degree, suggesting that FosB expression in PPN plays a role in L-dopa-induced dyskinesia. Glutaminergic and cholinergic neurons are the main types of neurons in PPN. The characteristics of these FosB-expressing neurons in dyskinesia mice were explored by double fluorescence immunohistochemical staining. We found FosB-expressing neurons in the PPN in Parkinsonian mice are mainly either cholinergic or glutaminergic. which correspond to the cell types in PPN, suggesting theses neurons in PPN play a role in L-dopa-induced dyskinesia in Parkinsonian mice.

Mentor: Dr Un Jung Kang

Summer Science Enrichment Academy

University of the Virgin Islands

Ehrlichiosis: Present and Future

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Mentors: Nailah Brandy⁵, Ms. Linda Wymer, Ed.S.⁵

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Ehrlichiosis: Present and Future is a research project that deals with Ehrlichiosis and the testing of a drug. For our project, we tested six different dogs for Ehrlichiosis and four tested positive for the disease. After being diagnosed, three dogs were then given Mefloquine-a drug used to treat malaria. One dog was omitted from the group. The Mefloquine was given once a day for nine days. At the end of this time the dogs were tested again for Ehrlichiosis. Our student research group had never heard of Mefloquine being used to treat Ehrlichiosis before, so wee were interested in the results.

Sites used were the Tutu Veterinary Clinic and the St. Thomas/St. John Humane Society.

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Modeling the Spread of Diseases

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Mentors: Meria Marcel, B.S. and Mr. Clive Wyatt, MA

The purpose of our project is to investigate the spread of a communicable disease. Since the disease is spread by contact with an infected person, the number of persons who have the disease at the end of any month depends on the number who had the disease the previous month. Our goal was to predict the number of persons with the disease in the future months. To achieve our goal we used three different models that involved different equations.

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Synthesis of the Antibiotic Sulfanilamide

Joseph, Jaheem; Christopher, Jamilya; Farrington, Porchia; Ranani, Aarti ;Robinson, Sheniqua (University of the Virgin Islands Summer Science Enrichment Academy 2005)

Professor: Akima Williams, A.S. and Dr. C. William Anderson, Ph. D.

The Sulfa Drug is a bacteriostatic drug that suppresses the growth of bacteria. Sulfanilamide was synthesized from inexpensive acetanilide by adding and removing functional groups. The acetanilide was recrystalized, followed by adding chlorosulfonic acid, hydrochloric acid, and ammonium hydroxide, sequentially. We each started with 2.0g of acetanilide, and produced approximately 0.1g of sulfanilamide. Sulfa Drugs are used world wide to treat malaria, respiratory infections, and meningitis. They provide an example from which we can learn how other drugs work.

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Does Human Disturbance Affect Relative Abundance of Birds on the UVI Campus?

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Mentors: Gaetan Gentius and Dr. Jim Corven Ph,D.

The purpose of this study was to examine the relationship between abundance and distribution of birds with the type of habitat created by human intervention and the level of human disturbance occurring on the UVI campus. Field data was collected on the birds in specific habitat types that were subject to levels of human disturbance. The data was analyzed statistically for abundance, species richness and for correlation with habit. The results included the calculation of the mean number of birds at each site, species richness and the positive correlation value of birds and level of human disturbance. The six week time frame and the number of species considered limited research to one season's behavior. A longer time period, more species of birds, and perhaps more habitat sites would provide a more accurate study of the relationships between birds and human disturbance. Human habitat disturbance seems to have a beneficial effect on some birds species.

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