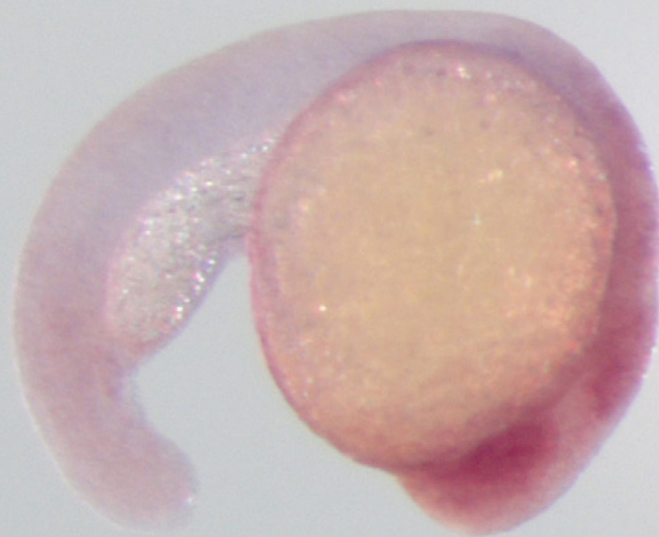


2020

DEPARTMENT OF

BIOLOGY

C A P S T O N E S Y M P O S I U M



MCDANIEL
COLLEGE



LITERATURE REVIEW POSTER PRESENTATIONS

3PM, APRIL 20, 2020

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MY'YAH MITCHELL
SARAH MAHMOOD
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CONCLUDING REMARKS

BY BRANDON ROZANSKI, BIOLOGY CLASS OF 2019 [15]

COVER: a zebrafish embryo containing the transgene *hsp70:venus-hes7UTR* was subjected to *in situ* hybridization with a riboprobe for *venus* (courtesy of Randy Hilleary, page 4.)

How Does the Environment Influence Constituents of the Endophytic Community?

JEFF CLARK

Endophytes are fungal symbionts of plants that have medicinal and agricultural potential. Endophytes confer benefits to their host, and these benefits can be utilized to create new medicines or increase crop yield. However, not all endophytes confer the same benefits, and not all plants have the same endophytes. Identifying the factors that determine endophytic constituents (EC) within a host plant is essential for discovering useful endophytes: studies have demonstrated that host plant species plays a role in the constituents of endophytic communities, but what about the environment? A study that reveals an approximate correlation between latitude and EC provided the first clue that location plays an important role in EC. To directly investigate whether local environment plays a role in determining EC, different subgenotypes of the same plant species were cultivated in three distant locations and EC were analyzed. Surprisingly, EC were similar in three different subgenotypes but distinct in three different locations. This establishes that local environment plays a pivotal role for EC, and raises the question of which factors within the environment affect EC. To determine which environmental factors influence EC, endophytes of *M. polymorpha* were sampled along a gradient of both rainfall and elevation to determine if these two factors affected EC. Both rainfall and elevation explained some of the variance of EC between host plants, but rainfall accounted for more variance. This shows that local environmental factors, especially rainfall, influence EC within a host plant. Not all environmental factors influence EC, though. A study of herbivory on *A. altissima* in three separate locations demonstrated that consumption of a host plant does not affect the EC of that plant. Rates of herbivory may vary between host plants and locations, but will have a negligible impact on EC. We can now say that both rainfall and elevation are two of the factors of variance between EC of different plants. Studies of temperature on EC are inconclusive: temp is or is not a factor of variance depending on the study.

Is Air Pollutant PM_{2.5} a Risk Factor for Diabetes?

MIKYAS BEKELE KISI

Diabetes affects many people and is a major medical and economic burden. Diabetes is characterized by either a lack of insulin, or a lack of insulin response, both of which lead to hyperglycemia. Given the prevalence and social cost of diabetes, identification of risk factors for diabetes remains a major research endeavor. Here, I review literature to evaluate whether the air pollutant PM_{2.5} constitutes a risk factor for diabetes. A broad survey indicated that there is an approximate positive correlation between concentration of PM_{2.5} and occurrence of diabetes in continental USA. A cross-sectional study done in India looked at two different areas within a city, areas that were highly affected and areas that were less affected by air pollutant PM_{2.5}. By obtaining blood results from random households, it was evident that there was a positive correlation between concentration of PM_{2.5} and occurrence of Hyperglycemia. To confirm whether hyperglycemia, the hallmark of diabetes, is indeed more prevalent in areas of higher PM_{2.5}, a 17 week experiment performed on two groups of wild-type mice was carried out by placing them in two different controlled environments, an area with filtered air, and an area with concentrated air (Air pollutant PM_{2.5}). Indeed, the mice that were placed in the concentrated air had a greater percent increase in blood glucose levels in comparison to the mice that were in the filtered air. The same experiment was performed on two groups of CCR2^{-/-} mice, and similar to the wild-type mice the CCR2^{-/-} mice placed in filtered air had a greater percent increase in blood glucose levels, but the increase wasn't as high in the CCR2^{-/-} mice, further establishing that Air Pollutant PM_{2.5} is a risk factor for diabetes.

Genetic Regulation of *Caenorhabditis elegans* Dauer Formation

KATELYN BOWEN

All organisms experience stress but the mechanism of how each organism copes with stress differs. The genetic model organism *Caenorhabditis elegans* forms dauers when it experiences stress, such as a lack of food. Dauer larvae are stress resistant because they are non-feeding; they are smaller than normal larvae at this stage, but are still mobile. There are many genes that regulate this mechanism. One key gene that has been identified to regulate dauer formation is *daf-16*. To establish that *daf-16* promotes dauer formation, loss of function mutants of *daf-16* were examined. *Daf-16* mutants fail to form dauers even under starvation, demonstrating that *daf-16* is a key promoter of dauer formation. Another gene known to regulate dauer formation of *C. elegans* is *pdk-1*. To test how *pdk-1* regulates formation of dauers, loss of function *pdk-1* mutants were obtained. It was observed that the *pdk-1* mutants did form dauers, concluding that *pdk-1* inhibits dauer formation under normal conditions. Other genes that regulate the dauer formation mechanism are *akt-1* and *akt-2*. To identify how they interact, RNAi was performed to knockdown both genes. Dauers only formed when both *akt-1* and *akt-2* functions were reduced simultaneously, demonstrating that both genes inhibit dauer formation in a redundant manner. To show that *pdk-1*, *akt-1* and *akt-2* interact with *daf-16*, wild type and *pdk-1* mutants were examined from kinase assays to determine the activity of the kinases on purified *DAF-16*. *Akt-1* and *akt-2* did not phosphorylate *DAF-16* when purified from the *pdk-1* mutant, establishing that *pdk-1* activates *akt-1* and *akt-2* which phosphorylates and therefore inactivates *daf-16*. The experiments conclude that the activation of the *akt-1/akt-2* complex by *pdk-1* inhibits *daf-16*, the key promoter of *C. elegans* dauer formation.

Alternative Polyadenylation of the *Hes7* 3' UTR Promotes the Oscillatory Expression Pattern Required for Somitogenesis

RANDY HILLEARY, KARA BRAUNREITER, AND SUSAN COLE

Somitogenesis is necessary for the proper development and segmentation of the axial skeleton in vertebrates. Importantly, when somitogenesis is disrupted in humans, it leads to the development of debilitating conditions such as spondylocostal dysostosis. During somitogenesis, the sequential budding of somites that lays the foundation for the axial skeleton is regulated by genes expressed in the presomitic mesoderm (PSM) of vertebrate embryos. The *Hes7* gene, conserved throughout the vertebrate lineage, regulates somitogenesis through its oscillatory expression pattern. Thus, *Hes7* expression acts as a genetic clock for somite formation to promote the segmentation of the axial skeleton. Interestingly, in mice embryos, *Hes7* is expressed in the PSM as three different transcript isoforms – termed *Hes7^{long}*, *Hes7^{middle}*, and *Hes7^{short}* – due to alternative polyadenylation of the 3' untranslated region (UTR). Although it is known that the oscillatory expression of all three *Hes7* isoforms is present during development, how these transcripts are regulated to properly time somite formation is not understood. When analyzing the decay rate of *Hes7* transcript isoforms using in situ hybridization, the transcript containing the *Hes7^{long}* 3'UTR decayed the fastest. This suggests that the faster decay rate of the longer transcript isoforms may promote the oscillatory expression pattern of *Hes7* during somitogenesis. Additionally, using a luciferase reporter system fused to each *Hes7* 3'UTR isoform, the amount of protein produced from each transcript was measured. However, there was not a significant difference in the protein produced. This suggests the need for further experimentation expanding upon this study to deduce whether it is the decay rate or translational efficiency of each of the *Hes7* transcript isoforms that regulates the oscillatory expression required for segmentation of the axial skeleton.

Preferences of the Invasive Spotted Lanternfly (*Lycorma delicatula*) Among Potential Host Plants

SUJI KIM , ANNA KUHN, MICHAEL J. RAUPP, AND
HOLLY MARTINSON

Spotted lanternfly (SLF, *Lycorma delicatula*; Hemiptera: Fulgoridae) is an invasive bug originating from China. This species first became problematic in the mid-Atlantic states around five years ago, and its range is rapidly increasing in this region. As of 2019, Pennsylvania, Virginia, New Jersey, and Delaware have quarantined counties with severe infestations to prevent the further spread of SLF. SLF are feeding generalists, with well-known preferences for tree-of-heaven (TOH, *Ailanthus altissima*), which is also invasive from Asia. In the US, SLF tend to feed on a variety of plants, including TOH, grapevine, and fruit trees. Because SLF were only recently introduced to the US and because the suite of available trees differs in their native and introduced ranges, the host plant use of SLF remains largely unknown. In this study, SLF host plant use was studied at multiple sites in Pennsylvania. At each site, timed counts of egg masses and nymphs were conducted, and the relative abundance of eggs and nymphs were assessed across tree species and as a function of tree size. The abundance of egg masses depended on tree size, whereas nymph abundance differed among plant species. Additionally, host plant use differed among sites. Specifically, SLF were abundant on TOH where it was present and on *Juglas nigra* (black walnut) and *Evodia danielii* (Korean evodia) where TOH was absent. Understanding the behavior and host plant use of SLF in the US will be beneficial for effective regulation and prevention. That egg masses and nymphs responded differently to tree size and species suggest that different management strategies must be employed to manage these different life stages. Finally, future studies investigating chemical similarities among host plants would clarify feeding behaviors and allow more specific management recommendations.

Captive Tiger Activity Levels Based on Seasonal Temperature Changes

HANNAH E. SMITH

Zoos are crucial tools for conservation and education, acting as genetic time capsules for thousands of different species. Through controlled breeding programs, zoos preserve and restore genetic diversity, keeping animal populations healthy and providing the potential to restore wild populations. Zoos also act as controlled stages to observe animal behaviors that would be next to impossible to observe in the wild. In this study, I investigate the seasonal and daily behaviors of captive Amur tigers, *Panthera tigris altaica*, at the Philadelphia Zoo. I hypothesized that the animals would be more active in the winter, because the temperature is more like that in their native habitat, whereas the tigers would be more lethargic in the summer because of the higher heat. Through six months of behavioral observations (4 in summer, 2 in winter), I found a 16.7% increase in activity from the summer to winter. This finding supports my hypothesis and suggests that the winter months in this zoo may be less stressful than the summer months. One clear recommendation from this study is that zoos allow captive tigers access to cool, indoor spaces during the hot summer months. Continued studies of captive animal behavior may allow improvements to captive animal husbandry and help us understand effects of climate on animal behavior in zoos. Such future research will be essential for maintaining the health and well-being of the 480 Amur tigers currently in captivity, especially as they represent 47% of the global population of this endangered subspecies.

Expression and Purification of Two Mutant Modified Vaccinia Virus Ankara (MVA) D10 mRNA Decapping Proteins

ERIC VON BERGEN, SUSAN PARRISH, JOHN SLATER,
LINDA WYATT, AND BERNARD MOSS

The Modified Vaccinia Virus Ankara (MVA) strain was developed for use as a viral vector to create a safer smallpox vaccine, as MVA is replication deficient in most mammalian cells. We recently identified mutant MVA viruses that could replicate in monkey, human, and HeLa cells and determined that the adapted viruses all contained one of three novel point mutations (termed D10mut25, D10mut226, and D10mut233) in the D10 protein. The D10 protein is a highly conserved mRNA decapping enzyme that plays a crucial role in the regulation of mRNA turnover at various stages of the viral replication cycle. Interestingly, the three point mutations identified in D10 occurred outside of the Nudix motif, which is the catalytic site needed for mRNA decapping activity. To understand the link between MVA host cell range extension and D10, we sought to express and purify D10mut226 and D10mut233. The respective mutant MVA DNA was amplified by PCR and cloned into the pmal-c5x plasmid to encode an MBP-D10-10X histidine fusion protein. The plasmids were transformed into *E. coli* and screened for the D10 insert by PCR and restriction enzyme digestion. Each mutation was subsequently confirmed by DNA sequencing. We induced protein expression and purified the fusion protein by affinity column chromatography using amylose and nickel resins. The purified mutant proteins were visualized by SDS-PAGE, confirming the successful expression and purification of the mutant D10 fusion proteins. Additional work will be performed to optimize fusion protein stability and purification. After experimental optimization, the purified D10mut226 and D10mut233 proteins will be assessed for mRNA decapping activity in comparison to wild type D10.

Investigating Gall Incidence on *Solidago altissima* at Organismal, Population, and Community Levels

SAMANTHA LONG AND HOLLY MARTINSON

Solidago altissima, a common flowering plant belonging to the Asteraceae family, is abundant in abandoned agricultural fields (old fields) throughout North America. *S. altissima* is the host for the galling fly, *Eurosta solidaginis*, which lays eggs on the stem of the host plant, inducing formation of a gall around those eggs. Gall formation occurs annually, but it is not known which environmental factors, if any, affect the incidence of galls in any given ecosystem. Here I examined gall incidence in an old field, mid-Atlantic ecosystem at three ecological levels: organismal, population, and community. To address potential mechanisms influencing gall occurrence at these three ecological levels, I assessed soil quality, *S. altissima* density, and plant species richness, respectively. I collected data within 5 x 5 m plots during the late summer, following gall formation in the spring. None of these environmental factors appeared to significantly influence gall occurrence within the study plots. Since galling is a specialized form of herbivory, I also examined the degree of general herbivory with respect to each level. Again, environmental factors at each level were not important. My observations suggest that galling by *E. solidaginis* is not affected by soil quality, abundance of host plant, or plant species richness in the community.

Comparative Genomics: Unique Genomic Pathways of Coral-Associated Bacterial Symbionts

DEVON MARANTO AND ALLISON KERWIN

Increasing environmental changes, often anthropogenic in origin, greatly threaten marine life and the intricate, symbiotic associations that exist, particularly in tropical corals. Studying the microbiomes directly associated with corals and their photosymbionts, Symbiodiniaceae, can provide insight into the coral response to environmental stressors. While the tripartite symbiosis between the coral host, Symbiodiniaceae, and bacterial microbiome has been well-studied, little is known about the bacterial microbiome functions since most studies to date have involved 16S rRNA microbial surveys. In this study, we use the bioinformatic tools KBase, RAST, and SEED to examine the genomes of one coral-associated bacterial isolate and one Symbiodiniaceae associated bacterial isolate. We compared these genomes with six previously published, symbiotic and free-living bacterial genomes from NCBI. We found that both free-living and symbiotic bacterial genomes contained genes potentially involved in glutathione metabolism, protein translocation, components of photosynthetic machinery, and oxidative stress regulation. However, genes for the specialized pathway of selenocompound metabolism were found only in the genomes of symbiotic bacteria, and not in the genomes of free-living bacterial isolates. Additionally, genes associated with the ability to denitrify nitrite to nitric oxide were found only in our Symbiodiniaceae-associated bacterial genome. These findings suggest that while symbiotic and free-living bacteria possess genes that function in conserved pathways critical for their own survival, symbiotic bacteria associated with either Symbiodiniaceae or directly with the coral host have some specialized pathways. Future investigations of more coral- and Symbiodiniaceae-associated microbial genomes, as well as comparisons between the genomes of the Symbiodiniaceae symbionts and coral host themselves, will help to identify genes in additional specialized pathways and to understand the complementarity of the coral tripartite symbiosis.

Buzzing around a Nutrient Network Site: Quantifying Relationships between Floral Abundance, Pollinator Visitation, and Herbivore Exclusion.

JESSIE WYATT, KAITLIN MURPHY, DR. HOLLY MARTINSON

Insect pollinators are important to the health and reproduction of wild and cultivated plants. Factors influencing the number and type of pollinators can therefore be crucial for maintaining pollination services. Old fields are herbaceous plant communities that develop on abandoned agricultural fields and include a diverse set of flowering plants. In this system, insect pollinators may be differentially attracted to these flowering plants, depending on specific characteristics such as floral abundance and floral height, or other conditions such as light availability or herbivory. To investigate the determinants of floral abundance and flower visitation, 18 field plots were established in May 2019 in an old field in central Maryland. Fences were installed around six of these plots to investigate the effects of deer on plant-pollinator interactions. Repeated surveys were performed to investigate the relationship between floral abundance, fruit abundance, and pollinator visitation. In total, 26 flowering plant species and 854 pollinator-plant interactions were observed. Investigating the full pollinator community, the number of pollinator visits varied among plant species and with floral abundance but did not differ with fencing treatment. Similarly, fruit abundance was positively related to floral abundance but was unaffected by fencing. In contrast, fencing influenced plant height, and the magnitude of this effect varied among species. These results suggest that characteristics such as floral abundance, floral height, light abundance, and herbivory can affect pollinator-plant interactions. Future studies can investigate the efficacy of particular pollinators and test the responses of plant-pollinator networks to additional environmental disturbances such as nutrient additions.

Characterization of the Role of D10 mRNA Decapping Enzyme in the Expansion of Modified Vaccinia Virus Ankara (MVA) Host Range

JOHN SLATER, SUSAN PARRISH, ERIC VON BERGEN,
LYNDA WYATT, BERNARD MOSS

The Modified Vaccinia Virus Ankara (MVA) strain has been developed as a vector for a safer poxvirus vaccine, in part because of its inability to replicate in most mammalian cells. However, certain MVA mutant strains have been isolated that have an extended host cell range and are able to replicate in monkey and human cells. These replication-extended MVA viruses were shown to possess single mutations in the D10 gene, denoted as D10mut25, D10mut226, and D10mut233. The D10 gene encodes an mRNA decapping enzyme that modulates mRNA turnover to promote viral infection. The three D10 mutations identified in the replication-extended MVA strains are all located outside of the Nudix domain, the active site of the D10 enzyme essential for mRNA decapping activity. To understand how mutations in D10 promote expansion of MVA host range, wild-type D10 and D10mut25 were amplified by PCR and cloned into the pmalc-5x plasmid to encode MBP-D10-10X histidine fusion proteins. The plasmids were then transformed into *E. coli* and the inserts were screened by PCR, restriction enzyme digestion, and DNA sequencing. Protein expression was induced and the resulting proteins were purified using affinity column chromatography using amylose and nickel resins. SDS-PAGE analysis of the purified proteins showed that wild-type D10 and D10mut25 were successfully expressed and purified. Future work will be performed to increase the solubility, stability, and purity of the MVA fusion proteins to subsequently determine if D10mut25 can decap mRNA *in vitro*.

Bugs and Bodies: An Investigation into the Volatile Organic Compounds Attractive to Flesh- and Fly-Eating Beetle

MEGHAN SADLER, DR. HOLLY MARTINSON,
DR. DANA FERRARIS

Forensic entomology is a field still in its infancy, with much to be characterized concerning how insects' roles in carrion ecology can be informative to forensics. Recently, a call has been made to standardize all scientific fields referred to in court cases, highlighting the need to define insect-carrion relationships and standardize methods used in forensic entomology. Much of the previous literature in forensic entomology has focused on fly species arriving shortly after death, with very few studies focusing on carrion-associated beetles, which arrive in the later waves of succession. This series of experiments used the species *Dermestes maculatus* (Dermestidae), *Dalotia coriaria* (Staphylinidae), and *Carcinops pumilio* (Histeridae) to understand Coleoptera-carrion interactions. This was done by measuring each species' affinity to different volatile organic compounds (VOCs) associated with various stages of decay. *D. maculatus* served as a model for carrion-feeding species, while *D. coriaria* and *C. pumilio* served as model species for beetles that prey on fly larvae. In this study, the compounds benzyl butyrate, dimethyl disulfide, dimethyl trisulfide, 1,2,4-trimethylbenzene, and hexanal were considered due to their distinctive physical properties and unique roles in carrion ecology. Olfactometer tests were used to assess VOC attractiveness or repellency for each beetle species. Differences are found in species responses, particularly their sensitivity to the presence of VOCs. Additionally, it was found that *D. maculatus* individuals are attracted to dimethyl trisulfide at a low concentration. The results of these experiments may be informative to forensic entomology studies, and future work should test a larger range of VOCs, mixtures of compounds, and lower concentrations to emulate naturally occurring conditions more accurately.

Design, Synthesis and Evaluation of Bicyclic Derivatives of PARP10

KAYLEE HOLLIGER AND DR. DANA FERRARIS

Poly-(ADP-ribose)polymerases are a family of enzymes that catalyze the transfer of ADP-ribose onto protein substrates. These enzymes are major regulators of cellular processes such as chromatin modulation, transcription, DNA repair, and stress responses, that can fuel oncogenesis. While all 17 members of the PARP family have some structural homology, each has slight differences around their active sites that may be exploited to design selective inhibitors. One of the members of this family, PARP10, has critical functions in chromatin remodeling, gene transcription, and tumorigenesis. The main focus of this research was to validate PARP10 as a drug discovery target and in doing so, design chemical probes that can be used as selective, potent inhibitors of PARP10. Inhibitory data from past research displayed that an aryl amide is an integral part of the pharmacophore of PARP10 inhibitors, and this portion of the molecule accounts for hydrogen bonding interactions and π -stacking within the active site of PARP10. These interactions stabilize the inhibitor within that active site. With this in mind, we focused on improving the H-bonding and π -stacking interactions between the inhibitor and the PARP10 active site by locking the amide into place with a bicyclic π -system, forming a quinazoline. Two series of quinazolines were created and analyzed for their inhibition potential, one series with a methyl group attached to the quinazoline, and one without. Inhibition potential was measured using a PARP10 assay. Both of which were overall very selective for inhibiting PARP10 over PARP1 and PARP14. But specifically, the addition of a methyl group on the quinazoline increased potency by 0.36 μM . These data indicate that future research should focus on more modifications to the quinazoline ring such as trying different R-groups instead of the methyl at that position, or modifying the 6-membered ring into a 7-membered ring.

Lipid Lost: Investigating Soybean Carbon Metabolism

COOPER HOSTETLER, SHRIKAAR KAMBHAMPATI,
SALLY BAILEY, AND DOUGLAS ALLEN

Soybean (*Glycine max*) is one of the most prevalent crops grown in the world today. Soybean is valuable because of its high lipid content, which is useful in biofuel production, as well as its high protein content, which makes it a top choice for livestock feed. However, traditional breeding efforts to increase lipid or protein content increase one at the expense of the other, thus not improving the overall productivity. We sought to investigate alternative carbon metabolism pathways to improve soybean productivity. It has been observed that lipid levels decline as the seed matures and that, at the same time, the levels of indigestible oligosaccharides increase. We hypothesized that the observed decline in lipids is associated with oligosaccharide production. For this study, a subset of mutagenized soybean lines with increased lipid content were thoroughly evaluated for lipid, protein, and oligosaccharide accumulation profiles using mass spectrometry and gas chromatography – flame ionization. Our results indicated that the decline in lipid content is not associated with oligosaccharide production. However, we discovered two mutant lines that seem to be unaffected by the previously observed inverse correlation between protein and lipid in soybean and thus have tremendous agricultural potential. These results indicate a promising avenue for further investigation that could lead to eventual improvements in the overall productivity of soybeans.

Dear McDaniel Biology Class of 2020,

Congratulations to making it to this point in your academic careers. I know many of you have looked forward to this moment for a long time and is a milestone that you should be proud of. Although COVID-19 has thrown an unexpected curveball into the end of your undergraduate careers at McDaniel College, it should not take away from the fact that you will be leaving McDaniel prepared to take on the world in which ever way you choose—teaching, medicine, research, biotechnology, etc.

As an alumni of McDaniel College Class of 2019, I proudly say that McDaniel was the best decision I ever made. McDaniel was the place where I grew as a young adult, created memories that I continue to look back on, and formed lifelong friendships and mentorships that are invaluable. Most importantly, McDaniel was where I fell in love with biology. Starting with Principles of Biology with Dr. Huang, I then branched out to several different domains of biology offered at McDaniel—Genomics with Dr. Parrish, Comparative Anatomy with Dr. Staab, and Ecology with Dr. Martinson. Although this was not an exhaustive list of the classes I had the opportunity to experience, each one highlighted some of my favorite memories. Perhaps this is a common theme amongst your class. I hope every single one of you fell in love with an area of biology that has led to your next step. For me, my love for biology led me to pursuing medicine at America’s Medical School, formally known as the Uniformed Services University of the Health Sciences.

Whatever your next step may be in the journey we call life; I have no doubt each and every single one of you will go on to achieve greatness in your own ways. The skills you developed throughout your four years at McDaniel will prove to be invaluable and separate you from others. Most importantly, I believe you have all faced unprecedented matters in your final year of schooling that will fuel the fire to accomplishments down the road. Albert Einstein could not have said it any better, “the midst of every crisis, lies great opportunity.” So go out there and take on the world with all you have and continue to make alumni such as myself proud!

Best Wishes,

Brandon Rozanski



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