Lymphoproliferative Disease Virus in Ruffed Grouse in Maine
Aleem, A.\textsuperscript{1}, Sullivan, K.\textsuperscript{2}, Howard, A.\textsuperscript{1}, Milligan, P.\textsuperscript{1}
\textsuperscript{1}University of Maine at Augusta, \textsuperscript{2}Maine Inland Fisheries and Wildlife
aaminah.aleem@maine.edu

Lymphoproliferative disease virus (LPDV) is a type C retrovirus that causes lymphoid tumor in turkey and chickens. It was first identified in domestic turkeys in Europe and Israel, but since 2009, it has been verified in wild North American turkeys. A recent study determined that 80% of Maine’s wild turkey population, between spring 2012 and fall 2014, tested positive for LPDV proviral DNA. LPDV is believed to spread horizontally amongst turkeys through direct contact. It is not predicted to cause human infection, however its transmission to other Galliform species is not known. Bonasa umbellus, ruffed grouse, shares the same terrain and comes in contact with wild turkey scat. Hence, Maine’s ruffed grouse population is being tested for the presence of LPDV. Results thus far, using PCR for detection of the gag gene, have not revealed any cross transfer of LPDV from turkey to grouse.

The Role of Neutrophil Cytosolic Factor 1 In Innate Immune Response to Influenza A Virus
Lucy Algeo
ldalgo@gmail.com

Influenza A Virus (IAV) causes over 21,000 deaths annually in the United States alone. The innate immune response to IAV includes the production of reactive oxygen species (ROS) via NADPH oxidase. ROS is known to impact signaling pathways and cellular processes in order to eliminate IAV, but can cause permanent damage to lung epithelial cells in the process. One gene involved in the production of ROS is Neutrophil Cytosolic Factor 1 (ncf1), which codes for a subunit of neutrophil NADPH oxidase. Mutations in ncf1 have been correlated with chronic granulomatous disease, chronic inflammation, and autoimmunity. Studying ncf1 in response to IAV infection could potentially lead to the discovery of novel therapies.
Characterizing the Neutrophil Response to an Influenza A Virus Infection in the Zebrafish Model

Bowman, A. 1, Jurczyszak, D.R. 1, Kim, C.H. 2

1 Department of Molecular and Biomedical Sciences, University of Maine, Orono, ME 04460, 2 Graduate School of Biomedical Sciences and Engineering, University of Maine, Orono ME 04469

alexis.bowman@maine.edu

The flu is a common contagious respiratory viral infection caused by the Influenza A virus (IAV). In healthy individuals, seasonal IAV infections are self-limiting, however, enhanced disease severity is seen in infants, elderly, and the immunocompromised. The innate immune system is responsible for recognizing and limiting viral infections. Neutrophils are a type of innate immune cell that play a role in the clearance of pathogens, however, current research suggests that an overly robust immune response may be detrimental to the host. The mechanisms responsible for neutrophil migration and behavior in response to an IAV infection remain unclear. The aim of this study is to utilize the zebrafish as a model to characterize the neutrophil response to IAV infections by modeling neutrophil migration in response to localized and systemic IAV infections, as well as investigating expression of how genes related to neutrophil migration and activity may be altered upon IAV infection.

The impact of imidacloprid on bumble bee gut bacterial communities

Conroy, D., Dobrin, S.E., Feinstein, L.
University of Maine at Presque Isle, Presque Isle, ME
Dylan.conroy@maine.edu

Human gut flora, a community of approximately 160 bacterial species in the digestive tract (gut), are associated with human health. We investigated the effect of pesticides on the diversity of bacterial communities found in the gut of bumble bees (Bombus impatiens). Due to their low bacterial species diversity, 9 species clusters, bees are an excellent model organism. We extracted gut DNA, amplified bacterial genes via PCR, and used TRFLP analysis to measure changes in the gut microbiota 6 hr, 24 hr, 4 days, and 7 days after exposure to imidacloprid. Feeders contained control or imidacloprid-laced sugar solutions providing ad lib access to foragers. For acute (<24 hr) treatments, bees were individually restrained and manually presented control, low, or high doses of sugar solution. Redundancy analysis was used to assess the effects of pesticide dose (control, low, high) and time of exposure (6 hr - 7 days) on TRFLP community profiles.

What is driving range expansion of vector deer ticks in Maine, USA -- climate, hosts, habitat, or human behavior?
In 2014 Maine had the highest incidence of Lyme disease among US states (105 case per 100,000). Incidence is higher in mid-coast counties (e.g., Knox County 268/100,000), and higher yet on off-shore Maine islands (up to 2,693/100,000). Lyme and other tick-borne illnesses (anaplasmosis, babesiosis) are correlated with deer tick abundance. On a univariate basis, deer tick abundance is correlated with warm winters, wet summers, deer, invasive plants (such as Japanese barberry), and human behavior, manifested as resistance to tick control strategies and clashing values over resource management. Climate change has already brought warmer winters and wetter summers. How do we adapt? The dissertation research will include 1) a Bayesian hierarchical spatiotemporal model to determine relative contributions of climate, hosts, and habitat, 2) weather forecasting models to predict future tick abundance, and 3) through an island case study, ascertain attitudes toward tick control in a time of abrupt climate change.

Building school and community collaborations to eliminate arsenic from drinking water in Maine and New Hampshire: a model for the US
Farrell, A., Bailey, D., Stanton, B., Borsuk, M., Lawlor, K., McGreavy, B., Bieluch, K., Disney, J.
1 MDI Biological Laboratory, Salisbury Cove, ME, 2 Geisel School of Medicine at Dartmouth, Hanover, NH, 3 Dartmouth College, Hanover, NH, 4 Mitchell Center for Sustainability Solutions, University of Maine, Orono, ME
afarrell@mdibl.org

Maine and New Hampshire have among the highest per capita reliance on private wells in the U.S. Significant numbers of wells (up to 40% in some towns) have elevated levels of arsenic, the number one contaminant of concern for human health worldwide. Numerous studies associate exposure to inorganic arsenic with health effects, including cancer, diabetes, heart disease, and reduced IQ. However, testing rates by homeowners are low. We are engaging teachers and students in ME and NH in projects that include monitoring wells for arsenic and charting a path to eliminate arsenic from drinking water in their communities. Preliminary results suggest up to 15% of wells tested for this project exceed the EPA arsenic standard of 10 ppb. “All About Arsenic”, the website at the core of our project, is being used to provide sample collection information, visualize and share arsenic data, and track and evaluate project progress.

Developing a Forest Carbon Sequestration Budget for the Aroostook Band of Micmacs
Larry Feinstein, University of Maine at Presque Isle
The purpose of this study was to determine the ability of different tree communities to sequester atmospheric carbon dioxide gas as soil organic carbon. This study focuses on tree communities at the Loring Air Force Base, the Powers Grid, and the Spruce Haven locations. The wood lots studied are owned by the Aroostook Band of Micmacs, and this study is meant to provide them with important data that will aid them in deciding which trees to cut for lumber, and which trees to leave standing for the purpose of sequestering carbon. To gather this data, leaves that fell from the trees in the woodlots were collected in baskets, and the amount of foliage gathered was weighed. Soil samples were also taken from these locations. The pH and percent organic matter in the soil was determined. This research demonstrates importance in both economic and climate science fields.

**Regulation of microRNA-199 upon Pseudomonas aeruginosa Infection in Zebrafish**

Gagne, EG¹, Sullivan, DK¹, Kim, CH¹,², Millard, PJ³

¹Department of Molecular & Biomedical Sciences, University of Maine Orono, ME 04469, ²Graduate School of Biomedical Sciences and Engineering, University of Maine Orono, ME 04469, ³Department of Chemical and Biological Engineering, University of Maine Orono, ME 04469

Eliot_Gagne@umit.maine.edu

Recent studies have shown that microRNAs (miRs) play a regulatory and “fine-tuning” role in the innate immune system of the Zebrafish (Jiang et. al. ISSN 2219-2840, 2014). We have found that the expression of miR-199 is significantly upregulated upon stimulation of the Zebrafish innate immune system with *Pseudomonas aeruginosa* infection. Knocking-down the expression of miR-199 using oligonucleotide morpholino injection led to a significant decrease in bacterial burden of fish infected with *P. aeruginosa* as well as a significant increase in overall survival. Overexpression of miR-199 using a mature miR-199 duplex led to a decrease in overall reactive oxygen species (ROS) within an infected organism. Recent data suggest that direct targeting of src family kinase *Lyn* by miR-199 may be responsible for these immunosuppressant characteristics of this specific microRNA.

**Calcitonin gene related protein antiviral role in LP-BM5 infection of astrocytes and microglia**

Grlickova-Duzevik, E.,¹,² Chiem, D.,² Vaughn, J.,¹,² Cao, L.,¹,²

¹Graduate School of Biomedical Sciences and Engineering, University of Maine, Orono, ME 04469, ²Biomedical Sciences, University of New England, Biddeford, ME 04005

egrlickovaduzevik@une.edu

CGRP (calcitonin gene related protein) is a neuropeptide expressed by primary sensory neurons upon infection. CGRP expression was increased in spinal cord upon infection with the LP-BM5,
a murine retrovirus that causes AIDS like disease in mice. In in vitro study, CGRP induced reduction of LP-BM5 viral loads in primary mixed glial cells. CGRP’s antiviral effects were present on individual astrocyte and microglia cell lines when infected with LP-BM5 virus. In our study we hypothesized that CGRP downstream signaling may be involved in the antiretroviral effect. Type I interferons and chemokines are known to interfere with retroviral infection. Currently, we are investigating the relationship between CGRP downstream signaling, interferon and chemokine production and the antiviral response in astrocytes and microglia.

Correlation between Pseudomonas aeruginosa Infection and Innate Immunity in CFTR Zebrafish Morphants
Soucy, A. 1,2, Hayes, J. 1,2, Kiidli, T. 1,2, Mansour, I. 1,3, DeBrock, S. 1,4, Kim, C. 2,6, Miller, C. 4, Longfellow, J. 4, Alsaday, T. 1,5, Averill, R. 1,4, Foley, J. 1,2, Gibula, A. 1,2, Knowles, S. 1,2, Kuun, S. 1,5, Roderka, M. 1,4, Soohey, R. 1,2, Thibault, E. 1,2, Wakeland, L. 1,2, Hutchinson, K. 2,6, Sato, D. 7, Stanton, B. 8

1Honors College; 2Department of Molecular and Biomedical Sciences; 3School of Marine Sciences; 4School of Biology and Ecology; 5College of Engineering; 6Graduate School of Biomedical Science and Engineering; University of Maine, Orono, 7MDI Biological Laboratory; 8Dartmouth Medical School
ashley.n.soucy@maine.edu, jordan.hayes@maine.edu, taaniel.kiidli@maine.edu, isaiah.mansour@maine.edu, spencer.debrock@maine.edu

Cystic fibrosis (CF) results from a mutation in the cystic fibrosis transmembrane conductance regulator (CFTR) which often correlates with severe Pseudomonas aeruginosa infections. Roughly 30,000 individuals in the United States currently suffer from CF. Because the affected population is so small, little research has been directed to understand the connection between CF and P. aeruginosa infections. To aid in defining this relationship, zebrafish were utilized as a model to determine the effect of CF on the innate immune response to systemic infection and by examining migration of neutrophils to the site of localized infection. Results showed a decrease in neutrophil migration in CFTR morphants. Bacterial burden was greater in CFTR morphants compared to controls. With a better understanding of zebrafish immune response to CF and infection, it may be possible to develop treatments for humans in the future.

Carbon Stocks in Eelgrass Areas around Mt. Desert Island: Implications for Eelgrass Protection in Maine
Hooper, M.L. 1, Schnorr, M.F.E. 1, Johnson B. 2, Disney, J.E. 3
1University of Maine, Orono, ME, 2Bates College, Lewiston, ME, 3MDI Biological Laboratory Salisbury Cove, ME
megan.hooper@maine.edu

Zostera marina, which is commonly known as eelgrass, is an important part of marine ecosystems around the world. This plant is able to store carbon in a process known as carbon sequestration,
resulting in the removal of carbon from the atmosphere and storage of it in sediments. This plant has experienced a significant worldwide decline, including the area around Mt. Desert Island, ME. We have determined the percent total carbon and dry bulk density in the above ground biomass, below ground biomass, and sediments in five remaining eelgrass areas. This involved sampling shoots, rhizomes, and ocean sediment with a coring device at each sample site. In addition we mapped the extent of the eelgrass beds in these areas. We calculated the total carbon stock with these data, comparing sites with and without recent declines. With this information, we are able to build a compelling case for protecting eelgrass in Maine.

Identification and characterization of 5-HT\(_2\) receptor calmodulin-binding domains in JC polyomavirus infection

Lajoie, C.R.\(^1\), Maginnis, M.S. \(^2\) The University of Maine, Orono, ME
Conner.Lajoie@maine.edu

JC polyomavirus establishes an asymptomatic infection in the kidney in the majority of the human population. In immunosuppressed individuals, virus can spread to the brain and cause PML, a fatal demyelinating disease. JCPyV internalization into host cells requires the serotonin 5-HT\(_2\) receptors, yet the mechanism by which these receptors mediate viral entry has not been characterized. The objective of this research is to determine whether calmodulin, a ubiquitous calcium-binding protein, and conserved calmodulin bindings sites in 5-HT\(_2\) receptors regulate JCPyV entry. Treatment of glial cells with a calmodulin inhibitor did not significantly decrease infection, suggesting that calmodulin is not required for JCPyV infection. The role of calmodulin in JCPyV infection will be further analyzed using additional inhibitors and 5-HT\(_2\) receptors with mutations in conserved calmodulin-binding domains. These data will provide a better understanding of how cellular factors regulate JCPyV infection.

C. albicans shape plays a crucial role in infection progression

Moore, J.\(^1\), Seman, B.\(^1\), Wheeler, R.\(^1\)
\(^1\)The University of Maine, Orono, Maine
jessica.1.moore4@maine.edu

*Candida albicans* is a common fungus which presents a risk for infection in immunocompromised individuals. In the U.S., *Candida* species are the number one cause of mortality from fungal disease and a leading cause of hospital-acquired bloodstream infections. Further understanding of how *Candida albicans* causes infection and interacts with its host may lead to improved treatment options. A vital aspect of *C. albicans* infection is its ability to switch between two morphologies; this change is due to several underlying factors. We investigated the relative power of morphology in dissemination and invasive growth by varying the orthogonal parameters of growth temperature and genetic regulation. We used temperature and phenotypically locked fungal strains in a zebrafish infection model to elucidate the characteristics of each factor. Observing the outcomes of these modifications with high resolution microscopic imaging, we confirmed the crucial level of importance of fungal shape in influencing the progression of infection.
Quantified expression of stress genes (HSP70, SOD and CYP) in Zostera marina (eelgrass) populations in Frenchman Bay and Casco Bay, Maine

Clukey, J.¹, Cote, J.¹, Bussiere, A.¹, Ehrenfeld, E.¹, Hayden, L.¹, James, K.², Kampoto, A.¹, Marnotte J.¹, Miller, D.¹, Moulton, J.¹, Mullen, C.¹, Seasholtz, L.¹, Seavey, G.¹, Tarbox, B.¹, Willey, C.¹

¹Southern Maine Community College, South Portland, Maine ²MDI Biological Laboratory, Salsbury Cove, ME
juliekmoulton@smccme.edu

Zostera marina is the dominant sea grass species in the Northern Hemisphere, yet over the past 20 years populations have decreased dramatically. To identify causes for this rapid decline we utilized qPCR to quantify expression of stress genes HSP70, SOD and CYP. These genes are associated with heat stress, oxidative stress and pathogen defense. During the summer and fall of 2015 samples were collected from a mixture of healthy and stressed eelgrass populations in the waters around Mount Desert Island, Maine, and from a dormant recovering eelgrass population in Casco Bay at Wolf’s Neck, Freeport, Maine. Many hypotheses exist as to the nature of stress in eelgrass populations including predation, disease, commercial fishing activity, inshore water quality and elevated temperatures within the Gulf of Maine. We predicted that three stress-related genes would exhibit increases in expression between stressed and healthy eelgrass populations.

Population identification of Hediste diversicolor (Annelida; Polychaeta) obtained from the Callahan copper mine estuary and nearby estuaries in Maine

Prentiss, N.¹, Bray, N.¹, LaPerriere, J.¹, Gibson, D.¹, Breton, T.¹, Brinegar, C.¹

¹University of Maine at Farmington, Farmington, Maine
prentiss@maine.edu

The marine polychaete worm, Hediste diversicolor (Annelida; Polychaeta), was used to detect possible genetic differences among populations obtained from heavy metal-contaminated sediments and from non-contaminated sediments. Worms were collected from sediments known to contain high levels of copper and zinc (following the Callahan Mine operations in Goose Pond, Brooksville, Maine) and from relatively clean sediments of nearby estuaries (Horseshoe Cove, Brooksville and Bagaduce River [North Bay], Penobscot, Maine). The five gene loci targeted for sequencing included the nuclear genes 18S rRNA, 28S rRNA and the ITS region, and the mitochondrial genes COI and 16S rRNA. X-Ray fluorescence was used to analyze the sediments for heavy metals. Learning more about the species’ physiological responses to heavy metal exposure should further understanding of the potential biomedical effects of heavy metals on humans.

Comparison of pathogenic antibiotic resistant genes from fall and winter
Antibiotic resistance is of great concern in the medical field because pathogens continually evolve resistance when exposed to newly-developed antibiotics. ARG (antibiotic-resistant gene) families evolved in the 1960s (TEM genes), mid-1980s (SHV genes), and early 2000s (CTX genes). We chose to investigate if human bacterial pathogen ARG distribution varies in conjunction with seasonal changes. We obtained fifty antibiotic resistant pathogenic bacteria isolated from wound, respiratory, and urinary tract infections from The Aroostook Medical Center during fall 2015 and winter 2016. We tested each pathogen for TEM, SHV, and CTX genes using DNA extraction, PCR, and gel electrophoresis. Each fall pathogen contained an average of 4.3 ARGs and each winter pathogen contained an average of 6.8 ARGs. The PCR product is now being sequenced. These results will be used in conjunction with soil ARG presence data to investigate gene distribution patterns between the environment and human pathogens.

Immune Recognition of *Candida albicans* in Zebrafish
Monique Theriault
Monique.theriault@maine.edu

*Candida albicans* is an opportunistic fungal pathogen that causes diseases ranging from oral thrush to candidemia in immunocompromised individuals. Recognition of pathogens, like *C. albicans*, during infection is poorly characterized primarily due to the difficulties in visualizing the host/pathogen interaction without killing the host. Transparent animal hosts, such as *Danio rerio* (zebrafish), are necessary to accomplish the task of imaging pathogen recognition while maintaining host viability. For pathogen recognition, zebrafish likely use immune receptors similar to mammalian receptors including C-type lectin receptors. Human C-type lectin receptors have already been shown to be crucial in recognition of fungal pathogens like *C. albicans*, and our goal is to identify and characterize cognate receptors crucial for fungal recognition in zebrafish. For my thesis I am purifying fusion proteins of recently identified receptors and characterizing their ability to bind different microbes, including *C. albicans*.

Role of SHIP1 in the Innate Immune System during an Influenza Infection
Traxler, S.¹
¹University of Maine Molecular and Biomedical Sciences, Dr. Carol Kim Laboratory, Orono, ME.
spencer.traxler@maine.edu

The *SHIP1* gene is a member of the inositol polyphosphate-5-phosphate (INPP5) and its expressed protein functions as a negative regulator of myeloid cell proliferation, survival, and migration. Mutations on this gene are associated with various defects and cancers of the immune system. Previous studies have shown that in response to wound formation, *SHIP1*-deficient
zebrafish have increased neutrophil motility while overexpression of SHIP1 resulted in decreased neutrophil migration (Lam et al. 2012). From this research, it is suggested that SHIP1 is a key brake that limits neutrophil motility through a P13K signaling-dependent pathway. While the role of SHIP1 has been categorized during a wound response, its function during a viral infection has been left uncharacterized. The goal of this present study is to examine the role of SHIP1 during an innate immune response to an influenza infection.

The Role of Macrophages in a Zebrafish Model of Mucosal Candidiasis
Trzilova D.¹, Archambault L.¹, Wheeler R¹.
¹Department of Molecular and Biomedical Sciences, University of Maine, Orono, ME 04469
dominika.trzilova@maine.edu

Candida albicans and Candida parapsilosis are opportunistic fungal pathogens that cause mucosal and systemic disease in immunocompromised individuals and neonates. This project aimed to further characterize the role of macrophages in a mucosal infection with Candida species in the larval zebrafish model. We hypothesized that infection with C. albicans would lead to a greater phagocyte recruitment than C. parapsilosis because only C. albicans can switch into the more invasive hyphal form. By non-invasively imaging transparent zebrafish, we observed that C. albicans indeed recruited more macrophages to the mucosa compared to C. parapsilosis. Macrophages were present in three distinct morphologies, all of which had the ability to phagocytose Candida cells. We believe that study of innate immune responses to Candida infections will help us better understand dynamics of phagocyte interactions with the fungus, how they recognize the switch from a commensal to a pathogenic organism, and how they clear the infection.

Triclosan is a proton ionophore mitochondrial uncoupler in living cells including primary human keratinocytes: Effects on reactive oxygen species production and on mitochondrial morphology using super-resolution microscopy
Weatherly, L¹,², Nelson, A³, Shim, J², Riitano, A², Hashmi, H², Sher, R¹,², Hess, S¹,³, Gosse, J¹,²
¹Graduate School of Biomedical Science and Engineering, Orono, ME, ²Department of Molecular and Biomedical Sciences, University of Maine, Orono, ME, ³Department of Physics and Astronomy, University of Maine, Orono, ME
lisa.weatherly@maine.edu

Triclosan (TCS) is an antimicrobial used so ubiquitously that seventy-five percent of the U.S. population is likely exposed to TCS via consumer goods and personal care products. TCS is readily absorbed into human skin and has been found in urine and in the plasma and milk from nursing mothers. Here, we show TCS disrupts ATP production in NIH-3T3 mouse fibroblasts and primary human keratinocytes cultured in glucose-free, galactose-containing media. The reduction in ATP with no change in plasma membrane integrity in multiple cell types indicates that TCS is a mitochondrial toxicant. We have successfully imaged mitochondrial ultrastructure using fluorescence photoactivation localization microscopy (FPALM) with the outer
mitochondrial membrane marker TOM20-dendra2 and have found that TCS induces changes in mitochondrial morphology. TCS also increases reactive oxygen species (ROS) production and decreases mitochondrial membrane potential in RBL-2H3 cells. These data show that TCS disrupts mitochondrial and cellular functioning in diverse cell types.

Developing a zebrafish model system for JC Polyomavirus infection
The University of Maine, Department of Molecular and Biomedical Sciences, Orono, Maine
michael.wilczek@maine.edu

JC polyomavirus (JCPyV) is a prevalent virus, infecting an estimated 50% of the population. In the majority of cases, it causes a lifelong, persistent infection in the kidney. During immunosuppression however, the virus can reactivate and disseminate to the central nervous system (CNS). The virus replicates in astrocytes and oligodendrocytes, destroying the myelin sheath, resulting in the disease Progressive Multifocal Leukoencephalopathy (PML). The development of PML severely impairs necessary CNS function and is almost always fatal when left untreated. Currently, there is not a successful animal model system for JCPyV studies, yet an in vivo model is crucial to gain a better understanding of viral pathogenesis and PML. Employing both in vitro and in vivo systems, we are currently characterizing JCPyV infection in zebrafish with the goal of establishing a viable model organism to study JCPyV pathogenesis and PML.