



24th BioFeedback 2017

Acadia University
Department of Biology
Honours Student Research
Presentations
15 & 16 February 2017
KCIC Auditorium

Wednesday 15 February 2017

1:15 Official Opening & Welcome
Dr Brian Wilson

Chair: Brent Robicheau

1:30 Michael Adams

1:45 Natalie Silver

2:00 Gina MacDonald

2:15 Hannah Gillam

2:30 Break

Chair: Lita O'Hallaran

3:00 Gillian Johnstone

3:15 Stephanie Haddad

3:30 Jessica Fahey

3:45 Bryanna Sherbo

4:00 Medina Velic

4:15 Alexia Hawkey-Noble

Thursday 16 February 2017

MIXED MATING AND ITS ECOLOGICAL IMPACT ON AN ENDANGERED PLANT

Emily Evans, Rodger Evans, and Kirk Hillier

Department of Biology, Acadia University, Wolfville, Nova Scotia

Through its links to demography and population genetics, the reproductive biology of a species is an important intrinsic factor that can be used to predict the future viability of a population. The objective of this study was to provide a comprehensive understanding of the unique mixed mating system utilized by an endangered plant, illuminating potential associated ecological repercussions. *Crocianthemum canadense* (Rockrose) is a small perennial herb listed as critically imperiled under the Nova Scotia Endangered Species Act. Rockrose exhibits a unique mixed mating system called dimorphic cleistogamy, whereby a single plant produces both cross-pollinating open flowers and self-pollinating closed flowers temporally separated within a single season. To assess the seasonal transition from one floral type to the other, several floral characteristics were compared using microscopic analyses and observations of seed production in field-based exclusion trials. Seed germination characteristics were also compared between flower types to determine if a difference in fitness existed between progeny from crossed versus self-pollinated flowers. Results indicate that flowers gradually change in morphology and reproductive strategy within a season, with transitional states between obligate cross-pollinating and obligate self-pollinating flowers. Seeds from late-season closed flowers had a lower percentage germination than open flowers, revealing a possible decrease in fitness of progeny from closed flowers. An increase in the infestation rate of open flowers by larvae of *Mompha capella* was also documented. By consuming seeds from open flowers pre-dispersal, the florivorous moth may decrease the proportion of genetically-recombined seeds distributed within infested populations and ultimately pose risks for the adaptability and success of future Rockrose populations.

Chair: Cate Little

- 8:15 Welcome
- 8:30 Mark Billard
- 8:45 Erica Gillis
- 9:00 Emily Evans
- 9:15 Emma Carmichael
- 9:30 Sarah Veinot
- 9:45 Tayler d'Entremont

10:00 Break

Chair: Erika Holland

- 10:30 Victoria Brown
- 10:45 Leah MacLean
- 11:00 Anik Obomsawin
- 11:15 Colton Burke
- 11:30 Cardy Saunders
- 11:45 Ersa Gjelij

12:00 Lunch

Chair: Eileen Haskett

- 1:15 Rielle Hoeg
- 1:30 Melissa O'Brien
- 1:45 Keelan O'Malley
- 2:00 Allie Flinn
- 2:15 David Albert
- 2:30 Sarah Fancy

Reception to follow, Biology Building, Room 324

Keith R. Porter

1912-1997

Regarded as one of the founders of modern cell biology, Keith Porter was one of a team of three who, in 1944, were able to take the first picture of a whole cell using the electron microscope. Many of the structures inside the cell were first observed and consequently named by him (e.g. “endoplasmic reticulum”). He continued to make substantial contributions to the field of cell biology throughout his long career and was recognized by numerous prestigious awards; one of them, the National Medal of Science, was presented to him by President Jimmy Carter.

Dr. Porter was an undergraduate in the Biology Department of Acadia University from 1930 to 1934. His career path took him to the Rockefeller Institute, to Harvard, and to the University of Colorado at Boulder where he was the first chair of the newly formed Department of Molecular, Cellular and Developmental Biology.

Having always retained an appreciation for the start to his academic career received here in Nova Scotia, on this death, Keith Porter left a bequest to Acadia to fund an annual speaking event in the Biology Department.

REINTRODUCTION OF SEED BANK DERIVED *GEUM PECKII* (EASTERN MOUNTAIN AVENS) ON LONG ISLAND, DIGBY COUNTY, NOVA SCOTIA

Sarah Fancy, Dr. Juan Carlos López, Dr. Robin Browne¹

Department of Biology, Acadia University, Wolfville, Nova Scotia, ¹K.C. Irving Centre, Acadia University, Wolfville, Nova Scotia

G. peckii is a small herbaceous plant listed as endangered with the federal Species at Risk Act. In Canada the plants are found in bogs on Brier Island, Digby County; globally the only other population is in the mountainous regions of New Hampshire. The Nova Scotia Department of Natural Resources supports a seed bank population of *G. peckii* at Acadia University. For this research the seed bank was used to establish a population of *G. peckii* in tissue culture, the plants were then put back into their natural habitat to evaluate plant success. The outplanting of endangered tissue culture material is the first for Nova Scotia and it is a relatively new technique globally. This study also documented that *G. peckii* grows in association with arbuscular mycorrhizal fungi (AMF). During outplanting plants received either a commercial mycorrhizal inoculum, a native mycorrhizal inoculum or were left untreated. Mycorrhizal treatments were used with the goal of enhancing plant success, though no significant differences were found. To date the field population boasts a 100% survival rate, showing that seed banking is a valuable tool for endangered native plant species.

COMPETITIVE GROWTH OF COMMENSAL ESCHERICHIA COLI AND SEROTYPE O157:H7 DEMONSTRATES ADVANTAGE FOR NON-PATHOGENIC STRAINS.

David Albert, H el ene D'Entremont, Timothy Ells¹

Department of Biology, Acadia University, Wolfville, Nova Scotia;
¹Agriculture and Agri-Food Canada, Research and Development Centre, Kentville, Nova Scotia.

Escherichia coli are common members of the enteric community found within a range of mammals. While most *E. coli* are non-pathogenic, a small subpopulation, such as serotype O157:H7, express virulence factors including attachment/effacement proteins, hemolysins and shiga-like toxins. Ruminants constitute a primary reservoir of the O157:H7 serotype (up to 30% carriers in some populations), and unlike humans, they exhibit commensalism with these bacteria; however, the specific role this serotype plays within the gut microbiome remains a mystery. The purpose of this study is to gain understanding of the growth kinetics of serotype O157:H7 and commensal *E. coli*. Specifically, our aim is to determine whether these bacteria compete for the same nutritional niche. Approximately 1000 *E. coli* isolates obtained from the feces of a local beef herd in Nappan, N.S., were screened by rep-PCR and pulsed field gel electrophoresis (PFGE). All commensal pulsotypes (58) were then tested for the presence of colicin, phage, and the aforementioned virulence factors. Impedance technology was used to determine monoculture growth kinetics of isolates representing each pulsotype and also a panel of 51 unique O157:H7 isolates. Competition studies pairing commensal and O157:H7 strains were then carried out by impedance coupled with end-point colony enumerations on differential media (BCIG). Results generally showed growth rates to be significantly greater for the commensal group over the O157:H7 serotypes thus providing a competitive edge. Data suggests a nutritional basis for this trend and competition studies involving different substrates (fucose, proline and N-acetylglucosamine) are ongoing.

APPLICATION OF A CUSTOMIZED DRIFTER AND NEW HYDROPHONE SOFTWARE FOR MONITORING ATLANTIC HARBOUR PORPOISE (PHOCOENA PHOCOENA) ACTIVITY IN HIGH FLOW ENVIRONMENTS

Mike Adams, Anna Redden, and Brian Sanderson¹

Department of Biology, Acadia University, Wolfville, Nova Scotia; ¹Acadia Centre for Estuarine Research, Acadia University, Wolfville, Nova Scotia

Atlantic Harbour Porpoises are small, echolocating toothed whales (Suborder Odontoceti) commonly found in coastal Atlantic waters. Click trains, produced during echolocation for the purposes of navigation and feeding, can be monitored using passive acoustic devices (e.g. hydrophones). In the upper Bay of Fundy, a small number of Harbour Porpoises occupy the high flow waters of the Minas Passage. Their presence and activities have been monitored since 2010. The naturally noisy conditions of the passage, however, can impede the collection of high quality porpoise click trains if hydrophones are bottom-moored and stationary (e.g. subject to flow noise). To mitigate flow noise and reduce the effects of surface waves, a customized drifter-hydrophone system was recently designed and assembled. The 20 m long drifter includes a surface GPS unit, sufficiently spaced flotation and weights, a wave dampening component, and two synchronized iListenHF hydrophones. Initial testing was conducted in Minas Passage in October 2016, one month prior to installation of a tidal turbine at FORCE. The hydrophone recordings were processed using three Harbour Porpoise echolocation detection procedures - a manual method (Audacity) and two automatic methods (PAMGuard and OceanSonics click detector). Preliminary comparison of the processing options suggests that the OceanSonics click detection software identifies more echolocation clicks than the other options. It also matches or exceeds the speed of PAMGuard. Audacity was the least effective method. Further drift studies will be conducted in spring 2017 to allow a pre- and post-turbine installation comparison of porpoise click train detections.

**MANAGEMENT OF *DELIA PLATURA*,
THROUGH TIMED PLANTING OF *PHASEOLUS
VULGARIS***

Silver, Natalie, Suzanne Blatt¹, and Kirk Hillier

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NS

¹Agriculture and Agri-Food Canada, Kentville, NS

All *Delia* spp. larvae feed on the roots of crop plants and the tissues of germinating seeds causing plant death and crop loss. Management of these species through selectively timed planting of *P. vulgaris* is not well understood. Three experiments were conducted to observe the impact of planting date and variety on the relationship between *P. vulgaris* and *Delia* spp. pests. Experiment 1 was a field study to evaluate planting date as a potential pest management practice. Of four planting dates, 14 June had the highest plant survival, realized the greatest number of beans per plant, as well as lowest damage ratings compared to other planting dates. Experiment 2 was a choice experiment in a growth chamber to explore *D. platura* female varietal oviposition preference. Based on egg counts, no difference was found in female varietal preference, but a significantly larger number of eggs were laid at early plant phenological stages. Experiment 3 was a growth chamber study to examine *P. vulgaris* response to *D. platura* infestation. Higher infestation levels resulted in higher damage ratings. Results in experiments 1 and 3 showed severely damaged plants survived and compensated for damage, growing up to six beans per plant, providing evidence that *P. vulgaris* exhibits tolerance to *D. platura* and other *Delia* spp. attack.

**INVESTIGATION OF *VARROA DESTRUCTOR*
MITE'S PREFERENCE OF ODORANTS FOR AL-
TERNATIVE MITE MANAGEMENT**

Allie Flinn, Dave Shutler, Kirk Hillier and Cate Little

Department of Biology, Acadia University, Wolfville,
Nova Scotia

The parasitic mite, *Varroa destructor*, are the most destructive disease to the European honey bees, making them of significant economic importance. Mite resistance to miticides, as well as detrimental effects on honeybees, have motivated exploration of alternative methods to safely manage these pests. Our research examines behaviorally-relevant compounds used in host-finding behaviour (attractants), or as potential deterrents to create a repellent for management. We tested various odour compounds associated with honey bees, including butyric acid, hexanol, methyl oleate, and 2-hydrohexanoic acid versus a hexane control, to mites in laboratory behavioral assays. Butyric acid and 2-hydrohexanoic acid at 100 ng/mL were favoured relative to the other compounds tested. Subsequently, all odour compounds were also tested on bees to quantify their reaction to these compounds. Even though limited numbers of mites could be tested on due to the difficulties retrieving them, this research did give insight on certain and refined methods that can be used in further testing.

**DOUBLY UNIPARENTAL INHERITANCE: SE-
QUENCING THE CONTROL REGION OF THE
MALE-TRANSMITTED MITOCHONDRIAL GE-
NOME OF THE RIBBED MUSSEL, *GEUKENSIA*
DEMISSA.**

Keelan O'Malley, Brent Robicheau, Dr. Donald Stewart

Department of Biology, Acadia University, Wolfville, Nova Scotia

A seemingly universal feature among metazoans is the strict maternal transmission of mitochondrial DNA (mtDNA). However, previous research has shown that this former dogma no longer holds within various members of the Bivalvia. For example, within the Mytilidae there is typically two distinct mitochondrial genomes, these are characterized as either F-types that are maternally transmitted to daughters via eggs, or M-types that are paternally transmitted through sperm to both daughters and sons – a concept called Double Uniparental Inheritance or DUI. Studies of the blue mussel, *Mytilus edulis*, which is in the same family as the ribbed mussel, *Geukensia demissa*, have shown that occasionally a fragment of an M-type genome can recombine with an F-type creating a so-called "recently-masculinized" or RM-type. RM genomes are subsequently transmitted paternally and consist of the F-type core and the control region (CR) of an M-type. The aim of my project is to sequence and analyze the CR of the ribbed mussel to determine if typical patterns of molecular evolution characteristic of DUI are also found in this species. Fragments of the paternally and maternally transmitted mitochondrial genomes of the ribbed mussel were characterized for the first time using Long-Range PCR, Next-Gen sequencing and mtDNA annotation. Sequences were obtained for the *cox 1* gene of the M-type, and the *rnl* and *cytb* genes of both M- and F-types. Our ultimate goal is to search for, and to classify, any conserved DNA motifs that are likely involved in cellular pathways that control paternal transmission of mtDNA in bivalves.

**EXPLORING THE RELATIONSHIP BETWEEN DNA
METHYLATION OF THE MATERNAL EPIGENOME
AND LARVAL PHENOTYPE IN THE MARINE
WORM *POLYDORA CORNUTA***

Gina MacDonald, Glenys Gibson, and Marlene Snyder

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Maternal environment plays a significant role in influencing offspring phenotype through epigenetic modifications such as DNA methylation. The marine invertebrate, *Polydora cornuta* (Annelida), has phenotypic plasticity in larval development, producing two distinct morphs of larvae: adelphophagic (non-dispersive) and planktotrophic (dispersive). The objective of my thesis work was to determine the relationship between larval morph and methylation of the maternal epigenome. Additionally, methionine was explored as a potential link between maternal environment and DNA methylation, as marine detritivores are thought to be methionine limited. I hypothesized that exposure of females to compounds that cause a shift in larval phenotype (e.g., BPA) would also cause a change to the maternal epigenome (DNA methylation). Adult *P. cornuta* were cultured in 6 groups: seawater (control), BPA, folate/B12, and three concentrations of methionine (10^{-5} , 10^{-6} , 10^{-7} M). Larvae produced from these cultures were collected and stored for analysis of phenotype. Gravid females were isolated after approximately one month in treatment and used for molecular analysis. Differences in maternal methylation were demonstrated through total genome digestion with isoschizomers *HpaI* and *MspI*. Gel electrophoresis was used to generate DNA smear patterns that indicated differential cleavage at methylated CpG dinucleotides. DNA smear patterns were analyzed using ImageJ software to generate intensity plots. Preliminary results indicate that maternal methylation pattern varies between treatment groups. Specifically, it appears that methionine may have concentration dependent effects on methylation. Further steps will be taken to establish a link with larval phenotype. This study has contributed to a greater understanding of the mechanism underlying epigenetic modification in invertebrates, and its relationship with phenotypic plasticity.

ROLE OF RELAXIN RECEPTORS IN RELAXIN-INDUCED NEUROPROTECTION

Hannah Gillam, Brian Wilson

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Nova Scotia

Stroke is the leading cause of disability and third leading cause of death in Canada. Recent studies have indicated various tissues including the brain are protected by relaxin during ischemic stress, making relaxin a potential candidate as a stroke therapeutic. H2 and H3 (relaxin) have been shown to protect rat brain tissue from ischemic conditions, but little is known about the receptor activity behind these processes. H2 and H3 interact with both RXFP1 and RXFP3 receptors, as both these receptors are present in brain tissue. The purpose of the experiment was to study RXFP1 and 3 roles in regulating relaxin during neural oxygen and glucose deprivation (OGD) stress, and to observe possible synergistic effects of H2 and H3. Brain slices from neonatal rats were cultured for two weeks. Subsequently, slices were subject to one of seven possible treatments: normoxic control (NC), OGD control, or OGD with: H2, H3, H2 and H3, H2 and RXFP3 antagonist, or H3 and RXFP3 antagonist. Propidium iodide (PI) fluorescence was used to highlight cellular damage and death. Mean PI +ve cell counts were compared among treatment groups. Western blots were performed to observe expression of both receptors in the neonatal rat neural tissue. NC and OGD with: H2, H3, and H2 with RXFP3 antagonist had significantly lower PI-positive cell counts compared to OGD control, and OGD with H3 and RXFP3 antagonist. Western blots showed expression of both receptors in neonatal brain slices. The data suggests H2 was acting through RXFP1 and H3 through RXFP3. Further research is required to determine cellular signaling mediated by RXFP1 and 3 and the role each play in tissue protection.

EFFECTS OF BPA AND BPS ON REGENERATION AND DNA METHYLATION IN THE MARINE WORM *Pygospio elegans*

Melissa O'Brien, Glenys Gibson and Marlene Snyder

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Scotia

Bisphenol-A (BPA) is a common environmental contaminant originating from manufacturing of plastics and epoxy resins. BPA is known to disrupt embryonic development by altering levels of DNA methylation. Bisphenol-S (BPS) is the "safe" replacement for BPA in plastics, but its health effects are understudied. The objective of my thesis was to investigate the effects of BPS on anterior regeneration in the spionid polychaete *Pygospio elegans* (Annelida), to compare its effects on regeneration to BPA (examined previously), and to identify a potential mechanism by which bisphenols disrupt regeneration, specifically, if they impact DNA methylation. My hypothesis was that BPS would disrupt regeneration as does BPA, and that this disruption would be associated with changes in levels of DNA methylation. Anterior regeneration was examined in regenerates cultured in seawater (control), BPA, and BPS by comparing growth and morphology using bright field microscopy. My results showed that BPS slowed regeneration, comparable to BPA. The effects of BPA, BPS, and folate/vitamin B₁₂ (a known DNA methyl donor) on DNA methylation levels were examined with agarose gel electrophoresis using *HpaII/MspI* digestion of whole genomic DNA. Using ImageJ gel analyses, preliminary results indicate that BPA and BPS alter DNA methylation levels in both intact adults and regenerates. Overall, this study helps contribute to a better understanding of the impact of bisphenols on regeneration and provides important preliminary evidence of a mechanism by which these environmental contaminants disrupt development.

HOUSE HUNTERS: PETREL EDITION

Rielle Hoeg, Ingrid Pollet, Dave Shutler

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Colonial living may be the result of patchy high quality habitat, and/or benefits of social behaviour. Benefits of colonial living may include enhanced foraging success, mate acquisition, and group defense. Costs may include fighting among adults, cannibalism in species with such tendencies, attracting predators, facilitating spread of parasites, and competition for nest sites. With respect to habitat, many seabirds avail themselves of islands that are free of mammalian predators. Within the former guild, some seabird species take advantage of stable, underground microclimates for nesting; these may also offer protection from aerial predators. If there are social benefits to living in close proximity to conspecifics, nearest neighbour distances should be short where nest success is high and vice versa. Water content of soil along with soil compaction and particle size can determine structural integrity and drainage capabilities of these nest sites. Burrowing animals should favour an intermediate soil moisture to avoid burrow crumbling or flooding. We investigated effects of living in close proximity to conspecifics and soil moisture on nest site selection and reproductive success. Leach's storm-petrels are colonial seabirds that nest in burrows on offshore islands. This study was conducted on Bon Portage Island, Nova Scotia, Canada. A total of 396 burrows was monitored. Burrows where a chick fledged were compared to those where they did not. Nearest neighbour distance and orientation of active burrows was measured from entrances to nearest active neighbour burrow entrances. Soil moisture was determined using a soil moisture meter at occupied and unoccupied burrows, and at control locations. Soil moisture was higher at control points than at burrows, but there was no difference in moisture at occupied versus unoccupied or successful versus failed burrows. Nearest neighbour distance and orientation did not appear to be associated with nest success.

EXPLORING STRESS BIOMARKERS IN AN AVIAN MODEL

Gillian Johnstone, Mark Mallory, Russell Easy

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Wolfville, Nova Scotia

Biomarkers are measurable substances that can be used as indicators of stress in animals. Presently, there is little understanding of stress biomarkers in birds. The objective of this study was to identify changes in unique proteins in an avian model to further our understanding of the stress response in aves. The models chosen in this study were the Herring Gull and Great Black-Backed Gull. Specimens were obtained from the St. John's International Airport, Newfoundland, CA. Samples of liver tissue were ground in a mortar and pestle under sterile conditions to extract soluble proteins. Protein concentrations were normalized and 1D SDS-PAGE followed by a modified silver staining method were used to identify individual protein profiles. Gels were imaged and digitized using a Fluor-S™ Multimager. Protein spots were excised from the gels and further analysed using tandem MS (LC-MS/MS). Preliminary MS data shows known stress proteins such as heat shock-70, heat shock-90, anti-oxidants such as superoxide dimutase and , pyridoxine phosphate oxidase which is an essential enzyme in vitamin B₆ metabolism. These proteins will be quantified using immunoblotting and used as targets to identify the effects of environmental stressors on the gulls. As our knowledge of stress in aves is limited, this data will contribute to the broader understanding of stress in aves.

FORMATION OF *LISTERIA MONOCYTOGENES* BIOFILM UNDER SIMULATED-MEAT-PROCESSING-CONDITIONS (SMPC) IN MIXED CULTURES WITH *LACTOBACILLUS PLANTARUM* AND *PSEUDOMONAS FLUORESCENS*

Haddad, Stephanie, Dr. Todd Smith, Dr. Timothy Ells¹

Department of Biology, Acadia University, Wolfville NS; ¹Department of Food Microbiology, Agriculture and Agri-Food Canada, Kentville NS

Contamination of food products with pathogenic microorganisms poses a serious health risk to consumers and can have major economic consequences for the food industry. Bacterial biofilms can form on surfaces and in hard to clean areas within food-processing facilities and subsequently lead to recurring contamination of food products. Consumption of foods contaminated with *Listeria monocytogenes* (*Lm*) can lead to the infection listeriosis, which can be fatal in susceptible subgroups within the population, including: infants, pregnant women, the elderly and immunocompromised individuals. To date, most research on *Listeria* biofilms has been conducted in monoculture, a situation rarely encountered in the real world. Therefore, the primary objective of this project was to study how the presence of two common meat spoilage organisms, *Lactobacillus plantarum* (*Lp*) and *Pseudomonas fluorescens* (*Pf*), influence the development of *L. monocytogenes* biofilms formed under a regimen of SMPC. Using CDC Biofilm Reactors®, biofilms with varying strain combinations (*Lm* only, duel *Lm+Lp*, duel *Lm+Pf* or triplex *Lm+Lp+Pf*) were grown in triplicate on two different surface types (Stainless Steel and High-Density-Polyethylene); over a 12-day period of alternating stress conditions (nutrient availability and desiccation) designed to mimic a possible two-week work cycle scenario in a meat processing plant. Results showed that biofilms preferentially developed on the plastic surface over the stainless steel for all strain combinations. Moreover, the viable numbers of *Lm* residing within biofilms was significantly enhanced when *Pf* was present. These results demonstrate the importance of species interactions in the development of pathogen-harboring biofilms which can ultimately influence the efficacy of downstream sanitization procedures.

FEMALE AUTODETECTION AND PLASTICITY OF PHEROMONE RESPONSE IN *HELICOVERPA ZEA*

Ersa Gjelaj, Kirk Hillier

Department of Biology, Acadia University, Wolfville, Nova Scotia

Most studies on insect olfaction have focused on males responding to female sex pheromones, due to assumptions that females were anosmic to sex pheromones. However, recently ‘autodetection’ (the ability for females to detect and respond to conspecific sex pheromones) has been observed in female moths in the family Heliothinae. The objectives of this study were to determine the functional importance of autodetection in *Helicoverpa zea* by determining what chemicals and concentrations result in a physiological response and behavioural response, determine whether there was a genetic basis correlating to responses and whether olfactory habituation contributes to compound sensitivity in females. Sensitivity to major and minor pheromone components was tested by behavioral bioassays and electroantennograms (EAG), and RT-PCR and qPCR techniques were used to determine and quantify the expression of olfactory receptors (ORs). All methods were carried out on two treatment groups; habituated, or ‘grouped’ females, and naïve, ‘ungrouped’ females. Behavioural trials suggest that females engaged in a ‘spacing behavior’ in response to both compounds, with greater response in the ungrouped females. EAG results showed statistical differences between concentrations of compounds, as well as grouped and ungrouped treatments. Finally, there is strong supporting evidence that there is greater expression of OR genes in the ungrouped treatment. Discovery that habituated females have a decreased peripheral sensitivity to compound due to downregulation of their olfactory receptors could have major implications in how laboratory experiments are conducted, as well as providing additional knowledge for use of pheromone based mating disruption strategies.

Analysis of Mercury Content of Lichens in Nova Scotia: Potential Use as Passive Air Samplers

Cardy Hallett Saunders, Biology Department, Acadia University Allison Walker, Biology Department, Acadia University Nelson O'Driscoll, Earth and Environmental Science Department, Acadia University Robert Cameron, Department of The Environment, Nova Scotia Government

ABSTRACT

Mercury is a global pollutant present in a wide range of ecosystems.

Atmospheric transport of elemental mercury gas enables this substance to be deposited far from its source. Quantifying mercury in biological indicator species, such as lichens, provides information about where mercury pollution may be an issue. Close to three hundred samples of *Hypogymnia* sp. and *Usnea* sp. lichens were collected from trees throughout mainland Nova Scotia and analyzed for total mercury using thermal degradation - atomic absorbance analysis. The resulting concentrations and geolocations were mapped using QGIS 2.12 and ArcGIS

10.4 to produce kernel density analysis hotspot maps. From these maps, it was possible to discern areas of higher mercury concentrations and trends across Nova Scotia. The presence of high concentration features in areas with known mercury problems such as Kejimikujik National Park suggested that lichens may be useful passive bioindicators of regional atmospheric mercury trends. Selected specimens were deposited in the E.C. Smith Herbarium (ACAD) at Acadia University and ITS rDNA barcoded to confirm morphological identities. Barcode sequences were deposited in NCBI GenBank (KY471545, KY471546, and KY471547) to increase the number of vouchered Nova Scotian lichen sequences available to science.

THE IDENTIFICATION OF SURFACE PROTEINS OF THERMOTOLERANT *CAMPYLOBACTER SPECIES* RECOGNIZED BY MAb M1169

Jessica Fahey, Hongsheng Huang¹, Russell Easy

Department of Biology, Acadia University, Wolfville, Nova Scotia; ¹Canadian Food Inspection Agency,

Ottawa, Ontario

Campylobacter spp. is a Gram negative, thermotolerant bacterium and a significant foodborne pathogen. Transmission is mainly through ingestion of undercooked and ready-to-eat poultry and mishandling of contaminated foods. While most individuals who have acquired campylobacteriosis recover rapidly (seven to ten days), individuals who are immunocompromised may develop rare, and potentially lethal complications, such as Guillain Barré Syndrome. Recently, a colony blot immunoassay has been developed at the Canadian Food Inspection Agency (CFIA) using a Monoclonal Antibody (Mab) M1169. In this study we characterized the surface proteins of *C. jejuni* and confirmed specificity of Mab to thermotolerant *Campylobacter* spp. After immunoprecipitation, M1169 successfully detected only thermotolerant *Campylobacter* spp. Protein bands were excised from 1D SDS-PAGE and analysed using mass spectrometry (MS). Proteins that were identified included major outer membrane protein (OMP) and flagellin. Other proteins included cytochrome C oxidase which is a large integral membrane protein found in prokaryotes. This work will help in advancing methods used in the detection and identification of *C. jejuni* in Canadian poultry, and in the development of a rapid detection method for thermotolerant *Campylobacter* spp.

FREQUENCY OF FOUR SPECIES OF SEALS KILLED BY POLAR BEARS AND HUNTERS IN WESTERN HUDSON BAY

Bryanna Sherbo¹, Don Stewart¹ and Stephen Petersen^{1,2}

¹Department of Biology and Earth and Environmental Science, Acadia University, Wolfville, Nova Scotia; ²Assiniboine Park Zoo, Winnipeg, Manitoba

Polar bear, *Ursus maritimus*, rely heavily on sea ice as their primary habitat for hunting their main food source, seals. The proportions of seals harvested by hunters versus seals killed by polar bears was unknown. This study compares 104 seal samples harvested by hunters between 2014-2016 and 12 seal samples killed by polar bears in 2014. All samples are from Western Hudson Bay near Churchill, Manitoba. The objective of this study is to quantify species-specific proportion of hunter killed seals versus polar bear killed seals. Samples were collected from freezers of hunters and conservation officers in Churchill, Manitoba. Analysis determined the species of unknown samples: harbour (*Phoca vitulina*), ringed (*Pusa hispida*), bearded (*Eignathus barbatus*), and harp (*Pagophilus groenlandicus*) seals. Eighteen highly polymorphic microsatellite markers and GeneCap technology was used to analyze samples, to identify any duplicates so that they could be removed from analysis. To determine the species of each sample, polymerase chain reaction (PCR) was performed on the mitochondrial cytochrome-*b* gene (*cyt-b*) to amplify the DNA. Following DNA amplification, a restriction enzyme digest was developed using the enzymes NsiI, AccI and AluI, which cut *cyt-b* PCR products of each seal species at different recognition sites. The resulting restriction fragment profiles visualized by agarose gel electrophoresis were used to determine seal species. Contingency tables and Fisher's exact test of proportions were used to determine significance between harvested seals compared to seals killed by polar bears. Overall, it was determined that the proportion of ringed, harbour and bearded seals harvested is not statistically significantly different from the proportions of species of seal killed by polar bears. This is significant because it indicates that hunters could be taking valuable food sources from polar bears potentially contributing to their decline.

AN EXPLORATION OF NORTHEASTERN COYOTE (*CANIS LATRANS* VAR.) MORPHOLOGY THROUGH THE USE OF MITOCHONDRIAL DNA (mtDNA) HAPLOTYPES AND NUCLEAR DNA (nuDNA) MICROSATELLITES

Colton Burke¹, Jason Power¹, Nathalie LeBlanc², Søren Bondrup-Nielsen¹, Mike Boudreau³, Mike O'Brien³, Brent Robicneau¹ and Don Stewart¹

¹Department of Biology, Acadia University, Wolfville, N.S.; ²University of New Brunswick, Saint John, N.B.; ³Nova Scotia Department of Natural Resources, Kentville, N.S.

Over the last decade, there have been various acts of aggression committed by coyotes (*Canis latrans* var.) in Nova Scotia. These attacks have led to injury, or even fatality, which promoted research on coyote morphology and behaviours. Many coyote carcasses from Atlantic Canada were received through the Nova Scotia Department of Natural Resources (NSDNR), which allowed various morphological measurements to be taken and tissue samples to be collected. A recent study demonstrated a trend among male coyotes in Atlantic Canada that contained an eastern wolf (*Canis lycaon*) haplotype and larger overall body size as measured by principal components analysis. The purpose of this study was to determine if there is a significant correlation between wolf mitochondrial DNA (mtDNA) haplotypes or nuclear DNA (nuDNA) (as assessed using microsatellite markers) and larger body size among male coyotes within Nova Scotia. To investigate this potential trend exclusively male samples were studied, and nuDNA was utilized to complement the mtDNA data. DNA was isolated from the skin (ear) of coyote carcasses obtained from NSDNR, and specific haplotypes of the mtDNA control region were amplified and sequenced. After sequencing, the mtDNA was identified to contain one of two coyote haplotypes, or an eastern wolf haplotype. Each coyote sample was assigned a haplotype through the use of phylogenetic analysis, and linked to its associated morphological meta-data. NuDNA was amplified through the use of eight microsatellite primers, and allele lengths were genotyped. Microsatellite PCR reactions are currently being optimized and the corresponding allelic data are being compiled for future analysis.

HABITAT FEATURES AFFECTING NEST-SITE SELECTION BY TREE SWALLOWS

Anik P. Obomsawin, Dave Shutler

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Nest-site selection is an important determinant of fitness because of its potential influence on reproductive success. To reduce metabolic costs of thermoregulation, and limit predation risks, adult birds must identify nest-sites with favourable habitat characteristics. I quantified habitat preference in cavity-nesting tree swallows. These birds will readily accept nest boxes, which facilitates manipulation of box type, surrounding vegetation density, and canopy cover. If predation avoidance influences habitat selection, adults may favour nest-boxes with smaller entrance holes because larger holes could render clutches more accessible to predators. Nest-boxes with sparse surrounding vegetation density and canopy cover may be favoured because birds can better detect and escape predators. However, adults may also favour nest-boxes with dense surrounding vegetation and canopy cover to make clutches less conspicuous to predators. I measured habitat surrounding 43 occupied and 136 unoccupied tree swallow nest-boxes in Port Williams, Nova Scotia. Vegetation density did not differ between occupied and unoccupied nest-boxes. However, occupied nest-boxes had significantly less canopy cover and smaller entrance hole diameters than unoccupied nest-boxes. Studies identifying habitat factors that influence nest-site selection may allow for conservation and management of suitable habitats and consequently, higher reproductive success for populations at risk.

ANTIMICROBIAL GROWTH PROMOTERS AND THEIR EFFECTS ON SWINE FECAL COMMUNITIES

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While antimicrobial growth promoters (AGPs) are widely used in North American animal production, there is a limited understanding of how they affect the animals which receive them. It is commonly claimed that animals fed AGPs have improved growth performance, reduced mortality, and improved overall herd health compared to animals not fed AGPs. These benefits are also commonly claimed to result from alterations of animal gut bacterial communities. More recently it has been suggested that AGPs work primarily as immune modulators, reducing host immune reactions in the gut, and rechanneling this energy into animal growth. The purpose of this research was to determine if AGPs alter fecal communities in swine and assess their possible immune modulation effects in pathogen challenged pigs. The experiment was a two block repeated design where swine in the first block were split into three treatment groups (control, tylosin-fed, Chlortetracycline-fed). Swine in the second block were treated the same, but were challenged at the study midpoint with a cocktail consisting of various species of *Salmonella*.

Fecal samples were collected every week and DNA was extracted. The extracted bacterial DNA was amplified using polymerase chain reaction (PCR) using primers that would target the V1-V2 region of the 16S rRNA gene. Denaturing gradient gel electrophoresis (DGGE) was used to fingerprint the fecal communities, and these fingerprints were analyzed by ordinal analysis. No effects due to feeding AGPs and/or pathogen challenge were observed. However, temporal change was observed in the gut communities, although this was independent of any treatment. The microbiota of the gut is known to change during growth and development in all mammals. This study indicates that AGPs do not alter bacterial fecal communities in swine, and raises questions regarding theories purporting to explain their underlying mechanism of action.

THE USE OF ANTIMICROBIAL GROWTH PROMOTERS IN FARM RAISED ATLANTIC SALMON (*SALMO SALAR*) FEED AND ANTIMICROBIAL RESISTANCE AMONG FLORA VS. WILD TYPE ATLANTIC SALMON

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The use of antimicrobials as growth promoters has become a common practice in the aquaculture industry (AI) and, as a result, the safety and quality of farmed Atlantic salmon (FS) has been brought into question. In Canada, as per veterinary regulation, only tetracycline antibiotics are allowed for use in the AI. This study aims to survey the flora of FS and wild type Atlantic salmon (WT) for antimicrobial resistance (AMR) by testing for AMR encoding genes and endogenous gene levels (EGL). Samples from the mouth, gills, skin, intestine, and intestinal contents were plated on neutral media and media containing 1 µg/mL chlortetracycline. DNA from the gut community and isolated bacteria were analysed using denaturing gradient gel electrophoresis (DGGE), and also tested for 19 known tetracycline efflux pump genes via polymerase chain reaction (PCR). Furthermore, EGLs will be measured using quantitative real time qPCR. To gain insight into the salmon gut bacterial community, we cloned and will analyze the 16S rRNA genes to determine phylogeny. Preliminary results show the presence of Tet-G, M, O, Q, S, W, Z, and OTR genes in FS samples and Tet-O, Q, S, T, W, and 30 genes present in WT community samples; suggesting an emergence of resistance in the AI salmon.

EXPLORING THE GENETIC BASIS OF AUTOIMMUNE DISEASE THROUGH THE DIFFERENTIAL EXPRESSION OF FOXP3 IN ZEBRAFISH (*DANIO RERIO*) FOLLOWING AN INCREASE IN DIETARY SODIUM

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Autoimmunity exhibits a female bias which is consistent across many disorders including multiple sclerosis (MS), systemic lupus erythematosus (SLE), and rheumatoid arthritis. However, much of the interplay between environment, gender and autoimmunity is not understood. FoxP3 is the primary transcription factor controlling the development of regulatory T cells (Tregs) which help control the immune system through suppressive effects. This study aims to evaluate how an increase in dietary sodium affects the expression of *zFoxp3*. Additionally, this study examines if *zFoxP3* is differentially expressed between males and females. Zebrafish (*Danio rerio*) have been well established as a reliable model for genetic studies as they share homologous genes with humans, including those of the immune system. Fifty-two zebrafish were separated into test and control groups with each group having equal numbers of male and female fish. Following an acclimation period, the diet of the test fish was modified to include a ten-times increase in the sodium concentration. One male and one female zebrafish were sampled at time 0 as a control. Three male and three female zebrafish from each group were sampled at 1, 2, 4, and 8 weeks following the introduction of the increased sodium diet. Total RNA was extracted from the gill tissue, followed by the generation of cDNA. The differential expression of *zFoxP3* and the reference gene *β-actin* was evaluated using real time PCR (qPCR). Analysis of the initial qPCR results confirm a single product generation, and suggest that *zFoxP3* is differentially regulated between test and control groups. Further analyses will aid to fill gaps in the relationship between causative factors and the development of autoimmunity.

**RELATIVE CONTRIBUTIONS OF SEXUAL
AND ASEXUAL REPRODUCTION IN
*LYSIMACHIA TERRESTRIS***

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Lysimachia terrestris (Myrsinaceae) is the only species within the genus *Lysimachia*, known to asexually reproduce via formation of bulbils. It is unknown if pollination and subsequent seed capsule production inhibits or reduces bilbil formation in *L. terrestris*. We examined whether there is a tradeoff between clonal and sexual modes of reproduction in this species. To examine this potential trade-off, populations were investigated in Middleton and Wolfville, Nova Scotia. Variation in bilbil production was monitored through application of pollinator exclusion bags prior to anthesis. Among treated plants, zero, two, six, twelve, or all flowers, were left open to determine if there was a threshold of pollinated flowers that dictated bilbil propagule formation. Counts of the number of bulbils, flowers, fruits, seeds and unfertilized ovules were analyzed among treated plants using light and scanning electron microscopy techniques. Results suggest that bilbil formation is limited, but not restricted by seed production. Additionally, investment in sexual reproduction was observed to be larger at the Middleton location, having anecdotal evidence of the presence of a specialist pollinator, *Macropis nuda* (Hymenoptera: Mellitidae). These findings provide new insight into the conditions influencing bilbil yield in *L. terrestris* and the ecological roles of both asexual and sexual colonization.

**AGE AND SIZE SELECTIVITY OF THE GASPHEREAU
RIVER ALEWIFE FISHERY: IMPLICATIONS FOR THE
ASSESSMENT OF THIS STOCK**

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Biological reference points (BRPs) are indices, based on the biological characteristics of a fish stock and the characteristics of its fishery, that are used to gauge whether specific management objectives are being achieved. They provide the link between stock assessment and management objectives. Age and size selectivity are important characteristics of the fishery because the impacts of a specific harvest rate can vary markedly depending whether younger, smaller or older, larger fish are being harvested. A fishery that is selective for certain age classes could have different BRPs for the alewife stock, and could require different management of the fishery. Alewife (*Alosa pseudoharengus*) is a diadromous, herring-like fish species that spawn in the rivers and lakes along the eastern seaboard of North America. Alewife are fished extensively throughout their range as they return to their natal rivers to spawn (ages 3-5). In this study, we examined the selectivity of a commercial alewife fishery on the Gaspereau River in Nova Scotia. In Spring 2016, biological characteristic data, including weight, fork length, sex, and age data were collected from alewife sampled at a commercial fisher's stand, and at a fish ladder 4km upriver. Data were used to reconstruct numbers at age for the total run, the commercial fishery and the spawning escapement after the fishery. A significant difference was found in mean fork length, weight, age, and numbers of repeat spawners between the two sampling locations, indicating that the fishery is highly selective. Selectivity was calculated for age classes three through seven, incorporated into the calculations for the BRPs for this fishery, and compared to existing BRPs based on the assumption the fishery is non-selective. BRPs were found to change slightly, but not enough to change the management for the Gaspereau River alewife stock.

COMPARATIVE GENETIC ANALYSIS OF *Crocantthemum canadense* (L.) Michx. PLANTS OF KNOWN PEDIGREE (CHASMOGAMOUS- OR CLEISTOGAMOUS-DERIVED SEEDS) USING AMPLIFIED FRAGMENT LENGTH POLYMORPHISMS (AFLPS)

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Crocantthemum canadense (L.) Michx., commonly known as Rockrose, is an endangered plant native to northeastern North America. Rockrose has a specialized dual breeding system that produces two types of flowers in different locations on the same plant in the same growing season. Chasmogamous (cross-fertilizing) flowers develop early in the season and are presented as large, open and yellow. Smaller, cleistogamous (self-fertilizing) flowers develop later, don't produce petals, and remain closed. A study by Yorke *et al.* (2011) showed that Nova Scotia populations of Rockrose are genetically distinct from other Rockrose populations in the NE USA and Quebec and, within NS populations, genetic variation was found to be the lowest of all those examined. These results left the question of whether reproductive influences contributed to the reduced genetic variation in Nova Scotia populations. A comparison of genetic diversity in plants raised from seed of known pedigree was performed using amplified fragment length polymorphism (AFLP) markers. Results showed no significant genetic variation between plants derived from cleistogamous- or chasmogamous-derived seeds. Additionally, several new populations of recently discovered Rockrose (along highway 201 and behind the Greenwood Air Force Base Museum) were analyzed. Genetic variability was also determined to be relatively low for these new populations. These conclusions imply that – as Rockrose is an endangered species – potential future breeding projects can use seeds derived from either flower without compromising the genetic integrity of the population.

EXAMINING ARBUSCULAR MYCORRHIZAL FUNGI IN *SPARTINA PATENS* AND *SPARTINA ALTERNIFLORA* IN THE MINAS BASIN, NOVA SCOTIA

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Saltmarshes are one of the most productive ecosystems found worldwide, providing habitat for a diverse group of organisms. Saltmarshes form the basis for many terrestrial and marine food webs, and also play a role in storm buffering and coastal stabilization. Critically they serve as nursery and refuge habitat for many estuarine fishes, crustaceans, and birds. Unfortunately, saltmarshes are in decline worldwide due to anthropogenic stressors, the warming global climate and sea level rise. In this study, we examined arbuscular mycorrhizal fungi (AMF) in the cortical root tissue of two dominant saltmarsh grasses *Spartina patens* and *Spartina alterniflora* (both Poaceae). AMF are known to colonize over 80% of land plants, providing nutrients like water and minerals in exchange for a carbon source. Nine samples were collected at the beginning, middle and end of the growth season for each species from the saltmarsh surrounding Wolfville Harbour, Nova Scotia. AMF root colonization counts were conducted through ink and vinegar staining to visualize the chitinous cell walls of the AMF microscopically, to determine the strength of the mycorrhizal mutualism for both *Spartina* species. DNA was extracted from the AMF and subjected to a nested PCR to amplify the ITS barcoding regions of the rDNA. This was later purified through agarose gel extraction and re-amplified using a single primer set for sequencing. *S. alterniflora* showed an overall colonization of 14.55%, while *S. patens* was much higher at 66.11%. One AMF species was identified (*Funneliformis geosporum*, Glomeraceae) using DNA barcoding. The data collected throughout this study may help with the conservation and restoration of saltmarshes in Nova Scotia. Knowing the strength of the mutualism and understanding the AMF species forming them will help with the production of local AMF inoculants to aid in *Spartina* establishment and increase their fitness in these stressful environments.

THE MITOCHONDRIAL H-ORF OF THE SWAN MUSSEL, *ANODONTA CYGNEA*: ANALYZING THE EXPRESSION OF A UNIQUE GENETIC REGION

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In most animal species mitochondria are inherited maternally, though some species of bivalves are capable of transmitting their mtDNA through a system called “doubly uniparental inheritance” (DUI). Under DUI, daughters still inherit their mother’s mtDNA maternally, but males inherit mtDNA from both parents and segregate it, creating two distinct male and female lineages. The genomes of freshwater mussels have been shown to contain unique gender-associated open reading frames (ORFs) called M-ORFs, F-ORFs, and H-ORFS that are strongly expressed within the gonad tissues of their respective sex. The H-ORF is of particular interest because previous work has shown that it is typically a highly divergent version of the F-ORF, and it is hypothesized that sequence divergence may have caused a change in gene function through differential expression. The H-ORF of the hermaphroditic species, the Swan Mussel, *Anodonta cygnea*, was sequenced and characterized; expression was examined using reverse-transcriptase PCR (RT-PCR) and various combinations of newly designed H-ORF primers. Early RT-PCR reactions showed promise for the H-ORF being present in the transcriptome, but the results have been non-duplicable and at this time inconclusive. If the H-ORF is present in the transcriptome, its expression levels should be compared to other mitochondrial genes to determine the likelihood that it is a functional protein. If it is functional, it is possible that it plays a role in the sex determination system of hermaphroditic mussels.

POPULATION DEMOGRAPHICS OF TWO CRYPTIC SKATE SPECIES, WINTER SKATE, *LEUCORAJA OCELLATA*, AND LITTLE SKATE, *LEUCORAJA ERINACEA*, IN THE BAY OF FUNDY AND THEIR SOCIAL IMAGE IN LOCAL FISHERIES

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Winter Skate, *Leucoraja ocellata*, and Little Skate, *L. erinacea*, are cryptic species found throughout the western Atlantic ocean. Due to historical exploitation and current bycatch levels from groundfish fisheries, Little Skate were assessed as ‘Near Threatened’ and Winter Skate as ‘Endangered’ by the IUCN. Both species exhibit life history characteristics of *Elasmobranchii* and these K-selected characteristics make populations highly susceptible to exploitation. Skates were sampled opportunistically at an intertidal herring weir located in Bramber, NS, and through targeted bottom trawls in both Scots Bay and the Minas Basin. Morphometric data were collected, and skates were tagged with uniquely coded dart tags. Population demographics were described for each of the sampled locations and population estimates were calculated using data collected between 2012 – 2016. Lastly, social image interviews were conducted to poll attitudes toward skates among recreational and commercial fishers, and to gauge knowledge of skate conservation needs and efforts. A total of 1237 skates were caught between May – Aug 2016, with tags applied to 704. One skate traversed the Minas Passage from the Minas Basin to Scots Bay, which provides the first confirmation of skate movement through the Passage. A total of 44 recreational angler interviews and 4 commercial fisher interviews were completed. Post-interview, 63% of participants reported that after gaining new information on status and ecological importance of skates, their views toward skates had changed. Due to continued fishing pressures and evident lack of knowledge of these species in local fishing communities, long-term monitoring and community involvement are especially important in ongoing conservation efforts.