# **ABSTRACTS FOR**

# **ORAL PRESENTATIONS**

(in order of presentation)

#### QUANTIFYING METHANE EBULLITIVE FLUX FROM SUBARCTIC ECOSYSTEMS

Jacqueline Amante and Ruth Varner Complex Systems Research Center & Department of Earth Sciences, UNH

Methane is a greenhouse gas demonstrated to be 23 times more potent than carbon dioxide and can have, even in small quantities, a large impact on the world's climate. Ebullition, the bubbling of methane after it has accumulated in the oxygen-poor anaerobic zone in wetland and aquatic sediments, results in the direct release of methane to the atmosphere. The spatial and temporal variability of ebullitive loss of methane to the atmosphere is largely unknown yet likely to be impacted by the warming climate and subsequent thawing in subarctic ecosystems. Therefore, a study was conducted to measure both the spatial and temporal pattern in ebullition events from a subarctic peatland and lake ecosystem in northern Sweden. Measurements were taken manually and compared to acoustic data gathered by the newly designed acoustic bubble sensors which identified both bubble size and methane concentration. Ebullition data were then compared to environmental variables (e.g. wind speed, peat and water temperature and atmospheric pressure) to identify controls on release of bubbles. The acoustic bubble sensors proved to be an efficient and accurate method for measuring the spatial and temporal variability of ebullition events.

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Adviser:

Ruth Varner

#### CLONING cDNA OF GONADOTROPIN-RELEASING HORMONE (GNRH) ISOFORMS FROM BRAINS OF BLACK SEA BASS, *CENTROPRISTIS STRIATA*

Scott Morin<sup>1</sup>, David Berlinsky<sup>2</sup>, Wayne Decatur<sup>1</sup>, and Stacia Sower<sup>1</sup> <sup>1</sup> Center for Comparative and Molecular Endocrinology, UNH <sup>2</sup> Department of Biological Sciences, UNH

Gonadotropin-releasing hormone (GnRH) plays the major role in the reproductive control of all vertebrates. The objective of this research is to determine the structure of the GnRH isoforms in a species of teleost (bony) fish, the black sea bass (Centropristis striata). Black sea bass are protogynous hermaphrodites; they are born as females and turn into males, which poses a problem for the aquaculturist. New data on the underlying neuroendocrine mechanisms of reproduction will provide key information to manage this important commercial fish species. To accomplish this objective, brains from black sea bass were obtained, extracted for RNA and followed by cDNA synthesis, 3' RACE and 5' RACE. The 3' untranslated region (3'UTR) was identified for GnRH2 and 3 of the black sea bass. The GnRH2 cDNA coding sequence contained 258 nucleotides, while the 3'UTR and poly-A tail contained 210 nucleotides, encoding the type II GnRH (QHWSHGWYPG). The GnRH3 cDNA coding sequence contained 273 nucleotides, while the 3'UTR and poly-A tail contained 183 nucleotides, encoding the type III GnRH (QHWSYGWLPG). In summary, we have identified two of the possible three GnRH isoforms in the brains of black sea bass. The identification of these GnRHs now allows us to perform functional studies of black sea bass during sex change and other reproductive stages.

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#### CELL STAINING TECHNIQUES FOR QUANTIFICATION OF ACTIVELY REPLICATING S-PHASE CELLS IN ARABIDOPSIS THALIANA

William Wyman and Kevin Culligan Department of Molecular, Cellular, & Biomedical Sciences, UNH

The quantification of actively replicating cells is an important descriptor in the study of gene mutations related to DNA damage repair pathways. Research conducted in the lab of Dr. Kevin Culligan involves identifying how these genes are related to cellular repair and replication in the model organism *Arabidopsis thaliana* when subjected to DNA damaging agents. Several fluorescent staining techniques were tested and optimized to identify a functional protocol for visualizing and analyzing root tip constitution of actively replicating S-phase cells. EdU staining was found to be effective for labeling actively replicating S-phase cells because it is a molecular analog to the DNA base thymidine. As DNA synthesis occurs, EdU is incorporated into the DNA of only cells that are replicating. These cells can then be detected by fluorescence. DAPI, To-Pro-3, and DRAQ-5 were tested as counterstains to contrast replicating and non-replicating cells.

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### AN INVESTIGATION OF *ZOSTERA MARINA* (EELGRASS) RESILIENCE THROUGH GENETIC DIVERSITY

Alyssa Mixon<sup>1</sup>, Jim Coyer<sup>2</sup>, Jeanine Olsen<sup>2</sup>, Sarah Weigel<sup>1</sup>, and Anita Klein<sup>1</sup> <sup>1</sup> Department of Biological Sciences, UNH

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A species' genetic diversity provides a buffer to counter the effects of environmental change, both abiotic and biotic. In clonal organisms such as eelgrass, diversity consists of two aspects: allelic diversity and genotypic diversity. Knowledge of both and the connectivity between populations are necessary for effective management policy of a declining species. Microsatellite analysis was used to measure genetic and allelic diversity of eelgrass populations along the New England Coast and compare the results with populations from other regions. The connectivity between these populations was determined in order to identify potential source populations for restoration projects. This study distinguished populations at risk because of low diversity. Research was carried out in part under Dr. Olsen and Dr. Cover at Rijksuniversiteit (Netherlands). New England populations (n=5) displayed high levels of allelic richness (6.3-7.3 alleles/locus), with high genetic diversity for four of them (0.84 to 0.97). Eight microsatellite loci strongly resolved (bootstrap = 90) these populations as a single cluster, distinct from the NE and NW Pacific populations. Connectivity (gene flow) within the Northern and Southern regions of New England seems to be limited. If eelgrass management policies in the New England area require restoration programs, the Quonochontaug Pond is an excellent source for transplants. These results are being integrated into an ongoing study under Dr. Klein.

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# EFFECTS OF KELP MEAL ON PERFORMANCE AND STRUCTURAL GROWTH OF CONVENTIONAL AND ORGANIC DAIRY CALVES

Gina Soule and Andre Brito Department of Biological Sciences, UNH

Kelp meal (KM) is a dried seaweed (Ascophyllum nodosum) product commonly used in dairy farms. This study examined the effects of KM on structural growth of 36 calves [18 conventional Holsteins (Trial 1) and 18 organic Jerseys (Trial 2)]. We hypothesized that the high mineral content and salty flavor of KM enhances diet palatability and intake in calves. Within breed, animals were blocked by calving date and randomly assigned to 2 treatments: Control (calf starter) or KM (calf starter + 25 g of KM). Approximately 5.7 and 3.8 L of milk were fed daily to Holstein and Jerseys, respectively. Grass hay was offered ad libitum and orts were collected daily. Body weight and growth were measured twice weekly. Results from Trial 1 showed no significant differences on intake and growth of calves. No differences in structural growth were observed for calves fed either diet in Trial 2 despite higher (P < 0.05) average daily weight gain and intake, suggesting that dietary energy was used for fat deposition. A 2-fold difference in ash concentration between the conventional (9.04%) and the organic starter (5.68%) suggests the latter was lacking minerals. Because KM is rich in minerals and adds a salty flavor, increased intake in Jerseys may be explained by enhanced diet palatability and/or an animal need to meet mineral requirements. Research is needed to investigate the interactions between KM and starter nutrient profile on body composition and structural growth of dairy calves.

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#### GENE FAMILY SIZE AND EXPRESSION LEVELS IN THE GENUS VIBRIO

Benjamin Decato<sup>1</sup>, Vaughn Cooper<sup>1</sup>, and Philip Hatcher<sup>2</sup> <sup>1</sup> Department of Molecular, Cellular, & Biomedical Sciences, UNH <sup>2</sup> Department of Computer Science, UNH

Understanding the relationship between gene expression and conservation across lineages is a topic of broad interest. This interest is compounded when families are comprised of both pathogenic and non-pathogenic lineages, and uncovering the basis for the differences between these lineages could lead to substantial advances in medicine. We derived homolog families from 15 fully sequenced *Vibrio* genomes and used expression data from two *Vibrio* lineages to compare expression levels with family size. We found that in both the non-pathogenic *Vibrio fischeri* and the pathogenic *Vibrio parahaemolyticus*, family size was a good predictor of gene expression, and that outliers in this trend tended to be clade-specific. This relationship was significantly more pronounced in the first chromosome than the second, suggesting that many genes responsible for variation and novelty could reside in the second chromosome. In addition, we determined that in homolog families containing duplicate genes, less duplicates led to higher gene expression.

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### QUANTITATING CHANGES TO GLOBAL HISTONE MODIFICATION PATTERNS FOLLOWING CAMPTOTHECIN TREATMENT

Ryan Finethy and Feixia Chu Department of Molecular, Cellular, & Biomedical Sciences, UNH

Through covalent modification of histone proteins at specific amino acid residues, cells are able to specifically regulate chromatin structure and function. Changes in modification patterns have been associated with cellular processes including cell cycle arrest and DNA damage response. Topoisomerase I enables DNA replication by relieving supercoiling ahead of replication forks and is a common target during anti-cancer chemotherapy. Topoisomerase I inhibitors bind both the DNA and topoisomerase and create a stable ternary complex that prevents DNA ligation and results in DNA single and double strand breaks. Little is known about the effect of treatment with topoisomerase I inhibitors on global chromatin structure. Using a combination of stable isotope labeling of amino acids in culture and liquid chromatography tandem mass spectrometry, I characterized changes to global modification patterns in response to treatment with topoisomerase I inhibitor camptothecin (CPT). HEK293 cells were treated with 300 nM CPT for 0/1/24 hrs and changes in modifications were quantitated. For all treatments an increase in histone H.2AX S139 phosphorylation was observed. This modification is characteristic of DNA double strand breaks. The 24 hr time point showed statistically significant changes (>30%) in many modifications. Many of the measured changes, e.g., a general decrease in acetylation on H3 and H4, typically correlate with decreased gene expression.

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### HIGH OR LOW ANTIBODY RESPONDER CHICKENS HAVE DIFFERENTIAL EMBRYONIC BURSAL GENE EXPRESSION AFTER TESTOSTERONE EXPOSURE

Tyler Burks and Robert Taylor Department of Biological Sciences, UNH

Chicken antibody responses depend on cells that develop in the bursa of Fabricius (BF). Embryonic exposure to hormones such as testosterone propionate (TP) affects the BF microenvironment. High (HAS) or low (LAS) antibody White Leghorn lines differ significantly in their antibody response against sheep red blood cells (SRBC). To assess hormonal impact on the BF, eggs from both lines were treated with a 2% TP ethanol solution or ethanol alone on incubation day 3. BF samples were collected from four embryos of each line and treatment at 15, 18, and 21 d of incubation. RNA was extracted from individual samples followed by reverse transcription to cDNA. Fluorescent labeled cDNA samples were hybridized to a microarray containing oligonucleotides from 320 immune-related genes. Growth hormone (GH), growth hormone receptor (GHR), and thyroid hormone receptor alpha gene expression were elevated in Line LAS controls compared with Line HAS controls. TP treatment also raised GH, GHR, and interleukin-1 (IL-1) gene expression in Line LAS vs Line HAS. In contrast, Line HAS TP embryos had higher fibroblast growth factor 1 and endothelin receptor expression than was found in LAS TP embryos. Markers associated with immune response pathways including IL-1 and NK2g were identified. Developmental growth hormone signaling was recognized as well. Differential antibody response, as well as the response to TP are influenced by the detected genes and their associated pathways.

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# ABSTRACTS FOR POSTER PRESENTATIONS

### DETERMINING THE PRESENCE OF CYTOPLASMIC SEQUESTRATION OF p53 IN SKBR3 BREAST CANCER CELLS

Sarah Yunes and Charles Walker Department of Molecular, Cellular, & Biomedical Sciences, UNH

In some human breast cancer cell lines, cytoplasmic sequestration of wild type p53 (wtp53) abrogates or eliminates its nuclear transcriptional function and blocks apoptosis. The molecular mechanism causing this phenotype is unknown, but two have been suggested. One involves a defective protein normally promoting nuclear import of wtp53. Another mechanism is cytoplasmic sequestrating (or tethering) of wtp53 by the mitochondrial heat shock (Hsp70) protein, mortalin. In this latter mechanism, mortalin is over-expressed and binds up wtp53 that cannot enter the nucleus and upregulate apoptosis related genes. In my research, I examine the idea that mortalin binding leads to cytoplasmic sequestration of wtp53 in the commercially available breast cancer cell line (SKBR3). Cells from this cell line were treated with MKT-077, a dye that competes with wtp53 for the p53 binding site in mortalin protein. Following this treatment, the nuclear translocation of wtp53 and subsequent apoptosis occurred after 24 hours following treatment with MKT-077. (Supported by a UROP grant to SY)

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#### EPIGENETIC REGULATION OF RETT SYNDROME IN A MOUSE MODEL

Taylor Hickman and Feixia Chu Department of Molecular, Cellular, & Biomedical Sciences, UNH

Rett Syndrome is a neurodevelopmental disorder that is thought to be caused by epigenetic dysregulation of the Methyl-CpG binding protein 2 gene (MeCP2). Epigenetic change involves heritable changes in phenotype that occur to an organism without a change to the genomic DNA sequence. One means of epigenetic change is through post-translational histone modifications that alter the structure of chromatin and consequently alter gene expression. Under normal function, MeCP2 binds methylated DNA and recruits protein complexes that repress transcription. Recent studies have suggested that loss of function MeCP2 results in transcriptional de-repression, resulting in a more open formation of chromatin. To investigate the state of chromatin, metabolic labeling and mass spectrometric analysis were utilized to identify and quantify post-translational histone modifications in MeCP2 wild type and knock out mouse brains. Analysis of two week and six week mouse brains was employed to determine if the structure of chromatin resulting from the post-translational histone modifications was altered by the onset of the Rett phenotype in the six week animals.

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### INFLUENCE OF CYTOSKELETAL STRESS FILAMENTS ON CELL DEATH IN HUMAN CERVICAL CARCINOMA

Benjamin Duckless and David Townson Department of Molecular, Cellular, & Biomedical Sciences, UNH

Epithelial tumors including cervical cancer are a leading cause of cancer death in women. The tumors are often composed of mixed cell types, which make them challenging to treat therapeutically. In this study cytoskeletal stress filaments of tumors known as cytokeratin 8/18 (KRT8/18) filaments were investigated. These filaments are considered diagnostic of cervical cancer, and their expression is postulated to protect cells from insults that otherwise would induce cell death. We hypothesized that cervical cancer cells (HeLa cells) expressing KRT 8/18 filaments (KRT+ cells) are more resistant to induced cell death than cells lacking these filaments (KRT- cells). Both types of HeLa cells were exposed to anti-Fas antibody (CH11; lug/mL) to induce cell death and/or an inhibitor of the mitogen activated protein kinase (MAPK) pathway (PD98059; 30µM) to evaluate the role of MAPK signaling. The KRTcells exhibited greater sensitivity to Fas-induced death than KRT+ cells (P<0.05; n=3 expts) as measured by a CellTiter 96<sup>®</sup> AQueous One-Step Cell Proliferation assay. Interestingly, treatment of the HeLa cells with MAPK inhibitor further augmented Fas-induced cell death in KRT+ cells (P<0.05), but had no effect in KRT- cells (P>0.05). The results supported our hypothesis, and suggest cytokeratin 8/18 filaments provide resistance to Fas-induced death in HeLa cells through mechanisms involving the activation of MAPK signaling. Supported by USDA, NRI #2007-35203-18074.

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#### THE EFFECTS OF PDBES ON LIPID ACCUMULATION IN LIVER TISSUE

Whit Durant and Gale Carey Department of Molecular, Cellular, & Biomedical Sciences, UNH

Environmental chemicals are a growing concern in public health. Chemicals like bisphenol A disrupt estrogen balance and phenobarbital inhibit phosphoenolpyruvate carboxykinase (PEPCK), a liver enzyme involved in redirection of carbon away from lipid synthesis. Our lab has shown that polybrominated diphenyl ethers (PBDEs), commonly used as flame retardants in electronic appliances, rugs, and furniture, inhibit liver PEPCK activity. The consequence of inhibited PEPCK on liver lipid accumulation is unknown. This research project tests the hypothesis that PBDEs promote the accumulation of lipids in liver. An increase in liver lipids can lead to non-alcoholic fatty liver disease and if untreated can progress to cirrhosis. To test this hypothesis, 8 rats were treated with 14mg PBDEs/kg body weight in corn oil, while 8 rats were treated with corn oil alone. After 28 days of treatment, rats were euthanized and livers were removed, weighed and frozen at -80°C. The percentage of total liver lipids will be determined by homogenizing in a phosphate buffer saline solution, extracting the homogenate with hexane: isopropanol (3:2), treating the extract with 0.5M sodium sulfate, and centrifuging to isolate the lipids in hexane. An aliquot of the hexane solution containing lipids will then be dried with nitrogen gas and the dried lipids will be weighed. We expect our results to support the hypothesis that PBDEs promote the accumulation of lipids in the liver.

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### A PILOT STUDY OF GENETIC RESILIENCE AND DIVERSITY AMONG EELGRASS (*ZOSTERA MARINA*) POPULATIONS IN THE NEW ENGLAND COASTAL REGION

Michael Cassella, Kati Kluck, and Anita Klein Department of Biological Sciences, UNH

Eelgrass (*Zostera marina*) is an estuarine and coastal plant species that serves as an important nutrient source and habitat to a variety of invertebrate marine fauna. As a result of human and environmental stressors, the species has experienced a steady decline in recent history. Theoretically, genetically diverse populations of this species have more resilience towards stressors and are more likely to support successful population restoration. As part of an ongoing project involving the Klein laboratory and its collaborators, this study investigates and compares the genetic diversity and resilience among *Zostera marina* populations in the New Hampshire Great Bay and Cape Cod coastal regions. Genetic variation within and between these populations will be measured using microsatellite genotyping and population genetic analyses to quantitatively determine both genetic diversity and allelic diversity. Both characteristics are associated with the genetic resilience required for effective restoration management of this endangered component of coastal ecosystems.

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### A SCREEN TO IDENTIFY MUTANTS WITH A SODIUM-INDUCED ROOT SKEWING PHENOTYPE

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The genome of the plant Arabidopsis thaliana encodes about 300 protein phosphatases and the functions of most of them are unknown. Phosphatases, along with protein kinases, play a major role in regulating many cellular processes by reversible phosphorylation. The Hrabak lab has identified plants with mutations in several Protein Phosphatase 2A (PP2A) genes. Two of these mutants have a root skewing or curling phenotype when grown on NaClsupplemented medium, indicating a role for PP2A in regulating root growth. The goal of this research is to identify additional mutants with similar root skewing or curling phenotypes to learn more about the pathway that causes this phenotype. To date, 170,000 random mutant seedlings have been screened. The primary screen was done by germinating seeds on medium containing 50 mM NaCl. About 2,500 putative mutant seedlings were identified that displayed root skewing; these were transplanted to soil, grown to maturity and their seeds were harvested. A secondary screen to verify that the root skewing is reproducible is now underway. Plants that pass the secondary screen will enter the tertiary screen to eliminate plants whose roots skew in the absence of NaCl or in response to ions other than sodium. Once additional sodium-induced root skewing mutants are confirmed, the identity of the mutated gene will be found using TAIL-PCR followed by DNA sequencing.

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Author:	Megan Thompson
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#### ASSAYING ANTHOCYANIN CAPACITY IN STRAWBERRY FRUIT

Jenny Jing, Lise Mahoney, and Thomas Davis Department of Biological Sciences, UNH

The cultivated strawberry fruit is an excellent source of antioxidants- micronutrients essential for human health. Cultivated strawberry is a hybrid of two very different wild species and antioxidant levels vary considerably among strawberry genotypes. The second-generation offspring of representative progenitor species of *F. chiloensis* ssp. lucida (FRA 1691) x BC Pink and *F. virginiana* ssp. glauca (BC6) x BC pink will be evaluated for the genetic controls of antioxidant capacity and levels of antioxidant compounds. Specifically, my aim is to alter the pelargonidin:cyanidin ratio to increase antioxidant properties while retaining other aspects of fruit quality, including flavor. Current research is funded in part by the 2009 USDA NIFA Specialty Crops Research Initiative RosBREED to deliver improved plant varieties more efficiently and rapidly in the Rosaceae family. By identifying the genes that control the healthful properties of fruits such as strawberries, to more effectively develop varieties with improved fruit qualities.

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Advisers:

Lise Mahoney and Thomas Davis

#### ASSESSMENT OF BACTERIAL ENDOSYMBIONT COMMUNITY POPULATIONS IN CAULERPA MEXICANA USING QUANTITATIVE PCR

Diego DelAlamo, Louis Tisa, and Wayne Fagerberg Department of Molecular, Cellular, & Biomedical Sciences, UNH

The subtropical coenocytic green alga *Caulerpa mexicana* hosts a complex community of endosymbiotic bacteria. Although the presence of these bacteria has been noted in the literature, the diversity and extent of the population remains to be determined. This study proposes to categorize a large segment of the bacterial community in the algal cytoplasm using qPCR. Determining the relative frequency and incidence of bacterial phyla will be a first step for future work in identifying the species and their possible roles within the cell.

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### AUXIN AND CYTOKININ CROSS-TALK AT THE LEVEL OF GENE EXPRESSION IN ARABIDOPSIS THALIANA

Steven Bench and Dennis Mathews Department of Molecular, Cellular, & Biomedical Sciences, UNH

Auxin and cytokinin are plant hormones that play a significant role in organ formation and cell division in plants. It is known that exposing a plant tissue cell culture to auxin will promote root growth whereas cytokinin will promote shoot growth. Less is known, however, about how these hormones interact with each other at the molecular and cellular levels. In order to discover more about this relationship, we have studied plants that are compromised in their cytokinin signaling ability because of specific mutations in certain transcription factor genes known to be regulated by cytokinin. We have also used reporter constructs (GUS and GFP) to visualize the expression of genes known to be activated by auxin. With these research tools we have been able to study the effect of cytokinin signaling on the sensitivity of plants to auxin. With this project, we intend to study the "cross-talk," or interaction, between auxin and cytokinin as well as other growth hormones that may be involved.

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### CARBOHYDRATE CANCER BIOMARKERS: A NEW TECHNIQUE FOR STRUCTURAL VERIFICATION

Phillip Munson, Matthew Reinhold, and Vernon Reinhold Department of Molecular, Cellular, & Biomedical Sciences, UNH

Glycosylation, the post-translational addition of sugar chains (glycans) to proteins, is necessary for protein function and stability. The occurrence of different glycan structures, or moleties, is indicative of many diseases. Consequently, aberrant glycosylation is a universal indicator of cancer progression. The identification and structural elucidation of altered aspects of glycans is therefore important in cancer research. Determining how these tumorspecific glycans are chemically constructed is significant to diagnostic and prognostic disease control. Some commonly used analytical methods for structural characterization of glycans include chromatography, nuclear magnetic resonance (NMR), mass spectrometry (MS), and glycan-specific antibody production. We investigated a modification of existing mass spectrometry and chromatography approaches by providing in depth fragmentation of methylderivatized sugar chains, incorporating both chromatography (LC/MS, MS) and MS<sup>n</sup>, to provide a simple and definitive technique for glycan moiety confirmation. This avenue of analysis delves deeper into the proposed sugar structure in an attempt to provide more accurate and conclusive data about the sugar chains' true chemical framework. Using Sialyl Lewis X, a known cancer biomarker, we find that this approach is simple and effective, more sensitive than NMR, and more definitive than prior mass spectrometry based methods.

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Career Interest:	Graduate School
Author:	Matthew Reinhold
Author's Major(s):	Physics
Year at UNH:	Recent Graduate
Career Interest:	Graduate School
Adviser:	Vernon Reinhold

### CYTOPLASMIC SEQUESTRATION OF p53 IN THE CELL LINE KG-1 AND A CLINICAL POPULATION

Hannah Eldred and Charles Walker Department of Molecular, Cellular, & Biomedical Sciences, UNH

Studies in Dr. Walker's laboratory have demonstrated that cytoplasmic sequestration of p53 in both cancerous clam hemocytes (CCH) and the human neuroblastoma cell line IMR-32. Experiments have shown the susceptibility of CCH to both genotoxic and non-genotoxic cancer therapies. When exposed to etoposide or MKT-077, p53 transcription is reactivated within the CCH. These methods have yet to be linked to acute myelogenous leukemia (AML) cells. Furthermore, this model has yet to be compared to any type of clinical population. The CCH model is an out-breeding population and is therefore comparable to patient derived AML cells we will employ in this study. The IMR-32 model suggests that cytoplasmic sequestration will be true of both AML cells and patient derived AML cells. In my study, I will repeat the experiments conducted by Dr. Walker using both AML cells and patient derived AML. This will be done to determine if cytoplasmic sequestration of p53 occurs in these two types of cells. Both cell types will be treated with genotoxic and non-genotoxic cancer drugs. The results gathered will be compared with both CCH and IMR-32 models to validate the use of these models.

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Adviser:

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### DEVELOPING THE TECHNIQUE FOR GENETIC ENGINEERING OF THE RED ALGA PORPHYRA

Caroline Wuest, Swathi Turlapati and Subhash Minocha Department of Biological Sciences, UNH

The red alga Porphyra (Nori) is one of the most commercially valuable seaweeds. In contrast to crop plants, most of which are genetically engineered and improved, a stable genetic engineering system has not been developed for *Porphyra* or other red algae, due to the lack of a reliable promoter, a component of the gene that regulates its expression. Genetic engineering of Porphyra would allow qualities of the red alga to be altered that will improve nutritional or phytoremediation properties and enhance polyculture with finfish, shellfish, and crustaceans. The specific objectives of my research are to clone promoters from one or more genes in Porphyra and test their activity in Porphyra cells using a reporter gene. This would be achieved through identifying and cloning a functional gene and using that information to clone its promoter, which will then be fused with a reporter gene (e.g.,  $\beta$ -glucuronidase or GUS) to test its functionality. I have amplified and cloned one of the several polyamine biosynthetic genes from P. umbilicalis into a TOPO vector and transformed Escherichia coli cells with the cloned gene. After confirmation of the gene sequence, I will perform a BLAST of the gene against the Porphyra genome in order to identify the promoter sequence. Primers will be designed to amplify the promoter sequence which will then be fused with a reporter gene. The promoter::reporter gene fusion will be tested for its expression in *Porphyra* protoplasts by electroporation.

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#### EXPRESSION OF FUNGAL LIPASES IN HIGHER PLANTS FOR BIODIESEL PRODUCTION

Jonathan Fogel<sup>1</sup>, Michael Gagnon<sup>2</sup>, Subhash Minocha<sup>1</sup>, and P.T. Vasudevan<sup>2</sup> <sup>1</sup> Department of Biological Sciences, UNH <sup>2</sup> Depatment of Chemical Engineering, UNH

In recent years, biodiesel has become a top contender as an alternative fuel. The use of enzymatic catalysts will drastically reduce the cost of biodiesel production by using lower quality non-edible feedstock as well as used edible oils, and environmentally safer product formation with fewer byproducts. We are investigating the feasibility of producing fungal lipases in large quantities in genetically engineered plants for use as catalysts in the continuous production of biodiesel from non-edible vegetable oils. Cloning and over-expressing the lipase genes in higher plants will decrease the cost of purification and immobilization of the enzyme in preparation for biofuel production. Currently, we have cloned the lipase gene sequences (TL1 and TL2) from complementary DNA (cDNA) of *Thermomyces lanuginosus*, a thermophyllic fungus, which have been shown to have high transesterification activity. The genes were amplified by PCR and inserted into plant vectors for eventual transfer into tobacco cells, from which genetically engineered plants will be produced. The plants will be tested for the production of these enzymes, and the recombinant enzyme will be purified, immobilized and tested for activity in an interdisciplinary study with Michael D. Gagnon, a Chemical Engineering Ph.D candidate (Dr. P.T. Vasudevan - adviser).

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### GENETIC ENGINEERING OF *DUNALIELLA* SPECIES TO IMPROVE USE IN BIOFUEL PRODUCTION

Alison Wirshing, Leland Jahnke, and Subhash Minocha Department of Biological Sciences, UNH

Biodiesel produced from algal lipids is a promising source of renewable energy. Reasons for using algae, as opposed to vascular plants, are numerous. Oleaginous algae do not require fertile land or fresh water to grow, and therefore do not compete with food crop resources. Algae have short generation times and higher growth rates than larger vascular plants allowing for synthesis and accumulation of large quantities of neutral lipids, about 20-50% of the dry cell weight, in a short period of time. The efficiency of biodiesel production from oleaginous algae could be improved by using transgenic (genetically engineered) algae. The goal of this research is to develop techniques for transforming algae species from the genus Dunaliella to increase its carbon sequestration capability and lipid biosynthesis. It has been learned in our lab that increased biosynthesis of polyamines can positively influence carbon sequestration; other labs have shown that enzymes involved in triacylolycerol (TAG) synthesis can enhance lipid production. To genetically engineer Dunaliella, in addition to genes, promoters are also needed to regulate the expression of these genes. Currently we are working on cloning the promoters for two highly expressed genes, i.e. actin and ribulose-1,5bisphosphate carboxylase oxygenase (RuBisCO). These promoters will be recombined with the polyamine biosynthetic genes already cloned and introduced into the algal cell for a high degree of expression.

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#### IDENTIFICATION OF NOVEL PROTEINS IN TRANSLATIONAL COMPLEXES IN SACCHAROMYCES CEREVISIAE VIA ANALYTICAL ULTRACENTRIFUGATION WITH THE FLUORESCENCE DETECTION SYSTEM

Mitchell Granoff and Clyde Denis Department of Molecular, Cellular, & Biomedical Sciences, UNH

All proteins made by eukaryotic organisms are synthesized in a process called protein translation. This process is regulated through protein interaction through the formation and degradation of complexes to perform necessary molecular tasks. However, the details as to how this complex forms and functions remain unknown. To learn more about which proteins make up certain complexes in eukaryotic organisms, we used Saccharomyces cerevisiae, or baker's yeast, as the subject of our experiments. We employed Analytical Ultracentrifugation with the Fluorescence Detection System (AU-FDS) to decipher this process by identifying complexes and the proteins they contain. By isolating protein complexes from crude extracts via genetically engineered Flag-tags, we then visualized proteins of interest via a genetically engineered Green Fluorescent Protein (GFP) tags. We ran multiple strains including eRF3, RPL6B, eIF2 $\alpha$ , and 4G1. RPL6B, eIF2 $\alpha$ , and 4G1 showed very low fluorescence yields in early trials. eRF3 showed substantial fluorescence at differing peaks depending on the presence of glucose. eRF3 associates with eRF1, but both associate within different complexes depending on the presence of glucose. RPL6B, eIF2 $\alpha$ , and 4G1 likely do not associate with eRF1 significantly. Subsequent runs will yield more insights into complex composition and interaction.

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#### IRON COMPETITION IN BIOFILM-EVOLVED BURKHOLDERIA CENOCEPACIA

Megan McLaughlin, Charles Traverse, and Vaughn Cooper Department of Molecular, Cellular, & Biomedical Sciences, UNH

A major challenge for bacteria colonizing host tissue is that hosts typically sequester iron as a resource, leaving little remaining for bacterial growth. During experimental evolution of *Burkholderia cenocepacia* under biofilm conditions, genomic sequencing revealed mutations in the promoter of an iron storage gene, bacterioferrin (*bfr*). In other bacteria, a protein known as ferric uptake regulator (*fur*) governs *bfr*, but this mode of regulation is unlikely in the *B. cenocepacia* strain under study. Thus how*bfr* regulates iron stores in *B. cenocepacia* remains unknown, although prior studies have shown that it lowers oxidative stress. This study examines how *bfr* expression varies in the presence of iron. To clarify the primary function of the *bfr* gene and to examine effects of different mutations in this gene, three different genotypes from the biofilm evolution experiment were grown in media with high and low concentrations of iron. The RNA was then extracted, reverse transcribed and quantitative realtime PCR was performed on the resulting cDNA. Preliminary results indicate that evolved mutations upregulated *bfr* expression in the low-iron selective environment, likely to improve iron scavenging. Ongoing work will reveal whether this expression is affected by increased iron concentrations and illuminate the precise function of *bfr* in *B. cenocepacia*.

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# IS THERE A LINK BETWEEN HETEROZYGOSITY AND MOBILITY, SIZE, OR SEX IN THE BLUE MUSSEL, *MYTILUS EDULIS?* – AN EPIC-PCR APPROACH

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Levels of heterozygosity of wild and cultured blue mussels *(Mytilus edulis)* were determined and correlated with mussel mobility, size, mussel sex, and collection location. Mussels of three size classes (early emergent = 28-30 mm; grow-out 1 = 31-35 mm; grow-out 2 = 36-45 mm) were collected from piers at the Wentworth by the Sea Marina (WSP) and the UNH Coastal Marine Laboratory (CLP). Mussels were buried under 5 cm of gravel and their emergence times were recorded. All mussels were measured, sexed, and genomic DNA was extracted. Heterozygous individuals were identified via EPIC-PCR of an intron length polymorphism of the calmodulin gene *(CaM-1)* intron 3 locus. No correlation was found between heterozygosity and either mussel mobility (p = 0.070) or sex (p > 0.05). However, a highly skewed sex ratio (4:1) for females was found. CLP mussels revealed a heterozygote deficiency (p = 0.003) possibly due to human disturbances at that site. Additionally, heterozygosities of 78 ropecultured mussels from Clarks Cove (CC), Damariscotta River, Maine were also determined. At all three sites, heterozygosity was negatively correlated with size when comparing the early emergent group and the grow-out 2 group (p=0.03) indicating a differential loss of heterozygotes over time.

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#### MEASURING TREHALOSE IN PORPHYRA USING ENZYMATIC ASSAYS

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*Porphyra* is an unique intertidal red algae. This commercially important seaweed has evolved high stress tolerance for multiple stressors. In some species, i.e. insects, trehalose has been shown to provide protection from stresses, while in others trehalose has been shown to be a signal molecule. Previous chemotaxonomic studies suggest that *Porphyra* does not contain trehalose. However, recent transcriptome sequencing of *Porphyra* has identified putative genes of trehalose metabolism. The purpose of this study is to re-examine whether *Porphyra* accumulates trehalose, perhaps at low concentrations, as a signal molecule in response to stress. This trehalose-specific enzymatic assay was used to determine if trehalose is present at low micromolar concentrations in *Porphyra* or accumulates during periods of stress.

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#### MOTILITY MUTANTS IN PHOTORHABDUS TEMPERATA

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Photorhabdus temperata is a bacterium that uses natural products to form mutualistic associations with the entomopathogenic nematode, Heterorhabditis bacteriophora. When the symbiotic relation has been made, the bacteria can be found within the nematode intestine. The nematode infects an insect and releases *P. temperata* into the insect hemocoel. The bacteria multiply and secrete different enzymes and toxins to kill the insect infected, allowing the nematodes to feed and reproduce in the insect cadaver. P. temperata produces antimicrobial agents, or natural products, that prevent other bacteria, nematodes, or fungi from entering the insect body. Once new nematodes reemerge, P. temperata reforms associations with the nematode, infecting more insects. Natural products are chemical substances that are produced by an organism. They are secondary metabolites that seem to have no defined role within the internal economy of the organism that synthesizes it. Natural products may have signaling effects supporting the bacteria-nematode association. This symbiosis acts as a biocontrol against insect pests in agriculture, supporting agricultural sustainability. Identification of genes involved in synthesis of natural products can allow for better understanding of the role they play in the environment, especially in the *Photorhabdus*-Heterorhabditis system. The purpose of this project is to identify putative genes involved in the production of antimicrobials in the bacterium *P. temperata*.

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#### POPULATION DIVERSITY OF NITRIFYING BACTERIA OF CHRONIC NITROGEN TREATED SOILS AT HARVARD FOREST, MA

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The nitrogen cycle is an important biogeochemical process by which bacteria in soil make atmospheric nitrogen available to plants. The impact of nitrogen deficiency and fertilization on the population diversity of bacteria involved in nitrogen cycling is important to understand the ecosystem level changes in nitrogen cycling. Nitrifying bacteria that oxidize ammonium to inorganic nitrites have the AMO operon, consisting of the *amoA* (ammonia monooxygenase), *amoB* and *amoC* genes. To study the diversity of nitrifying bacteria, the *amoA* gene is typically targeted, since it encodes the enzyme's active site. This project's goal was to use the DNA sequence of the *amoA* gene to demonstrate how soil nitrifiers may be affected by long-term soil treatments with nitrogen. DNA isolated from soil samples (control and nitrogen treated since 1989) was used to amplify the amoA gene sequences, which were cloned into a TOPO vector. The resulting plasmids were screened by restriction digestion to confirm the presence of inserts. The positive clones were sequenced and used to build clone libraries. Sequences were analyzed using NCBI blast and then aligned to generate a phylogenetic tree using MEGA 4.0. Some of the screened clones had the conserved amoA protein domain and shared  $\geq$  50% homology with the previously published amoA accession sequences. The clone library continues to be built to identify the bacterial populations involved in ammonia oxidation.

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#### THE EFFECT OF CYTOKININ ON PIN PROTEIN LEVELS IN ARABIDOPSIS THALIANA

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PIN proteins are present in all plants and play a role in plant growth and development by facilitating transport of the phytohormone auxin throughout the plant. Cytokinins are another class of phytohormones that contribute to cell growth and differentiation in plants. It is thought that auxin and cytokinin interact antagonistically. Cytokinin activity can be altered by adding exogenous cytokinin or by utilizing mutants defective in cytokinin signaling. Such alterations result in changes in root length that may result from effects on PIN expression through an unknown mechanism. The goal of my research is to examine the effect of cytokinin on the levels of different PIN proteins. The PIN proteins included in my study are PIN1, PIN2, PIN3, PIN4, and PIN7. These PIN proteins have a green fluorescent protein attached that allows the protein product to be observed when using fluorescent microscopy.

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#### THE EFFECTS OF FIRST STEP BENEFICIAL MUTATIONS IN MULTIPLE ENVIRONMENTS

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First step beneficial mutations are measured as increases in fitness relative to the ancestor, but do these first step beneficial mutations help the organism, if at all, when placed into a different environment, a process known as pleiotropy? The distribution of these first step beneficial mutations and their pleiotropic effects is an area of evolution that is poorly understood; yet remains critical in determining the success and failure of populations in a changing environment. We hypothesize that first step beneficial mutations will display positive pleiotropic effects. The collection of first step beneficial mutations is currently underway by evolving two oppositely marked ancestors that were added at a 1:1 ratio in a glucose minimal media. When a skew in the ratio is observed, we assume that a first step beneficial mutation has rise to frequency. This mutant (the winner) and its oppositely marked ancestor (the loser), are then frozen. The fitness values of the mutants that we collect will be determined by direct competition with their ancestor in different environments. Our findings will allow us to quantify the fitness increases of first step beneficial mutations and their pleiotropic fitness.

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### THE ROLE OF HAUSP IN THE MITOCHONDRIAL NON-TRANSCRIPTIONAL p53 APOPTOTIC PATHWAY OF IMR-32 HUMAN NEUROBLASTOMA CANCER CELLS

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In human neuroblastoma cancer cells, the transcription factor, p53, is responsible for initiating a response to stress-induced DNA damage by arresting the cell cycle, initiating the repair of DNA—or when DNA is severely damaged—initiating programmed cell death otherwise known as apoptosis. Aside from acting directly within the nucleus as a transcription factor to up-regulate apoptotic proteins, p53 can also rapidly initiate a cascade of molecular events at the mitochondria that induces apoptosis. To accomplish this latter function, p53 must be deubiquinated by an enzyme coded for by the HAUSP gene. If the resulting HAUSP deubiquinase is inhibited and nuclear access for p53 is blocked, non-transcriptionally induced apoptosis should not occur at the mitochondria in human neuroblastoma cells. My study will examine the effect of blocking HAUSP's activity on p53 at the mitochondria to determine if apoptosis is inhibited when access to the nucleus and deubiquination are blocked.

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### THE ROLE OF REPLICATION PROTEIN A (RPA70C) IN RESPONSE TO REPLICATION BLOCKS IN THE MODEL PLANT *ARABIDOPSIS THALIANA*

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The plant *Arabidopsis thaliana* is a model organism used to study DNA damage and repair pathways in eukaryotic cells. In the plant, internal and external cellular agents can cause DNA damage in cells that invoke repair responses. One chemical that affects *Arabidopsis* independently of DNA damage but causes a similar cellular response is hydroxyurea, which blocks DNA replication by negatively regulating ribonucleotide reductase in the synthesis of free nucleotides. In studies of *Arabidopsis thaliana* mutants, *atr* plants were found to be more sensitive than *rpa70c* plants to hydroxyurea as observed by their shorter primary roots, but the double mutant *rpa70c-atr* was found to be intermediary in sensitivity to hydroxyurea compared to both single mutants. This curious phenotype indicates that the *rpa70c-atr* double mutant suppresses the phenotype of the *atr* mutant and that the absence of RPA70C partially reverses the effect of hydroxyurea on primary root growth. These observations also suggest that RPA70C in conjunction with ATR plays a greater role in DNA replication than previously thought. Therefore, the goals of this study are to identify how RPA70C affects *Arabidopsis thaliana's* response to hydroxyurea and to determine how *rpa70c* and *atr* interact to cause the phenotype in the double mutant.

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# USE OF COMPARATIVE GENOMICS TO DETERMINE ORIGIN AND FUNCTION OF INTRONS IN CAENORHABDITES

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Introns are nucleotide sequences that interrupt eukaryotic genes. Many questions remain regarding the origin and function of these sequences. Introns continue to replicate with the rest of the gene, although their purpose has not yet been determined. Finding more about the origin of these components could help to better understand the function of the overall genome sequence. A comparative genomic approach is used to investigate the dynamics of intron gain and loss within genome sequences. This will be done using four complete genome sequences from four different species of Caenorhabdites (nematodes). The results to date reveal that introns are a very dynamic component of the genome.

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### WHOLE GENOME ANALYSIS OF MULTIPLE DIPLOID STRAWBERRY SPECIES USING READ DEPTH COVERAGE, SNP DENSITY, AND PERCENT HETEROZYGOSITY

David Wood<sup>1</sup>, Hailong Zhang<sup>2</sup>, and Thomas Davis<sup>1</sup> <sup>1</sup>Department of Biological Sciences, UNH <sup>2</sup>Department of Molecular, Cellular, & Biomedical Sciences, UNH

Improvements of the economically important cultivated strawberry, *Fragaria x ananassa*, along with its Rosaceae relatives, will become increasingly important to northern regions as the ideas of sustainability in agriculture expand. *Fragaria* has been shown to be an ideal genomics model for other *Rosaceae* families. A more complete genomics model will provide the insight that plant breeders need when working with these complex systems. From cytogenetic research, octoploid *Fragaria x ananassa* has been shown to exhibit the behavior of an allopolyploid, with subgenome types arising from distinct diploid ancestors. This project will focus on comparing massive sets of DNA sequences from the putatively ancestral *Fragaria inumae* and *Fragaria mandshurica* genomes, when mapped to the diploid *Fragaria vesca* reference genome. Analysis of read depth coverage, SNP density, and percent heterozygosity will provide a means to explore factors of sex compatibility systems and their inherent effects on genomic evolution, along with their roles in the octoploid genome. It will also provide a means to integrate existing comparative and functional genomics research, while being a useful tool for identifying future research areas.

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### PETUNIA (SOLANACEAE) FOR FLORA OF NORTH AMERICA PROJECT

Kathryn L. Fox and Janet Sullivan Department of Biological Sciences, UNH

The genus *Petunia* is native to South America and has been widely cultivated for home and garden use because it has large and beautiful flowers that now come in a variety of colors. The Garden Petunia, Petunia × atkinsiana, is the hybrid of Petunia axillaris and Petunia integrifolia, and is one of the most popular ornamental garden plants today. This hybrid has been grown in cultivation since its creation around 1834, and previously the parents of this hybrid were popular garden plants in their own right. Many populations of these three species have become established in abandoned fields, abandoned homesteads, and dump sites, and can become persistent sidewalk and roadside weeds such that the species are considered naturalized in the United States flora. Flora of North America (FNA) is an ongoing project aimed at documenting and providing information on all the native and naturalized plant species found in North America north of Mexico. The plant family Solanaceae, which includes Petunia, will be published in volume 14 (out of 30) in 2013. Many economically valuable plants are found within the Solanaceae, not only for human consumption (tomato, potato, peppers, eggplant, tobacco), but also for the garden. The FNA treatment will include corrected nomenclature, all synonyms, a key to the species, updated distribution maps, and detailed species descriptions.

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### DEVELOPMENT OF AN ON-LINE TAXONOMIC KEY TO STREAM MACROINVERTEBRATES

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Human disturbance on the landscape is of growing concern due to its impacts on the water quality of streams. As threats continue to develop, methods to measure water quality because increasingly important. Macroinvertebrates are often good indicators of water quality because pollution tolerance varies between families. Larger quantities of pollution intolerant families, found in a stream, can indicate water of good quality. One advantage of using aquatic macroinvertebrates to determine water quality is that these organisms represent an integration of stream condition over time, as they can spend over a year as larvae in the stream compared to a chemical test that represents only a "snapshot" in time. This project has furthered the development of an on-line, image-based, taxonomic key to freshwater stream insects in New England. The key has valuable applications to professionals in a variety of fields, especially educators and their students, as well as to citizen monitors, by having developed a user-friendly method of identification for stream insects. Videos have also been included, focusing on organism behavior. Bio-indicators were examined and related to water quality condition. Once it is made available on-line, this key will allow a wide audience to identify stream organisms, view behavior, and determine a water quality estimate.

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## DOES AN INVASIVE INTERTIDAL SHORE CRAB AFFECT THE POPULATION DEMOGRAPHY OF THREE INTERTIDAL PERIWINKLES?

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Introduced species have the ability to alter community structure through competition and predation changing population demography. Predation pressure by the invasive green crab *Carcinus maenas* alters the vertical distribution and morphology of three intertidal periwinkles, *Littorina littorea, L. obtusata,* and *L. saxatilis,* which are important in structuring the community through selective grazing of algae. The invasive shore crab *Hemigrapsus sanguineus* is now outcompeting *C. maenas* in rocky exposed habitats and little is known about how this could impact the *Littorina spp.* community structure. Density and morphology of both crabs and snails will be measured seasonally at three sites along three tidal heights (low, mid, high) and subtidally. Predation preference of *H. sanguineus* on *Littorina spp.* will be determined by pairwise laboratory feeding trials. To assess the predation pressure of *H. sanguineus* in the field, snails will be tethered inside cages (predator exclusion and inclusion) and outside of cages at three tidal heights (low, mid, high). If *H. sanguineus* exerts predation pressure on *Littorina spp.*, the snail population structure may alter in response to the differential habitat use of this new dominant predator. A changing snail population structure could have cascading effects on the community composition in the rocky intertidal.

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## SMALL FARM AGRO-FORESTRY DECISION MAKING IN CENTRAL AMERICA: A PRELIMINARY INVESTIGATION

Sarah McGraw and Theodore Howard Department of Natural Resources & the Environment, UNH

Central America has some of the most biologically diverse systems in the world, however forests are disappearing quickly and poverty rates are among the highest in the western hemisphere. A major cause of deforestation is the conversion of forest to agricultural use (FAO). Agro-forestry is a technique that mixes crops and natural ecosystems. Tscharntke et al. (2001) state that agro-forestry systems in tropical areas are designed to be part of the natural landscape. Agro-forestry works with natural forces, using the ecosystem services to benefit crops while preserving habitat. The purpose of this study is to show how linear and goal programming can be used to model land use allocation for small agro-forestry farms in Central America. A hypothetical farm of ten hectares that included forest, cattle and crop-land was used as the model. Initial age class structure, product prices and interest rates were tested for differences in land allocation. The results indicated that land allocation was sensitive to these three variables. The model used to make these decisions does not include mixed/shade trees as suggested by agro-forestry techniques but it does provide a basic structure for examining land use problems.

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### **COMMUNITY ACTIVISM IN THE GULF COAST AFTER THE DEEPWATER HORIZON OIL SPILL** Sara Gassman and Thomas Safford

Department of Sociology, UNH

On April 20, 2010 the Deepwater Horizon oilrig in the Gulf of Mexico exploded, spilling nearly 5 million barrels of oil over 86 days. Much of the spill response is directed by state and federal governments. Governmental control has left the ever-growing network of environmental and social justice activists feeling disenfranchised and even less in control. As a result, activists have been organizing to express concerns for their health, environment and livelihoods. On a larger scale, grassroots activism in the Gulf Coast is inspiring and empowering for those speaking out against new, potentially harmful energy development. This essay discusses the ability and responsibility of government to ensure environmental health and safety, and the power of citizen groups to take action when government fails to.

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## EFFECT OF FOOD SELECTION ON THE SURVIVAL AND GROWTH RATE OF THE BRYOZOAN *BUGULA NERITINA*

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The bryozoan *Bugula neritina* has recently become a subject of interest to many drug companies in the United States due to its harboring of its uncultured endosymbiont bacteria Candidatus Endobugula sertula. The bacteria are present throughout the bryozoan's life providing chemical protection from predation. The bryostatin chemicals, especially bryostatin 1 produced by Ca. Endobugula sertula are currently being researched due to their possibility of being used as anti-cancer drugs. In this experiment, five separate commercial diets and a mixture of all five diets was used to test the growth and survival of the B. neritina based on the food selection provided. If growth is seen and possible reproduction is observed, the aquaculture of *B. neritina* in large numbers could be possible. Since the growing Ca. Endobugula sertula separate from B. neritina has not yet been achieved, one way of getting bryostatins is taking it from the wild *B. neritina*. However thousands of *B.* neritina are needed to get just a usable amount of bryostatin. An alternative and easier way to get the *B. neritina* would be to culture *B. neritina* colonies in large numbers in a laboratory; however optimal growth requirements would need to be tested. In previous studies it was found the optimal growing temperature for *B. neritina* was between 15°C and 20°C. However in many of these studies the food was provided by using a flow through system from the ocean and not commercially available diets.

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Non-native plants used as ornamentals sometimes become invasive, establishing in and altering natural communities. Castor aralia (Kalopanax septemlobus), a tree native to China and occasionally planted as an ornamental in New England, has established wild populations on the UNH campus and adjacent College Woods as well as at the UNH Thompson Farm. Our objective was to assess the likelihood that these populations would grow and spread. All flower and shrub beds and natural woodlands on the UNH campus were censused for castor aralia. Transects were walked in College Woods and at Thompson Farm. Each plant was located with GPS, and its height, 2011 extension growth, and stem diameter measured. Age was estimated using ring counts and all plants were removed. Over 2,000 plants were found at the UNH campus where the population was all-aged, with seedlings dominant. Maximum age was 22 years; no plants were reproductive. At Thompson Farm, 93 castor aralia were collected; maximum age was seven years, age structure was unimodal, and there were no first year seedlings. The campus population is likely the result of seed production by a single tree on campus. The origin of the Thompson Farm population is unknown. Castor aralia plants were vigorous and height growth was rapid. We conclude that castor aralia can establish and grow rapidly in natural and human-modified habitats in New England. We recommend a major effort to find and eradicate populations of this species.

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Land trusts operate to protect privately-owned land from development or alternative uses unintended by the owners. Many owners in New Hampshire have opted to enroll their land in conservation land trusts to conserve the significant lands and natural resources, including farmland, working forests, water, wildlife habitat and natural areas, and community landscapes. Productive farmland is a significant contributor to maintaining integrity of the local food economy and the environment citizens opt to make active contributions toward. We will seek to find a land trust in New Hampshire that has a piece of open space land enrolled which is not currently productive but shows potential for being agriculturally productive. We aim to show the process of reintroducing a piece of currently unproductive, trusted land and the effects of it as a means of providing an opportunity for new farmers who lack land and capital.

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## LANDSCAPE AND LOCAL-SCALE EFFECTS ON STREAM SALAMANDERS IN SOUTHEASTERN NEW HAMPSHIRE

Adam Marquis and Kimberly Babbitt Department of Natural Resources & the Environment, UNH

The effect of local and landscape scale variables on stream salamanders (*Eurycea bislineata* and *Desmognathus fuscus*) were evaluated by gathering data on stream salamander population densities in 33 randomly selected first or second order streams located across southeastern New Hampshire. Leaf-litter bag sampling was conducted over a 6 week period during the summer of 2011. Species, total length, life-stage, and presence/absence of tail damage or regeneration was recorded for each salamander captured. General characteristics of each stream (e.g. siltiness, rockiness, density of coarse woody debris, leaf density) were visually estimated and recorded on a relative scale from 0-10 and the width and depth of each stream at the sample locations were recorded. Water quality measurements (pH, temperature, dissolved oxygen %, and conductivity) were taken at each stream at each sampling time. The primary landscape scale variable examined was human population density within the drainage of each stream. Initial analysis of the data does not show a significant correlation between human population density and stream salamander populations is ongoing, though initial tests show that local scale variables have a much greater effect on stream salamanders.

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### LEAF LITTER DECOMPOSITION IN BEAVER HABITAT OF THE IPSWICH WATERSHED

Clayton Hutchinson and Wilfred Wollheim Department of Natural Resources & the Environment, UNH

Stream restoration is often a costly process and often does not succeed in improving a stream's health. To restore a stream to a more natural state we must consider what missing factors were once natural to each individual stream. Before the European colonization of North America, beavers were prevalent in most stream ecosystems throughout what is now the United States. This study looks at beavers as a vital missing component to many natural stream ecosystems. We used leaf litter decomposition as an indicator of stream health in stream waters with and stream waters without beaver modifications in the Ipswich Watershed of Massachusetts. Leaf litter bags were placed at three different stream sites without beaver modifications as well as at three stream sites with beaver modifications. The rates of decomposition in stream waters with beaver modifications were then analyzed and compared to the three sites without beaver modifications. With more replications in other stream ecosystems we may find that beavers play an important natural role in restoring stream health. This could prove to be very useful in creating more successful restoration projects as well as save time and resources spent on improving stream habitats throughout North America.

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Year at UNH:	Senior
Career Interest:	Ecosystem Research and Restoration
Adviser:	Wilfred Wollheim

## SOLID WASTE MANAGEMENT IN URBAN DEVELOPING SOCIETIES: A COMPARATIVE STUDY OF HAITI, TANZANIA AND INDIA

Megan Murray and Mimi Becker Department of Natural Resources & the Environment, UNH

Solid waste is the trash we dispose of every day. It can include waste from households, the industrial sector, commercial and institutional establishments, markets, and even agriculture. The management of solid waste is a serious problem in urban areas where people are extremely concentrated. The problem is that a large amount of waste is produced but there is not a lot of space to put it. Developing countries especially struggle with solid waste management. This paper looks at urban solid waste in countries that do not have the funds or infrastructure to sufficiently tackle this problem. In particular this paper focuses on the capitals of three countries; Haiti, Tanzania, and India. These case studies help to understand how the cities and communities of developing nations are dealing with solid waste. The objectives of this paper are to see how the urban communities in these areas are affected by their country's waste management system and to see how they are dealing with solid waste, specifically looking at small-scale, community based initiatives. This paper also discusses other solutions that could be implemented. By comparing the situations of these three countries it can be seen what recommendations would work best in a developing city.

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Adviser:

Mimi Becker

## STANDARDIZING A NON-INVASIVE SURVEY METHOD TO ESTIMATE POPULATION DENSITY OF AMERICAN MARTENS IN NORTHERN NEW HAMPSHIRE

Peter Abdu, Alexej Siren, and Peter Pekins Department of Natural Resources & the Environment, UNH

The American marten (*Martes americana*) is a state threatened species that typically inhabits mature coniferous forests at high elevations. Martens are an "umbrella species" as their large range and sensitivity to disturbance make them indicators of land use, composition, connectivity, and ecosystem health. Population estimates of martens are generally anecdotal based on tracks, sightings, and accidental trapping; no structured estimate exists in New Hampshire. Identification of individual marten is key to develop a sound population estimate; therefore, this study was designed to assess a non-invasive and cost-effective method to identify individual marten by either marks on their radio-collar or their unique throat patches. Bait stations were created and 15 trail cameras were deployed for 3 weeks in an 8 mile<sup>2</sup> grid in Millsfield, NH in January 2012. Each site had a camera focused a set distance from bait (sardines) such that a feeding marten would present its collar or throat patch. A visit typically resulted in >15 pictures of a single marten. Pictures were assessed by ranking them relative to identification: 0 (not), 1 (probable), 2 (definite). In week 1, >3000 photos were taken of which 40% were ranked as 1 (35%) or 2 (5%); height of the bait station influenced identification success. This preliminary analysis indicates that marten can be identified from their throat patches, and camera trapping holds promise as a survey method of marten.

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### SUSTAINABILITY AND THE OYSTER RIVER COOPERATIVE SCHOOL DISTRICT

Miranda Cruz, John Doyle and Shelley Mitchell Department of Natural Resources & the Environment, UNH

By the end of this Spring semester, we intend to research and further implement the sustainability forum of the Oyster River Cooperative School District's Sustainability Committee that is based on the five principals of sustainability (Renewability, Substitution, Interdependence, Adaptability, and Institutional Commitment) in the areas of food/nutrition, transportation, energy, school curriculum, and community outreach. Our participation in the Committee's plans include assisting in the establishment and student involvement of school gardens in the high school, elementary, and middle school buildings in the towns of Durham, Madbury, and Lee. One intern will be working alongside school officials in constructing an ecological footprint on the school district's oil/natural gas consumption, emissions from its transportation fleet, waste and recycling patterns, and water and electricity use. Another intern will be working to establish an effective communication system and awareness effort for the transportation fleet's employees and further research effective methods to lower greenhouse gas emissions resulting from the fleet and commuting citizens. By the end of our internships, we aim to present our achievements in establishing the beginning efforts of the Committee's forum along with an inclusive ecological footprint, our methods to improve interrelations among employees and the community, and the processes that occurred among the school garden initiative.

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### ECONOMIC IMPACT ANALYSIS OF JACKSON LABORATORY

Nicole D'Alessio, Donna Pohli, Nicole Krukoff, and John Halstead Department of Natural Resources & the Environment, UNH

The purpose of this study is to examine the economic impact of the proposed Jackson Laboratory genomic research center in Farmington, CT. Although it has been mandated that 300 scientists be employed by the lab within the next ten years, this paper explores the overall direct and indirect economic impact of the lab. By using regional and national employment, trade, and socio-demographic data, economic base multipliers have been developed and location theory of the lab has been analyzed. The results of this research will instill confidence in future economic developments, provide rationale for the \$300 million state bond given to Jackson Lab and give town planners the projections needed to properly anticipate associated growth.

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Career Interest:	Risk Management or Graduate School
Author:	Nicole Krukoff
Author's Major(s):	Environmental and Resource Economics
Year at UNH:	Senior
Career Interest:	Sustainability
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### **PRODUCTION OF BARNACLE CEMENT IN PLANTS**

Matthew Marquis and Subhash Minocha Department of Biological Sciences, UNH

Barnacles are sessile, marine organisms that adhere to myriad surfaces (i.e. rocks, ship hulls, etc.) and compete with each other via fast growth and swamping techniques. Little is currently known about their novel adhesion mechanism whereby a protein matrix displaces water at the substrate, creating a strong bond under wet conditions. Commercial availability of biodegradable, wet-surface glue could be useful in biomedical fields, e.g. joining bones following surgeries. Understanding the properties of barnacle cement could also aid the shipping industry in preventing barnacle attachment to ship hulls – this fouling is known to increase drag and subsequently, fuel costs. In an attempt to produce the barnacle glue in plants, we have cloned one of the major cement protein genes (called BA5) from the barnacle *Semibalanus balanoides*. The cloned gene has been sequenced, recombined with plant-specific promoters and transferred into *Agrobacterium tumefaciens*, from where it is currently being transferred into tobacco cells. The transformed (genetically engineered) tobacco plants will be tested for the expression of the barnacle glue gene. Parallel attempts are also being made to clone genes for other component proteins of the glue so that they can be tested in different combinations to produce the actual barnacle glue in vitro.

Author:	Matthew Marquis
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Adviser:

Subhash Minocha

## THE EFFECTS OF FOREST MANAGEMENT ON MUSHROOM-EATING BEETLES

Jonas Insinga and Donald Chandler Department of Biological Sciences, UNH

Selective cutting of older trees in forest management creates a patchwork of different stages of forest succession. A result is that a more diverse environment is created that can bolster species diversity and abundance. The aim of this study was to determine if the practice affects the abundance and diversity of mycophagous beetles. Beetles associated with macroscopic fungal and myxomycete fruiting bodies were targeted due to the ease of observation of these beetle hosts. Two tree stands of different ages were chosen at West Foss Farm, in Durham, NH, where controlled tree harvesting has been practiced. From July 2<sup>nd</sup> to October 8<sup>th</sup>, 2009, two flight intercept traps were erected at each site to sample actively dispersing insects, while regular trips were taken to both sites to record the fungal species that were fruiting during the sampling period. In the comparison of these two tree stands, it was found that the older stand supported more fungal species that the younger stand. However, there was little difference in community composition of beetles, although abundances were clearly higher in the older stand than in the younger stand, likely due to the greater amount of dead wood and associated fungi in the older forest. This data supports the preservation of older growth forests over younger forests due to the ability for old growth to support a more diverse fungal community and in turn support a greater abundance of their associated beetles.

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Adviser:

Donald Chandler

### AGRICULTURAL IMPACTS ON SOIL AND PLANT LITTER MINERALOGY

Jessica Dick and Jo Laird Department of Earth Sciences, UNH

Through X-ray diffraction (XRD) analysis of several unconsolidated samples, impacts of agricultural techniques on soil mineralogy were analyzed. Techniques include crop legacy, rotation, tillage and location. All samples came from Great Lakes Bioenergy Research Center (GLBRC) in East Lansing, MI and include plant litter and soil. The litter samples were incinerated to burn off any organic material and then directly analyzed with XRD; soil samples were wet sieved with a 63 µg sieve and then coarse fraction and fine grain sediments were analyzed independently in XRD. After XRD analysis, EVA software produced a graph in which percent volume of a given mineral is determined, allowing for a comparison between sights and techniques. Texturally, all soils are sandy-clay-loam with a low percentage of clay (less than 7%). XRD analysis supported texture in that few clay minerals were found at low 2 $\Theta$ , even with the separation of fine grain sediments through wet sieving. Throughout all samples, quartz is the dominant mineral. Variation amongst samples exists in the presence of feldspars, be it sodium or potassium varieties as a result of chemical weathering and techniques applied to the system. With analysis, it also appears that location sample plots is important due to proximity to water and slope of sight. Overall, impacts of agricultural techniques causes variation amongst the sites and with further research, plant productivity could be further supported with knowing these impacts.

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Adviser:

Jo Laird

### ALPINE FLORA OF VALTALLINA AND VAL MALENCO, CENTRAL ITALIAN ALPS

Marianne Hromis and Janet Sullivan Department of Biological Sciences, UNH

During the summers of 2010 and 2011, the alpine flora was studied in Valtellina and Val Malenco within the Province of Sondrio in Lombardy Italy. The Valtellina region is situated north of Milan and south of the Swiss border. The river Adda flows through Valtellina from the east and empties into Lake Como on the west. The northern slopes of the Orobie Mountains are found to the south of the Adda. The mountains are divided by deep parallel valleys. In 1989, Parco Orobie Delle Valtellinesi was formed to protect the Orobie Alps that run through Valtellina. The alpine vegetation studied was found at the following locations: Campelli (Albosaggia) located between Val Venina and Val Livrio, Val Belviso, and Val Tartano. With permission, plant specimens were collected between Campelli (1316 m) and the alpine meadows below Pizzo Meriggio (2358 m) and in Val Tartano. Val Malenco is a lateral valley located north of the river Adda and south of Pizzo Bernina (4050 m). The alpine flora study sites were in the deglaciated foreland of the Ventina Glacier and between the dam at Campo Moro (1978 m) and Rifugio Bignami (2401 m). An understanding of the alpine flora requires knowledge of the geography and glacial geology of the region, environmental factors affecting plant distribution, plant adaptations to the alpine environment, and the sequence of primary succession following glacial retreat.

Marianne Hromis
Plant Biology
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Graduate School and Teacher

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Janet Sullivan

## IS p53 UPREGULATED DURING TEST REGENERATION OF THE SEA URCHIN, STRONGYLOCENTROTUS DROEBACHIENSIS?

Stephanie Moses and Charles Walker Department of Molecular, Cellular, & Biomedical Sciences, UNH

Regeneration is an important developmental process that occurs as a response to injury and cell damage. The ability to regenerate damaged cells and tissues occurs to some extent in many phyla. Although the regenerative response varies, the mechanism involves the same processes. These processes include formation of a blastema through cell proliferation, cell differentiation, and patterning. Apoptosis plays a critical role in development and maintaining tissue homeostasis, functioning as a quality control mechanism for cells. Previous studies in *Hydra, Drosophila*, and planarians have suggested that apoptotic cells trigger cell proliferation during tissue regeneration. These studies have also shown that p53 regulates cell proliferation in cells that have begun to undergo apoptosis. p53 is more commonly known as a tumor suppressor gene in vertebrates and may also have a function in stem cells and coordinating compensatory proliferation. Echinoderms display remarkable regenerative abilities, capable of regenerating external and internal parts due to autotomy and predation. The sea urchin, *Strongylocentrotus droebachiensis*, has the ability to regenerate its test. This experiment will explore the role of p53 in test regeneration by the use of qPCR. It is hypothesized that p53 will be upregulated in the blastema of the damaged tissues.

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### COMPARING THE STRESS MARKER CORTISOL IN PASTURE VS. TOTAL MIXED RATION-FED DAIRY CATTLE

Katherine Frazier, Danica Goodheart, and Thomas Foxall Department of Biological Sciences, UNH

Research demonstrates that milk from pasture-fed dairy cows contains fats that promote better health in humans. Minimal research has been performed to compare the health of dairy cattle fed on pasture vs. total mixed rations. This study used ten Jersey cows at both the UNH Organic Dairy and the conventional dairy for comparison of milk cortisol over a 12 month period. Cows were matched in terms of age and number of lactation cycles and regular milk samples were taken from individual cows. Milk samples were analyzed for percent fat and protein, somatic cell counts, milk urea nitrogen and total solids. Cortisol was determined by ELISA assay.

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### DO FOREST EDGES MEDIATE COMMUNITY INVASIBILITY IN PASTURES?

Lindsey Wright and Richard Smith Department of Natural Resources & the Environment, UNH

When considering agriculturally-maintained edges, there is some concern over whether the edge community provides a safe haven for weeds to grow and spread into the crop or pasture. This study focuses on the role of edge structure (plant architecture) in facilitating or mediating the presence of pasture weeds beyond the edge community. Consideration is also given to larger questions concerning edge influence in forests and the potential for edges to regulate flows across the edge boundary to neighboring forest or pasture patches. This study was conducted at two organic farms in Strafford County, New Hampshire during the summer of 2011. In this study, herbaceous vegetation at five pasture/forest sites was examined using edge structure as a context for analyzing plant community structure and distribution. Herbs across the pasture/edge/forest ecotone were surveyed using a line-intersect method at twelve sampling transects. Biomass analysis was also used to detect evidence of edge influence or interactions between the forest, edge, and pasture communities. Most edges in this study exhibited similar trends in edge influence on herb populations despite differences in edge structure, and edges did not appear to be significant sources of pasture weeds. Comparisons with a contrasting edge suggest that forest structure may play a greater role in governing edge structure and growth of herbaceous vegetation in the forest. (Supported by a SURF award to LW)

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Year at UNH:	Recent Graduate
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**Richard Smith** 

## FEEDING INCREMENTAL LEVELS OF KELP MEAL (*ASCOPHYLLUM NODOSUM*) DID NOT IMPROVE MILK PRODUCTION IN ORGANIC DAIRY COWS

Katie Velez, Samantha Werner, and Andre Brito Department of Biological Sciences, UNH

Kelp meal (Ascophyllum nodosum) is a seaweed product fed on dairy farms as a source of minerals to lactating cows. Anecdotal reports claim that kelp meal increases feed efficiency and overall animal health. There is limited scientific evidence to support the large popularity of kelp meal among dairy farmers. Sixteen lactating organic Jersey cows at the UNH Organic Dairy Research Farm were randomly assigned to four replicated 4 x 4 Latin squares to investigate the effects of incremental levels of kelp meal (0, 57, 113, and 170 g) on milk yield and concentrations of milk components. Each period lasted 21 days with 14 days for diet adaptation and seven days for sample collection. Cows were fed a total mixed ration consisting of (dry mater basis) 65% grass-legume baleage and 35% of a corn-soybean meal based concentrate twice daily. The kelp meal was mixed with 450 g of concentrate and offered to the cows prior to the morning feeding to ensure total consumption. No linear, guadratic, or cubic effects were observed for milk yield, which averaged 16.1, 16.2, 16.5, and 15.8 kg/day for the respective treatments. No significant changes were observed for yields or concentrations of milk components by feeding incremental dietary levels of kelp meal. It can be concluded that kelp meal did not improve milk production or composition in the current short-term, changeover design study. Research is needed to investigate the long-term effects of kelp meal on milk vield and animal health.

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Career Interest:	Orthopedic Medicine
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## FEEDING RED CLOVER CUT AT SUNDOWN AND HARVESTED AS BALEAGE DID NOT IMPROVE MILK YIELD IN LATE-LACTATION DAIRY COWS

Gina Soule and Andre Brito Department of Biological Sciences, UNH

Forages cut at sundown (PM) generally yield higher concentration of nonstructural carbohydrates (NSC = water soluble carbohydrates + starch) than those harvested at sunup (AM). In the current study, 12 multiparous and 2 primiparous Holstein cows averaging 238 days in milk received a total mixed ration containing, on a dry matter (DM) basis, either 65% PM- or AM-cut red clover (RC) baleage plus 35% of a common concentrate in a crossover design with14 d for diet adaptation and 7 d for sample collection. A well-established RC field was split in 2 with one half cut at sundown and the second half at sunup the next day. The PMand AM-cut RC baleages contained (g/kg DM): 103 vs.75.6 water soluble carbohydrates, 8.83 vs. 7.91 starch, and 112 vs. 83.5 NSC. Feeding late-lactation cows PM- vs. AM-cut RC baleage did not improve (P > 0.05) intake, milk yield, and yields and contents of milk components. However, milk urea N was lower (P < 0.001) in cows fed the PM- vs. the AM-cut RC indicating enhanced N utilization. Although cows were in late lactation, milk yield was relatively high (mean = 28 kg/d). Hence, the difference in NSC between RC baleages (28.5 g/kg DM) may not have been large enough to elicit a positive response in animal performance. Alternatively, the amount of concentrate fed may have offset the potential positive effect of PM-cut RC baleage on animal performance. Research is needed to examine the interaction of forage NSC and dietary levels of concentrate.

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## OVARIAN MAPPING: TRACKING THE RECRUITMENT, GROWTH, AND REGRESSION OF OVARIAN FOLLICLES IN DAIRY CATTLE

Andrew Clark, Rachel Fredette, and David Townson Department of Molecular, Cellular, & Biomedical Sciences, UNH

Reproductive health in cows is vital to the economic success of any cattle producer. Knowledge of the different stages of the bovine reproductive cycle is key to this success. The reproductive cycle (estrous cycle) in the cow is characterized as a 21-day cycle predominated by the growth and regression of ovarian structures known as follicles. These follicles each contain an egg or oocyte and are recruited in cohorts to ultimately yield a single, mature oocyte capable of being fertilized at ovulation. The corpus luteum (CL) is another structure commonly observed within the ovary that forms from the remnants of the ovulated follicle. The CL maintains pregnancy through its secretion of progesterone once fertilization occurs. The purpose of this study was to track and record the recruitment, growth and subsequent regression or ovulation of ovarian follicles throughout the estrous cycle of cows using transrectal ultrasounography. Follicles attaining >8.5 mm diameter and at least 2 mm larger than all other follicles were considered dominant follicles. Dominant follicles were characterized as early, late, or regressing follicles based upon their growth pattern over time, and were later sampled as part of a larger investigation examining the cellular mechanism(s) that lead to the dominance of follicles during the estrous cycle. Supported by Agriculture and Food Research Initiative Competitive Grant no. 2011-67016-

20041f rom	the	e USDA National Institute of Food and Agric	ulture.

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### PRODUCTION OF HUMAN $\alpha$ -GALACTOSIDASE A IN PLANTS

Katherine Lantz and Subhash Minocha Department of Biological Sciences, UNH

Many human genetic disorders involve changes in the production of a key enzyme, and changes in its homeostatic regulation cause ailments for which there is often no cure. Treatments, however, are available to manage the disease and relieve symptoms. One such treatment is Enzyme Replacement Therapy (ERT) in which artificially produced enzymes are injected into patients who do not produce enough of that specific enzyme. ERT has proven successful on several enzyme-deficiency disorders, such as Fabry Disease, in which the body does not produce the enzyme  $\alpha$ -galactosidase A. Management of this disorder includes regular supply of this protein to the patient; however, its production for clinical use is rather expensive, thus limited. In recent years, success has been achieved to produce human and animal proteins in plants through genetic engineering which results in lower cost of production and potentially a better-designed protein. Plants can thus be used as passive, solar energy-driven bioreactors to produce the human  $\alpha$ -galactosidase A. After having cloned the gene for this enzyme and having transferred it into tobacco and Arabidopsis plants, I propose to study if the transgenic plants that I have generated are producing the enzyme and if the enzyme is biologically active.

This research was supported by SURF awards to K. Lantz.

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## PRESENCE OF A LIGHT SENSITIVE MOLECULE, CRYPTOCHROME, IN THE VENTRAL NERVE CORD OF LOBSTERS, *HOMARUS AMERICANUS*

Cody White, Kimberly Bixby, and Winsor Watson Department of Biological Sciences, UNH

Daily rhythms of crustaceans are characterized by daily variations in locomotor activity, typically with a preference for the night. These rhythms are under the control of endogenous circadian clocks and these clocks become entrained to natural light:dark cycles using a variety of photoreceptors. In 1934, Welsh and Prosser discovered extra-ocular, touch and light sensitive, neurons in the ventral nerve cord (VNC) of crayfish. More recent studies have provided some evidence that crayfish without eyes can entrain to light:dark cycles. The hypothesis we tested in this study was that the extra-ocular photoreceptors in the VNC of lobsters contain the UV/blue light sensitive molecule cryptochrome (CRY) and thus might serve as a means for entraining circadian clocks to a light:dark cycle. Immunohistochemistry was used to stain these neurons with *Drosophila* anti-CRY (Alpha Diagnostics Intl Inc.) primary antibodies and goat anti-rabbit secondary antibodies bound to Alexa Fluor 488 (Invitrogen). We visualized these tissues with Zeiss LSM 510 Meta confocal microscopy (UNH Instrumentation Center), and found CRY in 2 clusters, of approximately 18 neurons per cluster, in the 6<sup>th</sup> abdominal ganglion. In addition, CRY was observed in 2-4 neurons in each other abdominal ganglion and in axons extending the length of the VNC.

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Author:	Kimberly Bixby
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### PUBLIC HEALTH PARTNERSHIPS

William Wyman and Rosemary Caron Department of Health Management and Policy, UNH

The Institute of Medicine concluded that keeping the public healthy required a well-educated public health workforce, thus leading to its recommendation that "all undergraduates should have access to education in public health." In response to this call, we investigated the status of collaborations between public health practitioners and public health academics. Local and regional health departments in New England were surveyed to: (1) establish a baseline of existing working relationships between them and nearby two- and four-year colleges; (2) examine their actual or perceived role as public health educators for future health professionals; (3) assess how they collaboratively promote public health workforce development; and (4) analyze how local practitioners employ the common essential public health services. The authors suggest how effective collaborations facilitate a broad-based, universal appreciation of public health among undergraduates.

Author:	William Wyman
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Year at UNH:	Senior
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Adviser:

Rosemary Caron

### A CORRELATIVE STUDY OF PERCEIVED STRESS AND EMOTIONAL SUPPORT WITH WEIGHT GAIN IN COLLEGE STUDENTS: EXPANDING THE YOUNG ADULTS EATING AND ACTIVE FOR HEALTH PROJECT

Margaret Donovan<sup>1</sup>, Kendra Kattleman<sup>2</sup>, and Jesse Morrell<sup>1</sup> <sup>1v</sup>Department of Molecular, Cellular, & Biomedical Sciences, UNH <sup>2v</sup>Department of Health and Nutritional Sciences, South Dakota State University

Obesity and unhealthy weight gain contribute significantly to the development of numerous chronic diseases, including diabetes and cardiovascular disease. Research shows that college adults experience high rates of weight gain during their undergraduate years. The Young adults Eating and Active for Health (Y.E.A.H) project is a 10-week tailored, web-based intervention designed to prevent weight gain among college adults by targeting physical activity, fruit and vegetable intake, and stress management. This undergraduate research project evaluates the effectiveness of the stress management component of the intervention as well as the correlation between stress management and weight maintenance. In the spring of 2011, UNH students between 18-24 years of age (n=163) were recruited (77% women) for the ongoing study. At baseline, participants had a mean body mass index of 24.0±4.1. Survey responses revealed that 34% of participants reported feeling nervous and stressed often within the last month and 25% felt that they were unable to handle their personal problems. Follow-up survey and anthropometric data, collected at 3- and 15-month assessments, will determine if the intervention increased stress management and weight maintenance as compared to the control group. This information will contribute to informing future similar interventions and assist campuses in making changes to improve their students' emotional and physical health.

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### AN ADAPTIVE WALK IN A BIOFILM COMMUNITY: REDUX

Leslie Mayo-Smith, Charles Traverse, and Vaughn Cooper Department of Molecular, Cellular, & Biomedical Sciences, UNH

Burkholderia cenocepacia is an opportunistic pathogen that infects cystic fibrosis patients. Chronic infections are caused by rapid adaptation to a biofilm lifestyle inside the lungs that generates ecological diversity. A natural isolate of *B. cenocepacia* was evolved for 1050 generations in conditions favoring biofilm formation, at which point five mutations were shared by most cells in the population, likely representing a series of adaptive steps. Using the frozen fossil record of the evolution, an individual clone harboring each mutational step was isolated. To understand effects of each mutation on fitness and the surrounding biofilm ecology, we competed individuals containing each mutational step against the ancestor. Each of the first four mutations increased fitness relative to the ancestor, but the last mutation was paradoxically less fit than the preceding mutation. However, this fifth mutation was associated with a nearly complete remodeling of the biofilm community, and a nearly identical mutation occurred on a minority alternative lineage. These two mutations are related to iron storage. When competing the adaptive steps against the ancestor in iron limited media, we found that although they remained more fit, fitness declined with each of the first four mutations but increased significantly for the fifth mutation. These results imply that adaptation in complex communities is increasingly driven by environmental changes caused by residents of the community.

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### A COST-BENEFIT ANALYSIS OF WATER QUALITY

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Water quality is positively correlated to real estate values, as well as other factors, such as tourism, employment, economic health, and human capital. Economics is an important factor when deciding on regulations and policies to be implemented. The focus of this presentation is to conduct a cost-benefit analysis of water quality regulation and policy, and to educate those who are unaware of the effect that water quality has on their wallets.

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## ARE MATRIX METALLOPROTEINASES (MMPs) PRESENT IN SKELETAL MUSCLE OF THE SPINY DOGFISH SHARK, *SQUALUS ACANTHIAS*?

Bradley Chamberlain and Paul Tsang Department of Molecular, Cellular, & Biomedical Sciences, UNH

The MMPs are proteolytic enzymes whose functions are associated with tumor angiogenesis, metastasis, and with the physiological remodeling of the female reproductive tract. During the 22-month gestation period of the spiny dogfish shark, the ovaries and uteri undergo dramatic tissue remodeling to accommodate the growth of follicles and fetuses, respectively. These changes correlate to morphological and biochemical endpoints obtained following autopsy. With declining populations of sharks as a growing concern, and the difficulty in obtaining blood samples from large pelagic sharks, we seek to develop non-lethal approaches to assess shark reproductive status. Because MMPs are involved in tissue remodeling of the reproductive tract, and because of the relative ease in obtaining muscle samples, our goal was to determine if MMPs are found in shark skeletal muscle. Muscle samples were collected from spiny dogfish of four gestational stages: A (n=6), B (n=6), C (n=5) and D (n=9). Gelatin zymography was performed to assess gelatinase (MMP) activity. Visual observations of zymograms revealed low expression of gelatin-degrading enzymes in dogfish skeletal muscle. Although we have not yet established a relationship between muscle MMPs and reproductive status, we showed that dogfish shark muscle tissue expressed MMPs.

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### BAI VERSUS BMI AS A PREDICTOR OF BODY FAT IN COLLEGE STUDENTS

Jennifer Roloff and Gale Carey Department of Molecular, Cellular, & Biomedical Sciences, UNH

The United States is currently suffering from a national obesity epidemic. Billions of dollars of health care expenditures are spent on conditions associated with obesity each year. Body Mass Index (BMI) is the current method of body fat reflection used in public and private health care systems. Its calculation is based on the ratio between weight and height but is criticized for its extreme inaccuracy. Body Adiposity Index (BAI) is a reflection of body fat first described in 2011. Its calculation is based on the ratio between height and hip circumference. The accuracy of BAI as a reflection of body fat has not been studied in college students. The purpose of this study is to evaluate BAI as a better reflection of body fat than BMI. Thirty college students, 15 males and 15 females, will be subjected to weight, height hip circumference, waist circumference and body adiposity measurements. BAI is an easier measurement to take and can be assessed in all types of settings as it only requires a tape measure. Replacement of BMI with BAI could also better target at risk individuals, those with a higher body fat, for more efficient direction of health care expenditures on obesity related conditions.

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## DEVELOPING ELECTROPHYSIOLOGICAL PROCEDURES TO RECORD NEURONAL RESPONSE PROPERTIES FROM THALAMIC NUCLEI

Amanda Craig, Rikki Miller, and Brett Gibson Department of Psychology, UNH

The thalamus is a structure in the brain that is involved in memory. Evidence suggests that nuclei within the thalamus serve as a relay for many larger processing areas such as the prefrontal cortex, hippocampus, and brain stem. Previous studies have indicated that damage to this area is involved in memory impairments and related disorders such as schizophrenia. Evaluation of thalamic neuronal response properties in rats has not yet been well characterized. This experiment used custom built tetrode assemblies (electrode bundles) to record thalamic nuclei activity of awake rats during a memory task. Presented here are the initial results from the procedures and analysis. These findings will aid in further understanding of the memory encoding process and in turn how dysfunction affects this process.

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### DEVELOPMENT OF A HIGH-SENSITIVITY METHOD TO DETECT ACCUMULATION OF CYANOBACTERIA TOXINS, MICROCYSTINS, IN TISSUES OF CHERRY TOMATOES AND BUSH BEANS.

Bethany Lefebvre, Amanda Murby, and James Haney Department of Biological Sciences, UNH

The effect of irrigation with water containing the toxic cyanobacteria *Microcystis* on the accumulation of their toxin, microcystins (MC), and fruit development in the agricultural crops, cherry tomato and bush bean was investigated in a greenhouse experiment. The plants were grown under natural light and temperature. Mature plants received treatments of toxic cyanobacteria, *Microcystis aeruginosa*, applied twice weekly to the soil. In order to simulate a scenario of naturally contaminated irrigation water, the culture was applied to plants as intact cells. Microcystins were extracted from fruit tissue with 80% methanol and analyzed by ELISA. The first extraction method tested indicated a likely presence of MC; more sensitive methods are currently being tested in order to yield lower limits of detection from the ELISA. To determine if the contaminated water affected the growth of the fruiting bodies, all harvested beans and tomatoes were individually measured, weighed and photographed before processing their tissue for ELISA. The presence of cyanobacteria showed some stimulating effect on bean growth (t-Test P<0.05), although there was no effect on tomatoes. The total number of fruit harvested from treated plants was greater, however not statistically significant. The high-sensitivity method of MC extraction allows for detection of the cyanotoxins Microcystis in the fruiting bodies of plants and an assessment of the health risk to humans and livestock.

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#### **DO ENVIRONMENTAL CHEMICALS PREDICT WEIGHT GAIN IN COLLEGE-AGED FEMALES?** Michaela Barhite and Gale Carey

Department of Molecular, Cellular, & Biomedical Sciences, UNH

Research has shown that low of levels of persistent organic pollutants (POPs), such as flame retardants (PBDEs), electrical insulators (PCBs) and pesticides (DDT) migrate into the human body through environmental exposure. These chemicals can have endocrine disrupting properties once in the body, possibly promoting obesity in adults. There is little information on the potential obesity-promoting tendencies of these POPs in college-aged females. This study explores the relationship between serum levels of POPs and biochemical, clinical, and anthropometric health markers in this unexplored population. Twenty female subjects, ages 18-20, all enrolled in an introductory nutrition class at UNH, volunteered for this study. Six mL of blood was drawn from each fasted subject via venous puncture by a phlebotomist. Serum was separated from packed cells by centrifugation, transferred to a hexane-rinsed amber vial and stored at -80°C until analysis. Serum is currently being analyzed for POPs using automated pressurized liquid extraction, a multi-column cleanup and mass spectrometer. Health markers including blood cholesterol, glucose and triglyceride levels, blood pressure, height, weight, BMI and waist circumference were measured as part of the nutrition class. These health markers will be correlated with serum POP levels in order to assess the relationship between POPs and obesity.

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### EFFECTS OF DIABETIC CONDITIONS ON VASCULAR WOUND HEALING

Siobahn Evans and Thomas Foxall Department of Molecular, Cellular, & Biomedical Sciences, UNH

Obesity is an increasing health concern that is related to higher incidences of Type II diabetes. Diabetes is known to promote cardiovascular disease and to inhibit wound healing. Endothelial cells that line blood vessels play an important role in these pathologies. This investigation measured the effects of two diabetic conditions, hyperglycemia and dyslipidemia, both independently and in combination, on the healing rate of wounded endothelial cell monolayers in vitro. The closing of the wound consisted of the complete coverage of the wounded area by endothelial cells. Results showed that wounds exposed to 200 mg/DL glucose, 400 mg/Dl glucose, and 2% dyslipidemic serum healed more slowly than the normal control. When in combination, healing rate of wound healing in endothelial cells in vitro with amplified affects when in combination.

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## EFFECTS OF EVIDENCE-BASED NUTRITIONAL STRATEGIES ON BODY COMPOSITION AND STRENGTH IN COLLEGIATE FOOTBALL PLAYERS

Michael MacArthur and Dennis Bobilya Department of Molecular, Cellular, & Biomedical Sciences, UNH

Recent research has yielded new information regarding optimal nutrition strategies to support athletic performance which has not become available to many athletes. This project attempts to demonstrate the benefits of evidence-based nutritional strategies to optimize health and athletic performance. Eight UNH football players followed nutritional guidelines through an off-season strength and conditioning program. The athletes received group guidance and each completed a diet diary for one training day each week that was analyzed to monitor adherence to the nutritional guidelines. Athletes were individually provided with a daily recommended caloric intake, with 60% of dietary calories from carbohydrate sources, 25% from fat and 15% from protein. Athletes were advised on proper pre- and post-workout nutrition. They were also taught how to select safe and effective dietary supplements. At the beginning of the trial each athlete was tested for several power and strength measurements including 3 repetition maximums in bench press, squat and power clean. A Bod Pod was used to estimate body composition. These parameters will be measured again at the end of this ongoing project. Changes in body composition and strength measurements will be compared with those of 'pair-matched' teammates following the same strength and conditioning program without nutritional guidance as well as comparison with their own changes in these parameters in previous years.

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## MITOCHONDRIAL DNA AND REAL-TIME PCR TO DETECT FECAL SOURCES IN SURFACE WATER

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Fecal pollution from mammalian waste in surface waters is considered a non-point nitrogen source that impacts coastal ecosystems worldwide and in particular, the Piscataqua River Watershed. Leaks in urban sewers, agricultural runoff, and residential septic system leaks can allow untreated influent to leach into rivers and streams. Mitigating these detrimental effects of nutrient and fecal contamination in surface water requires identification of its source. Bacterial indicators have not been successful in differentiating human, livestock and canine sources of fecal waste. The objective of this study was to develop a technique using real-time PCR to quantify mammalian host mitochondrial DNA (mtDNA) in surface water tributaries. A simplex and multiplex assay was developed with species-specific primers and dual-labeled probes for human, bovine, and canine to detect and quantify eukaryotic mtDNA in stream and ground water samples. Evidence of mtDNA concentrations from human and canine hosts could be used to inform managers of domestic wastewater contamination in the Great Bay Watershed.

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The nudibranch, *Melibe leonina*, is often used to study the neural basis of behavior because it has a simple nervous system with extremely large, identifiable, neurons. Its swimming behavior has been well characterized and swims more at night than during the day. The first goal of our project was to determine if the daily pattern of swimming is controlled by a circadian clock. We videotaped 25 animals in individual arenas while exposing them to a light dark (LD) cycle for 3 days, and to constant darkness (DD) for 5 days. In LD they consistently swam more in the evening, and some animals continued this pattern in DD, indicating that this daily rhythm of activity was under the control of an endogenous circadian clock. The second goal of the study was to identify the location of this circadian clock in the brain. We partially sequenced the Melibe genome and then identified sequences that matched known proteins found in clock neurons of other species. Next we used antibodies against two of those proteins, Timeless (*tim*) and Cryptochrome (*cry*), to identify the location of potential clock neurons in the Melibe brain using immunohistochemistry. Two-four neurons with cry immunoreactivity, and 6-8 tim immunoreactive neurons, were localized on each side of the cerebropleural ganglion. Therefore, it appears that the circadian rhythm of swimming expressed by Melibe is controlled, in part, by a circadian rhythm of gene expression in identifiable clock neurons.

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### SEEING STARS

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"Guiding Stars" TM (GS) is a point of purchase, consumer tool developed in 2006 that uses the nutrient density of foods to categorizes items as good (1 star), better (2 star) or best (3 star). In 2010, GS was implemented in the UNH dining halls by placing zero, one, two or three stars next to the food item. The goal of this project is to determine if implementing GS in the Union Court (UC) at the UNH Memorial Union Building influences patrons' choice of healthy foods. To meet this goal an anonymous baseline survey of patrons was conducted during November 2011 (n=200), GS was implemented in the UC during 2012 spring semester, and a follow-up survey will be conducted in April 2012. The baseline survey revealed that price, taste, convenience and hunger were the primary factors influencing patrons' food choices, 7% of surveyed patrons identified GS as a factor that influenced their food selection and 22% reported that healthy choices were not easily identified at UC. Flyers, menus and a "Gus (the GS icon) comes to UC" event will promote starred approved meals, drinks and snacks. The followup survey will determine if the presence of GS at UC increases the percent of patrons who identify GS as a factor that influences their food selection, and decreases the percent of patrons who report that healthy food choices are not easily identified at UC. Future work will examine food sales at UC before and after the implementation of GS.

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# TESTS FOR NUMBER DISCRIMINATION IN CLARK'S NUTCRACKERS (NUCIFRAGA COLUMBIANA)

Emily Callahan and Brett Gibson Department of Psychology, UNH

The ability to make optimal decisions regarding food choices is imperative to the foraging Clark's nutcracker, *Nucifraga columbiana*. The nutcracker is a species of bird that is well known for caching large numbers of seeds each season, and then recovering them as a food resource for survival in their harsh alpine habitat. Past studies have mainly focused on the spatial cognition of these birds, yet little else is known about the cognitive abilities of this species. The current study explores numerical cognition in nutcrackers. We tested the ability of 5 birds to choose the larger of two different quantities of pine nuts, presented simultaneously. The tested number contrasts include: 1v2, 1v3, 1v4, 1v5, 2v3, 2v4, 2v5, 3v4, 3v5, 3v8, 4v5, 4v6, 4v8, 5v6, 6v7, 6v8, 6v9, 6v12, 8v9, 8v10, and 8v16. Of the 21 combinations tested, the nutcrackers chose the larger quantity significantly more times than chance in 20 of these combinations. The ability of these birds to discern between a large variety of numbers suggests that the evolutionary pressures of caching and recovering food may have led to the development of advanced numerical cognition in this species.

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## THEY ALSO SERVE: SHELTER DOGS AS POTENTIAL ALTERNATIVES TO PURPOSE-BRED SERVICE DOGS

Amy Reichenbach and Jessica Bolker Department of Biological Sciences, UNH

This study explores the use of shelter dogs as a potential alternative to breeding programs in the service dog industry. Agencies produce an enormous population of puppies every year, half of which fail the training program for which they were bred. Meanwhile, according to the ASPCA, approximately 1,800,000 to 2,400,000 dogs are euthanized in the United States every year. Although most service dog organizations take pride in their closely controlled breeding programs, this traditional emphasis on bloodline may not be fully supported by current scientific knowledge. Not only breeding programs, but also standard training practices among service dog agencies, might profit from a new approach founded more strongly in the science of canine behavioral biology, genetics, and evolution. Recruitment of shelter dogs could benefit service dog organizations and their clients, as well as offer some shelter dogs a chance at a life of service.

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## DIRECT CHARACTERIZATION OF VIBRIO PARAHAEMOLYTICUS VIRULENCE USING FRUIT FLIES

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Vibrio parahaemolyticus is a gastric pathogen that is often acquired through consumption of raw or undercooked shellfish. With an estimated 4500 cases each year in the United States and outbreaks occurring world-wide, it has become an emergent pathogen of concern. An obstacle to preventing disease is our lack of understanding of the virulence mechanisms of the organism which requires an experimental model to directly assess disease on a host. A mouse model has been used, but it is laborious, and disease is highly variable. Our goal was to evaluate whether fruit flies which have been used for studying the related gastric pathogen *Vibrio cholera* will be useful for characterizing *V. parahaemolyticus*. Methods for propagating wild-type Oregon-R flies (Drosophilia melanogaster) on suitable growth substrates were optimized, and the colonization by pathogenic V. parahaemolytics was confirmed by direct plating. Flies were treated with either wild-type V. parahaemolyticus, a strain with attenuated virulence in mice (GacA mutant), or a bacterial-free media control. The flies were then monitored for weight loss and survival over 72 hours which are both indications of disease. Flies colonized with either wild-type or the attenuated strain showed greater weight loss at 24 hours, and decreased survival at 48 hours than the control. For both these traits the attenuated strain caused less severe symptoms. The model shows promise for use in identifying virulence factors in this organism.

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