

# PROCEEDINGS

## ABSTRACTS OF PAPERS, 84th Annual Meeting of the Virginia Academy of Science, May 25-26, 2006, Virginia Polytechnic Institute and State University, Blacksburg, VA

### Aeronautical and Aerospace Sciences

A LOGISTIC AIRCRAFT CONCEPT. M. Leroy Spearman, NASA-Langley Research Center, Hampton, VA 23681 & Katie Klein, MITRE Corp., McLean, VA 22102. Airlift and sealift capability could be useful as a means of providing the logistic support of manpower, supplies and equipment in the event of natural disasters or in the event of warfare. Such events could be within the homeland area or could be at distant locations. Such support may be required rapidly and to locations inaccessible by normal means of transportation. Conventional aircraft can provide the need for speed but the load capacity may be limited. The use of aircraft is also dependent on the basing for take-off and landing. Water-based vessels can provide large load-carrying capability but the speed is limited and water ports must be available. An effort to combine the requirements for capacity, speed and basing, has led to some research relative to unconventional designs. The concept considered has a large rectangular wing surface mounted inboard of large bodies that are attached to each wing tip. The use of two bodies of a conventional aircraft design results in doubling the capacity of the conventional aircraft. The large area of the rectangular wing provides adequate lift to sustain flight. In addition, if the wing was positioned near the surface, a cushion of air would be provided that would permit operation as a wing-in-ground (WIG) effect vehicle. Another possibility considered was the use of a portion of the bodies as containers for helium gas that would permit operation as a hybrid airship with vertical take-off and landing (VTOL) capability. Thus, the inboard wing arrangement potentially provides for large load carrying capability with a vehicle that could operate in free-air as an airplane, or near the surface in a WIG mode, or have greater basing freedom in a VTOL mode.

A CONCEPT FOR A LARGE TRANSPORT AIRCRAFT. M. Leroy Spearman, NASA-Langley Research Center, Hampton, VA 23681 & Karen Feigh, GA Inst. of Technology, Atlanta, GA 30332. The basic wing-body-tail arrangement of conventional transport aircraft has remained essentially unchanged over the years. Increased capacity has been achieved simply by increasing the overall size of the aircraft. However such an approach may be limited for aircraft beyond the size of the current jumbo jets such as the Boeing 747. Limitations may occur in the manufacturing process. There may be limitations in ground handling and in access to the boarding gate. A serious problem may occur from the trailing tip

vortex, which would be much stronger than that for current transports because of the increased lift required for the larger aircraft. In an effort to alleviate such problems some research has been done with an unconventional design for a large aircraft. The design has a large rectangular wing surface mounted inboard of large bodies attached to each wing tip. There are no outboard, cantilevered wing panels such as those that are found on conventional aircraft designs. The use of two bodies of a conventional aircraft design results in doubling the capacity of the conventional aircraft. The large area of the rectangular wing provides adequate lift to sustain flight. The tip-mounted bodies act as end plates for the wing and the formation of a trailing vortex is precluded. Wind tunnel tests have been made of such a concept using two B-747 fuselages. The resulting concept provides a payload capacity twice that of a B-747 with no increase in length, less span, and no tip vortex.

SOME CONCEPTS IN THE HISTORY OF FLIGHT VEHICLE DESIGN – PAST, PRESENT, FUTURE. M. Leroy Spearman, NASA-Langley Research Center, Hampton, VA 23681 & Jill Harper, GWU, King George, VA 22485. Since the mind of man first considered the prospect of flight, there has been remarkable growth in the field of aviation. Leonardo Da Vinci, the Italian artist and inventor, designed what appeared to be a practical airplane in 1490. Da Vinci also observed that birds had a wing surface that had a greater curvature on the upper surface than on the lower surface. He deduced that forward motion was necessary to produce airflow over the wing and that the difference in local velocity over the curved wing would create a pressure difference that had an upward force. The airflow over the wings could be achieved by gliding flight down a slope or flight into the wind. Thus a number of glider designs began to appear that included those by Lilienthal, Langley and the Wright brothers. Rotating the wings like a fan could also induce the airflow over the wings. Thus some rotary-wing designs similar to today's helicopter also appeared. It was about 400 years after Da Vinci that Langley's "Aerodrome" model flew in the United States in 1896. Then, in December 1903, the Wright Brothers in the U.S were credited with the first flight of a manned, powered aircraft. Over the years there have been numerous aircraft designs that have included variations in wing shape and location, tail shape and location, body shape and location, propulsion system arrangement and so on. However, the specific shape is relatively unimportant provided that the lifting surface area and the propulsive airflow system is able to produce enough lift to overcome the weight of the vehicle.

### **Agriculture, Forestry and Aquaculture Science**

CENTRAL ADMINISTRATION OF NEUROTENSIN DECREASES FEED INTAKE IN GOLDFISH. Marissa L. Smith and Mark A. Cline, Department of Biology, Radford University, Radford Virginia, 24142. Neurotensin (NT) is an anorexigenic tridecapeptide found in the hypothalamus and intestinal tracts of mammals and birds. The effects of NT in fish are unreported. Thus we conducted an experiment to determine the effects of NT on feed intake in goldfish. Goldfish (n=39) were centrally injected with 0, 0.25, 2.5, 25  $\mu$ g NT dissolved in teleost saline

as vehicle. Following injection, fish were fed a 4% body weight ration of fish feed pellets and digital overhead photographs were taken every 10 min for 90 min and cumulative feed intake was later quantified from the photographs. The treatment effect began at 40 min post feeding and lasted through the end of the observation period at 90 min. NT linearly decreased feed intake from 50-90 min post feeding. These results indicate similarities in the neurotensin-regulating feeding system between mammals and fish.

EFFECTS OF CENTRAL AND PERIPHERAL XENIN ADMINISTRATION ON APPETITE, FEED PASSAGE TIME, AND BRAIN CHEMISTRY IN 4-DAY POST HATCH CHICKS. Wint Nandar, J. Orion Rogers and Mark Cline, Neurophysiology & Behavior Lab, Dept. of Biol., Radford Univ., Radford, VA 24142. The 25 amino acid peptide xenin is found in the gastric mucosa and central nervous system of mammals. In mammals, xenin plasma concentration increases after a meal, reduces feed and water intake and causes altered intestinal contractility. The biological role of xenin in avian is unknown. We conducted a study to determine the effects of xenin on appetite and food transit time in 4-day post hatch chicks. Intraperitoneal injection of either 0.2, 2.0 or 20  $\mu\text{g}$  of xenin caused a quadratic reduction in feed intake after 150 min post injection with no effect on water intake in chicks. Intracerebroventricular (ICV) injection of 0.75, 1.5 or 3.0  $\mu\text{g}$  of xenin caused a linear dose dependent decrease in feed intake after 30 min post injection with no effect on water intake and food transit time through the total alimentary canal. ICV injection of 3.0  $\mu\text{g}$  of xenin and c Fos cells expression in chicks hypothalamus was detected immunocytochemically. Xenin activates ventromedial hypothalamus but not lateral hypothalamus. This study was supported by Virginia Academy of Science Undergraduate Research Grant.

WHITE LUPIN: A NEW ALTERNATIVE WINTER LEGUME CROP FOR VIRGINIA. Harbans Bhardwaj, Virginia State Univ., Petersburg, VA 23806. White lupin (*Lupinus albus* L.) has the potential to meet N needs of succeeding crops and to provide high protein grains to meet the food needs of increasing world population. However, detailed information about  $\text{N}_2$  fixation (BNF), and forage and grain crop performance of lupin, in the mid-Atlantic region, is not available. We studied BNF as related to lupin seed composition and conducted field experiments at three locations (Orange, Petersburg, and Suffolk) with 20 lupin lines in Virginia during 2003-04 season. Evaluation of 126 accessions indicated that alkaloid content affected root nodulation score. In general, bitter-seeded lines had higher nodulation score as compared to sweet-seeded lines. The fresh forage yields (kg/ha), averaged over 20 lines, varied from 12624 (Petersburg) to 5352 (Orange). The fresh yields averaged over three locations, among 20 lupin lines, varied from 1276 to 10714. The seed yield varied from 127 to 3008 kg/ha, harvest index varied from 9 to 49 percent, seeds per pod varied from 2.6 to 4.4, 100-seed weight varied from 18.7 to 21.6 g, and plant height varied from 58.9 to 71.7 cm. The mean seed yields at Orange, Petersburg, and Suffolk locations were 2488, 1975, and 1928 kg/ha, respectively. Seed yield exhibited a significant positive correlation with plant height, pods/seed, and harvest index. The results indicated that lupin can be potential alternative grain crop in Virginia and the mid-Atlantic region.

GROWTH COMPARISON OF CAGE-REARED RAINBOW TROUT FED SALMON VERSUS CATFISH DIET. B. Nerrie, C. Jones, T. Boyd, and R. Booker. School of Agriculture, Virginia State University, Petersburg VA 23806. Winter cage culture of rainbow trout (*Onchorhynchus mykiss*) is an important component of agriculture diversification in Virginia. This study coincides with the academic year and provides an opportunity for undergraduate aquatic science students to benefit from practical knowledge of daily aquaculture production management in addition to research methodology. Trout (58 g) were stocked at 50 fish per cage in four cages on December 2, 2005 and raised for 139 days. Trout were fed daily at a rate of 225 g of feed per cage. Fish in two cages were provided a catfish fingerling floating feed pellet (36% protein). Fish in the other two cages were provided a floating salmon fingerling feed pellet (42% protein). Fish were harvested on April 25, 2006. Water temperature and dissolved oxygen concentration were measured daily. Temperature ranged from 5.8-22.3 °C. Supplemental aeration maintained the dissolved oxygen concentration at >90% saturation over the entire production period. Mean harvest size for individual fish and feed conversion for the catfish feed was 236 g and 1.55. Mean harvest size for individual fish and feed conversion for the salmon feed was 257 g and 1.4. Based on feed cost alone, use of lower cost feed would provide higher returns. However, the slower growth rate would require purchase of extra trout adding to costs.

THE BIOLOGICAL SIGNIFICANCE OF BIOACTIVE PEPTIDES. Ali I. Mohamed, Jennifer Hill, & Hee-Jeong Bahng, Department of Biology, Virginia State University, Petersburg, VA 23806. Soy proteins have many bioactive components including protein subunits, and bioactive peptides (BPs). Interests in BPs grown since potential anti-carcinogens and other therapeutic agents have been identified. Using soy protein hydrolysis by several processing methods, numerous BPs with various specific biological activities have been found. These include BPs with the following properties: antihypertensive, hypocholesterolemic, antiobesity, opioid agonistic and antagonistic, antioxidant, anticancer, immunomodulatory and antimicrobial. Recent report showed that peptides containing 5-20 amino acids are the most potent hypocholesterolemic components of soy protein in animals and humans. BPs showed antimicrobial activity. These new antimicrobial peptides are of great practical importance given the rapid evolution of bacteria that are resistant to multiple antibiotics overuse by humans and livestock. Plants produce cysteine-rich antimicrobial peptides such as thionins, and purothionins, defensins and lipid transfer proteins either a priori or in response to microbial invasion. Their activity is based on the inherent amino acid composition and sequence. Peptides classified as prebiotics generally exhibit 1. limited hydrolysis and absorption in the upper gastrointestinal tract; 2. selective growth stimulation of beneficial bacteria in the colon; and 3. potential to repress pathogens and limit virulence through stimulation of a beneficial flora. Therefore such nondigestible peptides can beneficially affect the host animal by selectively stimulating the growth and/or activity of beneficial bacteria in the colon.

THE STATUS AND CONDITION OF VIRGINIA'S FORESTS, 2001. Anita K. Rose, USDA Forest Service, Southern Research Station, Forest Inventory and Analysis, Knoxville, TN 37919. Between 1997 and 2002, the USDA Forest Service's Forest Inventory and Analysis (FIA) program conducted the 7<sup>th</sup> inventory of the forests of Virginia ( $n_{\text{plots}}=4404$ ). Approximately 15,844,000 acres, or 62%, of Virginia was forested. The majority (12,102,000 acres) of Virginia's forestland was in nonindustrial private forest ownership. Public ownership and forest industry ranked 2<sup>nd</sup> and 3<sup>rd</sup>, with 2,718,000 and 1,024,000 acres, respectively. Red maple (*Acer rubrum* L.) dominated the number of live stems ( $\geq 1.0$  inch dbh) with 1.5 billion stems (13 % of total). Loblolly pine (*Pinus taeda* L.) was 2<sup>nd</sup>, with 959 million live stems, of which, 72% were in stands classified as planted. Yellow-poplar (*Liriodendron tulipifera* L.), sweetgum (*Liquidambar styraciflua* L.), and blackgum (*Nyssa sylvatica* L.) ranked 3<sup>rd</sup>, 4<sup>th</sup>, and 5<sup>th</sup> by number of stems. Yellow-poplar dominated the total live-tree volume with 5.5 billion cubic feet (13% of total). Loblolly pine was the 2<sup>nd</sup> most dominant species, with 4.7 billion cubic feet (11% of total). Chestnut oak (*Quercus prinus* L.), white oak (*Q. alba* L.), and red maple ranked next in total live-tree volume. Across Virginia, 95% of forest health plots ( $n_{\text{plots}}=110$ ) had an average crown dieback  $\leq 7.5\%$ . Scarlet oak (*Q. coccinea* Muenchh.) and sourwood (*Oxydendrum arboreum* (L.) DC.) had the highest percentage of trees with  $\geq 7.5\%$  dieback. On sites with soils samples ( $n_{\text{plots}}=74$ ), most had a pH  $< 5.0$ .

USING VOLATILES FROM THE LEAVES OF *VERNONIA GALAMENSIS* TO CONTROL INSECT PESTS. F. Favi & M. Kraemer. Agricultural Research Station, P.O. Box 9061, Virginia State University, Petersburg VA 23806. Insecticidal properties of plants belonging to the *Vernonia* genus has been well documented, including centuries of use by African tribes. We tested volatiles from the leaves of *Vernonia galamensis* (Cass.) Less as an alternative to methyl bromide, a fumigant rescheduled to phase out by 2010 due to its capability to deplete atmospheric ozone. Plant volatiles from leaf extract were released at room temperature and tested against adult whiteflies and confused flour beetles. One and two hundred microliters of our extract significantly killed adult whiteflies within an hour ( $F = 7.86$ ,  $df = 7$ ,  $p = 0.0022$ ) and continued to be active for twenty hours ( $F = 10.60$ ,  $df = 5$ ,  $p = 0.0010$ ). There was significant mortality of confused flour beetles fumigated with 3 mL of plant extract within twenty hours ( $F = \text{infinity}$ ,  $df = 4$ ,  $p = \text{infinity}$ ) whether they were under one gram or ten grams of flour. However, no mortality was observed by the same quantity of plant extract when beetles were under 500 g of flour.

EFFECT OF LOW WINTER LIGHT INTENSITY ON GREENHOUSE TOMATO PRODUCTION, PLANTING SCHEDULES AND ENERGY COSTS. Mark Kraemer & Françoise Favi, Agricultural Research Station, Virginia State University, Petersburg, VA 23806. Winter greenhouse tomato production provides supplemental income to limited-resource producers during the late winter and spring. Seeds are planted as early as late October or as late as February. Early planting has become popular because it allows earlier harvest, but it also incurs additional heating costs and the potential for blossom drop and other problems

related to low light levels. Photosynthetically available radiation (PAR) was monitored within two greenhouses, one in Virginia and the other in North Carolina. This data was used to calculate the maximum fruit load a tomato plant could support, and compared with actual fruit load. The greater the number of developing tomato "hands" (fruit clusters) per plant, the greater the amount of PAR light that is required to maintain this fruit production. We found that mid-winter light levels in our region were sufficient for good early development of tomato plants. However, light levels in late winter and early spring were sometimes not high enough to support plants with large fruit loads. Blossom drop and poor ripening was observed after periods of cloudy weather and this later resulted in poor harvests. We concluded that early planting of tomatoes was risky because it allowed plants to develop faster and produce more fruit than the available PAR light could support during periods of cloudy weather in late winter and early spring. Later planting could avoid this problem and reduce winter heating costs. New techniques of greenhouse temperature modification may allow earlier planting for more sophisticated greenhouse operations.

COMBINATION STOCKING OF TROUT SPECIES FOR CAGE CULTURE IN FARM PONDS. Scott H. Newton, Virginia State University, Petersburg, VA 23806. Since 1985, research has been conducted at Virginia State University on winter culture of trout in farm ponds in the Central Piedmont Region of the state. Studies have focused on use of floating plastic cages to rear fish in confinement similar to the practice of growing rabbits and chickens. Research experiments, along with industry results, have shown that winter cage culture of rainbow trout, golden trout, and brook trout is biologically feasible. A test demonstration, based upon documented research, was conducted to evaluate the combined aspects of a practical production and marketing scenario. Four different kinds of trout, totaling 500 fish, were stocked into one cage in the fall, 2005. In early spring, 2006, trout were randomly divided into two cages. Periodic harvests from the cages made beginning mid-March into early May. Production and sales data were summarized for farmer/producer information as it relates to small-scale agricultural diversification in existing farm ponds.

VIRGINIA FRESHWATER AQUACULTURE INDUSTRY: 1993 – 2003. Scott H. Newton, VSU, Petersburg, VA 23806. The first Virginia Aquaculture Survey was conducted for the production year 1993. Aquaculture survey forms and schedules were developed for producers along with the State Aquaculture Plan that was published in 1995. The 1993 survey established the status of both freshwater and marine commercial industries. The Virginia Agricultural Statistics Service (VASS) has published four survey reports for the years 1993, 1995, 1997, and 2003. These surveys have been conducted to track industry developments relevant to economic value of the Virginia industry. An analysis is provided on Virginia's freshwater aquaculture industry over a ten-year period along with recent comparisons with the North Carolina aquaculture industry.

WATER QUALITY FROM MULTI-BATCHING CATFISH PRODUCTION PONDS. David Crosby. Cooperative Extension VSU, PO Box 9081, Petersburg, VA 23806. Multi-batching catfish production project was initiated to simulate seine through water shed ponds in June of 2005. Five ¼ acre ponds were stocked with catfish weighting 55 lbs./1000 fish. Catfish were stocked at 5000 fish per acre. Fish were fed at 3% body weight daily with weekly feed adjustments. One of the objects of the project was to monitor and collect water quality data from June to September. Water samples were collected from each pond once a week in the afternoon before 3 pm. Water quality parameters that were tested included pH, TAN, and Nitrite. The pH ranged between 6.5 to 9 for all ponds. TAN were below 1 ppm for the entire study except for one pond spiking to 2.2 ppm briefly. Nitrites were consistently below 0.2 ppm for the summer. However, one pond did spike to nearly 1 ppm, but fell to 0.2 ppm level the following week. The overall water quality (TAN & Nitrite) for the first summer was considered very acceptable for this type of catfish production system.

**Astronomy, Mathematics and Physics  
&  
Materials Science**

COMPUTATION AND PHYSICS ASPECTS OF NON-EQUILIBRIUM MICROSYSTEM FLOWS. H. HU, Hampton University, Department of Mathematics, Hampton, Virginia 23668. In conventional fluid mechanics, the fluid behaves as a continuum and “sticks” to the surface of the boundary. The classical Navier-Stokes equations with no-slip boundary conditions for momentum and heat transfer in fluid flows work very well in modeling everyday flow situations where fluids maintain conditions of thermodynamic equilibrium. However in micrometer-length scale devices, flow behaves change and the continuum hypothesis may break down. Some unusual physics effects occur in microsystem flows. Microsystem flows are typically in the regimes of slip-flow and transition-flow. Fluid flows in transition-flow regime are too dense for molecular models, such as Molecular Dynamics Simulations or Direct Simulation of Monte-Carlo, and too rarefied for continuum models, such as Navier-Stokes models. The physics and computation aspects of flows in microsystems are examined. Extended hydrodynamics methods are discussed. Work-in-progress of developing numerical simulation schemes using extended hydrodynamics method of Burnett model for micrometer-scale flows is presented. The study focuses on some key issues of microsystem flows, such as the capability of the Burnett model and its numerical stability.

DEVELOPMENT OF A CONTROL SYSTEM FOR THE CLAS12 DETECTOR USING FPGA'S. Michael K. McGrath & Kevin L. Giovanetti, Dept. of Physics, Dept. of Physics and Astronomy, JMU. Control is an important element in large detection systems. These systems require complex control of detector voltages, readout and digitization, and calibration instrumentation. This ensures replication of the desired signals, proper mimicking of actual experiments, and proper timing and coordination. As experiments have become too complicated for manual

control, there is a need for electronic control over all aspects of the processes. Members of the James Madison University Physics Department have begun development of FPGA's for use in the 12 GeV upgraded CLAS detector at Jefferson Lab. The CLAS12 detector measures the momentum and velocity of various particles as they are emitted from electron-proton collisions. The goal of the CLAS12 detector is to study the structure of nucleons and nuclei. The FPGA will be used to control calibration systems for the experiment. It sends out various signals at specific time intervals and communicates and reacts with the detector. This allows for more elements of the calibration process to be automated, thus enabling more accurate detection due to the ability to mimic experiments. As a first step, an FPGA has been programmed to set the light output of an LED. Details of the program and associated electronics will be presented.

**SOME INTERESTING SYMMETRIES OF THE GRAVITATIONAL STRESS ENERGY TENSOR.** Joseph D. Rudmin, James Madison University. An isotropic metric for a black hole and a better vacuum condition ( $\tilde{N}V_G^2 = 0$ ) are presented which yield distinct terms for the energy densities of ordinary matter and gravitational fields in the Einstein tensor ( $G^{44} = -g^2(2\tilde{N}^2V_G + (\tilde{N}V_G)^2)$ ). This model resolves an inconsistency between electromagnetism and gravity in the calculation of field energy. Resolution of this inconsistency suggests a slight modification of the Einstein equation to  $gG^{\mu\nu} = 8\pi GT^{\mu\nu}$ . Initial work on a general relativistic model for calculating electron mass will be briefly presented.

**AXISYMMETRIC FIELD CALCULATIONS AND THE COMPLETE ELLIPTIC INTEGRAL OF THE FIRST KIND.** Joseph W. Rudmin, Dept. of Physics and Astronomy, JMU. The Complete Elliptic Integral of the First Kind, designated  $K(m)$ , frequently arises in the calculation of axisymmetric fields and potentials. One such example, which has widespread application, is the finding the magnetic field of a solenoid. It can be seen that this can be reduced to calculating the potential of a uniformly charged circular disk. Rapid precise calculation of this potential requires a computation of  $K(m)$ , but in spite of its widespread use, it is still a poorly understood function. Current methods of computation (infinite series, the method of the arithmetic-geometric mean, and Hastings' Approximation) each have drawbacks. These will be discussed. A new infinite series for  $K$  will be presented, and certain beautiful symmetries, which have enabled some progress, will be shown.

**THE DETERMINATION OF RELATIVE CONCENTRATIONS OF HYDROGEN ISOTOPES.** Laurence A. Lewis & C. Steven Whisnant, Dept. of Physics, James Madison Univ., Harrisonburg VA. 22807. The JMU photonuclear group operates a hydrogen distillery to purify hydrogen deuteride to concentrations greater than 99.99% for use in producing polarized nucleon targets. Initially, the gas sample is approximately 98% HD with small admixtures of  $H_2$  and  $D_2$ , undesirable contaminants in the target production process. Using low-temperature gas chromatography, samples of the distilled gas are analyzed to determine the relative concentrations of the isotopes  $H_2$ , HD, and  $D_2$ . The result is a chromatogram

containing two small peaks (the H<sub>2</sub> and D<sub>2</sub>) not well resolved from much a larger peak (the HD). The gas chromatography software does not allow a detailed analysis of the curves in such a situation. Hence, a separate peak fitting procedure is being developed. One method being explored represents the peak shape using the HD line shape taken from a chromatogram of very pure HD. By shifting and scaling this empirical line shape, a general peak can be characterized with three parameters: the centroid, width, and normalization. This method provides an efficient model for fitting the chromatograms. Once a viable parameterization is established, a code will be developed to fit the model to the data and estimate the uncertainties of the free parameters by minimizing  $c^2$ . From the resultant peak areas, the relative concentrations are computed. These will be correlated with the polarization decay times of polarized targets produced from this gas at Brookhaven National Laboratory.

USING NEURAL NETWORKS TO MODEL ELECTRON-NUCLEON CROSS-SECTION. John A. Telfeyan, Gabriel Nicolescu, Ioana Nicolescu, Dep. of Physics and Astronomy, JMU. Jefferson Lab is currently producing data on the nucleon cross section. The cross section affects how their electron beam scatters off of nucleons. It is convenient to use artificial neural networks, also known as multi-layer perceptrons, to analyze this data. Neural networks simulate the activity of a few neurons in the brain. With a set of test data, a user can train them to find a pattern or polynomial equation that represents the data. This talk will outline the theory behind neural networks and how they apply to the problem of the neutron's cross section.

## Biology

EFFECT OF EXOGENOUS CORTICOSTERONE ON RESPIRATION IN A REPTILE. S.E. DuRant<sup>1</sup>, W.A. Hopkins<sup>1</sup>, L.G. Talent<sup>2</sup>, & L.M. Romero<sup>3</sup>. <sup>1</sup>Virginia Polytechnic Institute and State University, Department of Fisheries and Wildlife Sciences; <sup>2</sup>Oklahoma State University, Department of Biology; <sup>3</sup>Tufts University, Department of Biology. Glucocorticoids (GCs), or stress hormones, increase in animals after exposure to stressors and enable organisms to meet energy requirements by increasing metabolic activity (e.g. protein catabolism and gluconeogenesis). Increases in metabolism should translate to increases in whole animal metabolism, and therefore energy expenditures by organisms. Few studies have examined the effects of GCs on energy expenditure and have produced conflicting results, which could be attributable to species-specific differences, differences in methodology and the levels of GCs examined (physiological vs. pharmacological). We examined the effects of exogenous administration of corticosterone (CORT), the primary GC in reptiles, on metabolism in western fence lizards (*Sceloporus occidentalis*) by frequently measuring changes in respiration for 48 hours after dosing. Low levels of CORT resulted in plasma CORT concentrations similar to controls, however injections of 0.40 $\mu$ g/g body weight of CORT produced a ten-fold increase in plasma CORT concentrations 3 hrs post

dose. Interestingly, increases in respiration were also noted in the highest dose group during this time interval. Respiration rates were returning to baseline 6 hours after dosing which coincided with decreases in plasma CORT concentrations. Our results suggest that frequent measures of metabolism facilitate detection of subtle and dynamic changes in respiration caused by GCs that may be important for understanding the overall energetic implications of stress.

EFFECT OF CENTRAL NEUROTENSIN ON APPETITE IN 4-DAY POST-HATCH CHICKS. Wint Nandar & Mark Cline, Neurophysiology & Behavior Lab, Dept. of Biol., Radford Univ., Radford, VA 24142. Neurotensin, a 13-amino acid peptide, was first isolated from the bovine hypothalamus and later was identified in the intestinal tracts of mammals and chickens. Intracerebroventricular or intraperitoneal injection of neurotensin suppresses food intake in rats. It also regulates the digestion and gastric motility in humans, dogs and guinea pigs. Intraperitoneal injection of neurotensin inhibits gastrointestinal motility in chickens. The biological role of central neurotensin in avians is unknown, thus we conducted an experiment to investigate the effect of central neurotensin on appetite in chicks. Four-day post-hatch chicks, *Gallus gallus*, randomly received intracerebroventricular injection of neurotensin (0, 15, 30 or 60  $\mu\text{g}$ ) and feed consumption was monitored for 90 minutes post injection. Our results demonstrated that central neurotensin caused a linear dose dependent decrease in feed intake from 15 min to 60 min post-injection in chicks. Neurotensin may regulate the appetite through both central and peripheral mechanisms.

EFFECTS OF AROCLOR 1254 ON UDP-GT ACTIVITY AND THYROID FUNCTION IN JAPANESE QUAIL. Catherine M. Webb & F. M. Anne McNabb, Department of Biological Sciences, Virginia Tech, Blacksburg, VA, 24061. Polychlorinated biphenyls (PCBs) decrease thyroid function in mammals as indicated by decreased circulating thyroxine ( $T_4$ ). PCBs increase the excretion of  $T_4$  by two mechanisms: (1) induction of uridine diphosphate-glucuronosyltransferase (UDP-GT), a liver enzyme that glucuronidates  $T_4$ , and (2) displacement of  $T_4$  from transthyretin, a thyroid hormone binding protein in the blood, creating free  $T_4$ . There are many studies in which rats or mice were exposed to a mixture of PCBs, Aroclor 1254, and it was found that UDP-GT activity toward  $T_4$  was increased, and circulating  $T_4$  concentrations were decreased. Compared to mammals, there have been relatively few such studies in birds. In the present study, Japanese quail were exposed to vehicle or 500 mg/kg body weight Aroclor 1254 for 5 or 21 days. UDP-GT activity was increased significantly compared to controls at both exposure times. Plasma  $T_4$  concentrations tended to decrease, but not significantly. Thyroid gland  $T_4$  content was not altered. This was the first study to demonstrate induction of UDP-GT activity toward  $T_4$  in quail. We conclude that the dose of Aroclor 1254 was not large enough to cause decreased thyroid gland  $T_4$  content or decreased plasma  $T_4$  concentrations, and that we would see significant decreases if the dose were increased. (Supported by a Virginia Tech Graduate Research Development Project grant.)

CENTRAL AMYLIN INCREASES WHILE ENTEROSTATIN DECREASES FEED INTAKE IN LARGE MOUTH BASS. Marissa L. Smith and Mark A. Cline, Department of Biology, Radford University, Radford, VA 24142. Amylin, a 37-residue peptide, and enterostatin, a 5-residue peptide, are secreted from the pancreas and affect ingestive-related processes in mammals. The effects of amylin and enterostatin in carnivorous fish are unknown; therefore, we conducted the following experiments using large mouth bass. Juvenile fish were injected with 0, 12.5, 25, or 50 micrograms rat amylin (Exp 1) or 0, 1, 10 or 100 micrograms human enterostatin (Exp 2) into the third ventricle using a modified free-hand technique. Amylin-treated large mouth bass responded with increased feed intake, which is similar to the effect we previously observed after injecting rat amylin in goldfish. However, when amylin is centrally administered to various mammalian species a reduction in feed intake is reported. Enterostatin-treated large mouth bass responded with decreased feed intake. This effect is similar to that reported in mammals. These results support evolutionary differences between fish and mammals for the amylin- and similarities for the enterostatin-gut-brain axes. Additional research is warranted to better understand the biological reason for these differences.

NOCTURNAL THERMOREGULATION IN THE AMERICAN ROBIN, *TURDUS MIGRATORIUS*, DURING WINTER. Raiza Vega-Candelario, Dept. Biological Sci., Univ. of Mary Washington, Fredericksburg, VA 22401. Thermoregulation is a costly biological activity for endotherms such as birds, especially when food is limited and temperatures are cold. When non-energy requiring heat conservation mechanisms are not adequate, birds may use facultative hypothermic responses. Although shallow rest-phase hypothermia has been identified in passerines, most research has been conducted in laboratories under controlled conditions. However, little is known about nocturnal thermoregulation in free-range passerines. One such passerine is the American Robin. I measured skin temperatures of five free-ranging American Robins between 1400-1500 and 0200 and 0300 hours for three to seven 24-hr cycles per subject. Nighttime ambient temperatures ranged from -4°C to 16°C during the study period. For all pooled observations (n = 40), skin temperature reductions ranged from 2.5°C to 6.4°C, while the average nightly skin temperature reduction per individual was 3.1°C. To our knowledge, these data represent the first evidence of facultative rest-phase hypothermia in a free-ranging member of the thrush family.

A STUDY OF THE PROTEOME OF THE APICAL SEGMENT MEMBRANES OF GUINEA PIG SPERMATOZOA. Sara S. Hirsch & James A. Foster, Dept. of Biol. Randolph-Macon Coll., Ashland VA 20005. The membranes over the acrosome play a key role in sperm-egg zona pellucida interactions in mammals, but few of the integral membrane proteins there have been characterized. The apical segment (AS) of guinea pig sperm, containing the plasma and outer acrosomal membranes along with the acrosomal matrix, can be isolated in sufficient quantities to perform biochemical analysis. Following the isolation of apical segments, AS proteins were separated into aqueous and detergent phase proteins by Triton X-114

phase partitioning. SDS-PAGE zymography detected acrosin activity concentrated in the aqueous phase with some partitioning into the detergent. Additionally, western blot analysis showed that the acrosomal matrix proteins SP-10 and AM50 partitioned mostly into the aqueous phase with a small amount resolving in the detergent phase. Thus, several known acrosomal matrix proteins appear to have some association with the membranes. One-dimensional SDS-PAGE analysis demonstrated that there are clear differences between the protein contents of the aqueous and detergent phases and identified a set of putative membrane-associated proteins. Proteomic analysis of 14 prominent detergent phase bands identified several proteins known to be associated with the AS membranes as well as many that have not yet been identified in mammalian sperm, including several adhesion proteins, numerous vesicle-associated proteins and signaling proteins, and two G-protein coupled receptors. Funded by a Virginia Academy of Science Undergraduate Research Grant to SH and a Small Project Research Grant to JF.

THE INFLUENCE OF EASTERN HEMLOCK, *TSUGA CANADENSIS*, ON ORGANIC MATTER AND NUTRIENT DYNAMICS IN HEADWATER STREAMS. Kate Morkeski, Julie E. Frank, Jackson R. Webster, & E. F. Benfield, Dept. of Biol. Sci., Va. Polytechnic Inst. & State Univ., Blacksburg, VA 24061. In the southern Appalachians, eastern hemlock is the only conifer that commonly occurs along streams, and it is currently experiencing widespread mortality due to an introduced insect. We added hemlock sticks to one headwater stream and hardwood twigs to another to determine whether the finely branched structure and decay resistance of hemlock twigs make them particularly effective contributors to leaf retention and thus to nutrient uptake. Ammonium demand in the two streams showed a strong seasonal pattern, rising with autumn leaf fall and decreasing as leaves were eliminated, but showed no apparent effects of twigs. Organic matter standing stocks, leaf transport distance, and hydraulic characteristics of the two streams have yet to be determined. Stream incubations showed that hemlock twigs support lower rates of microbial respiration and less fungal biomass and have lower breakdown rates than birch twigs, suggesting that the elimination of hemlock trees will result in the loss of a recalcitrant organic matter source to headwater streams. Retention of leaves by hemlock or hardwood twigs appears to be less important than other factors in regulating ammonium uptake, but the removal of long-lasting hemlock twigs may subtly alter structural characteristics and microbial activity in headwater streams.

THE TVN0703 PROTEIN FROM *THERMOPLASMA VOLCANIUM*, A PROBABLE ARCHAEAL METAL-DEPENDENT SERINE/THREONINE PROTEIN PHOSPHATASE (PPM). AbdulShakur H. Abdullah, Dept. of Biochem., Virginia Polytechnic Institute and State University, Blacksburg, VA. 24060. In order to survive and flourish, living organisms must sense and react to changes in their internal and external environments. Two major classes of potential cellular responses to environmental change are: alterations in gene expression and modifications to existing proteins. Changes in gene expression are generally caused by activation or repression of transcription, which is a slow response (minutes to

hours). Modifications to existing proteins are typically mediated by enzymes and constitute fast responses (seconds to minutes). Protein phosphorylation-dephosphorylation represents one of nature's major mechanisms for regulating protein function. The dawn of genome sequencing has created an opportunity to utilize phylogenetic diversity as a resource for exploring the similarities in the phosphorylation/dephosphorylation mechanisms of different organisms, including parallels between relatively simple organisms and more complex organisms that are generally more difficult to study. Most archaeal genomes encode potential protein kinases and protein phosphatases. However, unlike other archaea, we have found that *T. volcanium* has a potential PPM protein phosphatase based on DNA-derived amino acid sequence that thus far shows a kinetic profile similar to known PPMs. In addition, we have shown zinc to be a potent inhibitor of the *T. volcanium* PPM in vitro. This opens the way to ask whether zinc may be a physiological inhibitor of the *T. volcanium* PPM in vivo since zinc plays a role in the stress response in eukaryotes by inhibition of PPM.

ARABIDOPSIS THALIANA MYO-INOSITOL PHOSPHATE SYNTHASE MUTANTS VARY IN THEIR RESPONSES TO ABIOTIC STRESSES. Pyae P. Hein, Javad Torabinejad, & Glenda E. Gillaspay, Dept. of Biochem., Va. Polytechnic Inst. & State Univ., Blacksburg VA 24061. The *myo*-inositol metabolic pathway produces substrates for the synthesis of second messengers in plant cells. Second messengers are molecules that organisms use to communicate with their neighbors and respond to other biotic and abiotic stress stimuli. *Myo*-inositol phosphate synthase (MIPS) is the rate limiting enzyme in the *myo*-inositol metabolic pathway and three genes (MIPS1, MIPS2, and MIPS3) encode MIPS enzyme in the model plant, *Arabidopsis thaliana*. Loss of function MIPS-1, MIPS-2, and MIPS-3 *Arabidopsis* mutants were previously isolated and are useful in addressing the importance of MIPS in plants. We hypothesized that the *mips* mutants, compared to the wild type plants, differ in their responses to various stresses that may use inositol-containing second messengers. To test the hypothesis, we analyzed the responses of *mips1*, *mips2*, and *mips3* mutants to salt, abscisic acid, sorbitol, and lithium. We measured the seedling responses to these stresses by evaluating seed germination and root elongation. The analysis of transgenic seedlings revealed that *mips1* mutants were the most sensitive to most of the stresses tested. In contrast, *mips-2* and *mips-3* mutants were similar to wild-type seedlings in their responses to these stresses. These results suggest that the MIPS1 gene plays an important and unique role in synthesizing substrates for second messengers used during the stress response in plants.

CHARACTERIZATION OF TWO ARABIDOPSIS BETA-GALACTOSIDASES. Dashzeveg Gantulga<sup>1</sup>, Y. Turan<sup>1</sup>, B. S. Winkel<sup>1</sup>, D. R. Bevan<sup>2</sup>, and A.Esen<sup>1</sup>. <sup>1</sup>Department of Biological Science, Virginia Polytechnic Institute and State University and <sup>2</sup>Biochemistry Department, Virginia Polytechnic Institute and State University, Blacksburg, VA, 24061. The *Arabidopsis thaliana* genes At1g45130 and At3g52840 encode  $\beta$ -galactosidase isozymes Gal-5 and Gal-2, respectively. Microarray data analysis (Genevestigator tool) shows the highest expression of

At1g45130 in stem node and root elongation zones while that of At3g52840 in the petiole of adult leaves. These two isozymes share 38-81% sequence identity with other plant  $\beta$ -galactosidases, which are believed to be involved in modification of cell wall polysaccharides. Phylogenetic analysis reveals that Gal-5 and Gal-2 belong to the glycosyl hydrolase (GH) family 35, which includes most plant  $\beta$ -galactosidases. Recombinant Gal-5 and Gal-2 were expressed in *Pichia pastoris* and purified to electrophoretic homogeneity. Both enzymes hydrolyze such artificial beta-galactosides as pNPGal, oNPGal, 4-MUGal, X-Gal and 6-BNGal at a pH optimum of 4.0-4.5. This acidic pH optimum for enzyme activity is similar to the pH optimum of other characterized plant  $\beta$ -galactosidases and cell wall hydrolases. Gal-5 and Gal-2 exhibit highest specificity for the galactose moiety of substrates followed by fucose among five different glycosides tested. In addition, both isozymes cleave  $\beta(1-4)$  and  $\beta(1-3)$  linkages of galacto-oligosaccharides and release free D-galactose.

THE COMPLETE MITOCHONDRIAL GENOME OF *OLIGOCLADO FLORIDANUS*: IMPLICATIONS FOR PLATYHELMINTH PHYLOGENY. Nitin Bhagi, Virginia Commonwealth University, Richmond VA 23220. Platyhelminthes, or flatworms, are a very diverse group of simple invertebrate animals that despite their varying differences have been classified into two major taxa, the Acoelomorpha and the Rhabditophora. The Rhabditophora taxon is further divided into three general classes including Trematoda, Cestoda, and Turbellaria. The class Turbellaria, in particular, contains a wide range of flatworms that are not easily classified into any of the other two classes and hence there is some inconsistency within the current Platyhelminth classification system. Therefore, phylogenetic analysis based on mitochondrial gene order, can be used to correctly define how closely related two or more species are, despite what their current strict taxonomy indicates. Consequently, in this experiment the mitochondrial gene order of *Oligoclado floridanus* was determined using ten specific sequencing primers so that this species could be compared to the flatworms that have their mitochondrial gene order already determined and published. Finally, a preliminary phylogenetic tree was constructed from the data obtained using a parsimony analysis of the gene relative positions, which helps to validate the usefulness of gene order comparisons for phylogenetic reconstruction.

MAPPING OF BGAF BINDING REGIONS ON THE MAIZE  $\beta$ -GLUCOSIDASE ISOZYME GLU1. H. Y. Yu<sup>1</sup>, F. Kittur<sup>1</sup>, D. R. Bevan<sup>2</sup>, and A. Esen<sup>1</sup>, <sup>1</sup>Dept. of Biological Science and <sup>2</sup>Dept. of Biochemistry, Virginia Polytechnic Institute and State Univ., Blacksburg, VA 24061. In maize, two  $\beta$ -glucosidase isozymes (Glu1 and Glu2) have been identified and studied with respect to structure and function. In null maize genotypes,  $\beta$ -glucosidase fails to enter the gel and cannot be detected on zymograms. A specific  $\beta$ -glucosidase aggregating factor (BGAF) is responsible for the "null" phenotype. Domain swapping between maize  $\beta$ -glucosidase isozymes Glu1 and Glu2, to which BGAF binds and sorghum  $\beta$ -glucosidase (dhurrinase) isozyme Dhr1, to which BGAF does not bind, suggested that an N-terminal region (Glu50-Val145) and an extreme C-terminal region (Phe466-Ala512) together form

the BGAF binding site. We swapped the N-terminal (Ile72-Thr82) and C-terminal (Phe466-Ala512) regions of Glu1 with those of Dhr1. The results of binding assays suggest that the amino acids essential for BGAF binding are located in the N-terminal region (Ile72-Thr82) whereas the C-terminal region plays only a minor role. To identify the specific amino acids involved, we mutated the unique amino acids in the N-terminal region of Glu1 (Ile72/Val73, Asn75/Asp76, Lys81/Ala82, and Thr82/Glu83) to those in Dhr1. Of these single amino acid substitutions, the replacement of Thr82 in Glu1 with the corresponding residue (Glu83) in Dhr1 completely abolished binding to BGAF, suggesting that Thr82 plays a critical role in BGAF binding. This research is supported by Grant MCB-0417342 from the National Science Foundation.

PRE-FRACTIONATION BY LIQUID ISOELECTRIC FOCUSING OF THE *PENICILLIUM MARNEFFEI* SECRETOME. Kara G Segna<sup>1</sup>, Tim D. Kim<sup>2</sup>, Chet R. Cooper<sup>2</sup> & Gary R. Walker<sup>2</sup>, <sup>1</sup>Christopher Newport University, Newport News VA 23606, <sup>2</sup>Youngstown State University, Youngstown OH, 44555. The fungal pathogen *Penicillium marneffeii* is a significant opportunistic infection in Southeast Asia among immuno-compromised individuals. Although it is related to other *Penicillium* species, *P. marneffeii* shows dimorphism unique to its genus. At 25°C, *P. marneffeii* grows as a monomorphic mold, exhibiting the characteristics of other members of its genus. However at 37°C, *P. marneffeii* grows in a yeast-like form, known as arthroconidium. The secretome of an organism comprises all proteins secreted into the surrounding environment. The yeast form of *P. marneffeii* secretes different proteins than that of the mould form. We analyzed the secretome by means of 1 and 2 DGE electrophoresis after prefraction by liquid isoelectric focusing. An identification and understanding of these form-specific proteins will further our understanding of the pathogenic processes in this and other fungi.

UTILIZATION OF *SACCHAROMYCES CEREVISIAE* SCREENS TO ANALYZE TYPE-III SECRETED PROTEINS OF *SHIGELLA FLEXNERI*. Kumkum Sarkar, Virginia Commonwealth Univ., Richmond, VA 23284 & Cammie F. Lesser, Massachusetts General Hospital and Harvard Medical School, Cambridge, MA 02139. In the developing world today, diarrheal diseases like shigellosis continue to exist as an eminent problem, as they account for over 1 million deaths worldwide. *Shigella flexneri* uses a type-three secretion system to translocate toxic proteins, invade host cells, and spread intracellularly in the gut epithelium. The yeast screens were analyzed through non-inducing and inducing media to examine protein-protein interactions. It was hypothesized that through these genome-wide yeast screens, one will be able to further characterize the molecular roles of IcsB, IpgB2, and IpaJ in pathogenesis. After testing stock plates 104-150, 170, 171, and 172 using *Shigella* proteins IcsB, IpgB2, and IpaJ, the strains of yeast that showed growth in all four spots of yeast on inducing media were documented and retested on the 3 separate master plates that contained 26 yeast strains. IcsB had 10 hits in galactose, IpgB2 had 7 hits in galactose, and IpaJ had 3 hits in galactose. It was found that gal 3 and YJL051W suppressed all three proteins. Both IcsB and IpgB2 were suppressed by bud13 and sus1. Thus, *Saccharomyces cerevisiae* screens have been essential in modeling the interactions between eukaryotic cell proteins and

*Shigella* virulence proteins such as IcsB, IpgB2, and IpaJ. By utilizing yeast screens, one can study other pathogens like *Pseudomonas aeruginosa* in host cell environments as well. These studies can all lead to further knowledge in basic bacterial pathogenesis.

BOUNDARY EFFECTS UPON POPULATION DISTRIBUTION: THEORETICAL MODE AND CASE STUDY. Steven A. Hand, N. Bly & L. Espinasa, Natural Sciences Dept., Shenandoah University, Winchester VA, 22601. This study investigated the effect of a major environmental boundary, the Grand Canyon in Arizona, on the population distribution of the locally dominant tree species. We found that both transect studies and satellite images showed reduced tree population density in the proximity of the canyon edge. We explored the environment-tree system dynamics using a computational model incorporating coupled multi-generational dynamics, locally variable environmental carrying capacity, and a porous seed dispersal barrier. The model demonstrated that diffusion of seeds in the vicinity of the canyon edge is sufficient to account for the observed population distribution independent of other environmental parameters. We believe our study has relevance for the introduction of artificial boundaries into established ecosystems and their potential impact upon established populations.

DISPERSION AND PLACEMENT OF PRAYING MANTID EGG CASES IN AN OLDFIELD. R. K. Rose & A. S. Bellows, Dept. of Biol. Sciences, Old Dominion Univ., Norfolk, Virginia 23529-0266. We studied the introduced Chinese praying mantis, *Tenodera aridifolia sinensis*, in an oldfield converting to a pine stand through secondary succession in Chesapeake, Virginia. In each 6.25 X 6.25 m sub-cell in our 1-ha grid, we recorded the number of egg cases (oöthecae), the plant species to which each egg case was attached, its height on the plant, its approximate compass orientation, and the estimated percent of open area with herbaceous vegetation. In winter/spring 2005, 586 egg cases were found in the 324 sub-cells (mean = 1.81). The highest mean (2.70) was found in sub-cells with 25% open/75% trees and lowest mean (0.40) was found in areas with few or no trees (100 % open). Forty percent of egg cases were oriented southward. To test the hypothesis that south-facing egg cases might hatch sooner, we collected 192 egg cases on January 28, 2006 in the same field, recording orientation on the plant (0-359°, using a compass). Egg cases were removed from their host plant, weighed, placed separately into quart mason jars in an 18 °C room, and examined daily until all young had hatched. Three egg cases yielded young on the third day, and another on the fourth, but regular hatching did not begin until day 32, after which one or more egg cases hatched almost daily until day 89. After placing into eight 45° orientation sectors, *Chi*-square analysis revealed a significant ( $p < 0.001$ ) difference in egg case orientation, with large numbers facing south and southeast and many fewer than expected in the northern sectors. ANOVA revealed no significant differences among orientation sectors for days until hatching. We gratefully acknowledge The Nature Conservancy for the use of their land for our studies.

SPATIAL MODELING OF MOSQUITO HABITAT IN COASTAL PLAIN ENVIRONMENTS. A. S. Bellows<sup>1,2</sup>, T.R. Allen, Jr.<sup>2</sup>, & R. K. Rose<sup>1</sup>, <sup>1</sup>Department of Biological Sciences & <sup>2</sup>Department of Political Science & Geography, Old Dominion University, Norfolk, VA 23529. We created a set of predictive, spatially explicit classification models capable of ranking suitable mosquito habitat in heterogeneous landscapes with an emphasis on vectors of West Nile Virus and eastern equine encephalitis and species that were particularly abundant. The study area was the City of Chesapeake, Virginia. Life-history processes dictate organismal distributions, and are a function of spatial land cover patterns (*i.e.*, land cover composition and configuration). Thus, we hypothesized that local mosquito abundance can be predicted from a parsimonious set of measurable environmental factors represented as thematic layers in a GIS. Layers represented soil (e.g., runoff potential, water capacity) and vegetation (*i.e.*, Tasseled Cap transformations [TC]: wetness, greenness, brightness) characteristics. Soil moisture is closely associated with mosquito life histories and spatial composition and configuration of vegetation are closely linked with species diversity and abundance. TC has been useful for inferring photosynthetic activity and vegetative structure. All thematic layers were derived via direct interpretation of Landsat ETM+ imagery. Analyses included a comparison of the ability of linear regression models (parametric) and artificial neural networks (ANN: non parametric) to accurately predict habitat suitability and subsequent mosquito abundance. These HSI's were derived from spatially explicit empirical data, thus, making them useful within real landscapes. Future research will include metapopulation implications and subsequent spatial strategies of chemical mosquito control.

EFFECTS OF CELEBREX (CELECOXIB) ON THE FREQUENCY OF PREGNANCY LOSS INDUCED IN CD-1 MICE BY LIPOPOLYSACCHARIDE INJECTIONS. Kristin Cooper<sup>1</sup>, Carolyn M. Conway<sup>2</sup>, and Arthur F. Conway<sup>1</sup>, <sup>1</sup>Dept. of Biology, Randolph-Macon Coll., Ashland, VA 23005 and <sup>2</sup>Dept. of Biology, Virginia Commonwealth Univ., Richmond, VA 23284. Lipopolysaccharide (LPS), a component of the outer membrane of the cell wall of Gram-negative bacteria, triggers pregnancy loss in mammals. General non-steroidal anti-inflammatory drugs as inhibitors of LPS-induced pregnancy loss have produced mixed results. A specific cyclooxygenase-2 inhibitor, Celebrex (celecoxib), was evaluated in this study. Pregnant CD-1 mice were given one of three doses of Celebrex (0, 18, 36  $\mu\text{g}/\text{ml}$ ) from day 7 to day 12 in drinking water and one of three dose of LPS (0, 2, 5  $\mu\text{g}$ ) by intravenous injection on day 9 of gestation. Pregnancy loss was evaluated by sacrifice on day 12 of gestation. LPS treatment significantly increased frequency of pregnancy loss, significantly reduced maternal body weight and significantly increased maternal spleen weight. The high dose of Celebrex (36  $\mu\text{g}/\text{ml}$ ) decreased pregnancy loss in some of the mice treated with 5  $\mu\text{g}$  LPS, but the effect was neither consistent nor statistically significant. Celebrex treatment had no apparent effect on maternal body weight loss in response to LPS treatment, and very little effect on maternal spleen weight increase in response to LPS treatment. Thus, Celebrex appears to have limited, if any, use for prevention of pregnancy loss or control of inflammation resulting from Gram-negative infections during pregnancy.

EFFECTS OF DEXAMETHASONE ON THE FREQUENCY OF PREGNANCY LOSS INDUCED IN CD-1 MICE BY LIPOPOLYSACCHARIDE INJECTIONS. Jonathon Farmer<sup>1</sup>, Carolyn M. Conway<sup>2</sup>, and Arthur F. Conway<sup>1</sup>, <sup>1</sup>Dept. of Biology, Randolph-Macon Coll., Ashland, VA 23005 and <sup>2</sup>Dept. of Biology, Virginia Commonwealth Univ., Richmond, VA 23284. Lipopolysaccharides (LPS) cause strong inflammatory responses and can cause pregnancy loss at low doses. We studied the effects of daily intraperitoneal injections of dexamethasone on frequency of LPS-induced pregnancy loss. Intravenous injection of two or five micrograms of LPS on day 9 of gestation significantly increased frequency of pregnancy loss, decreased weight of implantation sites, caused significant maternal weight loss, and slightly increased maternal spleen weight. Dexamethasone administered on days 8 through 11 inhibited all LPS effects, with the reduction in LPS-induced pregnancy loss being statistically significant. One hundred micrograms of dexamethasone per day was as effective as 200 micrograms in reducing LPS-induced maternal weight loss and was more effective than 200 micrograms in reducing LPS-induced pregnancy loss. Dexamethasone caused a statistically significantly dose dependent reduction on spleen weight independent of LPS treatment status.

EFFECTS OF INDOMETHACIN ON THE FREQUENCY OF PREGNANCY LOSS INDUCED IN CD-1 MICE BY LIPOPOLYSACCHARIDE INJECTIONS. Joseph Greenwood<sup>1</sup>, Carolyn M. Conway<sup>2</sup>, and Arthur F. Conway<sup>1</sup>, <sup>1</sup>Dept. of Biology, Randolph-Macon Coll., Ashland, VA 23005 and <sup>2</sup>Dept. of Biology, Virginia Commonwealth Univ., Richmond, VA 23284. Lipopolysaccharides (LPS) cause strong inflammatory responses and can cause pregnancy loss at low doses. The effects of orally administered indomethacin on the frequency of lipopolysaccharide-induced pregnancy loss were studied in CD-1 mice. Indomethacin (0, 5, or 10 µg/ml) was administered in drinking water on days 7 through 12 of gestation and LPS injections (0, 2 or 5 µg) were given intravenously on day 9 of gestation. Mice were sacrificed on day 12 of gestation and examined for pregnancy loss. LPS induced pregnancy loss in a dose-dependent pattern. The indomethacin treatments tested had no effect on the frequency of LPS-induced pregnancy loss. These results indicate either that LPS-induced pregnancy loss does not depend on prostaglandin synthesis or other processes sensitive to indomethacin, or that orally administered indomethacin fails to achieve a concentration at critical locations which is sufficient to inhibit pregnancy loss.

EFFECTS OF PRETREATMENT WITH LIPOPOLYSACCHARIDE ON THE FREQUENCY OF PREGNANCY LOSS INDUCED IN CD-1 MICE BY LIPOPOLYSACCHARIDE INJECTIONS. Daniel Hudgins<sup>1</sup>, Carolyn M. Conway<sup>2</sup>, and Arthur F. Conway<sup>1</sup>, <sup>1</sup>Dept. of Biology, Randolph-Macon Coll., Ashland, VA 23005 and <sup>2</sup>Dept. of Biology, Virginia Commonwealth Univ., Richmond, VA 23284. Lipopolysaccharides (LPS) cause strong inflammatory responses and can cause pregnancy loss at low doses. A report that exposure to a low dose of lipopolysaccharide (LPS) earlier in pregnancy reduced the frequency of pregnancy loss induced by exposure to a larger dose of LPS later in pregnancy was systematically evaluated. Treatment of mice not previously exposed to LPS with two or five micrograms of LPS intravenously on day 9 of gestation significantly

increased pregnancy loss in a dose-dependent pattern. Intraperitoneal injections of LPS on day 7 followed by intravenous LPS on day 9 resulted in less pregnancy loss than resulted from the day 9 treatment alone. Maternal bodyweight was reduced in a dose-dependent pattern as LPS was increased but all mice showed a decrease in weight associated with handling stresses. As day 7 LPS dose was increased, implant weight was reduced relative to PBS-treated mice, indicating growth retardation in surviving implantation sites. Granulocyte abundance in the mesometrial region and total peroxidase staining (index of tissue destruction) at the placental margins were not correlated with frequency of pregnancy loss. If repeatable, these results argue very strongly against either of these patterns being heavily involved in pregnancy loss.

EFFECTS OF SINGULAIR (MONTELUKAST) ON THE FREQUENCY OF PREGNANCY LOSS INDUCED IN CD-1 MICE BY LIPOPOLYSACCHARIDE INJECTIONS. Roberta Monnier<sup>1</sup>, Carolyn M. Conway<sup>2</sup>, and Arthur F. Conway<sup>1</sup>, <sup>1</sup>Dept. of Biology, Randolph-Macon Coll., Ashland, VA 23005 and <sup>2</sup>Dept. of Biology, Virginia Commonwealth Univ., Richmond, VA 23284. Lipopolysaccharide (LPS) is a Gram-negative bacterial cell wall component that causes a strong inflammatory response and can cause pregnancy loss at low doses. A major component of inflammatory responses involves prostaglandin and leukotriene secretion. General non-steroidal anti-inflammatory drugs (NSAIDs) which block the cyclooxygenase enzymes involved in prostaglandin synthesis have been used as inhibitors of LPS-induced pregnancy loss with mixed results. This study examined the effect of Singulair on LPS-induced pregnancy loss in CD-1 mice. Singulair specifically blocks the action of leukotrienes, another cause of inflammation. Three doses of Singulair (0 µg/ml, 1 µg/ml or 2 µg/ml) were given to mice in drinking water on gestation days 7-12. Mice also received 0, 2 or 5 µg LPS in tail vein injections on day 9 of gestation. Two µg/ml Singulair resulted in a slight reduction in the pregnancy loss induced by LPS, but the effect was not statistically significant. Spleen weight increased slightly with increased Singulair dose, but the effects were not statistically significant. Singulair treatment had no statistically significant effects on the reduced implantation site weight induced by LPS. The partial inhibition of LPS-induced pregnancy loss by Singulair indicates that leukotrienes are probably involved but are apparently not the primary mechanism of pregnancy loss.

EFFECTS OF VITAMIN C (ASCORBIC ACID) ON THE FREQUENCY OF PREGNANCY LOSS INDUCED IN CD-1 MICE BY LIPOPOLYSACCHARIDE INJECTIONS. Inger Reres<sup>1</sup>, Carolyn M. Conway<sup>2</sup>, and Arthur F. Conway<sup>1</sup>, <sup>1</sup>Dept. of Biology, Randolph-Macon Coll., Ashland, VA 23005 and <sup>2</sup>Dept. of Biology, Virginia Commonwealth Univ., Richmond, VA 23284. Lipopolysaccharides (LPS) cause strong inflammatory responses and can cause pregnancy loss at low doses. We studied the effects of oral and intraperitoneal administration of ascorbic acid on frequency of LPS-induced pregnancy loss. Intravenous injection of two or five micrograms of LPS on day 9 of gestation significantly increased frequency of pregnancy loss, decreased weight of implantation sites, and caused significant maternal weight loss. Oral administration of 15 mg ascorbic acid per day in

drinking water resulted in no changes in any of the LPS effects. Intraperitoneal injection of 15 milligrams of ascorbic acid 45 minutes prior to the LPS injection significantly inhibited LPS-induced pregnancy loss and maternal weight loss and increased implantation site weights. Intraperitoneal injection was clearly more effective than oral administration of ascorbic acid, but we are unsure of the reasons.

THE EFFECTS OF ELECTROMAGNETIC FIELDS ON MOUSE BRAINS AND BEHAVIOR. Katherine M. Farrell & Deborah A. O'Dell. Dept. of Biological Sciences, Univ. Mary Washington, Fredericksburg, VA 22401. Enzymes in the melatonin biosynthetic pathway are inhibited by applied magnetic fields resulting in a decrease of melatonin. Tryptophan hydroxylase (TPH) is an enzyme important in melatonin biosynthesis and can be measured to indirectly observe melatonin levels. The purpose of this experiment was to investigate the effects of magnetic fields on tryptophan hydroxylase levels in the mouse brain and on mouse behavior. Two experimental groups, exposed to different magnetic field strengths (+0.5G & +0.75G), and one control group were tested over a 12 week experiment. Experimental groups showed a decrease in the area of TPH positive cells in the pineal gland and in TPH levels in blood serum and homogenized brain samples over time. Activity levels among experimental groups increased, while feeding habits decreased. These results support the hypothesis that exposure to magnetic fields leads to a decrease in melatonin levels, with longer exposure and stronger magnetic fields having a greater effect. This work was supported by an Undergraduate Research Grant from University of Mary Washington.

THE IMPORTANCE OF HEMLOCK WOOD INPUT INTO STREAM ECOSYSTEMS. Julie E. Frank, K. Morkeski, J.R. Webster, & E.F. Benfield, Dept. of Biological Sciences, Va. Polytechnic Inst. & State Univ., Blacksburg, VA 24061. Eastern hemlocks (*Tsuga canadensis*) are being rapidly killed by an insect, the hemlock woolly adelgid (*Adelges tsugae*). This insect infests hemlock trees and feeds on xylem ray parenchyma cells of the needles. The death of hemlock will lead to the growth of species such as black birch (*Betula lenta*) in riparian forests previously dominated by hemlock. Forested streams are influenced by inputs from hemlocks because this is the only riparian conifer in the southern Appalachians and it has been suggested that the decay resistance of hemlock wood makes it a critical structural component of streams. To explore the impacts of hemlock death, bundles of hemlock and birch twigs were placed in two small streams at Coweeta Hydrologic Laboratory in the southern Appalachian Mountains in North Carolina. Bundles were collected approximately monthly from October 2005 through April 2006 and used to measure breakdown rates and fungal biomass. For most of the study, fungal biomass was not significantly different between the two types of twigs; however, fungal biomass was significantly higher on birch twigs for collections from March through April. Also, breakdown rates of birch twigs were higher than breakdown rates of hemlock twigs. Based on this study, the widespread mortality of hemlock trees will result in the loss of critical physical structures, which are known to have a significant role in nutrient cycling, retention of organic matter, and in providing invertebrate habitat in headwater streams.

BACTERIAL COMMUNITY PROFILES AT ENVIRONMENTAL SITES CONTAMINATED WITH HEAVY METALS FROM MINING OPERATIONS. Julie Brooks, Katelyn Christopher, Shanley Ignacio, Tarah Brown, & Georgia Hammond, Department of Biology, Radford University, Radford, VA 24142. Microorganisms, due to their unique metabolic properties, play significant and key roles in remediation of polluted environments. The purpose of our project was to develop molecular profiles of bacterial communities present in sediments from abandoned mines contaminated with heavy metals. Using a culture-free method of acquiring DNA, we generate PCR products that correspond to a specific genetic region (from the ribosomal RNA cassette) from each of the bacteria present in the community sampled. The DNA products generated are variable in both length and nucleotide sequence. Agarose gel electrophoresis of the amplified DNA yields a ladder of fragments that constitutes a bacterial profile or fingerprint for each sediment sample. Profiles from samples allow us to compare bacterial communities, and to track changes in the communities over time. These data can be correlated with changes in communities of other organisms, or physical characteristics of the environment such as concentration of the heavy metals present, to yield a comprehensive view of remediation at our study sites.

EFFECT OF DIETARY WHEY PROTEIN SUPPLEMENTATION ON GROWTH OF *EUBLEPHARIS MACULARIS* (LEOPARD GECKOS). Breanna Hargbol, Adrienne Betancourt, Tommy Camp & Nick Snyder, Neurophysiology & Behavior Lab, Dept. of Biol., Radford Univ., Radford, VA 24142. We conducted an experiment to determine if supplemental dietary protein via gavage would accelerate the growth rate of leopard geckos. Geckos, 12 weeks of age, were randomly assigned to receive either 0 or 120 mg whey protein administered by gavage. Treatments were administered every other day with tap water serving as a vehicle. All geckos had *ad libitum* access to crickets, a calcium supplement, and water during the experiment. Daily body weights were recorded. Geckos treated with 120 mg whey protein responded with increased body weight 17 days following the start of treatment. Although the data collection ceased after 23 days, we propose that the magnitude of differential body weight would increase at day 24 and beyond. Thus, supplementing a leopard gecko's diet with whey protein is an effective method to accelerate body weight gain. This technique may be used by commercial reptile supply companies to increase production efficiency.

RNAI AND TRANSPOSABLE ELEMENT ACTIVITY IN *Aedes Aegypti*. Edward A. Mead & Zhijian J. Tu, Department of Biochem., Va. Polytechnic Inst. & State Univ., Blacksburg, VA 24061. Transposable elements make up a significant portion of the genome of most animals, and active transposable elements have demonstrated promise as vectors for genetic engineering. Transposition is often a rare event, making it difficult to identify currently active transposable elements. It has been shown in *C. elegans* and other systems that transposition is suppressed through several routes, including RNA interference. We have begun to target components of the siRNA pathway by transfecting mosquito cell lines with dsRNA against particular RNAi pathway components to remove the RNAi-mediated suppression of transposable elements. We have observed success with an

Argonaute-2 dsRNA knockdown of RNA interference, monitored through the use of a luciferase assay. We will monitor the change in transposable element activity by microarray and by examining the expression of rasiRNA. Successfully identified active transposable elements may then be used as vectors for introducing refractory genes into mosquitoes as a means to combat deadly epidemics such as malaria, West Nile, and dengue fever.

EFFECTS OF CENTRAL NEUROTENSIN ON BEHAVIOR AND ALIMENTARY CANAL TRANSIT TIME IN BROILER CHICKS. M. Meissner, M. Smith, W. Nandar & M. Cline, Department of Biology, Radford University, VA 24142. Neurotensin (NT), a 13 amino acid peptide, has been identified in the bovine hypothalamus and the intestinal tract of bovines, rats, and chickens. Central NT inhibits feed intake, affects uterine contraction, and causes duodenum relaxation in rats and ileum contraction in guinea pigs. The role of NT in chickens is unknown, thus we conducted experiments to determine the effect of NT on behavior and alimentary canal transit time in broiler chicks. In Experiment 1, chicks received central injections of NT and were placed in an observation arena. The chick's behavior was videorecorded for 15 min and later analyzed in 5 min intervals. The distance the chick traveled, the number of pecks, jumps, and escape attempts, and the amount of time the chick spent standing, sitting, preening, and sleeping were quantified. In Experiment 2, chicks received central injections of NT, followed by gavage of a known amount of chicken feed slurry. Gastric transit time was determined as the time post injection at which the marker was visually detected in feces. Our results demonstrated that central NT affected the total distance the chick traveled, but had no affect on total alimentary canal transit time in broiler chicks.

A COMPARISON OF THE NEUROANATOMY OF EGG-LAYING WORKERS OF *APIS CERANA* AND *APIS MELLIFERA*. Elena M. Quintiliani & Deborah A. O'Dell, Dept. of Biological Sciences, Univ. of Mary Washington, Fredericksburg, VA 22401. Egg-laying in female worker honeybees is suppressed by pheromones from the queen bee. In the absence of this suppression, workers show ovariole development and begin to lay eggs. Egg-laying *Apis mellifera* workers show an increase in size due to process growth within the ganglia controlling egg-laying compared to non-laying workers. This suggests that additional neuronal development must occur prior to egg-laying. The rate at which egg-laying begins after removal of suppression varies with different races of honeybees which suggests that some races may have more developed ganglia. Compared with the European honeybee (*Apis mellifera*), the Asian honeybee (*Apis cerana*) has a more rapid on-set of egg-laying behavior. We examined whether growth occurred in the abdominal ganglia of egg-laying workers of *Apis cerana*. A6 ganglia of *A. cerana* were measured in laying and non-laying workers and the sizes compared. Results show that in laying *A. cerana*, only an increase in ganglion length (26% increase) is seen, as compared to the A6 ganglia of laying *A. mellifera* in which an increase in both width (39%) and length (22%) is seen. This suggests that *A. cerana* has a more completely developed nervous system than *A. mellifera* although neuronal growth could not be assayed. This work was supported by an Undergraduate

Research Grant from University of Mary Washington and an Undergraduate Research Grant from Virginia Academy of Sciences.

CHRONIC EXPOSURE TO ENDOSULFAN ALTERS BEHAVIORAL PATTERNS IN WOOD FROG (*RANA SYLVATICA*) TADPOLES. Amanda K. Simmons, Jamie M. Leonard & Melissa Zwick, Department of Natural Sciences, Longwood University, Farmville, VA 23909. Endosulfan is an organochlorine insecticide used in agricultural systems to control a wide variety of insect pests. Endosulfan exerts neurotoxic effects by targeting both cholinergic and GABAergic systems. Non-target aquatic organisms, such as amphibians, are particularly susceptible to endosulfan exposure via runoff from agricultural fields following application of the pesticide. The effects of endosulfan on wood frog (*Rana sylvatica*) phenotype were explored by exposing *R. sylvatica* to either 10 µg/l or 20 µg/l endosulfan, acetone (vehicle control), or water (control) beginning in the egg stage and extending into the larval stage. Tadpoles exposed to endosulfan were significantly more active than control animals, but there was no difference in tadpole activity level in the two endosulfan treatments. There were no main effects of treatment on time to settle following a disturbance; however, there was a significant time by treatment interaction. This indicates a differential response between treatments over time. Following pesticide removal at day 7, behavior of tadpoles in endosulfan treatments began to resemble tadpole behavior in acetone and water treatment groups. Tadpoles exposed to endosulfan did not develop as rapidly as those in acetone and water treatments as measured by Gosner stage. These results clearly indicate that endosulfan alters the behavior of non-target organisms at environmentally realistic concentrations, possibly by affecting the development of the sensory and/or motor nervous systems.

IMPACTS OF AGRICULTURAL PESTICIDES ON THE SURVIVAL, GROWTH AND DEVELOPMENT OF THE WOOD FROG (*RANA SYLVATICA*). Allison M. Smith, Philip C. Klineburger, Ellen M. Simpson, Krystal L. Freels, Amanda K. Simmons, Jamie M. Leonard & Melissa Zwick, Department of Natural Sciences, Longwood University, Farmville, VA 23909. Global amphibian populations are currently experiencing significant decline and agricultural pesticides in aquatic ecosystems are likely one of the causative factors. Amphibians are often exposed to a combination of agricultural insecticides and herbicides during critical developmental stages. Endosulfan is an organochlorine insecticide that inhibits acetylcholinesterase and GABAergic systems and atrazine is a powerful herbicide used to control weeds by inhibiting photosynthesis. The effects of endosulfan and atrazine on wood frog (*Rana sylvatica*) phenotype were explored by exposing *R. sylvatica* to either 10 µg/l endosulfan, 20 µg/l atrazine, 10 µg/l endosulfan and 20 µg/l atrazine, or water (control) in the larval stage. Endosulfan had significant negative effects on wood frog tadpoles. Tadpoles were underdeveloped, weighed less, and experienced higher mortality when exposed to endosulfan, relative to tadpoles exposed to atrazine or water. Activity levels in *R. sylvatica* tadpoles were lower in endosulfan-treated tanks. Following both internal and external disturbances, tadpoles took longer to settle in tanks treated with endosulfan. Atrazine had no effect on any variables tested, and there were no interactive effects

of atrazine and endosulfan. These findings are consistent with previous research on the effects of pesticides on amphibian populations and future studies will explore the underlying neuroanatomy of tadpoles exposed to pesticides.

EFFECTS OF AMMONIUM PERCHLORATE ON THYROID FUNCTION AND PLASMA CORTICOSTERONE IN JAPANESE QUAIL. Eric R. Weigel, F. M. Anne McNabb, & Ignacio T. Moore, Dept. of Biol., Va. Polytechnic Inst. & State Univ., Blacksburg, VA 24061. Ingestion of perchlorate has been shown to inhibit the thyroid gland's production of thyroid hormones, tri-iodothyronine (T3) and thyroxine (T4) in various species. In this experiment, the effects of varying doses of ammonium perchlorate (AP) upon thyroid function and plasma corticosterone were investigated. Forty adult birds were separated into six experimental groups, with equal numbers of males and females. The groups received the following doses of dissolved AP in their drinking water: 0 (control), 100, 250, 500, 1000, and 2000 mg AP/L of drinking water. Treatment was administered for 2 weeks, after which blood samples were taken, birds were sacrificed, and thyroid glands were removed. Thyroid function was assessed by T4 and T3 concentrations in the thyroid glands, T4 levels in plasma, and thyroid gland masses. Dose-dependent effects were observed, but only in male animals. In males, thyroidal T4 declined with increasing AP treatment, and thyroid gland hypertrophy was also evident, as expected. However, females did not respond significantly to any level of AP treatment. We hypothesize that some of the AP ingested by females may be accumulating in their eggs, thereby decreasing their effective thyroidal dose as compared to males.

THE EFFECT OF ANTIOXIDANT VITAMINS AND NSAID DRUGS ON PLAQUE FORMATION IN ALZHEIMER'S DISEASE. Kristen M. Zach & Deborah A. O'Dell. Dept. of Biological sciences, Univ. Mary Washington, Fredericksburg, VA. 22401. Alzheimer's Disease (AD) is characterized by the deposition of neurofibrillary tangles (NFTs), neuritic plaques composed of  $\beta$ -amyloid, the release of inflammatory molecules such as TNF- $\alpha$ , and severe lipid peroxidation. Although the exact mechanism responsible for causing this cascade of events is unclear, it is clear that they are interrelated and act synergistically to cause neuronal death. Recent therapeutic strategies attempt to limit the neurotoxicity of oxidative and immune responses through the use of antioxidant vitamins and non-steroidal anti-inflammatory drugs (NSAIDs). To determine the efficacy of Collect brand vitamins and Ibuprofen in performing this role, we fed mice predisposed to plaque development (J10, JAX), rodent chow supplemented with either vitamins, Ibuprofen, or both for either 4 or 7 months. Mice from two litters were sacrificed and their brains removed and analyzed for presence of inflammation (TNF- $\alpha$ ) and  $\beta$ -amyloid protein. The level of TNF- $\alpha$  in mice treated with supplements for 7 months was much lower than that of the control (0.046 ng/mg tissue vs 5113.38 ng/mg tissue, respectively). Analysis using light microscopy revealed that mice treated with a combination of Ibuprofen and vitamins for 7 months had the least amount of  $\beta$ -amyloid protein in the brain. This study indicates that a long-term diet supplemented with both vitamins and NSAIDs may help prevent the onset of the hallmark pathologies associated with AD. This

work was supported by an Undergraduate Research Grant from the University of Mary Washington.

ASSESSMENT OF A NATURALLY OCCURRING FATTY ACID AS AN ANTIMICROBIAL PRODUCT. James R. Doherty, Gregory J. Sproull & Barbara B. Kreutzer, Dept. of Integrated Science and Technology, James Madison Univ., Harrisonburg, VA 22807. Naturally occurring antimicrobial compounds are often less expensive to produce and deemed safer by the public than synthetic pharmaceutical products. We assessed a fatty acid abundantly present in some commercially produced plants for its use as an antimicrobial product. Chemical properties of the compound were examined and effectiveness was tested against bacterial growth using two methods, a sensitivity disc experiment and a liquid culture experiment. We found the compound to be strongly inhibitory to *Staphylococcus epidermis* growth and to have multiple properties which make it useful as an antibacterial product.

INFECTION OF HEPATOCYTE AND NON-HEPATOCYTE CELLS BY *FRANCISELLA*. A.P. Belsches-Jablonski<sup>1</sup>, S. Pylypko<sup>2</sup> & M.L. van Hoek<sup>2</sup>, <sup>1</sup>Biol. Program, Lynchburg Coll., Lynchburg, VA 24501, <sup>2</sup>Dept. of Molecular and Microbiology, National Center for Biodefense and Infectious Diseases, George Mason Univ., Manassas, VA 20110. *Francisella tularensis* is a gram-negative, coccobacillus, aerobic organism and is considered a Category A bioweapon due to its potentially high infectivity and ease of transmission. The predominant modes of transmission include inhalation and intradermal inoculation. Both have been shown to initiate severe infection in mammals with as low as 10 bacilli when aerosolized. Once *F. tularensis* enters the host, it multiplies within phagocytes as well as in other cells including hepatocytes. Our hypothesis is that *F. tularensis* LVS, *F. tularensis novicida*, and *F. philomiragia* infect and subsequently cause apoptosis in hepatocytes leading to liver failure contributing to tularemia pathogenesis. Our data suggest that *F. tularensis* LVS, *F. tularensis novicida*, and *F. philomiragia* effectively adhere to and given time, invade and replicate intracellularly within TIB-73, J774A.1, and HEPG2 cells. We observed a greater abundance of recovered bacteria in both the adherence and invasion/replication assays in HEPG2 cells versus murine embryonic liver (TIB-73) and macrophage (J774A.1) cell lines, suggesting that the human hepatocyte cell line has greater uptake ability and therefore may be more prone to infection than other cell types. In addition, infection of J774 macrophage cells by *F. tularensis* LVS results in early phosphorylation events in several downstream signaling molecules involved in apoptosis and/or cell proliferation. This study was funded by the Commonwealth Health Research Board of Virginia.

BIOFILMS OF *FRANCISELLA* & MIXED BIOFILMS WITH AMOEBA. A. B. Verhoeven & M. L. van Hoek. Department of Molecular and Microbiology, National Center for Biodefense and Infectious Diseases, George Mason University, Manassas, VA 20110. *Francisella* are pleomorphic, gram negative coccobacilli bacteria that have a thin lipopolysaccharide capsule and have recently been found to have pili. We are exploring the interaction of *Francisella* species and amoeba (such

as *Acanthamoeba castellanii*), and their role in biofilms. Establishing *Francisella* as a biofilm-forming organism may help to explain the historical findings of close association with water and water-dwelling mammals, and may point to one of the natural reservoirs of this organism. In order to begin to address this question we demonstrate that biofilms are formed by some subspecies of this genus. Biofilms were detected using the standard crystal violet staining assay. Preferences were noted for surfaces that were positively charged such as polyvinyl chloride (PVC). This might imply that organic material found in waterways could serve as surfaces for biofilm formation and that *Francisella* in the wild would exhibit biofilm formation instead of platonic conformation. We also co-cultured amoeba and *Francisella* and have shown that they form a mixed biofilm when grown together. This could imply a symbiotic relationship with amoeba in the functioning biofilm, whereby the release of nutrients from the amoeba increases biofilm formation by the bacteria and the amoeba feeds on the bacteria (including *Francisella*) in the biofilm.

THE CHARACTERIZATION OF OUTER MEMBRANE VESICLES PRODUCED BY *Francisella* SPECIES. D. Matrakas<sup>1</sup>, V. N. Morozov<sup>2</sup>, W. Zhou<sup>3</sup> & M. L. van Hoek<sup>1</sup>, <sup>1</sup>Department of Molecular and Microbiology, <sup>2</sup>National Center for Biodefense and Infectious Diseases, <sup>3</sup>Center for Applied Proteomics and Molecular Medicine, George Mason University, Manassas, VA 20110. The phenomenon of outer membrane vesicle (OMV) production occurs in many Gram-negative bacteria. OMV production by the genus *Francisella*, a Gram-negative bacterium, has not been reported to date. This work demonstrated the presence of OMVs from *Francisella* and a preliminary characterization of these OMVs. The characterization was done by the use of atomic force microscopy (AFM) and transmission electron microscopy (TEM). AFM studies showed that the OMVs from *Francisella* species were within the range of 50-250nm in diameter, as described for other Gram-negative bacteria. In addition to this characterization, we tested the cytopathic and hemolytic effects of OMVs on cells. Western blots were performed to detect the presence of lipopolysaccharide (LPS) and outer membrane proteins on the surface of the OMVs. This research was partially supported by grant funding from the Virginia Academy of Science Small Project Research Fund.

### **Biomedical and General Engineering**

IDENTIFICATION OF THE EFFECTS OF LOCAL MUSCLE FATIGUE ON POSTURAL SWAY THROUGH WAVELET ANALYSIS. Hong B. Zhang & Maury Nussbaum, Dept. of Industrial and Systems Eng., Virginia Tech, Blacksburg VA 24061. Occupational falls result in nearly 13% of U.S. workplace fatalities. Among several identified contributing factors, loss of balance has been indicated as an important precipitating event. Localized muscle fatigue (LMF) has been demonstrated to compromise the postural control system, and is a suspected risk factor for falls via decreases in muscular control and proprioceptive ability. Here, fatigue was induced, through voluntary exertions, in the ankle, knee, torso, and shoulder of experimental participants, and the effects on postural control quantified

from observed sway during quiet erect stance (eyes closed). Certain frequency bands in sway data correspond to specific postural control mechanisms (i.e. proprioception and central processing). A new algorithm was developed to estimate the entropy in these bands. Specifically, a wavelet transform was applied to obtain the frequency domain characteristics of sway in both the AP and ML directions. Total power was determined in the bands associated with the two control mechanisms. Finally, Tsallis entropy was calculated for each band both pre- and post-fatigue. Reduced entropy was interpreted as a decrease in complexity, which was used in turn to infer a disruption to the associated control mechanism. Such effects were found for the proprioceptive control of ML sway resulting from ankle and torso fatigue, and central control of bilateral sway from fatigue at all joints. This method has potential future utility in that it can ascertain effects on control mechanisms without the need for more complex perturbation methods. This work was supported by NIOSH Grant #R01 OH007882.

AN AFFORDABLE VIBROTACTILE DISPLAY DEVICE FOR THE BLIND AND VISUALLY IMPAIRED. David Burch and Dianne Pawluk, Department of Biomedical Engineering, Virginia Commonwealth University, Richmond VA. Currently, there are several systems that display graphical information to the blind and visually impaired; however, these systems are often too expensive and/or cumbersome for practical use by most individuals. Thus, there is a need for an affordable display device capable of rendering graphical information through stimulation of working sensory systems. To further facilitate individuals, the device must be portable, as to enable them to use it in many different settings. However, to keep the scope of the device within reasonable limits, the primary goal will be to create a device for rendering charts, graphs, and blueprints to blind and visually impaired individuals. The device I developed utilizes photo-detectors to produce a signal corresponding to areas of low photonic reflectivity. This signal then triggers a vibratory motor, stimulating tactile receptors in the user. By combining this tactile sensation along with a sense of device position, the user receives haptic feedback relating to the position of lines and shaded areas on charts, graphs, and blueprints.

A FINITE ELEMENT EXAMINATION OF THE SACROPLASTY PROCEDURE. Dennis E. Anderson & John R. Cotton, Department of Engineering Science and Mechanics, Va. Polytechnic Inst. & State Univ., Blacksburg, VA 24061. A new procedure called sacroplasty has recently been used as a treatment for sacral insufficiency fractures. Sacroplasty, which is similar to the well established vertebroplasty procedure, consists of the injection of polymethylmethacrylate (PMMA) bone cement into the cancellous core of the sacrum. This stabilizes fractures in the bone and provides rapid pain relief for most patients. Finite element models of the upper pelvis, including the sacrum, were used to examine the mechanical effects of cement in the sacrum. The geometry and nonhomogeneous material properties of the models were based on computed tomography (CT) images of cadavers on which sacroplasties were performed. The effect of the cement was examined by comparing models with and without the cement present. Because the stiffness of PMMA cement is higher than that of bone, inclusion of the cement increased the overall model stiffness by 1 to 5%. In the

locations of the cement, the principal strains were reduced by 40 to 60%. Thus, the effect of cement injection on the sacrum is primarily localized. This indicates that sacroplasty may be able to treat sacral insufficiency fractures without significantly altering the stiffness of the whole sacrum. This is in contrast to studies of the vertebroplasty, where cement injections into a vertebral body have been found to increase the vertebral body stiffness by 50% or more.

**CHANGES IN BALANCE WITH LOW BACK FATIGUE.** B.S. Davidson, M.L. Madigan, & M.A. Nussbaum, Virginia Tech Wake Forest School of Biomedical Engineering and Sciences, Blacksburg VA 24060. The purpose of this investigation was to characterize changes in postural sway and postural control during quiet standing using joint kinematics. Twelve physically active males participated in the experiment. Body position and center of pressure (COP) data during quiet standing were collected for 30 seconds both before and after a lumbar extensor fatigue protocol. Posture in the anterior-posterior (AP) plane was described using mean center of mass (COM) and COP position and mean joint angles. Postural sway was described using standard deviation (SD) of COM and COP position, SD of ankle, knee, hip, and back angles, and SD of ankle, knee, hip, and back angular velocities. Cross correlations of ankle angle and COM position, hip angle and COM position, ankle angle and hip angle were also performed to quantify AP postural strategy in terms of ankle strategy and hip strategy. A two-way repeated measures ANOVA was used to determine significant effects of fatigue. Three main findings emerged from this investigation. First, participants adopted a slight forward lean when fatigued. Second, changes in sway involved increased variability in joint kinematics at different joints. Despite these changes, ankle angle correlated well with AP COM position. Third, global measures of COM and COP did not reveal localized changes in sway. The results of this study indicate that additional or multivariate measures of sway are necessary to understand how fatigue affects movement patterns during quiet standing. This work was supported in part by Jeffress Memorial Trust, Richmond, VA and grant # R01 OH007882 from the Centers for Disease Control and Prevention.

**THE DESIGN OF AN AUTOMATED PIEZOACTUATOR-BASED SCOPE SYSTEM.** Joshua L. McCroskey, Daniel M. Hambright, Allan Bishop, Karla K. Mossi & Kam K. Leang, Department of Mechanical Engineering, Virginia Commonwealth University, Richmond VA, 23284-3015. We present the design of a piezoelectric-actuator-based system that enables automated and accurate positioning of the optics in a sight device. It is noted that current devices require manual adjustment (by hand) and the accuracy of the system depends on the operator skills. Additionally, the manual adjustment provides limited precision. However, by automating the process and using a piezoelectric actuator to position the optics, quick response and precise target location with respect to target distance can be achieved. In particular, the relatively-high positioning bandwidth of the piezoelectric actuator ensures fast target location. We describe the design of the mechanical system as well as the feedback controller. We show experimental results to demonstrate proof-of-concept.

TRIP RECOVERY: CAN WE IMPROVE IT THROUGH PRACTICE TO PREVENT FALLS?. K.A. Bieryla<sup>1</sup>, M.L. Madigan<sup>2,4</sup> & M.A. Nussbaum<sup>3,4</sup>, <sup>1</sup>Mechanical Engineering, <sup>2</sup>Engineering Science and Mechanics, <sup>3</sup>Industrial and Systems Engineering, <sup>4</sup>School of Biomedical Engineering and Sciences, <sup>1,2,3</sup>Virginia Tech, Blacksburg VA 24061, <sup>4</sup>Virginia Tech Wake Forest University, Winston-Salem, NC 27157. The purpose of this study was to determine if repeatedly exposing older adults to a simulated trip would result in motor learning. Six older adults (mean age 71.6, SD 5.5) participated in the study. The experiment consisted of two identical testing sessions separated by one week. While in a safety harness, participants stood on a treadmill that was then quickly accelerated to simulate a trip. Subjects were instructed to step over an obstacle with their right foot and recover their balance. Ten simulated trips were performed. Whole body kinematics and lower extremity EMG were collected. A two-way repeated measures ANOVA was conducted on each dependent measure of trip recovery performance with trial and session as independent measures. Several measures showed a main effect of trial, indicating short-term adaptation including maximum trunk angle, time to reach maximum trunk angle, maximum trunk angular velocity, time to reach maximum trunk angular velocity, and onset times of the swing leg tibialis anterior (TA), vastus lateralis, and medial hamstring (MH). Several measures showed a main effect of session indicating motor learning including trunk angle at toe off, and onset times of the swing leg TA, MH, medial gastrocnemius (MG), and stance leg MG and MH. In conclusion, older adults were able to improve their ability to recover from a simulated trip, and these improvements can be attributed, at least in part, to motor learning.

QUANTIFICATION OF TREMOR IN PARKINSON DISEASE. C.R. Dosier, S. Hijaz, P.A. Wetzel, Biomedical Engineering Department, Virginia Commonwealth University, Richmond VA 23298. Parkinson disease (PD) is movement disorder caused by a degradation of dopamine levels in the brain resulting in a variety of symptoms including tremor. A frequent problem during clinical diagnosis and evaluation of PD is that measurements of tremor are often subjective and difficult to quantify. To address this problem we developed a portable device that can accurately provide quantitative, objective measurements of tremor behavior. Our system utilizes MEMs based accelerometers which are used to provide quantifiable measurement of tremor position (amplitude) and tremor frequency. The accelerometer signals are acquired through a data acquisition module (National Instruments, NI USB-6009) which is interfaced to a notebook computer via a USB port connection. Control of the data acquisition module, signal processing algorithms, real-time display of the tremor waveforms and storage of waveform data were developed using LabVIEW Version 8 (National Instruments). Future work includes quantification of patient tremor during clinical assessment.

COMPUTATIONAL MODELING OF THE TURTLE UTRICLE. Julian L. Davis & John W. Grant, Dept. of Engr. Sci. & Mech., Va. Polytechnic Inst. & State Univ., Blacksburg VA 24061. The utricle is an organ in the inner ear that senses tilt and linear acceleration of the head. The utricle is composed of a layered structure and operates through shear deformation. Hair cells are contained in the rigid

neuroepithelial layer whose upper surface is referred to as the macula. The macula serves as the supporting surface for the remaining layers (arranged from most to least compliant): the column filament, compact gel, and otoconial layers. Hair bundles extend from the cuticular surface of hair cells, located at the macula, into the upper layers of the utricle. As the layers of the utricle deform in response to an inertial excitation, bundles deflect which in turn depolarizes hair cells. This depolarization then generates neural action potentials that travel to the central nervous system, and are interpreted as head tilt or head movement. The utricle has been modeled with simple geometries in an effort to understand how its layered structure effects the stimulus of hair bundles. This research investigates the effect of three common simplifying geometries on the stimulus of hair bundles: 1) Curvature of the macular surface, 2) Macular perimeter and 3) Varying layer thickness. By modeling the utricle in its full three dimensional geometry, we can systematically remove each of these variables to investigate their effects on the stimulus of hair bundles. (Supported by: NIH-R01 DC 05063)

NONINVASIVE BLOOD PERFUSION MEASUREMENT ON THE KIDNEY OF AN ANESTHETIZED RAT. Ashvinikumar V. Mudaliar<sup>1,2</sup>, Brent E. Ellis<sup>1,2</sup>, Patricia L. Ricketts<sup>1,2</sup>, Otto I. Lanz<sup>1,3</sup>, Elaine P. Scott<sup>1,2</sup> & Thomas E. Diller<sup>1,2</sup>, <sup>1</sup>Virginia Tech Wake Forest University School of Biomedical Engineering and Sciences, <sup>2</sup>Department of Mechanical Engineering, <sup>3</sup>Department of Small Animal Clinical Sciences, Virginia Tech, Blacksburg, VA 24061. Blood perfusion represents the local, multi-direction blood flow through capillaries and intracellular spaces of living tissue, and it is crucial in the assessment of many physiological phenomena, such as skin grafts, burn injuries, head traumas, and tumor detection. A noninvasive probe has been developed to determine blood perfusion. It consists of housing and a 1cm x 1cm heat flux sensor that is attached directly to the tissue using double-sided tape. To validate the qualitative performance of the blood perfusion probe, the kidney of an anesthetized rat was used as an experimental model to provide controlled perfusion in living tissue. The kidney is highly perfused organ and the blood supply to the kidney can be controlled easily by occluding and opening the renal artery and vein pair. The measured thermal event is created by convective cooling on the top surface of the sensor using an array of impinging jets through the holes in the housing. The jet impingement onto the sensor causes a measurable heat flux response from the tissue. The air is then exhausted through an outlet provided in the housing. The blood perfusion and contact resistance are estimated using a parameter estimation code, which employs a Gauss minimization technique. The code compares the recorded heat flux data to numerical solutions of the Pennes bioheat equation. The rat kidney test clearly demonstrates good repeatability and sensitivity of the probe and that the probe could be used in vivo.

DYNAMIC MECHANICAL ANALYSIS OF INDUCED WEAR ON ARTICULAR SURFACES. K. J. Shields & J. S. Wayne, Orthopaedic Research Laboratory Department of Biomedical Engineering, Virginia Commonwealth University, Richmond, VA 23298. The development of a suitable method for repair and replacement of damaged cartilage has been a challenging area of orthopaedic research. Healthy cartilage experiences relatively low wear despite demanding

loading patterns. Injured or diseased cartilage experiences increased wear and frictional properties. Repaired surfaces may also have increased frictional characteristics that compromise the success of the reparative technique. Typical quantitative techniques for analyzing the effectiveness of a cartilage repair have been characterizing the mechanical response in static compression and/or tension tests. The current study develops a method for analyzing static stress relaxation data from articular surfaces to predict dynamic characteristics (storage and loss modulus and  $\tan \delta$  for a range of frequencies) and apply the method to tests on bovine femoral condyles before and after inducing wear. Wear is induced on the specimens with a polished aluminum plate applying 20% compression to the tissue and allowing a translation of 10mm at 1Hz for 1 hour. After equilibration for 1 hour, the stress relaxation and dynamic cyclical testing is repeated. The dynamic cyclical testing at 1Hz allows for a comparison to the predicted dynamic characteristics at 1Hz from stress relaxation data. Future endeavors include measuring the coefficient of friction ( $\mu$ ) of normal and repaired surfaces in real time while inducing wear. These quantitative measures may help tailor the properties of tissue engineered cartilage to increase the chance of surviving normal articulation without the continuation of fibrillation and extracellular matrix degeneration.

**MODELING AND CONTROL OF A SHAPE MEMORY ALLOY ACTUATOR.** Guy N. Tchoupo<sup>1</sup>, Karla M. Mossi<sup>2</sup>, & Kam K. Leang<sup>3</sup>. Department of Mechanical Engineering, Virginia Commonwealth University, Richmond, VA 23284. In this work, we present the control of a shape memory alloy actuator (SMA). SMA is a smart material with the ability to remember its shape even after experiencing significant deformation. For example, at low temperature an SMA can be deformed and it remains in this state until it is heated. This unique property can be exploited to create SMA-based actuators, and compared to other smart material-based actuators (such as piezoelectric ceramics and magnetostrictive devices), SMA actuators offer relatively large strains (up to 8%) and high strength-to-weight ratio. These advantages make SMAs ideal for a wide variety of advanced actuators designs and miniature applications, such as tools for minimum invasive surgery. But unfortunately, SMA actuators exhibit significant hysteresis effect that can lead to loss in positioning precision. In this work, we design a controller to minimize positioning error due to hysteresis. We present experimental results and discuss the performance of the controller, in terms of achievable precision and implementation challenges.

**MECHANICS AND STRUCTURE OF VESTIBULAR HAIR BUNDLES.** Corrie E. Spoon & John W. Grant, School of Biomedical Engineering and Sciences, Virginia Tech-Wake Forest Univ., Blacksburg VA 24061. The structure of sensory hair bundles in the utricle is highly varied and organized. Characteristic bundle morphologies are observed in the different regions, striola and extrastriola, of the utricle. The functional significance of this variation and its impact on mechanotransduction remains unknown. In efforts to further this understanding, we have measured the steady-state stiffness of hair bundles of varying morphology and location in the turtle utricle. All measurements were performed under a light

microscope with a 100X liquid immersion objective (NA = 1.0) with DIC optics. Bundles were deflected in their excitatory directions with a flexible glass fiber positioned at the tip of the kinocilium. Bundle stiffness was determined from measurements of the applied displacement at the base of the whisker, the whisker stiffness, and deflection of the kinocilium tip. To characterize bundle morphology, we measured array length (distance from the tall to short end of the bundle) and heights of the kinocilium, tallest stereocilia and shortest stereocilia. From these values the KS ratio (height of the kinocilium/height of the tallest stereocilia) and slope ((tallest – shortest stereocilia height)/array length) were calculated. The stiffness was found to vary with location on the macula. The bundle stiffness measurements in the striolar and extrastriolar regions were  $42 \pm 25$  (n=28) and  $10 \pm 0.9$  (n=21)  $\mu\text{N/m}$ , respectively. In the striola, stiffness declines systematically from the line of polarity reversal to the medial extrastriola. Bundle stiffness and structure were shown to covary. The stiffest bundles demonstrated a characteristic morphology including short kinocilia, low KS ratios and steep slopes.

A HUMAN COMPUTER INTERFACE THAT OPERATES WITH GAZE AND EYE-BLINKS. Federico A. Puma & Paul A. Wetzel, Dept. of Biomedical Eng., Virginia Commonwealth University, Richmond VA. A method for a real-time communication system is presented. The system uses the user's gaze to control cursor movement over a computer screen. The creation of an innovative algorithm, allows an efficient and accurate determination of gaze position. This algorithm combines eye position data and head position data in real-time to output the direction of the user's line of sight. Eye movements are measured with a pupil-corneal reflection device and head movements are measured with a magnetic tracking system. Another important feature of the interface is the ability to detect and classify the user's eye blinks in order to provide input to the computer in the form of mouse clicks. Voluntary eye-blinks are recognized when the duration of a blink event surpasses a pre-fixed value. Voluntary blinks are then used to trigger mouse clicks and therefore, enable interaction. The system is intended to provide an alternative input modality for human computer interaction systems. Preliminary tests and results prove that the system is capable of individuals whose only channel of interaction is through their eye movements or to individuals that, for any reason are unable to use their hands to control cursor movement.

IN VITRO FLOW VISUALIZATION STUDY OF THE INTERFACE BETWEEN OUTFLOW GRAFT OF VENTRICULAR ASSIST DEVICE AND AORTA. Suhas M. Thatte & Gerald E. Miller, Department of Biomedical Engineering, Richmond VA 23298. For left ventricular failure, heart transplant is the best treatment but due to limited donor hearts and ineligibility of some patients to get a transplant, heart assist devices are considered as an alternative to assist the failing ventricle and even as destination therapy. Numerous in vitro and animal trials are used to study the flow fields in the artificial ventricle to avoid hemolysis and thrombosis, but the effect of the flow fields due to the angle of the LVAD insertion into the aorta are neglected. We have used 30, 60 and 90 degree glass models to signify different angles of the surgical end to side anastomosis. Particle Image Velocimetry is used

to study the flow fields at the interface. 30 degree angle was determined to be the optimum angle amongst the three as it kept stagnation zone, recirculation patterns, flow reversal, high blood shear rate and other flow irregularities to a minimum. This may lead to reduced blood trauma, hemolysis and thrombosis in an in vitro setting.

### Botany

BETULA UBER, THE VIRGINIA ROUND-LEAF BIRCH: THE BEGINNING OF THE END, OR THE END OF THE BEGINNING? Kevin G. Jones & Carnita Owens, Department of Natural Sciences, UVA's College at Wise, Wise, VA 24293. As the first woody taxon to be granted inclusion on the Federal List of Endangered Species, *Betula uber* (Ashe) Fernald, the Virginia round-leaf birch, occupies a unique niche in the floral history of south west Virginia. Given the resources devoted to its preservation, it is perhaps surprising that the species-level status of *B. uber* remains controversial, and some regard it merely as a variety of *B. lenta*, a species from which it differs morphologically only in leaf shape. The taxonomic difficulties afforded by *B. uber* are typical of those found across the genus *Betula* as a whole, where extensive hybridization and introgression events have confused definition of taxa within the genus. To address this issue, we have sequenced the ITS regions of rDNA for *B. uber* and six other species of *Betula* present in Virginia. Aligned sequences show no length variation, and differ at between three and twelve nucleotide positions for the species considered. Significantly, sequences obtained from four specimens of *B. uber* and three specimens of *B. lenta* are identical, and do not support species-level recognition for *B. uber*. The impact this data may have on further funding for the preservation of *B. uber* is unclear, but we argue that the taxon still merit attention. Preliminary genetic fingerprinting data discriminate *B. uber* and *B. lenta*, and this genetic diversity, potential economic value, and historical significance all justify continuation of a support program for *B. uber*. In addition, *B. uber* represents a useful model system for subsequent study of the genetic basis for leaf morphology, and the impact of leaf shape on colonization by fungal endophytes.

SOROCARP FORMATION IN *POLYSPHONDYLUM*: ALTRUISM, OR SOCIAL STRIFE? Carla D. Glass, Brendan G. Hunt & Kevin G. Jones, Department of Natural Sciences, UVA's College at Wise, Wise, VA 24293. Dictyostelid slime molds are a group of protists, with a unique lifestyle. Most of the time, dictyostelids live as single-celled amoebae, that divide asexually. When their bacterial food supply becomes depleted, amoebal cells aggregate to form a multicellular 'slug', in which the component amoebae never fuse but retain their individuality, while co-operating as members of an organized 'society'. Subsequently, the slug differentiates into a sorocarp, in which the anterior 20% of the slug convert into a non-viable stalk, supporting the remaining cells which become viable spores, capable of hatching into new amoebae. The stalk cells have often been seen as being altruistic in behavior, but data from *Dictyostelium discoideum* has shown that when different

clones contribute to sorocarp formation, some clones 'cheat' and contribute less than their fair share of cells to the stalk. We are interested in whether cheating behavior also occurs in the genus *Polysphondylium*. In contrast to *Dictyostelium*, this genus is capable of forming microcysts, which might provide an alternative strategy for coping with starvation, rather than risking being cheated on, in a chimaeric sorocarp. The sorocarp of *Polysphondylium* has whorls of branches, each bearing spores. This also suggests the potential for more complex patterns of social strife than occurs in *Dictyostelium*. Here, we report the isolation of clones of *P. violaceum* from forest soil, and their genetic discrimination using PCR and random primers. These fingerprinted isolates will allow testing for 'cheating' through forced chimaeric sorocarp formation, and the spatial localization of clones within them.

ISOLATION AND CHARACTERIZATION OF ROOT ENDOPHYTES FROM HERBACEOUS PLANTS IN THE APPALACHIAN FOREST. T. L. Neece, C. Fultz & K. G. Jones, Department of Natural Sciences, University of Virginia's College at Wise, Wise VA, 24293. This work is preliminary research for a fellowship study this summer. The research is an investigation into the diversity of root endophytes of various herbaceous plants in the Appalachian region. Endophytes are microbial organisms that colonize healthy plant tissue without apparent damage to the host. Some bacteria produce antibiotics and have been identified as being plant endophytes, suggesting a mutualistic relationship. The species of plants used in this research were *Galax aphylla*, *Lycopodium flabelliforme*, *Chimaphelia maculata*, *Tussilago farfara*. Their roots were surface sterilized, sectioned and plated onto tap water yeast extract (TWYE) agar. Bacterial and fungal isolates were collected from the *G. aphylla* and the *L. flabelliforme* samples while the *C. maculata* and *T. farfara* did not yield any isolates. Even though fungal isolates were obtained, the main focus was given to the bacterial isolates. The bacteria from *G. aphylla* and *L. flabelliforme* were Gram positive rods hypothesized to be of the genus *Bacillus*, which commonly inhabits ground soil. Due to a budget freeze, the final stages of the investigation were not concluded. These steps included DNA extraction and amplification of a 730 bp fragment from the 16S rRNA gene. Then the isolates would have been submitted for sequencing and then differentiated through BLAST searches. Even though these steps were not reached, the project showed that the isolation and sterilization procedure will be successful for the summer fellowship research where funding has already been appropriated.

THE *FLORA OF VIRGINIA* PROJECT: A 2005-2006 UPDATE. Marion B. Lobstein, Dept. of Biology, Northern Virginia C.C., Manassas, VA 22205. Virginia, for its landmass, has the most diversity of vascular plant species of any state in the United States. It had the first flora, the *Flora Virginica* in 1739 yet does not have a modern flora. The Virginia Academy of Science for over eighty years has supported efforts to produce a modern *Flora of Virginia*. In 2001 the Foundation of the *Flora of Virginia*, Inc, was formed in 2001 and in May 2002 received 501(c) 3 status. Progress continues to be made on the efforts to develop a *Flora of Virginia* including fund-raising and public outreach efforts. Work on the

content of the Flora of Virginia including the nearly 600 illustrations have been commissioned, completed, and funded by VAS funds. Funds awarded from the Gwalthmey Memorial Trust for 2005 will be used to support continuing work on illustrations. The Academy, including the Fellows, continues to provide essential support including financial for this Project. Other progress includes completion of treatments of the dichotomous keys of 101 of the 205-210 vascular plant families in Virginia and the first step in developing species descriptions for approximately 50% of Virginia's 3800 vascular plant species. The second step of species description development has also begun. The University of Virginia Press has offered a contract to publish the Flora of Virginia in 2011-2012.

PHYLOGENETIC ANALYSIS OF *matK*-PSEUDOGENE FORMATION IN THE SUBTRIBE AERIDINAE (ORCHIDACEAE). Eric K. Latourelle and David C. Jarrell, Dept. of Biol. Sci., University of Mary Washington. Fredericksburg VA. 22401. This study was part of an ongoing project examining the sequence of the chloroplast gene *matK* from species in the subtribe Aeridinae. The purpose of this study was two fold: first, to create a more accurate evolutionary model of the subtribe and second, to understand the origin of an apparent *matK* pseudogene. In earlier analyses, a clade appeared in which the sequences contained frameshift deletions, a diagnostic event in pseudogene formation. Analysis with the addition of a significant number of new sequences supports previous conclusions that the pseudogene arose once and also shows that the pseudogene most likely resulted from gene duplication and a subsequent loss of function. This pseudogene also seems selectable by apparent PCR primer bias; PCR product from one set of primers (K118F&K1581R) yield sequence which appears to be the functional form, while product from a different set (trnk3914&K1581R), yields sequence with the frameshifts present which indicate a loss of function. Current results and future prospects for this project will be discussed.

PHYLOGENETIC UTILITY OF *PHALAEOPSIS* (ORCHIDACEAE) USING CHLOROPLAST SEQUENCES – AN UPDATE. Y. Lazdun and D.C. Jarrell, Dept. of Biol. Sci., Univ. of Mary Washington, Fredericksburg VA 22401. *Phalaenopsis* is a genus of Orchidaceae, tribe Vandeeae, and is currently divided into five subgenera composed of 63 species. Past classification schemes were based on morphology, largely floral, which can be problematic since such characters may be subject to convergent evolution. This study targets regions of the chloroplast genome to develop a more evolutionarily accurate phylogeny. The *matK* and *rps16* introns were used to construct an initial phylogeny of the genus including representatives from all subgenera. This phylogeny showed that the subgenus *Phalaenopsis* was not monophyletic, however the large subgenus *Polychilos* appears monophyletic. The subgenera *Aphyllae* and *Proboscidioides* also appear as sister subgenera in this analysis. Improved resolution will require an increase in informative characters and additional species sampling, particularly from underrepresented subgenera. The *rpl16* intron and the *rpoB-trnC* spacer were analyzed using percent variability tests to assess their utility for phylogenetic analysis. PCR products have been generated for both the *matK* and *rps16* introns

have been collected from underrepresented subgenera but have not been sequenced as of yet. Sequencing these additional regions and phylogenetic analysis of a combined chloroplast dataset will help determine species relatedness and improve our understanding of the evolutionary history of this genus. (Supported by: UMW Summer Science Institute and Univ. of Mary Washington Research Grant).

PHYLOGENY OF THE GENUS ARACHIS. Sheena A. Friend, Chelsea M. Black, & Khidir W. Hilu, Department of Biological Sciences, Virginia Tech, Blacksburg, VA 24061. *Arachis* (Fabaceae, legume family) contains approximately 70 species, including the peanut crop (*A. hypogaea* L.). The genus has been divided into nine sections based on morphology, geography and cytogenetics. The largest section, sec. *Arachis*, has been further subdivided based on morphology and cytogenetics into three genome groups. Previous studies were either limited in species/section representation or based on molecular data that could not be analyzed phylogenetically. Thus, the systematics of the genus remains in need of an in-depth study. We are using DNA sequence information from plastid *trnT-trnF* and the nuclear ITS to examine species relationship in the genus. The resulting phylogeny will be compared to the morphology-based taxonomic treatment. Information on the evolution of the tetraploid crop will also be investigated. Preliminary data from the sequence information of the nuclear ITS confirms that the B and D genome species are more closely related to each other than to the A genome species of section *Arachis*. Also, sequence data has brought into question the validity of two sections of the genus by placing them in other sections. (Supported by the Virginia Academy of Science, and the Graduate Research Development Project, Virginia Tech)

MOLECULAR STUDY OF PEANUT ALLERGEN ARA H 2 ACROSS WILD PEANUT SPECIES. C. M. Black, S. A. Friend & K. W. Hilu, Dept. of Biol., Virginia Tech, Blacksburg VA 24061. Ara h 1 and Ara h 2 are considered the most important genes that contribute to peanut allergy because of 90% serum recognition in peanut sensitive patients. *Ara h 2* has been found to be the more important of these two allergens because *Ara h 2* has a higher frequency of patient recognition, and a smaller amount is needed to initiate a reaction. In the linear form of *Ara h 2*, ten IgE binding epitopes have been identified through amino acid sequence analysis and immunoblotting. Two of the ten binding epitopes contain a DPYSPS amino acid motif which is considered to be an immunodominant epitope. *Ara h 2* is found in two isoforms, in the smaller isoform, *Ara h 2.01*, two of the ten epitopes contain the DPYSPS domain. In the larger isoform, *Ara h 2.02*, a twelve amino acid insertion that contains a third DPYSPS epitope was detected. This additional DPYSPS domain rendered *Ara h 2.02* more potent than *Ara h 2.01*. Based on this finding, wild species having an *Ara h 2* sequence fewer or no DPYSPS epitopes could potentially be less allergenic. In this experiment, gene sequencing and ELISA immunological assays will be used to look at wild species closely related to the peanut plant in order to find less harmful genes that could potentially be inserted into the peanut crop in order to reduce the risk of anaphylactic shock in consumers allergic to peanuts.

THE VIRGINIA MASTER GARDENER PROGRAM: MORE THAN YOUR AVERAGE GARDEN VARIETY. David D. Close, Dept. of Horticulture, Virginia Polytechnic Institute and State Univ., Blacksburg VA 24141. The Virginia Cooperative Extension (VCE) Master Gardener program has existed since 1979. Horticulture is used as a platform for training volunteer community educators. Once trained, these volunteers deliver programs and offer training to their fellow citizens through a variety of means and at a variety of venues. These specially trained volunteers contribute to projects focused on sustainable landscape management practices through outreach, teaching, and research. In 2005, more than 4,000 active, certified Maser Gardener volunteers contributed more than 309,500 hours of service and worked with more than 502,000 fellow citizens. The volunteer time was equal to 154 full-time equivalents and had an economic value of \$5.58 million. The impacts of this effective program include: promoting and implementing good land stewardship practices, actively engaging youth and the leaders working with youth, emphasizing sustainable landscapes that provide economic returns, providing an avenue for improved nutrition and food security, and contributing to an overall higher quality of life. The VCE Master Gardener volunteers truly epitomize the mission of Cooperative Extension in that they deliver cutting edge information to their family, friends, and neighbors through an educational process that is based on community needs and issues.

ANOMALOUS “PERFORATIONS” IN SECONDARY XYLEM OF THE *ACALYPHA VIRGINICA* COMPLEX (EUPHORBIACEAE). Alexa Jahdi, Carolyn B. Marks, & W. John Hayden, Department of Biology, University of Richmond, Richmond, VA 23173. Previous light microscope-based studies of wood structure of local species of the *Acalypha virginica* complex revealed structures interpreted as anomalous perforation plates. Anomalous “perforations” were re-examined by means of scanning electron microscopy and laser scanning confocal microscopy, techniques that reveal more detail than found in the earlier studies. Whereas ordinary perforations form by hydrolysis of primary cell walls, we show that primary wall material is retained in anomalous “perforations.” Anomalous “perforations” are thus comparable to very large simple pits with pit membranes and are not truly perforated. We hypothesize that retention of primary walls in anomalous “perforations” permits random synthesis of secondary wall material in the “perforation” region, resulting in the various partial bars, loops, and serpentine outlines observed. Regular and frequent occurrence of vessel element-like cells with anomalous “perforations” in *Acalypha deamii*, *A. rhomboidea*, and *A. virginica* suggest functional/adaptive significance, perhaps forming water reservoirs interspersed among the ordinary conducting vessels.

THE GENUS *ACALYPHA* (EUPHORBIACEAE) IN YUCATAN, MEXICO. Nina Bhattacharyya & W. John Hayden, Department of Biology, University of Richmond, Richmond, VA 23173. Recent progress in the floristic botany of *Acalypha* in the Yucatan Peninsula is reported. The regional scope of this effort encompasses the Mexican states of Campeche, Quintana Roo, and Yucatan, and

corresponds closely to a natural floristic region defined by low elevation and limestone bedrock. Information compiled for each species is synthesized from the literature, study of herbarium specimens, and study of many species in the field. Useful characters for distinguishing Yucatan species of *Acalypha* include: woody versus herbaceous habit; presence and type of pubescence on aerial organs; leaf size and shape; stipule size and morphology; pistillate inflorescence architecture; morphology and size of pistillate bracts; and the absence or presence and form of allomorphic flowers. Current knowledge supports the recognition of ten native and two cultivated species of *Acalypha* for Yucatan. Several names in frequent use in the region must be reduced to synonymy. One species, *Acalypha gaumeri*, is endemic to the region. Results will contribute towards two ongoing floristic projects, an illustrated guide and a detailed ethnoflora.

QUALITY CONTROL AND ASSURANCE REGARDING A STANDARDIZED METHODOLOGY IN PHYTOPLANKTON AND PRIMARY PRODUCTIVITY MONITORING IN CHESAPEAKE BAY AND VIRGINIA TRIBUTARIES. T.A. Egerton, H.G. Marshall, & K. Nesius. Dept. Biology, Old Dominion Univ., Norfolk, VA. The Chesapeake Bay Phytoplankton Program in Virginia began in 1985 monitoring phytoplankton composition and abundance. Consistency in all procedures with strict QA/QC standards have been maintained throughout the program. Monthly collections of 2 15 l composite carboy samples are taken above and below the pycnocline at each station (14), followed by replicate sub-samples from each set, combined, reduced to a 40 ml concentrate through a settling/siphoning protocol. The samples are fixed in Lugol's solution, preserved with buffered formalin. Cells are identified and counted using modified Utermohl protocol and inverted plankton microscopy. Random fields are examined at 300 and 600x, and the entire settling chamber scanned at 150x. Autotrophic picoplankton analysis was added in 1989, using epifluorescence microscopy, with productivity (C-14) measurements added in 1989, both taken during the phytoplankton collections, along with samples for water quality analysis. Data are submitted to the Chesapeake Bay Monitoring Program and made available online ([www.chesapeakebay.net](http://www.chesapeakebay.net)). Supported by Virginia Department of Environmental Quality and USEPA.

ANTIOXIDANT CONTENT OF FRESH AND PROCESSED STRAWBERRIES. Kelley K. Gibbons<sup>1</sup>, Michael H. Renfro<sup>2</sup>, Patricia B. Brevard<sup>1</sup> & Robert E. Lee<sup>3</sup>, Dept. Health Sci.<sup>1</sup>, Dept. Biol.<sup>2</sup>, & Dept. Math. & Statistics<sup>3</sup>, James Madison University, Harrisonburg, VA 22807. Berries are a rich source of antioxidants which are thought to help prevent some chronic diseases and have multiple health benefits. We determined the antioxidant concentration of fresh, frozen, and freeze-dried strawberries, and strawberry jam, with the aim of identifying which contains the highest antioxidant level. Antioxidants were extracted and the hydrophilic antioxidant activity (HAA) and lipophilic antioxidant activity (LAA) were measured using the ABTS/H<sub>2</sub>O<sub>2</sub>/HRP decoloration method. Differences in HAA and LAA content were analyzed using a one-way analysis of variance (ANOVA), and

pairwise comparison of means was completed using Dunnett's T3 due to heterogeneous variances. Differences in the TAA were compared using 95% confidence intervals. Mean TAA for freeze-dried strawberries based on "as consumed" weight (CW) was significantly higher than fresh strawberries, frozen strawberries, and strawberry jam. Mean TAA based on dry weight (DW) for fresh strawberries was significantly higher than freeze-dried strawberries, frozen strawberries, and strawberry jam. This study suggests that strawberries, especially freeze-dried and fresh strawberries, are valuable sources of antioxidants.

## Chemistry

A PIROUETTE DANCE ON A METALLOFULLERENE SPHERE: SYNTHESIS AND CHARACTERIZATION OF REGIO-INTERCONVERTIBLE N-TRITYLPYRROLIDINO DERIVATIVES OF  $Sc_3N@C_{80} I_h$  ISOMER. Ting Cai<sup>1</sup>, Liaosa Xu<sup>1</sup>, Mark R. Anderson<sup>1</sup>, Carla Slebodnick<sup>1</sup>, Harry W. Gibson<sup>1</sup>, Harry C. Dorn<sup>1</sup>, Alan Balch<sup>2</sup> and Marilyn Olmstead<sup>2</sup>, <sup>1</sup>Dept. of Chem., Virginia Tech., Blacksburg, VA. 24060 and <sup>2</sup>Dept. of Chem., Univ. of California, Davis, Davis, CA. 95616. Trimetallic Nitride Templated Endohedral Metallofullerenes (TNT EMFs), the most abundantly formed endohedral metallofullerenes, have attracted much attention since their discovery. Functionalization of the TNT EMFs provide unique materials useful for a number of diagnostic (MRI and X-ray contrast agents) and therapeutic medical applications. In this paper, we reported the synthesis and characterization of N-tritylpyrrolidino derivatives of the  $Sc_3N@C_{80} I_h$  isomer. The  $I_h$  isomer predominantly forms two monoadducts as well as a little of the bisadduct. The two monoadducts are a 6,5 ring junction adduct and a 6,6 ring junction adduct, which are fully characterized by NMR spectroscopy and X-ray crystallography. A kinetic study suggests that the 6,6 ring junction adduct is the kinetically controlled product, it can interconvert into thermodynamic product 6,5 ring junction adduct at elevated temperatures. The difference in the thermal stabilities of two monoadducts will be also discussed as well as the kinetic parameters of the interconversion process, the electrochemical properties, and computational studies. In addition, these products are useful precursors to N-(3-maleimidopropionyl)-3,4-fulleropyrrolidine which can be used as the adapter for coupling proteins for targeted therapies utilizing  $Lu_3N@C_{80}$  encapsulated with the radionuclide, Lu-177.

OPTIMAL EXPRESSION OF THE CLONED HUMAN CAP METHYLTRANSFERASE IN *E. COLI*. Jeanhee Chung, Michael Pickup, Kevin D. Kim and Thomas O. Sitz, Dept. of Biochemistry, Virginia Tech, Blacksburg, VA 24061. The 5'-cap structures in eukaryotic mRNAs are methylated in the N-7-position of the guanine base by the guanine-7-methyltransferase (GMT). This modification is very important in gene expression because if the cap is not methylated the mRNA is not translated into protein. Dr. Shuman at Sloan-Kettering sent us two plasmids, one containing the coding sequence for the full length human GMT and the other coding for the deletion mutation with 120 amino acids deleted

from the N-terminus ( 120). These plasmids were transformed into the E. coli host cells, BL21(DE3) codon plus. These bacteria were streaked on agar plates and clones containing a high level of enzyme activity were isolated. We were able to use the his-tag system to isolate the human GMT. When the enzyme was applied to a Ni<sup>+2</sup> column the his-tagged protein bound to the column. The enzyme was eluted with 1 M imidazole. With both the full length and especially with the deletion mutation significant precipitation of the enzymes occurred. When the non-ionic detergent, Triton-X 100 was added to the elution buffer the enzyme activity was almost zero. However when EDTA (a chelating agent) was added to the collection tube high levels of enzyme activity were found. It would appear that high levels of imidazole used to elute the enzyme from the column were also removing Ni which allowed the enzyme to form aggregates. We were able to use lower concentrations of imidazole to elute the enzyme, 0.2 and 0.3M. Large amounts of active GMT were isolated when EDTA was added and low concentrations of imidazole were used to elute the human methyltransferase from the Ni-column.

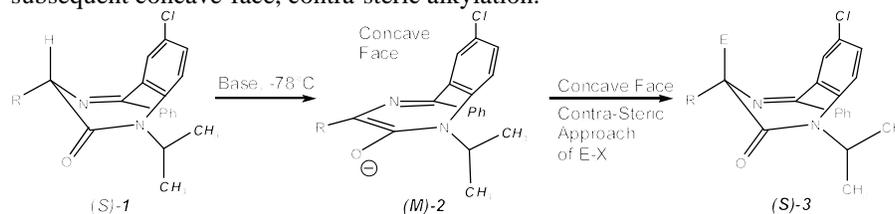
PREDICTING THE SINGLET OXYGEN QUANTUM YIELD OF HUMIC SUBSTANCES FROM SPECTROSCOPIC PROPERTIES. Renee M. Dalrymple and Charles M. Sharpless, Dept. of Chem., University of Mary Washington, Fredericksburg VA 22401. Upon absorbing solar radiation humic substances (HS) produce <sup>1</sup>O<sub>2</sub> which degrades organic pollutants such as phenols. It is hypothesized that the quantum yield of <sup>1</sup>O<sub>2</sub> ( $\Phi_{so}$ ) for a humic substance can be predicted from the ratio of the absorbance at 250nm to the absorbance at 365nm (E2/E3 ratio) since spectroscopic properties of HSs should be related to their photochemical properties. The quantum yield was determined by monitoring the rate of reaction of HS with furfuryl alcohol (FFA), which reacts specifically with <sup>1</sup>O<sub>2</sub>. Phosphate buffered solutions of Suwannee River NOM, pH 4, 7, and 10, Nordic NOM, pH 4 and 7, and Nordic humic and fulvic acid (HA and FA), which are the photoreactive subfractions of NOM, were prepared. The solutions were placed in a petri dish and exposed to ultraviolet light while stirring. Samples were taken periodically and analyzed for FFA content using High Pressure Liquid Chromatography (HPLC). Absorbance spectra were collected and the E2/E3 ratios were calculated for the HS solutions at each pH. The  $\Phi_{so}$  values were plotted versus E2/E3 and appear to have a linear trend. These results indicate that it may be possible to predict the quantum yield of <sup>1</sup>O<sub>2</sub> from natural samples by simple absorbance measurements.

THERMAL DECOMPOSITION OF M(NH<sub>3</sub>)<sub>2</sub>C<sub>2</sub>O<sub>4</sub>(M= Cu, Zn). Maura Goodrich and T.C. DeVore, Dept. of Chem., James Madison Univ. MSC 4501, Harrisonburg, VA 22807. Copper ammine oxalate and zinc ammine oxalate dihydrate were prepared by placing the corresponding metal oxalate in contact with the vapor over a saturated ammonia solution for 24 hours. The IR spectrum indicated that ammonia had been added to each compound and the observed mass change indicated that it was the diammine complex. The thermal decomposition of both compounds was investigated using thermal gravimetric analysis (TGA) and differential scanning calorimetry (DSC). The copper complex decomposed in two

steps. The ammonia was lost between 200 and 250 °C to form copper oxalate which decomposed at ~ 300 °C. The decomposition of the zinc complex was more complicated in that 5 decomposition steps were observed. The observed mass losses are consistent with the intermediates being  $\text{Zn}(\text{NH}_3)_{1.75}\text{C}_2\text{O}_4$ ,  $\text{Zn}(\text{NH}_3)\text{C}_2\text{O}_4$ ,  $\text{Zn}(\text{NH}_3)_{0.33}\text{C}_2\text{O}_4$ , and  $\text{ZnC}_2\text{O}_4$ . The enthalpies of each compound have been estimated using the DSC data and the balanced chemical equations for each decomposition step.

PREPARATION AND CHARACTERIZATION OF HYDROGENATED TRIMETALLIC NITRIDE ENDOHEDRAL METALLOFULLERENES. Wujun Fu, Harry W. Gibson, and Harry C. Dorn, Dept. of Chem., Virginia Tech, Blacksburg, Va 24060. Since the discovery and isolation of trimetallic nitride templated endohedral metallofullerenes (TNT EMFs),<sup>1</sup> the chemical modification of this new material has attracted widespread interests. Due to the unique molecular structure of fullerenes and endohedral metallofullerenes, the carbon cage is considered to be the only allotropic form of carbon that can be hydrogenated and dehydrogenated reversibly.<sup>2</sup> Thus, the hydrogenation of fullerenes and metallofullerenes is of interest for hydrogen storage and new battery applications. In this paper, we will report the hydrogenation of the carbon cage surface of TNT EMFs, such as  $\text{Sc}_3\text{N}@C_{80}$  by the Benkenser reduction reaction. A highly reduced TNT EMF was synthesized and characterized by HPLC, MALDI-TOF MS, <sup>1</sup>H NMR and UV-vis spectroscopies.

EVIDENCE AGAINST A ‘DOUBLE INVERSION’ MECHANISM IN MEMORY OF CHIRALITY ALKYLATIONS OF 1,4-benzodiazepin-2-ones. Danny C. Hsu, Polo C. H. Lam, Joe C. DeGuzman, and Paul R. Carlier, Dept. of Chem., Virginia Tech, Blacksburg, VA 24061. We have previously reported the enantioselective synthesis of “quaternary” 1,4-benzodiazepin-2-ones **3** via memory of chirality. The high enantiomeric excess realized in the retentive synthesis of **3** has been attributed to the formation of enantiopure, *conformationally* chiral enolate (*M*)-**2**, and subsequent concave-face, contra-steric alkylation.



However, another possibility is that deprotonation of (*S*)-**1** affords the enantiomeric enolate (*P*)-**2**; subsequent convex-face, sterically controlled alkylation would then yield (*S*)-**3**. In this paper we report NMR studies that establish concave-face alkylation. Thus, the ‘double inversion’ mechanism can be ruled out.

ANALYTICAL STRATEGIES RELATED TO ACCURACY AND PRECISION IN AN ICP-MS TRACE ANALYSIS PROBLEM IN INSTRUMENTAL ANALYSIS. Dana M. Edwards, Ashley M. Lakner, Daniel M. Downey, and James J. Leary, Dept. of Chem., MSC 4501, James Madison Univ., Harrisonburg, VA 22807. Students in Instrumental Analysis at JMU routinely analyze commercial (OTC) multivitamin/multimineral supplements for the trace elements chromium, molybdenum, and selenium using an inductively coupled plasma mass spectrometer (ICP-MS). Questions have persisted concerning both the accuracy and the precision of determinations. This work summarizes some of the analytical strategies that have been used to minimize both random and systematic errors associated with this experiment. The merits associated with using one or more internal standards will be presented. Results will be presented for determinations of samples that have been "spiked" with known amounts of the analytes. Finally, a comparison will be made between the results obtained when a calibration curve is used and the results that are obtained when the experiment is performed utilizing the standard addition approach. Proprietary considerations have made it impossible to know the scatter associated with the formulation and the manufacturing of any component in OTC supplements. However, it is possible to document the random error contributions from other sources (e.g. lab technique, calibration curve, instrument fluctuations); having these values, it becomes possible to estimate the total standard deviation associated with the analytical protocol.

SYNTHESIS AND CHARACTERIZATION OF PEROXYNITRITE PREPARATIONS FOR PHOTOCHEMICAL STUDIES. Jennifer M. McKay and Charles M. Sharpless, Dept. of Chem., University of Mary Washington, Fredericksburg, VA 22401. The photolysis of nitrate is known to produce, among other things, hydroxyl radicals ( $\cdot\text{OH}$ ), a highly reactive species that can destroy hazardous organic pollutants in natural waters by initiating a series of oxidative degradation reactions. The exact mechanism by which this  $\cdot\text{OH}$  formation occurs is still under debate. Some of the answer may lie in the study of peroxynitrite ( $\text{ONO}_2^-$ ), a reactive intermediate in nitrate photolysis and key precursor of peroxynitrous acid, which decomposes to produce  $\cdot\text{OH}$ . Additionally, few studies of peroxynitrite photochemistry have been conducted and more needs to be done to better understand this aspect of the overall nitrate photolysis mechanism. Peroxynitrite suitable for photochemical studies was synthesized, therefore, by reacting nitric oxide with alkaline hydrogen peroxide ( $\text{H}_2\text{O}_2$ ) in the presence of  $\text{O}_2$ . The amount of peroxynitrite generated was determined from the absorbance of the solution at 302 nm. To account for any interferences, a solution of the background matrix was created by acidifying the final solution. Peroxynitrite's true absorbance equaled the difference between the two mixtures' absorbencies. The calculated peroxynitrite concentration was 4.58 mM. The acidified solution, readjusted to  $\text{pH} \approx 7$ , was used to analyze for nitrite and nitrate. The nitrite concentration was determined to be 218  $\mu\text{M}$  using a colorimetric analysis via the method of standard additions. Nitrate was determined to be 193  $\mu\text{M}$  using the cadmium reduction method. Peroxide was determined to be 19.5  $\mu\text{M}$ . The nitrite and nitrate observed were probably due to peroxynitrite decomposition.

STABILITY AND ACTIVATION OF THE CLONED HUMAN CAP METHYLTRANSFERASE. Amanda J. Misiewicz and Thomas O. Sitz, Dept. of Biochemistry, Virginia Tech, Blacksburg, VA 24061. The 5'-ends of eukaryotic mRNAs are highly modified with a structure that is called a "cap". It caps the end of the molecule and prevents it from attack by 5'-exonucleases. The most important function of this "cap" is to allow the binding of ribosomes to allow the mRNA to be translated into protein. The most important of the modifications of the cap structure is the methylation of the guanine base in the N-7 position. If the cap is not methylated in this position it is not functional. The human cDNA coding for the guanine-7-methyltransferase (GMT) was cloned in a pET expression system that allowed the expression of the his-tag GMT with 10 histidines at the N-terminus. When enzyme preparations were assayed with low activity the enzyme became more active with time when assayed over 30 minutes. When enzyme with high activity was assayed the kinetics were linear. However when preparations of enzyme with high activity were diluted 30 fold and assayed for 3 hours a dramatic increase of activity was seen in the last 15 or 30 minutes of the incubation. This increase in activity was not affected by incubating the enzyme with either substrate alone (SAM or RNA) for 90 min and then adding the missing substrate. Even when the enzyme was left out of the incubation the same acceleration of apparent activity was seen! Therefore the increase in activity at long time intervals was not a biochemical event. Addition experiments demonstrated that an artifact of the assay system was responsible---specifically the washing of the DEAE-filter paper. However, the increase in activity seen at early time intervals up to 30 minutes is a real biochemical phenomenon.

THE SYNTHESIS OF SUBSTITUTED MOLYBDENUM CARBONYL COMPLEXES AS POTENTIAL CATALYSTS. Carolyn Segerdell, Taryn Cummins, D.S. Amenta, and J.L. Gilje, Dept. of Chem., MSC 4501, James Madison University, Harrisonburg VA 22807. We are interested in molybdenum complexes that may be used as catalysts in epoxidation reactions. In this study we have sought to prepare complexes with the general form  $\text{CpMo}(\text{CO})_3(\text{CH}_2)_n\text{---R}$ , where n ranges from 4-6 and where R is the tosyl group (OTs) or  $\text{CpMo}(\text{CO})_3^-$ . The anion  $\text{CpMo}(\text{CO})_3^-$  was allowed to react in a 2:1 ratio with  $\text{T}_s\text{O}(\text{CH}_2)_n\text{OT}_s$ , n = 4-6. The ditosylates as well as the molybdenum complexes resulting from these reactions were characterized using  $^1\text{H}$  and  $^{13}\text{C}$  NMR spectroscopy as well as infrared spectroscopy.

TARGETING SERINE HYDROXYMETHYLTRANSFERASE FROM THE HUMAN PARASITE *TRYPANOSOMA CRUZI*. Kamran Shahzad, Shivan Desai, Brittany Gettleman and Daniel G. S. Capelluto, Dept of Chem., Virginia Tech, Blacksburg, VA 24061. The human parasite *Trypanosoma cruzi* is the causal agent of the Chagas' disease. Possible therapeutic targets are proteins involved in the metabolism of amino acids and vitamins. Folates are precursor of vitamins that function as a family of cofactors by carrying one-carbon units that are required for the synthesis of thymidylate, purines, methionine and methylation reactions in mammalian cells. The parasite *T.cruzi* is folate auxotroph and thus it depends of the

availability of these molecules in the host and a proper system to transport folates from the environment. Serine is the major source of one-carbon units that are generated by the enzyme serine hydroxymethyltransferase (SHMT). This enzyme catalyzes the interconversion of serine and tetrahydrofolate (THF) to glycine and methylene 5,10 THF. The active site of the enzyme is located at the interface of two monomers in an obligated dimer of dimers. In mammals, SHMT is a tetrameric protein present in both the mitochondria and cytoplasm compartments. Our previous studies indicate that the single *T.cruzi* SHMT presents structural differences compared with the mammalian counterpart. However, the native *T.cruzi* SHMT is unstable and thus making difficult its characterization as a target for drug design. We have cloned and expressed the recombinant *T. cruzi* SHMT in *E. coli*. Initial purification yielded a highly stable and soluble protein. Folate analogs will be tested as specific *T.cruzi* SHMT inhibitors using circular dichroism, isothermal calorimetry, X-ray crystallography and NMR spectroscopy.

METHYLATION OF A LARGE ALKALINE STABLE RNA FRAGMENT IN *E. COLI* tRNA. Nicole M. Tellmann and Thomas O. Sitz, Dept. of Biochemistry, Virginia Tech, Blacksburg, VA 24061. The "cap" structure in eukaryotic mRNA is important for gene expression and is highly modified. The most important of these modifications is the N-7-methylation of the guanine base. Without the methylation of the guanine base in the cap the mRNA will not be translated as ribosomes will not bind to the mRNA. Previously we developed a rapid assay for the determination of the amount of non-methylated cap structure in RNA isolated from various tissues. When I used total *E. coli* RNA as a negative control the level of methyl-accepting activity was very low as expected. However, when *E. coli* tRNA was assayed the level of methyl-accepting activity was elevated. When the tRNA was isolated from a methyl-accepting assay, hydrolyzed with base, and then analyzed on a DEAE-Sephadex column a large alkaline stable fragment (-5 charge) was found. This is the same size as the "cap" structure found in methyl-deficient RNA isolated from ethionine treated mouse liver. Transfer RNA from two commercial samples (type XX and type XXI) and a methyl deficient tRNA sample that I prepared were analyzed on DEAE-Sephadex columns and the -5 charge fragment was not observed. The inability to reproduce the methylation of a large alkaline fragment may have been due to an artifact in the first experiment or due to the aging of the enzyme sample on storage at -20°. The first assays were performed when the enzyme preparation was about three months old and the last set of data was generated when the enzyme had been stored for eight months. In future experiments, I will isolate the methyltransferase and use it to analyze the tRNA without storage of the enzyme.

THE PREPARATION AND STRUCTURE OF CeSc<sub>2</sub>N@C<sub>80</sub>: An ICOSAHEDRAL CARBON CAGE ENCLOSING AN ACENTRIC CeSc<sub>2</sub>N UNIT WITH A BURIED f ELECTRON SPIN. Xuelei Wang<sup>1</sup>, Tianming Zuo<sup>1</sup>, Thomas E. Glass<sup>1</sup>, Frank Cromer<sup>1</sup>, Harry C. Dorn<sup>1</sup>, Marilyn M. Olmstead<sup>2</sup>, Alan L. Balch<sup>2</sup>, and James C. Duchamp<sup>3</sup>, <sup>1</sup>Dept. of Chem., Virginia Tech, Blacksburg, VA, 24061, <sup>2</sup>Dept. of Chem., University of California at Davis, Davis, CA, 95616, <sup>3</sup>Dept. of Chem.,

Emory and Henry College, Emory, VA, 24037. In this paper we report the preparation, purification, and characterization of a mixed trimetallic nitride endohedral metallofullerene,  $\text{CeSc}_2\text{N}@C_{80}$ . We will show the single-crystal X-ray diffraction structure which consists of a four atom trigonal planar cluster ( $\text{CeSc}_2\text{N}$ ) offset with a N atom offset by 0.36 Å inside a  $C_{80}(I_h)$  carbon cage. Surprisingly, at ambient temperatures, the  $^{13}\text{C}$  NMR spectrum exhibits isotropic motional averaging yielding only two signals (3 to 1 intensity ratio) for the icosahedral  $C_{80}$  cage carbons. At the same temperature, the  $^{45}\text{Sc}$  NMR exhibits a relatively narrow symmetric signal (2700 Hz) with a small temperature dependent Curie shift. A rotation energy barrier ( $E_a = 79$  meV) was derived from the  $^{45}\text{Sc}$  NMR line-width analysis. Finally, the XPS spectrum for  $\text{CeSc}_2\text{N}@C_{80}$  confirms a +3 oxidation state for cerium,  $\text{Ce}^{3+}(4f^15d^0)$ , this oxidation state, and the Curie shift, are consistent for a weak paramagnetic system with a single buried f electron spin.

AN EXPERIMENTAL AND THEORETICAL STUDY OF  $\text{Sc}_3\text{N}@C_{68}$ : ELECTROCHEMICAL AND RAMAN VIBRATIONAL MODE ANALYSIS. Liaosa Xu<sup>1</sup>, Ting Cai<sup>1</sup>, Mark R. Anderson<sup>1</sup>, Harry C. Dorn<sup>1</sup>, Ling-Ling Wu<sup>2</sup>, Nathan Swami<sup>2</sup>, and Keith Williams<sup>3</sup>, <sup>1</sup>Dept. of Chem., Virginia Tech, Blacksburg, VA 24061, <sup>2</sup>Dept. of Electrical Engineering, and <sup>3</sup>Dept. of Physics, University of Virginia, Charlottesville, VA 22908.  $\text{Sc}_3\text{N}@C_{68}$  was produced by encapsulation of  $\text{Sc}_3\text{N}$  in a  $C_{68}$  cage that disobeys the isolated-pentagon rule, namely, with  $D_3$  symmetry. The calculations are based on quantum chemical [density functional theory] methods. We first report electrochemistry study of  $\text{Sc}_3\text{N}@C_{68}$ , as a technique to characterize the electrochemical properties of this endohedral metallofullerene which was compared to the calculated HOMO-LUMO gap. Also, the vibrational modes of  $\text{Sc}_3\text{N}@C_{68}$  are currently of considerable interest. The calculated metal cluster-cage vibrational modes in far-infrared and Raman spectroscopic studies provide direct evidence for the bonding interaction between  $\text{Sc}_3\text{N}$  cluster and the carbon cage. Translational and rotational  $\text{Sc}_3\text{N}$  modes were found by both theory and experiment which induce the formation of the  $\text{Sc}_3\text{N}-C_{68}$  bonding.

DEVELOPING NEW ANTI-CANCER DRUGS. Shengliang Zhao, Matthew T. Mongelli, Brenda S. Winkel, and Karen J. Brewer, Dept. of Chem., Virginia Tech, Blacksburg, VA 24061-0212. The design and development of antitumor platinum-based complexes has become one of the most important areas of medicinal chemistry. These chemotherapeutic agents used in cancer therapy target DNA. Interactions are based on the four possible interaction modes: external binding, groove binding, intercalating and covalent binding. Platinum complexes are known to covalently bind to DNA, including the antitumor drug cis-diamminedichloroplatinium (cisplatin). The applicability of cisplatin is limited by toxicity, side effects, solubility and tumor resistance. Therefore, new platinum-based complexes have been designed and fabricated to improve the efficiency of the antitumor activity. This presentation will focus on the use of electrochemical and spectroscopic methods to probe platinum complex-DNA interactions. This work is supported by the NSF (CHE-0408445).

A NOVEL FAMILY OF TERBIUM-BASED TRIMETALLIC NITRIDE TEMPLATE ENDOHEDRAL METALLOFULLERENES:  $Tb_3N@C_{2n}$  ( $40 \leq n \leq 44$ ). Tianming Zuo<sup>1</sup>, Liaosa Xu<sup>1</sup>, Kim Harich<sup>1</sup>, Harry C. Dorn<sup>1</sup>, Marilyn Olmstead<sup>2</sup>, Alan Balch<sup>2</sup>, and James Duchamp<sup>3</sup>, <sup>1</sup>Dept. of Chem., Virginia Tech, Blacksburg, VA 24061, <sup>2</sup>Dept. of Chem., University of California, Davis, Davis, California 95616, <sup>3</sup>Dept. of Chem., Emory and Henry College, Emory, VA 24327. Trimetallic nitride template endohedral metallofullerenes (TNT-EMFs) have many special physico-chemical properties. In this presentation, we report the synthesis, isolation, and characterization of  $Tb_3N@C_{2n}$  ( $40 \leq n \leq 44$ ).<sup>3,4</sup> We have recently developed a chemical separation method that was successfully employed in the current study that is based on the chemical kinetic stability of TNT-EMFs relative to empty cage fullerenes and classic EMFs,  $M_x@C_{2n}$  ( $x = 1\sim 3$ ,  $n = 30\sim 50$ ).<sup>5</sup> These new carbonaceous nanomaterials were characterized by MS, UV-VIS, and X-ray crystallography as well as DFT computational approaches.

### Computer Science

THE REQUIREMENT ELICITATION OF SOFTWARE ENGINEERING. Jeff Zadeh, Department of Mathematics and Computer Science, Virginia State University, Petersburg, VA 23806. This paper describes the requirement elicitation of software development. Software is an increasingly important aspect of our daily life in government, banking and finance, education, transportation, entertainment, medicine, agriculture, and law. Unfortunately, there are severe problems in the cost, timeliness, and quality of many software products. The more serious problem is the effect that quality of software can have on the safety-critical issues that directly affect the health of humans. Today software engineering is widely accepted by industry, government, and academia. Software engineering is a rather young and somewhat immature discipline. Software requirements elicitation is the process where the customers' needs in a software project are identified. This process is one of the most important parts of building a software system because during this stage it is decided precisely what will be built. However, requirement gathering needs close interaction between developers and end-users of the system. Obtaining complete requirement information about large software systems is a key challenge in dealing with increasing complexity of a software product. Requirement information can be analyzed to provide a clearer picture about the problems concerning an individual feature or a set of features and over time exhibits a high potential to illustrate software product

## Education

STRATEGIES TO ENHANCE LEARNING IN PHARMACY TECHNICIANS. Mary Arnold<sup>1</sup>, Tricia Easterling<sup>1</sup> & J. Orion Rogers<sup>2</sup>, <sup>1</sup>School of Teacher Education and Leadership and <sup>2</sup>Dept. of Biol., Radford Univ., Radford VA 24142. The objectives of this project were to investigate a variety of instructional methods to facilitate learning, student attitude and performance of ten students enrolled in HLT 250 Pharmacy Technician Internship and Virginia Certification Review that was taught by Mary Arnold at Wytheville Community College during the spring 2006 semester. Authors obtained NIH certification to conduct research with human subjects, the Radford University IRB Committee on Human Subjects Research approved this research, and informed consent was obtained from students. Students were administered a pretest, posttest and attitudinal survey. Class demographics included: five students are 18-22 years old and five students are 23-53 years old, five students have children and five do not, and five students plan to pursue higher degrees while five plan for careers as pharmacy technicians. Methods of instruction included traditional lectures as well as interactive CD-ROMs to engage students in the learning process, to improve midterm and final exam scores in the course, and to improve scores on the Pharmacy Technician Certification Test for the Commonwealth of Virginia. Blackboard 6 instruction was used to provide a study guide on each chapter and six quizzes as well as a discussion board for questions and concerns. Results reveal that the posttest class average of 95% significantly increased ( $P < 0.0001$ ) when compared to the pretest average of 77%. Five students have already taken and passed the Virginia Certification Test, and three are scheduled to take the certification test. Four students earned final course grades of "A", five students earned grades of "B" and only one student earned a grade of "C".

USING THE SCIENCE MUSEUM AS A TEACHING RESOURCE David B. Hagan and Patricia D. Fishback,, Center for Science Education, Science Museum of Virginia, 2500 W. Broad Street, Richmond, VA 23220. The teaching of science at middle and high school levels requires use of laboratory and research equipment not often available to teachers in school buildings. The Science Museum of Virginia presents examples of exhibits and research programs specifically designed to support the teaching of science at this level. The Discover Virginia is an exhibit laid out in four quadrants based on the Virginia Science Standards of Learning for the Earth Science course, generally taught in the ninth grade. Theme areas are: Rocks, Minerals and Natural Resources of Virginia, Virginia on a Moving Continent (Tectonic Plates), Sea Level Changes in Virginia, and Watersheds and Rivers of Virginia. The room has 500 square feet of acoustic paneling covered with high-resolution color printing on cloth medium. Nineteen specimen cases line the room, illustrating examples of minerals, rocks and fossils. The room is designed as a teaching space for the geology and natural history of Virginia. The second program presented is the adaptation of large, expensive physical science exhibits for student research. These include a large diffusion cloud chamber for high energy particle tracks, a 4-meter Earth-moon gravity well, a 1.5 second microgravity drop tower, and a 60 gallon instrumented crystal growing chamber. High school physics

and chemistry students are invited to use these exhibits for Virginia Junior Academy of Science research projects.

DEVELOPING A NEW LABORATORY PROGRAM FOR COLLEGE-LEVEL INTRODUCTORY BIOLOGY. Ronald S. Mollick, Dept. of Biol., Chemistry, & Environmental Science, Christopher Newport Univ., Newport News VA 23606. CNU instituted new general education requirements at the same time the Department removed General Biology from the major requirements. This meant that the General Biology lab course needed to be restructured for non-majors only. The lab course was reduced to two hour sessions, and lab topics were chosen not to try to cover the scope of Biology, but to teach non-majors what science is and how science is done using biological topics. Each lab topic is now taught typically in two or three sessions per topic. In the first session, students are taught about a subject area and gain experience in how it can be studied in lab. At the end of the first session, they must construct testable hypotheses of their choosing, and design an experimental protocol. During the second session, they will test their hypotheses and gather data. The third session is when they orally present their work and hand in a lab report. This system therefore includes all basic elements of science: observation, creating and testing hypotheses, collecting and working with data, and reporting their findings both orally and in a written form. Most of the lab topics require hands on work, but two of them, Evolution and Ecology, are soft ware based. This partial inquiry based system provides the students with the opportunity to develop their interests and control their work which the literature tells us will lead to greater student satisfaction and better learning.

ENHANCING HIGH SCHOOL BIOLOGY REMEDIATION THROUGH SMART BOARD TECHNOLOGY. Brandi Norman<sup>1</sup>, Tricia Easterling<sup>1</sup> & J. Orion Rogers<sup>2</sup>, <sup>1</sup>School of Teacher Education and Leadership and <sup>2</sup>Dept. of Biol., Radford Univ., Radford VA 24142. Review of literature reveals that research regarding the use of SMART Board technology in education, particularly with biology remediation students, is relatively limited because it is a fairly new and expensive teaching tool. The literature suggests that the SMART Board interactive whiteboard can be a powerful tool to motivate and engage students in learning and to aid their retention by incorporating a wide range of information from various resources and by accommodating various learning styles. This research included seven students, three males and four females, at Abingdon High School ranging from 15 to 18 years of age. These students were in a biology remediation study hall because they had previously completed high school biology but had not passed the Virginia Biology Standards of Learning (SOL) test. Students were given the released 2004 Biology SOL test as a pretest before beginning remediation using the SMART Board and again as a posttest following eight weeks of remediation. Results reveal that the mean posttest scores were significantly higher after using the SMART Board for biology remediation. A learning preferences survey was also administered to each of these seven students before and after remediation to measure student attitudes about biology and use of technology. The mean of student attitudes and learning preferences did not differ significantly over the course of remediation, so no

correlation was seen between student attitudes/learning preferences and Biology SOL test achievement. Posttest score results indicate that the SMART Board is a valuable tool for remediation of struggling learners in biology.

DESIGN OF A HIGH QUALITY PROFESSIONAL DEVELOPMENT INSTITUTE FOR MIDDLE SCHOOL SCIENCE. J. Orion Rogers, Dept. of Biol., Radford Univ., Radford VA 24142. The No Child Left Behind (NCLB) Act of 2001 requires that experienced middle school teachers who are licensed in middle education or special education meet the definition of "highly qualified". One mechanism to fulfill this requirement is to meet the High Objective Uniform State Standard of Evaluation (HOUSSE) definition by completing an institute in the content area that meets high quality professional development (HQPDP) criteria established by the Virginia Department of Education. The objective of this project was to design and implement a HQPDP Institute for special education teachers of middle school science. Methods included conducting 30 clock-hours of classroom-focused instruction over three weekends at the Higher Education Center in Roanoke during the spring of 2006. Each weekend institute focused on the Virginia Science Standards of Learning (SOL) for either sixth, seventh or eighth grades, and one morning session discussed the Kansas State University Strategic Instructional Model (SIM). The institutes were designed to enhance teachers' knowledge of scientific investigation through hands-on activities in addition to their content knowledge of physical, life, Earth and space systems science. A pretest designed to simulate the Virginia Science SOL test for eighth grade was given before the first session, and a posttest was administered after the third HQPDP session. A formal assessment instrument was used after each of the three sessions to evaluate effectiveness, and modifications were enacted to stimulate engagement. Results revealed that teachers' knowledge was enhanced as evidenced by a pretest class average of 71% and a significantly higher ( $P < 0.0088$ ) posttest class average of 81%.

PRELIMINARY RESULTS FROM A MULTICENTER INVESTIGATION OF THE EFFECT OF NETWORK LATENCY ON PEDAGOGIC EFFICACY. J. C. Squire<sup>1</sup>, V. K. Walsh<sup>2</sup>, & H. F. Bush<sup>3</sup>, <sup>1</sup>Dept. of Electrical and Computer Engineering, <sup>2</sup>Dept. of Mathematics and Computer Science, and <sup>3</sup>Dept. of Economics and Business, Virginia Military Institute. Interactive web-based learning tools are becoming increasingly common to intuitively convey cause-and-effect relationships. There is little systematic investigation of the relationship between network delay and student learning. Our hypothesis is that student understanding is relatively insensitive to small network delays, but exhibits a sharp downwards knee for delays in excess of a few hundred milliseconds. If this is true, the time delay associated with that knee has implications for pedagogic software design, client-server computational distribution for web-based apps, and even university IT infrastructure. An interactive software application was designed purportedly to teach Fourier Analysis concepts, but actually embedding a variable delay between the time a student moves a control and the time the screen updates. Students were randomly assigned versions with different delays. Knee location was computed

using a nonlinear least squares fit to find the best-fit of a two-part piecewise linear function. Data using a subpopulation of 42 students from VMI using 8 equally-spaced delays from 0 to 420 milliseconds suggests a knee may exist ( $225 \pm 112$  ms), however the small population size results in a high standard error of the mean. As more data is collected, the SEM of the knee location will be reduced, enabling tests such as whether knee location is invariant with respect to age, gender, or major.

**REFORM OF INTRODUCTORY SCIENCE COURSES FOR NON-SCIENCE MAJORS: AN ASSOCIATED COLLEGES OF THE SOUTH INITIATIVE.** David W. Sukow, Department of Physics and Engineering, Washington and Lee University, Lexington VA 24450. A major issue confronting science education in this county is the scientific literacy, or illiteracy, of non-scientists. As science and technology continue to grow in importance in all aspects of our society, including economic and political arenas, it is crucial that science educators address this issue. The Associated Colleges of the South (ACS), a consortium of sixteen private liberal arts colleges and universities, has undertaken an initiative to improve science courses for students majoring in disciplines outside the sciences. Goals of this program are to increase the visibility of science education issues, provide faculty with expertise and resources to improve science literacy among non-science majors, improve scientific and technological understanding among these students, and to demonstrate the value of confronting this problem through collaborative efforts across institutions. This is accomplished through a program of workshops on course design and assessment, mentoring young science faculty, developing new courses and activities suitable for the target audience, assessing the results of the projects, and sharing widely the best and most effective models thus developed. This initiative is supported by the W. M. Keck Foundation.

**UNDERGRADUATE RESEARCH TEAMS: A STRATEGY FOR INCLUSION.** Lisa S. Webb, Dept. of Biology, Chemistry, & Environmental Science, Christopher Newport Univ., Newport News VA 23606. Undergraduate research teams are composed of groups of students of varying skill, ability and experience levels working collaboratively on an extracurricular research project. Each team member has a specific role in the project, and each team has a leader who coordinates task assignments and monitors results. The faculty mentor organizes and oversees the project and meets individually and collectively with team members and leaders. Because undergraduate research teams can accommodate students of all skill, ability and experience levels, they can be utilized to include students who have not traditionally been involved in scientific research projects. These include students with low grade point averages, underachieving and/or unmotivated students, students lacking self-confidence, students from underrepresented populations, and non-traditional students; the very students who stand to benefit most from a research experience. Therefore, undergraduate research teams can serve as an effective vehicle for inclusion.

### Environmental Science

EVALUATION OF FALL SOIL SAMPLING FOR PREDICTING SPRING INFESTATION OF SECONDARY SOIL PESTS IN CORN. T. Jordan<sup>1</sup>, R. Youngman<sup>1</sup>, C. Laub<sup>1</sup>, T. Kuhar<sup>2</sup>, & S. Tiwari<sup>1</sup>, <sup>1</sup>Virginia Tech University, Blacksburg, VA & <sup>2</sup>Virginia Tech University, Eastern Shore AREC, Painter, VA. A field study was started in fall 2005 to predict spring infestation levels of secondary soil pests, specifically white grubs (Coleoptera: Scarabaeidae) and wireworms (Coleoptera: Elateridae) in cornfields. Fifteen post-harvest soybean fields were sampled in late October and early November in several eastern Virginia counties using a randomized complete block design with 15 replicates. Current sampling procedures for secondary soil pests are done in the spring prior to planting by visually inspecting a 30-cm<sup>2</sup> by 15-cm deep (standard method) volume of soil for annual white grubs or by using some form of baiting method for wireworms and annual white grubs. This study compared a 20.3-cm<sup>2</sup> by 15-cm deep soil sample with the standard method. Initial results of fall 2005 sampling indicate abundant white grub densities ranging from 0.9-10.1 grubs per standard method. Of the 15 sampled fields, 12 exceeded the economic threshold of 2 grubs per standard method. The 20.3-cm<sup>2</sup> by 15-cm deep sample method was evaluated for its potential to correlate to the standard method after a 2.25 weighting factor (the standard method samples 2.25 x more volume of soil than the 20.3-cm<sup>2</sup> by 15-cm deep method). No significant differences were detected between the two methods after correcting for differences in sampling volume. Wireworms also were found, but numbers were generally low across all fields, averaging less than 1 wireworm per standard method. Sampling is planned for spring 2006, and again in fall 2006 and spring 2007, to evaluate how well fall sampling densities correlate with spring densities.

COMPARATIVE ANALYSIS OF THE IMPACT OF DEVELOPMENT ON THE HEALTH OF TWO STREAM TRIBUTARIES OF THE RAPPAHANNOCK RIVER. Justin Park and Michael Bass. Environmental Science & Geology, University of Mary Washington, Fredericksburg, VA. Monitoring of the ecological impact of the Celebrate Virginia project in Stafford Co. VA has progressed from 2001 thru 2005 by measuring water chemistry parameters and the health of the macrobenthic community. In 2005 water chemistry data showed variations but not many levels above the established EPA/ VDEQ regulations for any parameter. The most prevalent change was in the amount of sediment accumulation seen in the streams. The impact of this embeddeness was seen in changes to the macrobenthic community. Three evaluators of stream health in our study were: the collection of insects used to determine the Hilsenhoff Biotic Index(HBI) , the calculation of the Ephemeroptera/Plecoptera/Trichoptera richness(%EPT) and comparing trophic relationship proportions. The HBI indicated a decrease in stream quality at all stations except one, however the HBI's are within the good to very good range. Percent EPT decreased at all stations and the trophic level distribution became increasingly unequal from the previous year. Both the total organism and the total insect population collected showed a decrease.

POND MACROINVERTEBRATE COMMUNITY COMPOSITION ALONG ENVIRONMENTAL GRADIENTS. Sara A. Shakeshaft & Jeremy M. Wojdak, Department of Biology, Radford University, Radford, VA 24142. The number and composition of species that inhabit freshwater ecosystems can reflect current and historical land use in the watershed, physical and chemical characteristics, and biotic interactions. As a preliminary step to understand the invertebrate communities in ponds in southwest Virginia, we conducted a field survey of 14 ponds. Invertebrates were sampled with 10 dip-net sweeps in each pond, and the first ~100 animals were identified (most to family). Phytoplankton abundance was estimated as chlorophyll *a* by filtering, ethanol extraction, and narrow band fluorometry. The growth of algae in the absence and presence of macrograzers was determined by deploying artificial substrates in each pond, and determining chlorophyll *a* concentrations as above after 14 days. Measurements of depth, dissolved oxygen, temperature, conductivity, and pH were taken at five points in each pond, and each pond's circumference was measured. Ponds with higher dissolved oxygen, higher pH, or greater area had greater macroinvertebrate species richness. These ponds were typically in less disturbed landscapes than were small, turbid, high-nutrient farm ponds (cattle watering ponds). Farm ponds typically had greater phytoplankton and periphyton biomass and a greater proportion of pollution tolerant invertebrate taxa such as corixids, chironomids, and oligochaetes.

LETHAL AND SUB-LETHAL EFFECTS OF COPPER ON AN AQUATIC INVERTEBRATE (*LUMBRICULUS VARIEGATUS*). Jason Crolley & Jeremy M. Wojdak, Department of Biology, Radford University, Radford, VA 24142. Copper (and other heavy metal) pollution leaching from the abandoned Toncray Mine in southwest Virginia has been linked to biological impairment of the East Prong of Furnace Creek. To evaluate the efficacy of three remediation ponds built in 1994 to help reduce pollution runoff to the creek, we conducted chemical analyses and laboratory-based toxicological tests using the California Blackworm, *Lumbriculus variegatus*. Mortality and pulsation rates (an indicator of physiological well-being) of *L. variegatus* were examined after exposure to mine effluent, remediation pond water, water from the creek above and below the mine, and to copper solutions of known concentration. Water from the mine drainage and settling ponds was very toxic, exceeding the US EPA screening value for copper in freshwater by nearly 500 fold, and causing nearly 100% mortality within 48 hours. Mine drainage into the creek had no clear effects on worm pulsation rate or mortality (i.e. comparing upstream vs. downstream). Our data and previous data indicate the ponds effectively removed copper and increased pH in 1998, but not in 2004 or in 2006. Experiments with lower concentrations of copper than found in the mine area indicate that worm pulsation rates decrease with increasing copper concentration.

AN ASSESMENT OF THE WATER QUALITY OF CROOKED RUN, IN FREDERICK, CLARKE AND WARREN COUNTIES, VIRGINIA, BASED ON THEIR FISH POPULATIONS. Lyla H. Gray, Michelle S. Miller, Woodward S. Bousquet & Karen R. Andersen, Environmental Studies Program, Shenandoah

University, Winchester, VA 22601. Located in the northern Shenandoah Valley, Crooked Run is a tributary to the North Fork of the Shenandoah River. Its watershed comprises approximately 30,000 acres. A new 1930-unit housing development in the watershed's upper reaches surrounding Lake Frederick raised local concerns about impacts on the stream's water quality and biological diversity. In cooperation with the Friends of the Shenandoah River (FOSR, a regional citizens organization), and the Virginia Department of Game and Inland Fisheries (VDGIF), the researchers used electroshocking gear to collect fish from nine sites on Crooked Run's main stem and its stream and spring-run tributaries. A total of 1,352 fish of 32 species were captured, identified, weighed and released. The average fish Index of Biotic Integrity (IBI) score of 2.62 revealed that the water quality in the main stem of Crooked Run is only fair. Two wastewater processing facilities will eventually add their contents to this small stream.

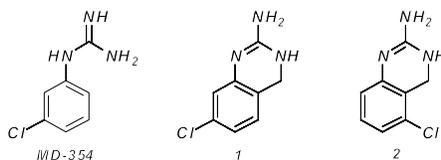
MONITORING THE PROGRESS OF A WETLAND MITIGATION PROJECT FOR CENTRAL PARK IN FREDERICKSBURG, VA. Michelle Arthur & Michael Bass, Environmental Science & Geology, University of Mary Washington, Fredericksburg, VA. The purpose of this study was to monitor the ecological development of a series of stormwater management ponds and their accompanying off-site wetland mitigation area which was to replace a destroyed forested wetland. The quality of the ponds and forested wetland site was monitored through chemical testing and a survey of the vegetation present. In addition the distribution of hydric soils was also monitored in the off-site wetland area. The water chemistry parameters measured showed no unusual highly elevated components. Manganese was the highest metal found but was still below the accepted regulated level. Zinc and copper concentrations were well below the limits. In the off-site area the number of woody stems per acre was the important parameter that required monitoring. VDEQ requires 400 stems per acre to meet the guidelines for a successful forested wetland. In 2003 the woody stem count was 557 per acre, in 2004 the woody stem count was 1096. However in 2005 the count had dropped dramatically to 334 woody stems per acre because of wildlife predation, primarily from a beaver. The herbaceous species identification yielded an increase with some new species added and some others from previous years being lost, exhibiting ecological succession.

### Medical Science

FGF23 BINDS TO FGFR1 TO DOWNREGULATE 1-ALPHA HYDROXYLASE IN HUMAN PROXIMAL KIDNEY CELLS (HK-2G). Megan Forster & M. J. Beckman. Orthopaedic Surgery and Biochemistry, Virginia Commonwealth University, Richmond, VA, 23298. The 1-alpha-hydroxylase (CYP27B1) is a p450 mitochondrial enzyme that is responsible for the conversion of vitamin D to its active form. CYP27B1 is highly expressed in the proximal tubules of the kidney and is regulated by many factors. Parathyroid hormone (PTH), which is released under low calcium conditions, is a key regulator of CYP27B1. PTH up-regulates

CYP27B1 leading to increased 1- $\alpha$ -hydroxylation of 25-OH-D at the carbon one position to form the active form, 1,25-dihydroxyvitamin D<sub>3</sub> (1,25 VD). 1,25 VD has the main role of restoring blood calcium. Recent findings have demonstrated that the novel fibroblast growth factor 23 (FGF23) is responsible to down-regulate CYP27B1 in response to high blood phosphate conditions or increased 1,25 VD. FGF23 is primarily released from the bone, but new evidence shows minor expression from other tissues such as the liver, and spleen. The goal of this study was to demonstrate the expression of FGF23 from renal proximal tubule cells. A human kidney proximal cell line (HK-2G) was used as the model. This study confirmed that PTH follows the PKA/CREB pathway in order to upregulate CYP27B1. Using Real-Time RT-PCR FGF23 and FGF receptor 1 gene expressions were also shown to occur in a time-dependent manner and in response to 1,25VD. In conclusion, this new pathway may represent a short feedback mechanism for modulating the synthesis of CYP27B1 and counteracting its increase by PTH.

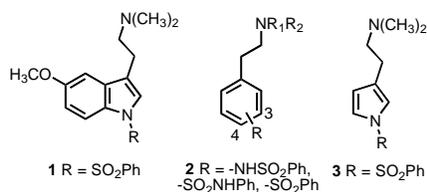
CONFORMATIONALLY-CONSTRAINED ANALOGS OF THE ANALGESIC ENHANCING AGENT MD-354. E. O. De Oliveira, S. Young, R. A. Glennon & M. Dukat, Department of Medicinal Chemistry, Virginia Commonwealth University, Richmond VA, 23298-0540. The severe side effects of the  $\alpha_2$ -adrenoceptor agonist clonidine generally limit its use as an analgesic to clinical settings where such effects can be closely monitored. *m*-Chlorophenylguanidine (*m*CPG; MD-354) is an agent that lacks antinociceptive actions of its own in the mouse tail-flick assay (i.e., <5% MPE – maximal possible effect), but is able to potentiate the antinociceptive actions of a low “inactive” dose of clonidine (13% MPE) such that when administered in combination significant (>80% MPE) antinociception is achieved. MD-354 has been shown to be a partial agonist at 5-HT<sub>3</sub> receptors, and elimination of the chloro group results in reduced affinity. To examine conformational preference for the binding of MD-354 at 5-HT<sub>3</sub> receptors, two rotamers of MD-354 (i.e., 7-chloro-3,4-dihydroquinazolin-2-amine (**1**) and 5-chloro-3,4-dihydroquinazolin-2-amine (**2**)) were prepared. Neither of them (i.e., **1** (K<sub>i</sub> = 680 nM), **2** (K<sub>i</sub> = 4,600 nM)) retained the affinity of MD-354 (K<sub>i</sub> = 90 nM). These findings might reflect a lack of tolerance by the receptors for the added methylene group, or indicate that either a specific NH contributes to binding or that some other conformer might be preferred for high affinity. (This work was supported in part by J-778 from the Jeffress Foundation.)



BINDING OF METHOXY-SUBSTITUTED *N*<sub>1</sub>-BENZENESULFONYLINDOLE ANALOGS AT HUMAN 5-HT<sub>6</sub> SEROTONIN RECEPTORS. R. Kolanos<sup>1</sup>, U. Siripurapu<sup>1</sup>, M. Dukat<sup>1</sup>, B. L. Roth<sup>2,3</sup> & R. A. Glennon<sup>1</sup>, <sup>1</sup>Department of Medicinal Chemistry, Virginia Commonwealth University, Richmond VA 23298 and Departments of <sup>2</sup>Biochemistry, <sup>3</sup>Psychiatry and Neurosciences, Case Western

Reserve University, Cleveland OH 44106. 5-HT<sub>6</sub> receptors, one of the seven families of serotonin receptors, are of interest because of their possible involvement in certain neuropsychiatric and neurological disorders. Two series of compounds, N<sub>1</sub>-(4-aminobenzene)sulfonylindole and N<sub>9</sub>-(4-aminobenzene)sulfonyl-1,2,3,4-tetrahydrocarbazole analogs, were utilized to determine if the effect of methylamine substituents was related to a direct receptor interaction with an amine binding site or whether they simply altered the electronic character of the molecules. Comparison of several amine-substituted indoles with their methoxy-substituted counterparts suggests that these substituents influence 5-HT<sub>6</sub> receptor affinity indirectly *via* their electronic effect on the indolic nucleus. The amine-substituted and methoxy-substituted tetrahydrocarbazole counterparts, despite their structural similarity to N<sub>1</sub>-(4-aminobenzene)sulfonylindoles, do not behave in the same manner; introduction of methylamine or methoxy substituents was not well tolerated and tended to decrease 5-HT<sub>6</sub> receptor affinity. Overall then, the presence of the methoxy group results in slightly enhanced affinity compared to unsubstituted and amine-substituted indoles but not tetrahydrocarbazoles, 4,6-dimethoxy-N<sub>1</sub>-(4-aminobenzene)-sulfonylindole ( $K_i=0.8$  nM) was found to bind with very high affinity at 5-HT<sub>6</sub> receptors, and the indolic substituents likely influence affinity *via* their electronic effect on the indole nucleus. [Supported by MH-60599]

PHENYLALKYLAMINE BENZENESULFONAMIDES AS 5-HT<sub>6</sub> RECEPTOR LIGANDS. D.M.N. Sikazwe<sup>1</sup>, M. Dukat<sup>1</sup>, B.L. Roth<sup>2</sup>, & R.A. Glennon<sup>1</sup>, <sup>1</sup>Dept. of Medicinal Chemistry, Virginia Commonwealth University, Richmond VA 23298 and <sup>2</sup>Depts. of Biochemistry, Psychiatry and Neurosciences, Case Western Reserve University, Cleveland OH 44106. Human 5-HT<sub>6</sub> receptors have been implicated in several types of neurological disorders. We previously reported sulfonyltryptamine MS-245 (**1**;  $K_i = 2.9$  nM) among the first 5HT<sub>6</sub> antagonists. We now report the synthesis, binding affinities, and structure-activity relationships (SAR) of a series of phenylalkylamine benzenesulfonamides and their sulfone derivatives. The agents were designed to test the hypothesis that sulfonamide-containing arylalkylamines bind to 5HT<sub>6</sub> receptors in an extended, ergoline-type conformation. If so, compounds such as **2** and **3** should retain affinity. The new derivatives displayed affinities ( $K_i$  values) for human 5-HT<sub>6</sub> receptors ranging from 15 to 70 nM. It appears that neither an intact sulfonamide moiety (*to wit*: sulfones) nor the position (i.e., 3- vs 4-position) of the sulfonamide, nor exchanging a phenylalkylamine with a structurally simpler pyrroloethylamine (i.e., **3**;  $K_i = 15$  nM), alters binding significantly. The results indicates that these ligands bind to human 5-HT<sub>6</sub> receptors in a similar, ergoline-type fashion, and provide a comprehensive model for the binding of multiple classes of 5-HT<sub>6</sub> receptor ligands. [Supported by MH-60599.]



ARYLOXYETHYLAMINES: BINDING AT  $\alpha 7$  NICOTINIC CHOLINERGIC RECEPTORS. H.M. Ragab<sup>1</sup>, J.S. Kim<sup>1</sup>, M. Dukat<sup>1</sup>, H. Navarro<sup>2</sup> & R.A. Glennon<sup>1</sup>, <sup>1</sup>Department of Medicinal Chemistry, Virginia Commonwealth University, Richmond VA 23298 and <sup>2</sup>Research Triangle Institute, NC 27709.  $\alpha 7$ -Nicotinic cholinergic (nACh) receptors are the second most abundant population of nicotinic receptors in brain after  $\alpha 4\beta 2$  nACh receptors. Very little is known about them but they are of interest because of a possible role in cognitive and perceptual disturbances. Gotti et al., reported that certain choline ethers (**1**; R=styryl, R'=CH<sub>3</sub> and X=CH) are  $\alpha 7$ -selective antagonists with little affinity for  $\beta 2$  containing nACh receptors. Because we have previously developed 6-(2-phenethyl)nicotine (6-PEN) and APXQ (**1**; R=R'=H, X=N) as nicotine ligands, and because the choline ethers might be viewed as an amalgamation of 6-PEN and APXQ, we examined the structure-affinity relationship for  $\alpha 7$  binding. We examined several aryloxyalkylamine analogs to determine the role of various structural features for binding at homomeric  $\alpha 7$  nACh receptors. In general, the presence of R = phenyl or styryl had little influence on affinity and the ring N atom might contribute to affinity but is not essential. In contrast, the N,N,N-trimethyl quaternary amine is a major contributor to  $\alpha 7$  binding. [Funded in part by DA 05274 and the Egyptian Channel Program].

THE SIGNALING PATHWAYS INVOLVED IN ACTIVATION OF VITAMIN D RECEPTOR REGULATION IN HUMAN PROXIMAL KIDNEY CELLS (HK-2G). Aparna Maiti<sup>2</sup> & M. J. Beckman<sup>1,2</sup>. Orthopaedic surgery<sup>1</sup> and Biochemistry<sup>2</sup>, Virginia Commonwealth University, VA, Richmond 23298. The vitamin D Receptor (VDR) is a steroid nuclear transcription factor responsible for mediating the biological activities of 1,25-dihydroxyvitamin-D<sub>3</sub>. Renal VDR content is an important factor in calcium homeostasis, and vitamin D inducible target genes. In these tissues, VDR expression is highly regulated by calcium level in the body. However, the signaling connection between extracellular calcium mediated VDR up-regulation is not been well studied. Here we report that calcium sensing receptor (CaSR) expressed endogenously in renal proximal tubules, is involved in VDR up-regulation in response to high extracellular calcium [Ca<sup>2+</sup>]<sub>e</sub>, following p38 mitogen activated protein kinase (MAPK) pathway. Western Blot results demonstrated that 3mM [Ca<sup>2+</sup>]<sub>e</sub> calcium triggers VDR activation within 1h, reached maximum at 6-18h, and declined after 24h. High [Ca<sup>2+</sup>]<sub>e</sub> activated both ERK1/2 and P38 phosphorylation, however specific p38 inhibitor at the dose of 10 $\mu$ M, abolished VDR induction. Essential role of CaSR is demonstrated by using gadolinium a known agonist, and further confirmed by using G-protein inhibitor, pertussis toxin, and PLC inhibitor, U73122. Pertussis toxin did not inhibit high [Ca<sup>2+</sup>]<sub>e</sub> mediated VDR up-regulation explains that CaSR is involved in Gq coupling system. This new evidence demonstrates how extracellular Ca plays a direct role in restoring cell-specific responsiveness of the kidney proximal tubule to effects of 1,25-dihydroxyvitamin-D<sub>3</sub>.

ISOLATION OF MESENCHYMAL STEM CELLS DERIVED FROM ADULT BONE MARROW AND UMBILICAL CORD BLOOD AND THEIR POTENTIAL TO DIFFERENTIATE INTO OSTEOBLASTS. Andrew P. Pacitti<sup>3</sup>, K. Wartella<sup>4</sup>, J. Wayne<sup>4</sup>, E. Mainali<sup>2</sup> & M. Beckman<sup>1,5</sup>, Depts. of <sup>1</sup>Orthopaedic Surgery, <sup>2</sup>Pediatrics, <sup>3</sup>Physiology, <sup>4</sup>Biomedical Engineering, and <sup>5</sup>Biochemistry, Virginia Commonwealth University, Richmond VA 23298. Mesenchymal stem cells (MSCs) are defined as pluripotent progenitor cells with the ability to generate cartilage, bone, muscle, tendon, ligament and fat. These cells have generated tremendous interest in their potential use to replace damaged tissues. Mesenchymal stem cells can be cultured to expand their numbers then transplanted to the injured site after seeding on biomimetic scaffolds to generate appropriate tissue constructs. An alternative approach for skeletal repair is the selection, expansion and modulation of osteoprogenitor cells in combination with a conductive scaffold together with appropriate bone growth factors to support and guide regeneration. We have focused on the isolation of mesenchymal stem cells derived from both adult bone marrow (ABM) and umbilical cord blood (CB) using a novel isolation technique based on the positive and negative selection of specific stem cell surface markers. These cells were induced to differentiate into mature bone producing osteoblast cells, which was confirmed using the techniques of RT-PCR, von Kossa staining, and confocal microscopy. Our results confirmed that highly homogenous MSC populations from both ABM and CB tissues were able to be obtained by an immuno-depletion method and that these cells were also capable of maintaining their stem cell nature until induced down a distinct cell lineage.

TEMPO AND REGULATION OF IFNG PRODUCTION IN EXPERIMENTAL RSV INFECTION. L. M. Pujanauski<sup>1</sup>, W. W. Stevens<sup>2,4</sup>, J. P. Castillo<sup>2</sup> & T. J. Braciale<sup>2,3,4</sup>, <sup>1</sup>Dept. Of Biology, <sup>2</sup>Beirne B. Carter Center for Immunology Research and Depts. Of <sup>3</sup>Pathology and <sup>4</sup>Microbiology, UVA. In previous efforts to develop a vaccine for Respiratory Syncytial Virus (RSV), infants vaccinated with Formalin-Inactivated RSV who were later naturally infected with RSV developed enhanced pulmonary injury. Murine models have been developed to show that mice vaccinated with the G protein of RSV and then challenged with RSV also developed pulmonary eosinophilia and a memory (m) CD4 T cell Th2 response with no mCD8 T cell response. Mice vaccinated with the M2 protein and then challenged with RSV produced no eosinophilia, a mCD8 T cell response, and a yet to be characterized mCD4 T cell response. In RSV-infected mice that were vaccinated with both the G and M2 proteins, no eosinophilia was observed while both a mCD4 and mCD8 T cell response was generated. These studies suggest that mCD8 T cells are modulating mCD4 T cells away from a Th2 response, preventing eosinophilia. IFNg is a cytokine secreted by activated mCD8 T cells and has been shown to prevent the development of a Th2 response. As a result, it is hypothesized that IFNg produced by mCD8 T cells could drive mCD4 T cells away from a Th2 response, thus preventing pulmonary eosinophilia. In order to test for the presence of IFNg, IFNg ELISAs were performed on lung samples from M2-primed RSV-infected mice. IFNg was detected in the lungs during the first 4 days following RSV

infection in M2-primed mice, but not seen in control mice. These results indicate that IFN $\gamma$  is present in the lungs of M2-primed RSV-infected mice and could potentially cause mCD4 T cells to differentiate away from a Th2 response.

THE EXPRESSION OF RANKL BY FIBROBLASTS WITHIN THE PERIPROSTHETIC MEMBRANE. Samuel C. Ramage<sup>3</sup>, Patrick E. Jones<sup>2</sup>, William A. Jiranek<sup>1</sup> & Matthew J. Beckman<sup>1,3</sup>, Departments of Orthopaedic Surgery<sup>1</sup>, Anatomy<sup>2</sup> and Biochemistry<sup>3</sup>, Virginia Commonwealth University, 23298. Aseptic osteolysis is the loss of bone surrounding a total joint replacement. This results in implant loosening and usually requires costly total joint revision surgery. A periprosthetic membrane composed primarily of fibroblasts and macrophages is usually found between the bone and implant surfaces at the site of resorption. Previous work in our lab has shown that the cytokine known to promote osteoclast activation, RANKL, is present at high levels in areas bone resorption and shows cellular expression patterns closely related to fibroblasts. We investigated this expression pattern using confocal microscopy and a variety of fibroblasts markers as well marker for other cell types. We saw strong colocalization of several fibroblast markers with RANKL and a lack of co-localization with the myeloid markers used. In addition, our study showed in all patient samples examined there was a large multinuclear cell or collection of cells that showed fibroblast markers as well as extremely high levels of RANKL.

ROLE OF SPP-24 AND RHBMP INTERACTION IN OSTEOBLAST CALCIFICATION. James M. Silcox, Samuel C. Ramage, Joanna Jeruzal, & Matthew J. Beckman, Departments of Orthopaedic Surgery, Anatomy, & Biochemistry, Virginia Commonwealth University, Richmond VA 23298. Marshall Urist first isolated what he called non-collagenous protein/bone morphogenetic protein (NCP/BMP) nearly forty years ago that caused ectopic bone formation. This was a mixture of proteins that included the now widely characterized bone morphogenetic protein (BMP) family. However, Urist has said that he believes what has been characterized so far is not responsible for the original NCP/BMP activity he observed. Behnam et al., based off of Urist's observations, produced a synthetic peptide that corresponds to a 19-amino acid sequence of secreted phosphoprotein 24 (Spp-24). This peptide has the characteristics of the peptide that Urist first described and attributed NCP/BMP activity. When used in conjunction with BMP-2, their synthetic peptide increased the over-all osteogenic activity of rhBMP-2, reduced the time required for rhBMP-2 to induce ectopic bone formation, and induces calcification by itself. We have taken the entire spp-24 gene, and ligated it into a FLAG vector. We then transfected osteoblasts (MG-63) with the vector, and treated the cells with 0, 5, and 50 ng/ml of rhBMP-2 three days after transfection. We let the cells grow for three days after treatment, and then performed von Kossa staining on each treatment. We were able to see significantly higher amounts of calcium deposition in the cells treated with spp-24 and rhBMP-2 as opposed to the cells that were treated with just rhBMP-2 or just spp-24.

THE EFFECTS OF CANNABIDIOL, A CONSTITUENT OF MARIJUANA, ON THE DISCRIMINATIVE STIMULUS AND CONDITIONED PLACE AVERSION EFFECTS OF  $\Delta^9$ -THC. Thomas F. Gamage, Robert E. Vann, Jonathan A. Warner, Ericka M. Marshall, Nathan L. Taylor & Jenny L. Wiley, Dept of Pharmacology VCU, Richmond, VA, 23298. Sativex, a drug containing equal parts of  $\Delta^9$ -tetrahydrocannabinol (THC) and cannabidiol (CBD) has recently been approved in Canada for the treatment of multiple sclerosis. CBD's subjective effects and reward-related stimulus as they occur in marijuana, at various ratios to THC, or in Sativex, at a 1:1 ratio, have yet to be studied. This study examined the effects of CBD on THC's discriminative stimulus effects in Long Evans rats trained to discriminate THC from VEH in a two lever drug discrimination paradigm. In addition, the effects of CBD on contextual cues associated with THC's stimulus effects were studied using the conditioned place preference/aversion (CPA) model in ICR mice. In the drug discrimination study, CBD failed to substitute for THC and did not alter its subjective effects at any ratio tested. During the CPA tests, CBD alone had no effects. In contrast, a 10 mg/kg dose of THC alone produced aversion whereas lower doses produced neither aversion nor preference. When administered in combination in a 1:1 ratio as found in Sativex, CBD (10 mg/kg) attenuated the aversive effects 10 mg/kg THC, but had no effects on lower doses of THC. In conclusion, CBD does not alter THC's discriminative stimulus effects, but it may alter THC's aversive effects. In addition, this research supports a view that the combined effects of cannabinoids such as CBD and THC may elicit different effects than when THC is administered alone. Research supported by NIDA grants DA-09789 and DA-03672.

THE GENETIC ANALYSIS OF ETHANOL-INDUCED ANXIOLYSIS IN BXD RECOMBINANT INBRED MICE. Alexander H. Putman & Michael F. Miles, Dept. of Pharmacology & Toxicology, Virginia Commonwealth University, Richmond VA 23298. Due to the high comorbidity between anxiety and ethanol abuse, there has been a long-standing interest in understanding the relationship between these two disorders. Although anxiety is hypothesized as a factor in the initiation of ethanol abuse and risk for relapse, the molecular mechanisms underlying anxiety, ethanol addiction, and their correlation are not well understood. The identification of ethanol-induced anxiolysis-like behavioral quantitative trait loci (QTL) will aid in understanding the neurobiology of ethanol-induced anxiolysis. Therefore, anxiety-related responses to ethanol were measured across the BXD recombinant inbred panel and C57BL/6J (B6) and DBA/2J (D2) progenitors using the light-dark transition model of anxiety. The progenitor strains exhibited a robust anxiolytic-like response following 1.8g/kg ethanol while preliminary QTL analysis identified potential genetic loci associated with various anxiety-related behaviors. A significant basal anxiety QTL mapped to chromosome 11 and a significant chromosome 12 QTL may influence the susceptibility to ethanol-induced anxiolysis. Additional analyses will include the identification of ethanol-responsive expression patterns in the prefrontal cortex (PFC) across the BXD recombinant inbred panel using Affymetrix oligonucleotide microarrays and identify expression QTL. This approach will allow us to correlate expression

patterns with behavioral data, ultimately aiding in defining the molecular mechanisms involved in ethanol-induced anxiolysis. Supported by NIH Grants AA013678, AA014717 to MFM and AA016052 to AHP.

ALTERATIONS OF THE IMMUNE SYSTEM DURING CRITICAL STAGES OF DEVELOPMENT FOLLOWING EXPOSURE TO 1,2:5,6 DIBENZANTHRACENE (DBA) IN B6C3F1 MICE. Denise M. Hernandez & Kimber L. White, Jr., Virginia Commonwealth University, Richmond, VA. DBA is an environmental contaminant classified on EPA's priority pollutant list and is formed by the incomplete combustion of carbon containing compounds. The objective of these studies was to evaluate the immunosuppressive effects of DBA in adult and juvenile B6C3F1 mice to determine whether exposure at different windows of development causes enhanced immunotoxicity. Juvenile mice were dosed beginning on postnatal day 21 (+/- 2 days) with DBA in corn oil (79.3, 250, 793, and 2500 ug/kg) subcutaneously for 28 days. Adult animals were dosed beginning at 8-10 weeks of age and received similar and higher doses up to 5000 ug/kg. Several immune parameters including the IgM antibody forming cell response, Natural Killer cell activity, and anti-CD3 antibody-mediated proliferation assay were used to evaluate humoral, innate and cell-mediated immune competence. These studies will provide insight into how environmental agents may impact the developing human immune system.

TIME COURSE OF THE ENHANCEMENT AND RESTORATION OF THE ANALGESIC EFFICACY OF CODEINE AND MORPHINE BY  $\Delta^9$ -TETRAHYDROCANNIBINOL. I. Jovan Williams, Sherita Edwards, Alex Rubo, Victoria L. Haller, David L. Stevens & Sandra P. Welch, Dept. of Pharmacology and Toxicology and School of Nurse Anesthesia, Virginia Commonwealth University Medical College of Virginia, Richmond VA 23298.  $\Delta^9$ -tetrahydrocannabinol ( $\Delta^9$ -THC) synergizes with morphine and codeine by releasing endogenous opioids. These studies determined 1) the duration of enhancement of morphine and codeine by  $\Delta^9$ -THC, 2) the effect of  $\Delta^9$ -THC on the time course of fully efficacious doses of opioids, 3) restoration of efficacy of morphine and codeine by  $\Delta^9$ -THC, and 4) duration of restoration. Sub-active combination doses of  $\Delta^9$ -THC/morphine or  $\Delta^9$ -THC/codeine are equivalent in duration of action and efficacy to high-dose opioids alone.  $\Delta^9$ -THC (20mg/kg p.o.) significantly restores the antinociceptive effects of both high-dose morphine and codeine (100 and 200 mg/kg p.o., respectively) at later time points at which morphine or codeine was no longer active (360- and 120-min post-administration, respectively). Thus, the cannabinoid/opioid combination might be useful in therapeutics to enhance opioid activity, as well as to restore the efficacy of opioids.

DESIGN OF A HUMAN HEART TRANSPORT UNIT. K. Garland<sup>1</sup>, G. Horton<sup>1</sup>, J. Olsen<sup>1</sup>, E. Roesch<sup>1</sup>, R. Grisso<sup>1</sup>, R. P. Wyeth<sup>2</sup> & the Via Virginia College of Osteopathic Medicine, Cardiac Transplantation Group<sup>1, 2</sup>. <sup>1</sup>Virginia Polytechnic Institute and State University, Blacksburg, VA 24061. <sup>2</sup>Edward Via Virginia College of Osteopathic Medicine, Blacksburg, VA 24060. The inability to extend

cold ischemic storage time during transport is the primary factor in loss of donated hearts before they can be used. We have designed a small self-contained portable heart transportation unit that will increase storage time of explanted hearts. All elements, devices, and perfusate, in addition to the donor heart, are contained in a lightweight, ergonomic, durable unit of three layers; an inner and outer layer of polypropylene and a middle layer of polyurethane foam. The transport unit is designed with monitoring devices for flow, temperature, oxygen level, pH, and pressure. It will store 5.0 L of perfusate, a discharge bag, and a container with the perfused heart surrounded by a small amount of superfusate. The unit will contain a piezoelectric heating/cooling device, pressure monitoring transducers, a pH and O<sub>2</sub> sensor, a variable flow metering pump and a dedicated microprocessor to integrate and control temperature, pressure, flow rate, pH and O<sub>2</sub> tension of the donor heart. The unit will be modular. It will have a small ledge attached to the bottom of the unit. The battery, pump controller, dual pressure indicator, and multifunction meter will be fastened to this ledge. The final design follows regulations set forth by the FAA, DOT, EPA, and FDA. In conclusion, a compact, lightweight cardiac transport unit has been designed to increase the storage time of explanted donated hearts. This design allows for continuous perfusion of myocardium and careful monitoring and adjustment of O<sub>2</sub> tension, pH and temperature. This design could significantly reduce, or eliminate ischemic time in explanted hearts. Thus, this device will significantly increase the number of donor hearts suitable for transplantation.

ALTERATIONS IN EXCITATORY AND INHIBITORY NEUROTRANSMISSION FOLLOWING MILD MECHANICAL INJURY. J.G. Pope, P.B. Goforth & L.S. Satin, Department of Pharmacology & Toxicology, Virginia Commonwealth University Medical Center, Richmond VA 23298. Traumatic brain injury (TBI) is a major health problem and a leading cause of death in individuals under 30. TBI leads to impaired cognitive and motor function, and may result from changes in inhibitory GABAergic, as well as excitatory glutamatergic synaptic transmission. Spontaneous, non-action potential driven miniature inhibitory postsynaptic currents (mIPSCs) and miniature excitatory postsynaptic currents (mEPSCs) were recorded in control and mildly stretch injured cultured cortical pyramidal neurons. Both mEPSCs and mIPSCs provide a simple and direct method to examine the function of postsynaptic glutamate and GABA<sub>A</sub> receptors respectively. Neurons were injured using a controlled puff of air produced with an injury control device to simulate forces that occur during mild brain concussion. This study shows that mild mechanical injury reduces mEPSC amplitude, slows their rise time and increases their half width. Longer mEPSC duration would be consistent with an increase in NMDA receptor contribution to the synaptic event possibly due to the relief of Mg<sup>2+</sup> block after injury and/or reduced AMPA receptor desensitization, both found in previous studies by our lab. In opposition to the findings for injury induced changes in mEPSCs, this study shows injury increases mIPSC amplitude, yet does not significantly alter their rise time or their half width. The change in mIPSC amplitude observed following injury is possibly due to phosphorylation of GABA<sub>A</sub> receptors by CAMKII, whose activity

has been found to increase after injury by our lab, possibly due to increased intracellular free calcium.

**TOLERANCE TO THE IMMUNOSUPPRESSIVE EFFECTS OF  $\Delta^9$ -THC IN B6C3F1 MICE.** Chris M. Sheth & Kimber L. White Jr., Dept. of Pharmacology and Toxicology, Virginia Commonwealth University, Richmond, VA 23298.  $\Delta^9$ -Tetrahydrocannabinol is isolated from *Cannabis sativa* and is responsible for its psychotropic effects. In the CNS, tolerance to THC's effects has been observed. We have seen a similar phenomenon in the immune system. Cell mediated immunity was evaluated in the delayed type hypersensitivity (DTH). 14 Days of THC exposure at 50 mg/kg suppressed the DTH response 43% compared to controls. With mice treated for 30 days responding identically to control mice. Our objective was to investigate THC's ability to produce immunologic tolerance using other functional assays. Mice were treated for 5, 14, or 28 days and were evaluated with the AFC response to the T-dependent antigen sRBC. Five days of dosing resulted in 47% suppression compared to controls. However, mice treated with for 14 or 28 days were suppressed 18% and 7%. To determine if the tolerance could be overcome, mice were treated for 14 days at 50 mg/kg and then at 200 mg/kg for an additional 4 days, which resulted in a 52% suppression, similar to the 5 day response. Tolerance was also demonstrated in the anti-CD3 mediated proliferation assay. At 6 hours post exposure the response was suppressed by 96%. However, there was no difference in the proliferative response in mice treated for 5 days when compare to vehicle controls. Following short periods of exposure THC is immunosuppressive; however, tolerance appears to develop after prolonged administration.

**COMPARISON OF ACUTE OPIOID DEPENDENCE IN THREE RAT STRAINS.** Richard W. Morgan, Robert L. Balster & Katherine L. Nicholson, Dept. of Pharmacology & Toxicology, Virginia Commonwealth University, Richmond VA 23298. Opioids are still among the most effective and widely used analgesic agents. Unfortunately, their clinical usefulness can be hindered by the development of physical dependence. While most models investigating physical dependence rely on chronic administration of opioids the Acute Opioid Antagonist Sensitization (AOAS) model can produce signs of withdrawal upon administration of an opioid antagonist (naltrexone) after a single dose of an opioid agonist (morphine) administered 4-hours earlier. Previous studies have used Sprague Dawley rats to demonstrate this phenomenon. The current study compares acute dependence in the outbred Sprague Dawley strain to the inbred Lewis and Fischer 344 strains. The latter two strains have been shown to have differing responses to opioid agonists. Relative to the other strains, Fischer 344 rats appeared to be more sensitive to the rate decreasing effects of naltrexone alone. While all three strains demonstrated sensitization to naltrexone following morphine, the degree of sensitization appeared greatest in the Lewis rats and least robust in the Fischer 344 rats. The ultimate goal of the study is to compare the ability of drugs from different classes to attenuate the development of acute dependence in the three strains, therefore, we have also completed dose effect curves in a separate group of Sprague Dawley rats showing

the ability of naloxone pretreatment to block the development of acute dependence. This work was supported by NIDA grants DA 01442 and DA 07027-30.

THE EFFECT OF TETRAETHYLAMMONIUM CHLORIDE ON THE KINETICS OF OSMOTICALLY-INDUCED HEMOLYSIS OF MAMMALIAN RED BLOOD CELLS. James W. Pickens & Stephen Gallik, Dept. of Biology, University of Mary Washington, Fredericksburg, VA. 22401. The specific objectives of this study are to describe the kinetics of osmotically-induced hemolysis of sheep red blood cells and the effect of tetraethylammonium chloride (TEA) on those kinetics using a comprehensive curve-fitting analysis designed to determine the simplest equation(s) that best describe the hemolytic process. The hemolysis of normal and TEA-treated sheep erythrocytes was tracked across a series of four hypotonic phosphate-buffered saline (PBS) solutions ranging from 0.40% to 0.55% PBS. The percent transmittance of 625nm light (%T625), used as a measure of the amount of hemolysis, was recorded continuously over a period of three minutes. The results show that the process of osmotically-induced hemolysis of sheep red blood cells can be described equally well by any one of five relatively simple kinetic equations, the simplest of the five being a second-order hyperbolic equation of the form  $y = (ax) / (b+x)$ , where a is the maximum %T625 (max % hemolysis) attained in the reaction and b is the time required to reach half-maximum %T625. The results also show that the initial instantaneous velocity of hemolysis of sheep red blood cells falls within a range of approximately 16 %T625/sec for cells submerged in 0.40% PBS to approximately 1.4 %T625/sec for cells submerged in 0.55% PBS. In addition, the results show that TEA, at concentrations of 62.5 mM and 125 mM, significantly reduces the initial instantaneous velocity of hemolysis in these four hypotonic solutions.

### Natural History & Biodiversity

TREES, TURTLES AND FISH: ASSESSING THE ECOLOGY OF THE CEDAR CREEK WATERSHED IN FREDERICK, SHENANDOAH AND WARREN COUNTIES, VIRGINIA. Woodward S. Bousquet, Lyla H. Gray, E. David Kocher, Arturo Oliverosamador & Gregory N. F. Spangler, Environmental Studies Program, Shenandoah University. Cedar Creek's watershed, part of the Shenandoah River drainage, is situated in the northern Shenandoah Valley and encompasses an area of approximately 157 square miles. Six sites for assessing fish assemblages and nine sites for describing terrestrial communities were selected to represent a diversity of ecological situations and geographic locations. Fish were electroshocked and released. Terrestrial communities were documented through the relevé procedure, which emphasizes physical features and vegetation. Thirty fish species in 8 families were collected. The average fish Index of Biotic Integrity (IBI) score of 4.25 showed that the creek's main stem is relatively clean and not degraded. Although a complete botanical survey was beyond the project's scope, this investigation, with additional data provided by Gary Fleming, Virginia Division of Natural Heritage (DNH), revealed 12 vascular plants not previously recorded for Frederick County

and 4 species new to Shenandoah County. Seven ecological communities were named using the DNH's classification system. Findings establish the watershed's environmental quality and biodiversity, providing a basis for its protection and management. (Supported by: Potomac Conservancy through the Potomac Watershed Partnership, the Virginia Foundation for Independent Colleges, and donations to Shenandoah University for environmental research, outreach and service-learning.)

THE 2005 VIRGINIA SOCIETY OF ORNITHOLOGY BREEDING BIRD FORAY IN RUSSELL AND WISE COUNTIES. Andrew S. Dolby, Dept. of Biological Sciences, University of Mary Washington, Fredericksburg VA 22401. The 2005 Virginia Society of Ornithology Breeding Bird Foray was conducted June 4-12 in Russell and Wise Counties. Twelve participants recorded species richness and abundance by both auto and foot. While effort was spread throughout Russell County, Wise County's high elevation High Knob Recreation Area and heavily forested Guest River Gorge were targeted. For both counties combined, 114 species were reported, representing 13 orders and 37 families. European Starlings were most abundant, followed by Common Grackles and American Robins. For 11 species, only one individual each was reported. Blue Jays were found along the greatest number of survey routes. In general, edge and disturbance-tolerant species predominated in Russell County, but more forest interior and high elevation species were detected at the Wise County sites. Direct signs of breeding activity were observed for 52 species, from sightings of male-female pairs to active nests containing viable nestlings. Similar species totals were reported during several previous southwestern Virginia forays spanning 1966 through 1992. However, many species were at far lower abundance in 2005. This apparent reduction in abundance may be attributable to habitat loss in both breeding and non-breeding ranges. Several species consistently detected during previous surveys in the region, but absent in 2005, were united by their dependence on spruce-fir forest. It is hypothesized that continued decline of this habitat type in the southern Appalachians has resulted in regional reductions in their populations, thus decreasing their overall likelihood of detection.

LARGE MAMMAL DISTRIBUTION, TRAP SUCCESS, AND HABITAT USE DETERMINED BY REMOTELY TRIGGERED INFRA-RED CAMERAS ON SALT POND MOUNTAIN, GILES CO., VA. Erika L. Holub & Marcella J. Kelly, Dept. of Fisheries and Wildlife Science, VPI & SU, Blacksburg VA 24061. In order to evaluate trap success across species and among camera types as well as predict occurrence of target species, we established 15 remotely triggered infra-red camera stations across a 27 km<sup>2</sup> study area comprised of Jefferson National Forest land, Mountain Lake Biological Station (MLBS) land, and other private land in Giles Co., VA. We monitored camera stations for 83 days (August to October 2005) for a total 891 trap nights (TN). Overall trap success for all animals combined was 51 per 100TN. As expected, white-tailed deer (*Odocoileus virginianus*), had the highest trap success (21.32) followed by all the target carnivores black bear (*Ursus americanus*) (1.97), coyote (*Canis latrans*) (1.01), bobcat (*Lynx rufus*)

(1.46), and gray fox (*Urocyon cinereoargenteus*) (0.67). Passive camera units had higher trap success than active camera units and digital cameras performed better than film cameras. We extracted percent cover type from a geographic information system (GIS) using circular buffers around each trap site and we determined the distance from each station to the main access road. Deer were common and showed little habitat preference. Black bears and coyotes exhibited a tendency to avoid coniferous forest, but only bobcats showed significant avoidance of coniferous forest and had higher trap success the further stations were away from the main access road. This study highlights the potential to use camera traps for numerous wildlife species (particularly carnivores) and to combine camera-trapping with GIS to predict animal occurrence across a landscape.

AN EXAMINATION OF FEED QUANTITY AND FEED QUALITY FOR *EPIOBLASMA SPP.* HELD AT WHITE SULPHUR SPRINGS NATIONAL FISH HATCHERY, WEST VIRGINIA. A. L. Bush<sup>1</sup>, S. R. Craig<sup>1</sup>, C. M. Gatenby<sup>2</sup>, D. A. Kreeger<sup>3</sup> & R. J. Neves<sup>1</sup>, <sup>1</sup>Dept. of Fisheries and Wildlife Sciences, Va. Polytechnic Inst. & State Univ., Blacksburg VA 24061, <sup>2</sup>White Sulphur Springs National Fish Hatchery, White Sulphur Springs WV 24986 and <sup>3</sup>Partnership for the Delaware Estuary, Wilmington DE 19801. Recovery of federally endangered oyster mussel (*Epioblasma capsaeformis*), northern riffleshell (*Epioblasma torulosa rangiana*), and Cumberlandian combshell (*Epioblasma brevidens*) depends upon present efforts to successfully propagate and rear juveniles, and hold adults in a captive environment. An understanding of food quantity and food quality requirements for *Epioblasma spp.* is vital to successful captive care. Neither an optimum food quantity, nor specific food quality requirements have been identified for adults of these species. An optimum feed ration for adults fed green-algae (*Neochloris oleoabundans*) will be determined via measurements of clearance rate and absorption efficiency. Clearance rates were measured for oyster mussel, northern riffleshell, Cumberlandian combshell, and snuffbox (*Epioblasma triquetra*). Average clearance rates among species ranged from 1.1 to 1.6 mg h<sup>-1</sup> g<sup>-1</sup> of wet mussel tissue. Clearance rates for all species were highest when fed a ration of 80,000 cells ml<sup>-1</sup>. Diet quality will be examined by targeting seasonal protein demand of specimens. Oyster mussels will be fed low or high protein diets of *N. oleoabundans* for 3 weeks in spring, summer, and fall 2006. O/N ratio will be measured before and after trial diets to assess seasonal protein demand.

LEAF MOVEMENT AND LEAF DAMAGE IN *CERCIS CANADENSIS*: DO LEAVES ACT DIFFERENTLY IN THE SUN AND SHADE? A. Smith & A. B. Griffith, Dept. of Biological Sciences, University of Mary Washington, Fredericksburg VA 22401. Past research has shown that plants growing under different light conditions (direct light, full shade, and partial shade) differ in photosynthetic rates. Two other processes, heliotropism and leaf damage, may lead to photosynthetic differences in sun and shade. Our objective was to measure leaf movement and leaf damage in different light conditions for *Cercis canadensis*. Forty plants were sampled from four sites. At each site five plants were in the sun and five in the shade. Leaf orientation (azimuth and altitude) and leaf damage were

measured on ten leaves. We found differences in leaf orientation of light and shade plants. Leaves in direct light oriented in the same direction while leaves in the shade oriented randomly. The percent of leaves damaged / plant was significantly different between light environments ( $F = 5.5$   $p = .026$ ) and between sites ( $F = 9.0$ ,  $p = <.001$ ). There was also an interaction between sites and light environment ( $F = 4.4$   $p = .011$ ). The percent of damage / leaf was significantly different between light environments ( $F = 31.69$ ,  $p < 0.001$ ) and between sites ( $F = 6.28$ ,  $p < 0.001$ ). Plants growing in a shaded environment tend to have a greater, amount of leaf damage per plant and per leaf than those in the full sun. Our results show that leaf damage is more prevalent in cool, damp environments. It is likely that disease is more damaging to photosynthesis in these conditions than in full sun.

INSECT HERBIVORES OF *AILANTHUS ALTISSIMA* (TREE OF HEAVEN) IN VIRGINIA AND THEIR POTENTIAL AS BIO-CONTROL AGENTS. Shicai Yan, T.J. McAvoy, S.G. Salom & L. T. Kok. Dept. of Entomology, Va. Polytechnic Inst. & State Univ., Blacksburg VA 24061. *Ailanthus altissima* (tree of heaven) is a deciduous woody tree introduced from China to North America in 1800s'. Currently, it has dispersed into 41 of the United States and is regarded as a pest weed. Our monthly surveys in nine sites around Virginia in 2004 and 2005 revealed that native insect herbivores have little impact on *A. altissima* and their potential utilization as biological control agents is minimal. The majority of herbivores we collected are foliage feeders with broad host range and low abundance. *Ailanthus* webworm, *Atteva punctella* Cramer, is the only herbivore species consistently present in all sites. It caused 50% or more defoliation for one-year aged seedlings, while less than 5% defoliation on larger trees (>3 cm diameter) with no visible impact. The only woodborer is ambrosia beetle, *Scolytinae* spp., which is collected from isolated dying trees. Their effects on health *A. altissima* are unknown.

EFFECT OF SIZE AND TEMPERATURE ON OXYGEN CONSUMPTION IN THE HISSING COCKROACH (*GROMPHADORHINA PORTENTOSA*). Jeffrey W. Streicher & Geoffrey F. Birchard, Department of Environmental Science & Policy, George Mason University, Fairfax VA. 22030-4444. *Gromphadorhina portentosa* (Blatteria: Blaberidae) is a cockroach species from tropical Madagascar. Despite its commercial popularity and availability, no physiological research has been conducted on this species. Herein we present the first examination of the rate of metabolism in relation to body mass and temperature in *G. portentosa*. Oxygen consumption rate of nymph and adult cockroaches ( $n = 95$ ) temperature acclimated to 28°C ranging in mass from 0.06-10.61g was measured. The relationship between body mass and oxygen consumption rate was shown to be highly significant ( $P = 0.0000$ ). Oxygen consumption rate scaled with an exponent of 0.51, which is lower than the values previously reported for insects. The oxygen consumption rate was also measured at four different temperatures over the range of 16-34°C. An analysis of covariance showed that the oxygen consumption rate decreased with temperature ( $F_3 = 50.56$ ,  $P = 0.0000$ ).  $Q_{10}$  values were determined between each of the temperature classes.  $Q_{10}$  values ranged between, 1.651-4.458. An arrhenius plot

of these data indicates a possible break in the relationship for *G. portentosa* between 21 and 16°C. This break along with observed behavioral reactions may indicate the onset of cold torpor around 16°C which would be consistent with the tropical ecology of *G. portentosa*.

A SURVEY OF FLORA AND FAUNA AT HANOVER WAYSIDE PARK HANOVER COUNTY, VA. R.A. Hawkins, K.D. Arnold, & R.S. Groover, Biology Department, J. Sargeant Reynolds Community College, Richmond VA 23285. As the ecology component of a Biology 102 class, a survey of flora and fauna at Hanover Wayside Park in Hanover County, VA was conducted in April of 2006. Eight teams collected data on located flora and fauna in the Park. The survey will be presented to the County as a community service project, to assist with upcoming environmental assessments. The list of identified species include: 17 tree species were located, 9 fungi and lichen; 30 shrubs, grasses and other plants; 15 mammal species, 18 birds; 6 reptiles and amphibians; 12 arthropods; and 20 protists. Species found were typical of a temperate deciduous forest, with no rare, threatened or endangered species found.

SYSTEMATICS OF OPISTHOBRANCH MOLLUSKS FROM THE EASTERN SHORE OF VIRGINIA (MOLLUSCA: GASTROPODA: OPISTHOBRANCHIA). Sara G. Lawrence & Deirdre Gonsalves-Jackson, Dept. of Biol., Randolph-Macon Woman's College, Lynchburg VA. 24503. Few studies exist documenting the diversity of opisthobranch mollusks (sea slugs) from the Eastern Shore of Virginia. To date only 21 species have been recorded. The goals of this research were to survey and document the current diversity of marine slugs on both the Eastern Shore and the Chesapeake Bay. This was accomplished through methods of collection such as snorkeling and wading followed by laboratory examination of collected specimens. Of the seven sites sampled (5 Atlantic and 2 Bay sites) two species in two families were collected during an eight week period from June – July, 2005. Both species were collected from the Eastern Shore and are new records for that area. Additional sampling is necessary to fully assess current diversity levels.

EXPERIMENTAL EFFECTS OF FOREST REGENERATION METHODS ON SALAMANDER POPULATIONS UP TO 11-YEARS POST-TREATMENT. Jessica A. Homyak<sup>1</sup>, Carola A. Haas<sup>1</sup> & Shannon M. Knapp<sup>1,2</sup>. <sup>1</sup>Dept of Fisheries and Wildlife Sciences, Va. Polytechnic Inst. & State Univ., Blacksburg VA 24061 and <sup>2</sup>Dept. of Statistics, Purdue University, West Lafayette IN 47907. In commercial and public forests, silvicultural systems are expected to maintain ecological sustainability in addition to reaching economic, social, recreational, and aesthetic objectives. Plethodontid salamanders are physiologically tied to microhabitat and microclimatic conditions and may play critical roles in energy and nutrient cycling and thus are model organisms for examining effects of forest management. To investigate effects of forest management on salamanders, a suite of 7 oak regeneration treatments, ranging from no treatment to clearcut harvest, were experimentally applied across 7 replicates in southwestern Virginia and West

Virginia during 1994-1998. Terrestrial salamander populations were monitored pre- and post-treatment with night-time, area-constrained searches from spring-fall 1994 through 2005. We report trends in salamander populations on 4 sites that were sampled nearly every year up to 11-years post-harvest with >4,500 salamanders captured. Plethodontid salamanders were less abundant on plots following all over-story removal treatments as compared to either control or herbicide treated plots ( $P < 0.05$ ), and abundances remained below 50% of pre-treatment levels 7-11 years since the initial harvest. Ongoing research will examine effects of repeated-stand entries to salamanders, potential mechanisms for the slow recovery of populations, and the ecological consequences of reduced densities of salamanders.

THE EFFECTS OF HERBIVORY ON A RARE, WETLAND LEGUME, *AESCHYNOMENE VIRGINICA*. C. Eck<sup>1</sup> and A.B. Griffith<sup>2</sup>. <sup>1</sup>Dept. of Environmental Sciences and Geology, University of Mary Washington. <sup>2</sup>Dept. of Biological Sciences, University of Mary Washington, Fredericksburg VA 22401. The Endangered Species Act grant endangered species the right to life regardless of any monetary value they may or may not possess. Apart from their intrinsic value, conserving rare plants helps maintain biodiversity that may aid in the long term viability of ecosystems. *Aeschynomene virginica* is a threatened, freshwater, tidal, wetland plant of the mid-Atlantic region. Little is known about the impact of herbivory on seed production of this plant. In September 2005, we conducted a census of all populations on the Cumberland Marsh Preserve, New Kent County, VA. The mean % of seeds eaten per plant for all populations was 9.4 %. The mean % incidence of herbivory per population was 66%. We found no significant relationships between % of seeds eaten or % incidence of herbivory and number of plants per population, distance to nearest population, or distance to nearest corn field. This year we found no relationships between seed predation and population size, population density, or distance from potential herbivore source. But, population size of *A. virginica* was the lowest since 1998 and random error in the small census could swamp relationships. The mean levels of herbivory suggest a possible important decrease in seed production, especially in low population years. We plan two more years of data collection to develop a broader picture of seed predation impact on this rare plant.

A COMPARATIVE STUDY OF THE HARVESTMEN OF THE FAMILY MANAOSBIIDAE (OPILIONES, LANIATORES) FROM TRINIDAD, W. I. Daniel N. Proud, Virginia Wesleyan College, 1584 Wesleyan Drive, Norfolk VA 23502. Prior studies of the harvestmen (Arachnida, Opiliones) of Trinidad, W. I. have documented the occurrence of 24 species on the island, including 2 species (*Cranellus montgomeryi* and *Rhopalocranaus albilineatus*) of the family Manaosbiidae. Relatively little is known about the distribution or natural history of these harvestmen. From 9 July to 5 August 2005, I collected adult specimens of *C. montgomeryi* and *R. albilineatus* from several locations. From 20 July to 5 August 2005, I hiked and camped along Morne Bleu Ridge and collected adults of a manaosbiid species that had not previously been reported for the island. I collected this species in montane rainforest and elfin woodland only at elevations above 600

m. This past year, I used light microscopy and scanning electron microscopy to examine important diagnostic characters of these harvestmen, including the armature of the pedipalps, legs, chelicerae, eye mound, and dorsum as well as the number of articles on the basitarsus (legs I-IV), the length of the segments of legs I-IV, and total body size (length and width). The results of my study indicate that the previously unreported species of harvestmen is a new species. Efforts are presently underway to complete a preliminary description of this animal and verify its status. (Supported by grants from the Virginia Academy of Sciences Undergraduate Research Fund and the Virginia Wesleyan College Undergraduate Research Fund).

IMPACT, DISTRIBUTION, AND BIOLOGICAL CONTROL OF HEMLOCK WOOLLY ADELGID IN VIRGINIA. T. J. McAvoy, S. M. Salom, A. Lamb & D. Mausel, Dept. of Entomology, Va. Polytechnic Inst. & State Univ., Blacksburg VA 24061. Surveys (90) of hemlock stands (*Tsuga canadensis*) were conducted from 1997 to 2005 from Bath and Rockbridge counties south to Lee and Grayson counties, Virginia and Alleghany, Ash, and Watauga counties North Carolina. Tree health and hemlock woolly adelgid (HWA) (*Adelges tsugae*) infestation rates, a serious pest of hemlock were recorded at each site. Sampling of HWA involved examining the terminal 30 cm of 50 branches at random from at least ten trees at each site. Presence or absence of HWA was noted and the percent of the 50 branches infested with at least one HWA was recorded. Hemlock stand health was determined by measuring: percent crown density, live crown ratio, live branches, live tips, new foliage, and live trees at each site. These six parameters were summed and divided by 6 to obtain a health index. The percentage of sites infested with HWA was 25, 36, 41, 53, 53, 57, 59, 62, and 77 in 1997, 1998, 1999, 2000, 2001, 2002, 2003, 2004, and 2005, respectively. Stand health index declined from 72 to 57 from 1997 to 2005. None of the sites had any tree mortality until 2000 when 8% of the sites had at least one dead tree and in 2005, 30% of the stands had at least one dead tree. Two biological control agents have been released in Virginia, *Sasajiscymnus tsugae* (Coleoptera: Coccinellidae) (8 sites) and *Laricobius nigrinus* Fender (Coleoptera: Derodontidae) (5 sites). *Sasajiscymnus tsugae* has not been recovered however; F<sub>3</sub> *L. nigrinus* has been recovered from most of the release sites. This species appears to be established at these release sites.

REDEFINING VIRGINIA'S PREHISTORIC INDIANS: THE NATIVE AMERICAN COLLECTION FROM THE VIRGINIA DEPARTMENT OF HISTORIC RESOURCES. Donna C. Boyd<sup>1</sup>, Cliff Boyd<sup>1</sup>, & Richard Guercin<sup>2</sup>  
<sup>1</sup>Dept. of Soc. and Anth., Radford Univ., Radford VA 24142, and <sup>2</sup>U. S. Forest Service, George Washington and Jefferson Nat. Forest, 5162 Valleypointe Parkway, Roanoke VA 24019. In 2004, the remains of 70 prehistoric (A. D. 300 – 1700) Native Americans from ten sites across the Commonwealth of Virginia were sent by the Virginia Department of Historic Resources (VDHR) to the Radford University Physical Anthropology and Archaeology Laboratory for analysis. Many of these remains, although excavated decades ago, had never been professionally analyzed using current standard osteological techniques. The goals of this study were to record as much as possible about the Native Americans from the VDHR collection

and to bring these collections up to current standards for curation and maintenance. Sixty-two prehistoric Native Americans are identified from the seven Southwest Virginia sites which are the focus of this paper. These include 19 males, 15 females, four adults of indeterminate sex, and 24 subadults. High frequencies of dental pathologies including dental caries, antemortem tooth loss, and enamel hypoplasias are noted across the collection, in addition to multiple evidences for non-specific infection. These observations are consistent with a pattern of declining health associated with an increased dependence on maize agriculture. Our research has also revised and corrected earlier analyses of these individuals and has shown the significance and worth of this collection for further study. We, therefore, strongly recommend continued curation of this collection. This study was funded by a grant from the Va. Dept. of Historic Resources, Richmond.

PLANT DISTRIBUTIONS ALONG CORRIDORS AT THE GRASSY HILL NATURAL AREA, FRANKLIN COUNTY, VA. Gregory D. Turner<sup>1</sup> & Marianne Demko<sup>2</sup>, <sup>1</sup>Dept. of Biol., West Chester Univ., West Chester, PA 19383 and <sup>2</sup>Va. Western Comm. Coll., Roanoke, VA 24038. Disturbance from human-made corridors influences plant distributions. Specifically, disturbance influences seed dispersal and microclimate, differentially affecting plant recruitment and survival. To better understand how corridors influence plants, a study was conducted to examine (1) whether plant abundance varies with corridor type and (2) whether plant abundance varies with corridor proximity. The study also assessed environmental factors to help explain distributions. To address these questions, the native grasses *Brachyelytrum erectum*, *Leersia oryzoides* and *Panicum clandestinum*, and the exotic *Microstegeum vimineum*, were quantified adjacent to one of three corridors (paved, gravel, & dirt roads) traversing the Grassy Hill Natural Area. Plants were quantified in transects varying in distance from the corridors. *B. erectum* and *P. clandestinum* were abundant across corridors and at all distances, while *L. oryzoides* and *M. vimineum* were only abundant at corridor edges. Corridor type and proximity were influential as we found significant effects of corridor type on native abundances, while *L. oryzoides* and *M. vimineum* abundance was also affected by proximity. Results suggest that the type of, and proximity to, corridors influences grass distributions, possibly due to microclimatic variation and disturbance itself, and that some species may actually benefit from corridor disturbance.

FECUNDITY OF THE SICKLEFIN REDHORSE, CAROLINA REDHORSE, AND HARELIP SUCKER. M. L. Henebry, R. E. Jenkins & E. Jorgensen-Earp, Dept. of Biol., Roanoke College, Salem VA 24153. The Sicklefin and Carolina redhorses are relatively rare sucker fishes, inhabiting a range hindered by dams. The Harelip Sucker, *Lagochila lacera*, is an extinct species of its monotypic genus, which is closely related to redhorses. Fecundity was studied for Sicklefin and Carolina redhorses and Harelip Sucker to determine reproductive capacity and ovarian cycles. Oocyte counts were taken gravimetrically by 5–6 subsamples of 0.50–2.77 g per Sicklefin and Carolina Redhorse specimen. Sicklefin fecundity ranged 6307–22,298, mean 14,165, in 12 specimens of 385–500 mm SL. Gravid

Sicklefin mean oocyte size ranged 2.58–3.15 g. The Sicklefin's annual pattern of ovarian development includes increase in GSI and oocyte size during March and especially April, the start of spawning and occurrence of some spent fish in mid-April, and onset of recrudescence in July or August. Diameters of 4 artificially fertilized, water-hardened, round eggs were 4.10–4.25 mm, mean 4.15 mm. Unspawned eggs taken from 2 ripe females ranged 3.00–3.40 mm, mean 3.17, 3.18 mm. Carolina Redhorse fecundity was 19,907 and 21,384, mean 20,646 in the 2 specimens imminent to spawn. Gravid Carolina Redhorse had oocyte diameters of 2.15–2.70 mm, mean 2.41 mm. Fecundity of the *L. lacera* specimen was 8926 maturing oocytes and an undetermined, manyfold greater number of apparently non-maturing oocytes. Maturing oocytes ranging 1.35–1.60 mm, mean 1.47 mm and non-maturing oocytes from the same subsamples ranged 0.25–0.45 mm, mean 0.323 mm.

IMPACTS OF TROUT STOCKING ON AQUATIC INVERTEBRATE DIVERSITY AND ABUNDANCE IN STREAMS. Amanda A. Crossett & Patrick W. Crumrine, Department of Natural Sciences, Longwood University, Farmville VA 23909. Non-native species can have significant and long-lasting impacts on native communities. Non-native species that replace top predators have the potential to disrupt food webs through predation and competition. Non-native species of trout are commonly introduced to streams in eastern North America to provide recreational fishing opportunities and function as top predators in these ecosystems. Trout species stocked in Virginia are brown trout (*Salmo trutta*) and rainbow trout, (*Oncorhynchus mykiss*). We sampled the aquatic invertebrate community in riffles from streams in Virginia before and after trout stocking events to better understand how these fishes, impact native aquatic invertebrate communities. Although we did not observe changes in species diversity, there were changes in community structure. The number of aquatic invertebrates in riffles increased after the stocking of trout. Mayflies and caddisflies were significantly more abundant in riffles after trout stocking relative to other orders collected from the riffles. These preliminary results suggest that non-native trout influence aquatic invertebrate communities in streams but their complete impacts remain to be fully described.

## Psychology

CLICK IT OR TICKET VS. FLASH-FOR-LIFE: A COMPARISON OF INTERVENTION TECHNIQUES TO INCREASE SAFETY-BELT USE OF STUDENTS AT A LARGE UNIVERSITY. Matthew G. Cox, E. Scott Geller, Christina L. Goodwin, & Steven W. Clarke, Department of Psychology, Va. Polytechnic Inst. & State Univ., Blacksburg, VA 24061. While safety-belt use in the U.S. is high (80%), non-use remains an ongoing issue requiring intervention. The most widely used technique to increase safety-belt use is the national Click it or Ticket (CioT) campaign. Another technique used, while less well known, is the Flash-for-Life (FfL). This intervention employs a person holding a sign asking drivers to buckle up on one side; then on the other side, thanks them when they do

so. This study examined the effectiveness of the respective sign for each intervention to increase belt use in 2 commuter parking lots at a large university. In addition, observations were made on positive/negative hand gestures and positive/negative facial expression made when drivers were exposed to the respective signs. Data were collected during 3 non-consecutive 1 hour shifts, 4 days a week for 2 months. Researchers targeted unbuckled student drivers leaving the parking lots and flashed the respective sign, alternating signs each day. A total of 1,822 student drivers (574 women, 1,248 men) were exposed to the signs. Of those exposed to the FfL sign, 34% buckled, vs. 25% compliance with the CioT sign. In addition, the FfL sign elicited significantly more positive and less negative facial expressions and hand gestures than the CioT sign. Overall, the FfL technique appears to be more effective at increasing safety-belt use among college students who seem to be more receptive to the FfL technique. Further research should examine the lasting effects of the FfL intervention.

**BETTER MEDICATION ORDERING THROUGH TECHNOLOGY: EVALUATING BEHAVIORAL RESULTS OF A SHIFT TO ELECTRONIC MEDICATION ORDERING.** Patrick A. Rhodes, David M. Harris, Thomas R. Cunningham, & Christina L. Goodwin. Virginia Polytechnic Institute and State University, Blacksburg, VA 24061. Nineteen percent of medications in U.S. hospitals are administered in error. Computerized Physician Order Entry (CPOE) potentially decreases these errors by eliminating problems with current handwritten systems. This data-based paper will present objective observations obtained at two regional hospitals. The research investigated the impact of a CPOE system on several physician medication ordering behaviors. Many people are injured each year as a result of adverse drug events, and CPOE is considered critical to reducing this number. The dependent measures are order compliance, time-to-first-dose of antibiotic, and reported medication incidents. Data were obtained by reviewing over 1,000 medication orders over four months. Data compare compliance and efficiency measures pre- and post-CPOE implementation, as well as between- and within-group comparisons of written and CPOE orders. Measures of reported medication incidents from the intervention hospital were compared to the control hospital. Greater rates of compliance and efficiency for electronic orders were found. Implications for future CPOE system implementation and patient safety will be discussed.

**IMPROVING MEMORY IN THE ELDERLY VIA POSITIVE FEEDBACK.** Kathryn Van Veen & David G. Elmes, Dept. of Psychology, Washington and Lee Univ., Lexington, VA 24450. Elderly people frequently complain about having difficulty remembering names. We report on two experiments testing the effect of feedback on learning and retention of names and occupations. College students and elderly participants had to remember the names and occupations of faces seen on a computer. After matching on first trial performance, half the participants received feedback informing them that they had done very well. The remaining participants received no feedback. On subsequent trials after feedback, the elderly who received feedback recognized names, but not occupations, better than the elderly who did not

receive feedback. Feedback did not influence the college students' recognition. Both experiments showed the same pattern of results. One way to interpret the results is to consider the name memory task as a stereotype threat to the elderly. Feedback lifts the stereotype threat, and the elderly recognize names better on subsequent trials. Recognition of occupations is not a stereotype threat to the elderly, so feedback did not influence recognition of occupations. Since stereotype threat was irrelevant to the college students, feedback and stereotype lift did not occur for the students. Removing stereotype threat might be an effective addition to memory remediation programs for the elderly.

THE EFFECT OF PFIESTERIA TOXIN ON LEARNING IN RATS: REPEATED ADMINISTRATION AND PERFORMANCE IN THE RADIAL-ARM MAZE. Shawn L. Dickerson, Perry M. Duncan, & Rebecca G. McBride, Dept of Psychology, Old Dominion Univ., Norfolk, VA 23508. *Pfiesteria piscicida* is a toxic dinoflagellate with lethal effects on fish and cognitive effects on humans. The cognitive effects consist of impairment for memory and learning which has been previously demonstrated in rat models. This experiment utilized twenty-three male Long-Evans rats, twelve of which were injected intraperitoneally with a *Pfiesteria* culture and eleven which were injected with a non-toxic water culture. This study used repeated administration of the *Pfiesteria* toxin for three weeks to be more similar to human exposure. The rat subjects were tested with a radial-arm maze (RAM) twice each week, where three of the eight arms were baited. The researcher recorded total errors for each rat over each week. Results indicate minimal support for the hypothesis that the experimental group would progressively get worse at learning the RAM over several weeks after repeated exposure to *Pfiesteria*. The results were not found to be significant but the mean total errors did suggest an emerging difference between the two groups. Additional research is required to demonstrate clearly that *Pfiesteria* toxin interferes with learning upon repeated exposure.

THE EFFECT OF PFIESTERIA TOXIN AND ETHANOL ON RAT SPONTANEOUS MOTOR ACTIVITY AND PERFORMANCE IN THE ELEVATED-PLUS MAZE. Jammie M. Abbott, Department of Psychology, Old Dominion University, Norfolk, VA 23529. *Pfiesteria piscicida* is an ambush predator dinoflagellate that has been implicated in multiple health problems. To fully understand and mitigate the impact of *Pfiesteria* this research attempts to determine if *Pfiesteria* exposed rats have significantly different spontaneous motor activities and anxiety-related behaviors than non-exposed rats. In order to evaluate these previously documented behaviors fully two interwoven experiments were conducted. In the first experiment a total of 32 rats were randomly separated into either a control or an experimental group. In this part of the experiment the anxiety level of the *Pfiesteria* exposed group and control group participants were evaluated utilizing an Elevated-Plus Maze (EPM). Anxiety for this experiment was operationally defined as greater time spent in the closed arms of the EPM. Data obtained from this portion of the experiment and its subsequent analysis with mixed ANOVAs indicated that participants in the *Pfiesteria* exposed group were

significantly more anxious than participants in the control group. One day after initial experimentation was completed, Spontaneous Motor Activity (SMA) was evaluated in the same participant groups utilizing three different doses of ethanol. In this part of the experiment participants' motor activities as denoted by ambulation and rearing were detected and recorded by IR light-sources, photocells, and computer systems. Data obtained from this portion of the experiment was inconclusive producing similar ethanol effects on Spontaneous Motor Activity for both the *Pfiesteria* exposed and control groups.

AN INVESTIGATION OF CONTROL CONDITIONS IN ASCH-TYPE EXPERIMENTS: VII. Joshua M. Taylor, Alexander R. Titus, Zachary A. Philbrick, E. JoAnn Boyce, Ahmad J. Bah, Rodolfo E. McIntyre, & James P. O'Brien, Tidewater Cmty. Coll., Virginia Beach, VA 23453. For almost 60 years replications of Solomon E. Asch's classic group pressure paradigm have typically failed to conduct adequate control groups. This neglect reflects a prevalent assumption that high stimulus clarity, an essential feature of the "Asch dilemma," obtains for all participants in all experimental treatments. However, this 2x2x2x2 control replication (N=689) confirms that Experimenter-Participant contextual factors interact complexly to produce stimulus ambiguity among female participants and most men. This factorial design systematically manipulated Experimenter gender and status (authoritative vs. peer) and participant gender and institution of origin (4-yr. vs. community college). All Es and male participants were White (as in Asch) but, since there is little stimulus clarity data for women, female participants were not restricted on ethnicity. Of the 16 conditions, only the direct replication of Asch's controls – 4-yr. White male undergraduates with an Authoritative White male Experimenter (n=37) – closely approximated Asch's standards for stimulus clarity. Since the baselines of other participants emanate from stimulus ambiguity, the vast literature (including Asch's group pressure treatments with female Experimenters) must be reevaluated regarding issues such as the persistent sex difference, internal validity, population validity, cultural relativism, the nature of the cognitive conflict, and corruption by informational influence (in addition to normative social influence). Abstracts of previous interim reports appear in the *Proceedings* issues of *Va. J. Sci.* 1999-2005.

ETHNICITY IN ASCH CONTROL REPLICATIONS: AFRICAN-AMERICAN PEER EXPERIMENTERS AND COMMUNITY COLLEGE PARTICIPANTS: II. James P. O'Brien, E. JoAnn Boyce, Alexander R. Titus, Joshua M. Taylor, Zachary A. Philbrick, Ahmad J. Bah, Rodolfo E. McIntyre, Sulaiman T. Bah, Neysa L. Isler, Natalie A. Clouser, Lauren H. Compton, Tiffany Blake, Gayle P. Lentz, Andrea L. Atwell, Lauren K. Burt, Denise A. Lugenbeal, Elisa M. Ramirez-Feliciano, Danial J. Kim, Adam J. Stinson, Elizabeth S. Martin & Sakeenah T. Abdullah, Tidewater Cmty. Coll., Virginia Beach, VA 23453. Danso and Esses (2001) found evidence for stereotype threat activation among White participants on a test of mathematical reasoning; participants high in social dominance orientation performed significantly better when the test administrator was Black rather than White. Since the Asch stimuli are relatively neutral, would E's ethnicity affect stimulus clarity measures?

The procedure is identical to that described above (Taylor, et al), with two exceptions: (1) E (male or female) is of African-American descent and (2) groups were mixed genders and ethnicities. Comparing the stimulus clarity measures for these White male Ss with Black male Es (n=78) or with Black female Es (n=26) with similar cells in the main protocol (White male or White female peer Es), there is no evidence of stereotype threat activation. For White female participants and Black male Es (n=59), the hypothesis of stereotype threat activation is supported. Since 79% of Danso and Esses' Ss were women, sex x ethnicity interactions are suggested. Many sample sizes are still too small for analysis and data using Black authoritative Es (instead of peers) have not yet been acquired. An abstract of the first interim report of this protocol (Bah, et al.) appeared in *Va. J. Sci.*, 54 (2), Summer 2003, p. 108.

THE EFFECTIVENESS OF AUDITORY COLLISION AVOIDANCE WARNINGS IN REDUCING FATIGUE-RELATED CRASHES. Jennifer F. May<sup>1</sup>, Carryl L. Baldwin<sup>1</sup>, & Raja Parasuraman<sup>2</sup>. <sup>1</sup>Old Dominion University, Department of Psychology, <sup>2</sup>George Mason University, Department of Psychology. The goal of this study was to examine the effectiveness of two auditory collision avoidance system (CAS) warnings for reducing crash rates in fatigued drivers faced with a potential rear-end collision. Forty-five participants between the ages of 18 to 82 ( $M = 46.56$ ,  $SD = 22.52$ ), including 19 between the ages of 18-35 and 23 between the ages of 60-82, were included in the analysis. Participants completed a 1.5 hour fatigue inducement process followed by a potential collision scenario. Fatigue was determined from the continuous measure of lane position variability (LPV), and was calculated at 1 standard deviation above the participant's baseline LPV. Once the LPV reached 1 SD above baseline during the collision scenario, an event was triggered where the lead car suddenly and rapidly decelerated to a stop. Participants either heard a verbal warning "danger," a 1000 Hz tone or no warning. A binary logistic step forward regression indicated that CAS warning type was the only predictor of CAS response,  $\beta = -.975$ ,  $p = 0.037$ , Nagelkerke  $R^2 = .151$ . Eight of the 13 crashes occurred in the no warning condition. The presence of a CAS warning was particularly beneficial to reducing crashes in older drivers,  $\beta = -2.207$ ,  $p = 0.05$ , Nagelkerke  $R^2 = .392$ . Following distance was significantly greater in the older group,  $F(1, 36) = 13.57$ ,  $p = .001$ ,  $\eta^2 = .25$ . Stopping distance was also greater for older drivers,  $F(1, 23) = 6.6$ ,  $p = .017$ ,  $\eta^2 = .22$ . Results of this study indicate that auditory CAS warnings may help reduce fatigue-related rear-end crashes, particularly among older drivers.

JUGGLING MULTIPLE ROLES WHILE MAXIMIZING LIFE SATISFACTION. Marian L. Troutman & Barbara A. Winstead, Department of Psychology, Old Dominion University, Norfolk, VA 23529. The present study examined the demands of multiple roles and the coping strategies that individuals use to maximize their work, parenting, and general life satisfaction. Participants included 31 college students who were both workers and parents. They completed questionnaires that assessed family and work demands, coping strategies, conflict, spillover, and parent, work, and life satisfaction. The researcher hypothesized that for participants who

face multiple demanding roles, the use of coping strategies and positive spillover would positively correlate with levels of parent, work, and life satisfaction. Also, levels of conflict would negatively correlate with levels of parenting, work, and life satisfaction. Pearson's  $r$  was used to test hypotheses. Partially supporting one of the three hypotheses, researchers found work-family conflict to be negatively related to work satisfaction. Using a larger and more representative sample, future research should examine job and family involvement to see how levels of role demands moderate the relationships such that correlations will be stronger when role demands are higher.

THE EFFECTS OF RELATIVE SYSTEM RELIABILITY AND PRIORITIZATION ON ALARM REACTION TIME. Elizabeth T. Newlin, Ernesto A. Bustamante, James P. Bliss, Randall D. Spain, & Corey K. Fallon, Department of Psychology, Old Dominion University, Norfolk, VA 23529. Alarm system operators often manage multiple alarm systems concurrently. Because such situations frequently accompany cascading events, it is important to know how operators sequence responses. We examined how relative reliability and priority of two concurrent alarms affected alarm reset patterns and response times. We hypothesized that operators would respond first to an alarm with higher reliability or higher priority when the other variable was held constant. We expected that participants would respond to alarms with higher priority and that response times would increase when one alarm was more reliable and the other alarm had higher priority. Sixty-one Old Dominion University undergraduates performed a tracking task and responded to concurrent alarms. A between-subjects ANOVA revealed that participants responded to alarms with higher priority first when reliability was constant and to higher reliability alarms first when priority was constant. Participants did not respond significantly more often to the higher priority alarm when one alarm was more reliable and the other alarm had higher priority. A mixed ANOVA demonstrated there was no difference in response times across conditions. Our results suggest that relative priority and reliability may be useful parameters to control in complex task sequencing.

DETERMINANTS OF BODY IMAGE ATTITUDES AND EATING DISTURBANCES IN BLACK COLLEGE WOMEN. Sharnail D. Bazemore & Thomas F. Cash, Department of Psychology, Old Dominion University, Norfolk, VA 23529. The present study examined the effect of family and peer groups, ethnic identity, and media images on body image ideals and eating disturbances among Black women. Participants included 186 Black female psychology students from Old Dominion University. The current study merged an existing dataset of 115 participants with data collected from an additional 69 participants to attain desired sample size. Participants completed a series of online questionnaires that evaluated self-attitudes related to body image, eating habits, past and present social groups, ethnic identity, and personal experiences with racial diversity. Pearson correlations revealed all variables, with the exception of family and peer groups, had a significant impact on the formation of body image and eating attitudes in Black women. Past peer groups were moderately related to body image and eating

attitudes. Standard multiple regression analyses revealed Black Media Internalization was the only significant predictor of Self-Evaluative Salience, and Self-Hatred and Anti-White were the only significant predictors for Eating Attitudes. Our study also confirms that additional research is needed to identify variables within the Black culture that are pertinent to the development and prevention of eating disturbance and negative body image. This is particularly relevant to clinical populations. Limitations and implications for future research are discussed.

### Statistics

GENE NETWORK INFERENCE VIA GENETIC ANALYSIS OF EXPRESSION PROFILES IN SEGREGATING POPULATIONS. Bing Liu<sup>1</sup>, Alberto De la Fuente<sup>2</sup> & Ina Hoeschele<sup>1</sup>, <sup>1</sup>Dept. of Stat. and Va Bioinformatics Institute, <sup>2</sup>Va Bioinformatics Institute, Va. Polytechnic Inst. & State Univ., Blacksburg, VA 24061. Genetical genomics experiments are multifactorial perturbation experiments in which a segregating population is expression profiled for ten thousands of genes and DNA marker genotyped for hundreds or thousands of markers. Quantitative Trait Locus (QTL) mapping identifies which genomic regions influence the expression of which genes, and which genes have causal, regulatory effects on other genes. This information is used to construct a causal, encompassing network of regulatory relationships among genes and QTL. Subsequently, the network is sparsified using Structural Equation Modeling (SEM), which retains only direct regulatory relationships. As opposed to current Bayesian network analyses, SEM analysis does not require discretization and is able to model networks with cycles or feedback loops. QTL mapping is performed by genome-wide and localized searches of individual expression profiles and principal components. For each QTL region, we obtain a list of genes whose expression is affected and a list of genes physically located in the region (candidate regulators). The candidate regulator list is reduced using local structural models and additional biological information. SEM analysis is being implemented in Maximum Likelihood and Bayesian frameworks. The SEM contains expression profiles, QTL and certain types of interactions. Typically, these models have been implemented for tens of variables. Here we aim for applications involving at least hundreds of variables based on a factorization of the likelihood and a strongly constrained network topology space.

MODEL-BASED CLUSTERING IN A BROOK TROUT CLASSIFICATION STUDY WITHIN THE EASTERN UNITED STATES. Huizi Zhang, Samantha Bates Prins & Eric P. Smith, Dept. of Stat., Va. Polytechnic Inst. & State Univ., Blacksburg, VA 24061. When dealing with data compiled over a large spatial region, a single model may not be appropriate to describe relationships between variables. We developed a model-based clustering method to group categorical response data by their empirical stressor-response relationships with the goal of better classification performance after clustering. Voronoi tessellations techniques are implemented to subdivide a region and the area under the receiver operator

characteristic curve is used as the criterion when searching for the optimal clustering. This method is applied to a carefully brook trout absence/presence data within the eastern United States. Results indicate fairly strong stressor-response relationships that vary spatially and show significant improvement over the conventional single model approach.

RANGES OF MEASURES OF ASSOCIATIONS FOR FAMILIAL BINARY VARIABLES. N. Rao Chaganty & Yihao Deng, Department of Mathematics and Statistics, Old Dominion University, Norfolk, VA 23529. Familial binary data occur in a wide range of scientific investigations. Numerous measures of association have been proposed in the literature for the study of intra-family dependence of the binary variables. These measures include correlations, odd ratios, kappa statistics, and relative risks. In this talk, we will study the permissible ranges of these measures of association such that a joint distribution exists for the familial binary variables.

DOES PAIR-MATCHING ON BASELINE MEASURES IMPROVE POWER IN A PRE-POST CLUSTER RANDOMIZED TRIAL? Misook Park & Robert E. Johnson, Dept. of Biostatistics, Va Commonwealth U., Richmond, VA, 23298. The variance between cluster units may be controlled in part by matching prior to randomization. Clusters may be placed into blocks by matching on known characteristics such as demographics, cluster size, etc. The baseline measure of the study primary outcome is oft recommended for matching. One matching scheme is to first sort clusters on their baseline means and match the two clusters with the smallest means into a block, match the two clusters with the next smallest means, etc. Does this matching strategy decrease the variance and improve the power to detect treatment effects? Standard methods involve using the baseline mean as a covariable or analyzing pre-post differences. Does the addition of matching improve these methods? We investigate this question in light of the variance components, ICC, pre-post correlation, and allocation of within-cluster sample size across time points.

GENETIC CONSTRAINED K-MEANS ALGORITHM. Jianmin Zhao & Robert E. Johnson, Department of Biostatistics, Virginia Commonwealth University, Richmond, VA 23298. Inspired by Krishna and Murty Genetic K-means Algorithm (GKA), we propose a novel Genetic Constrained K-means Algorithm (GCKA). In our work, each cluster is constrained to contain at least two subjects. The Constrained K-means Operator (CKO), rather than K-means Operator, is used in GCKA. A solution that violates the constraint constitutes an illegal string which must be accounted for in GKA. The constrained K-means algorithm generates the initial population; hence we do not need to consider the illegal string issue in this stage. CKO greatly speeds up the convergence process. CKO first performs the standard K-means algorithm, then relocates the subjects so that there are at least two subjects in each cluster. Lastly subjects are relocated in order to reduce the total objective value while satisfying the constraint. The objective function used in this paper is Root Mean Squared Error (RMSE). Based on simulated and experimental

data, we demonstrated that GCKA be able to detect the correct clusters when clusters exists. We also showed that GCKA always converge to global optimum, though the convergence may be slow.

AN IMPROVED GENETIC ALGORITHM WITH THE METHOD OF STEEPEST ASCENT/DESCENT. Wen Wan, Jeffrey B. Birch & G. Geoffrey Vining, Dept. of Stat., Va. Polytechnic Inst. & State Univ., Blacksburg VA 24061. The genetic algorithm (GA) is an important tool in optimization area. However, the general GA is usually computationally intensive. It has to perform a large number of evaluations of an objective function. This paper presents a computationally efficient genetic algorithm by applying the method of steepest ascent/descent from the response surface methodology to improve the general GA. During the process of a GA, on the whole, successive generations tend to find improvement in terms of the values of an objective function. Our modified GA utilizes numerical information from the GA process itself to try to obtain further possible improvement. The modified GA focuses on the best offspring among the current population and its offspring population. Similar to the method of steepest ascent/descent, the modified GA finds three appropriate directions so that further improvement may be found by collecting data directly and economically along those appropriate paths until no further improvement can be found. Then by replacing the best offspring by the best point found, the GA process is continued until the next best offspring better than the current population is found. The proposed method can greatly improve the performance of the general GA. Several examples, such as low-dimensional versus high-dimensional cases, and smooth response surface versus bumpy response surface cases, are employed to illustrate the improvement of the proposed method. This paper also explores varying the GA by considering several different levels of various GA operators through a Monte Carlo simulation study using a split-plot design.

SAMPLING RARE AND ELUSIVE SPECIES: THE SEPARATION OF COMPONENTS OF DETECTION PROBABILITY IN WILDLIFE AND FISHERIES POPULATION ESTIMATION. Kenneth H. Pollock, Departments of Zoology and Statistics, North Carolina State University, Raleigh, NC 27695-7617. Population abundance estimation for rare and elusive species critically depends on the estimation of detection probability under a particular sampling method. I discuss in detail two components of detection probability. These are the probability of an animal being available for detection and the conditional probability of an animal being detected given that it is available. Methodology for estimating these two components of detection probability is illustrated with three very diverse examples involving aerial surveys of marine mammals (dugongs), point counts of terrestrial birds, and capture-recapture studies of terrestrial salamanders. The statistical methodology used in the three examples is very different. If we ignore the issue of animals not being available, then we obtain an estimate of the size of the available component of the population rather than the total population size. The available component may be only a small proportion of the total population. In addition, this component may vary with time and with important auxiliary variables in ways that

are so complex that it is unsatisfactory for monitoring the population. I will conclude my talk with some general remarks on the challenges of interdisciplinary research, the future of statistics as a field and the training of quantitative students who work at the interface of Statistics, Biomathematics and Wildlife Ecology.

AN ASYMPTOTIC VIEWPOINT ON HIGH-DIMENSIONAL BAYESIAN TESTING. Dan Spitzner, Dept. of Stat., Va. Polytechnic Inst. & State Univ., Blacksburg, VA 24061. Bayesian testing is studied asymptotically on a high-dimensional normal means model, in which the null hypothesis of zero means in all dimensions is tested against general alternatives. This is known to serve as a canonical model for smooth goodness-of-fit testing. The asymptotic setup is such that prior mass placed on the null hypothesis is allowed to decrease as dimensionality increases, while at the same time the dispersion of the prior placed on the alternative is allowed to increase, thereby tending toward a noninformative specification. When geometric constraints representing smoothness are imposed it becomes sensible to weight the prior, for which high-dimensional asymptotic evaluation provides a simple weight specification that leads to favorable properties.

MULTIVARIATE ASSAYS WITH VALUES BELOW THE LOWER LIMIT OF QUANTITATION: PARAMETRIC ESTIMATION BY IMPUTATION AND MAXIMUM LIKELIHOOD. Robert E. Johnson, Dept. of Biostat., Va Commonwealth U, Richmond, VA, 23298 & Heather J. Hoffman, Biostatistics Center, George Washington University. Laboratory assay data often include left-censored values that are reported to be below the lower limit of quantitation (BLLOQ or  $\leq$  LOQ). While simple imputation of a specific value such as LOQ/2 is commonly implemented in practice, maximum likelihood methods accounting for censoring provide a more accurate way of analyzing the data. Assay measures such as biomarker levels or concentration levels of contaminants in water are typically modeled with a normal or lognormal distribution. The corresponding maximum likelihood estimate of means and variances in univariate analyses can easily be obtained from an assortment of standard software packages; however, a multivariate analysis may be more appropriate when multiple assays are measured on the same subject. For example, total nicotine intake may be represented by a linear combination of the amount of nicotine and its five metabolites present in some physiological fluid. Especially in unexposed nonsmokers, one or more of these measures may fall below the LOQ. In this case the total nicotine cannot be directly measured. Simple imputations will suffice when the proportion of data BLLOQ is small. When this is not the case, we propose an algorithm that provides maximum likelihood estimates of mean and unstructured (co)variance parameters corresponding to a multivariate (log)normal distribution in the presence of left-censored and missing values. This work is based in part on Heather J. Hoffman PhD dissertation.

OBJECTIVE PRIORS FOR THE MULTIVARIATE NORMAL MODEL. Dongchu Sun, Va. Polytechnic Inst. & State Univ., Blacksburg, VA 24061 & James O. Berger, Duke University, Durham, NC 27708. Objective Bayesian inference for the multivariate normal model is illustrated, using different types of objective priors (Jeffreys, invariant, reference and matching), different modes of inference (Bayesian and frequentist), and different criteria involved in selecting optimal objective priors (ease of computation, frequentist performance, marginalization paradoxes, and decision-theoretic evaluation). A variety of surprising results were found, including the availability of objective priors that yield exact frequentist inferences for many functions of the parameters, such as the correlation coefficient. The prior that most frequently yields exact frequentist inference is the right-Haar prior, which unfortunately is not unique. Two natural proposals are studied for dealing with this non-uniqueness: first, mixing over the right-Haar priors; second, choosing the empirical Bayes right-Haar prior, that which maximizes the marginal likelihood of the data. Quite surprisingly, we show that neither of these possibilities yields a good solution. This is disturbing and sobering. It is yet another indication that improper priors do not behave as do proper priors, and that it can be dangerous to apply understandings from the world of proper priors to the world of improper priors.