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## A New Long-tailed Weasel County Record in Shenandoah National Park

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### ABSTRACT

Though abundant throughout much of its range, the ecology and local geographic distribution of *Mustela frenata* (Long-tailed Weasel) is not well-known, especially in the central Appalachian Mountains. In 2015, we conducted a camera study in rock outcrop habitats within Shenandoah National Park, Virginia. Our objective was to determine the presence of mammals considered uncommon in these habitats. After 2,016 trap nights, we report eleven photographic captures of Long-tailed Weasels at eight sites. Two of these sites represent the first record of this species in Rappahannock County, Virginia. These detections represent the first record of Long-tailed Weasels in Shenandoah National Park in 60 years and extend their known range within the Park.

*Mustela frenata* Lichtenstein (Long-tailed Weasel), is abundant throughout most of its geographic range, which extends from southern Canada to northwestern South America (Chapman 2007). Long-tailed Weasels are considered habitat generalists, as they have been found in low-elevation agriculture areas to high-elevation (2,133 m) forests of Colorado (Chapman 2007, Quick 1949). However, their ecology and geographic distribution throughout the eastern United States as compared to other furbearer species is essentially unknown (Richter and Schauber 2006). According to the International Union for Conservation in Nature (IUCN), they are considered Stable-Least Concern throughout their geographic range (Reid and Helgren 2008). In the Central - Southern Appalachian Mountains, they are considered vulnerable in North Carolina and uncommon in West Virginia, but secure in Maryland, Tennessee and Virginia (Webster et al. 1985, Chapman 2007, WVDNR 2001). In the Great Smoky Mountains National Park (GRSM), Linzey (1994) states that this weasel is “fairly common” but lists only eight locations in a park that is nearly twice the size of Shenandoah National Park (SHEN). In SHEN, only five confirmed records of Long-tailed Weasels exist (Manville 1956). Only one other nearby record outside of the Park (e.g. Warren County, Smithsonian Conservation Center, Front Royal; Lat 38 53 42.6, Long -78 09 49.6; live capture – released alive) exists (VDGIF 2013).

Over the course of 18 weeks during the spring-early fall 2015, we conducted a camera study at 48 rock outcrop sites in SHEN. The overall objective of this project was

to investigate the presence and distribution of Long-tailed Weasels, *Spilogale putorius* (L.) (Eastern Spotted Skunk), *Neotoma magister* Baird (Allegheny Woodrats), *Mustela nivalis* (L.) (Least Weasel), and *Martes pennanti* Erxleben (Fisher). In the paper, we confine our results to new records of Long-tailed Weasels. Camera sites were primarily located in rock outcrop habitat types (e.g., talus slopes, boulder fields, cliff lines & faces, and rock barrens) in both Mesophytic *Quercus* sp (L.) (Oak) – *Carya* sp. Nutt. (Hickory) and Central Oak – *Pinus* sp. (L.) (Pine) Forests (USGS 2011). We detected Long-Tailed Weasels at eight sites in Warren, Page, Madison and Rappahannock Counties in the North and Central districts of SHEN (Figure 1). Previously recorded locations of Long-Tailed Weasels in SHEN (Manville 1956) include 3 sites in Page County (Park Headquarters, Gravel Ridge, and Rock Spring Shelter) and 2 in Madison County (Limberlost and near Hawksbill Gap).

In Warren County, a Long-tailed Weasel was detected on 9 June 2015 at 0323 h and again on 16 September 2015 at 0003h at Dickey Hill (Elevation 744 m; 38°85'381"N, 78°20'601"W) in a Central Oak-Pine Forest near the edge of a field. Due to the inability to identify individual Long-tailed Weasels, we are uncertain whether these were the same or different individuals. In Page County, a Long-tailed Weasel was first detected at Miller's Head Viewpoint (Elevation 991 m; 38°59'315"N, 78°39'506"W) on 3 June 2015 in a Mesophytic Oak – Hickory Forest at 0343 h. A Long-tailed Weasel was detected at a second site in Page County on 18 June 2015 at 0410 h in a closed-canopy Oak-Hickory Forest located along Skyline Drive 140 m from Hazeltop Overlook (mile marker 52) (Elevation 1006 m; 38°47'824"N, 78°45'644"W).

In Madison County, a Long-tailed Weasel was recorded at three sites. The first detection was a location in a closed canopy Oak-Hickory Forest intermixed with *Kalmia latifolia* (L.) (Mountain Laurel) (Elevation 1009 m; 38°61'762"N, 78°34'817"W) where weasels were photographed on 19 July 2015 at 0433 h, 6 September 2015 at 0021 h and 9 September 2015 at 0053 h (Figure 2). The second detection was located along a trail that cuts across an exposed talus slope on Hawksbill Mountain (elevation 1113.1 m, 38°55'683"N, 78°39'360"W) and occurred on 23 July 2015. Interestingly, this was also the same location where we recorded the first known record of an Eastern Spotted Skunk in Madison County almost two months later (Lombardi et al. In Review). The third site was located under a *Pinus rigida* Mill (Pitch Pine) on a steep talus slope near the summit of Stony Man Mountain (Elevation 1637 m; 38°59'889"N, 78°37'388"W) and occurred on 8 September 2015 at 2101 hr.

In Rappahannock County, we detected a Long-tailed Weasel at two sites, which represents the first evidence of Long-tailed Weasels in the county. The first record occurred on 3 July 2015 at North Marshall Mountain (Elevation 973 m; 38°77'382"N, 78°20'788"W) along the Appalachian Trail at 0721 h in a closed canopy Oak-Pine Forest near the edge of a cliff-face heavily dominated by Mountain Laurel and *Rhododendron* sp. (L.) *Rhododendron*. The second record was located just below the Mt. Marshall Overlook (mile marker 20) (Elevation 852 m; 38°25'140"N, 78°25'140"W) at the bottom of a steep boulder field near the edge of an Oak-Hickory Forest on 12 July 2015 at 2202 h.

The observations reported here represent the first documentation of Long-tailed Weasels in SHEN in sixty years, a new county record in Rappahannock County, VA and

extends the known range of this species within the National Park. Given this species' wide habitat breadth and the spatial extent of the sampling, it suggests that Long-tailed Weasels are more common in the Park than previously thought and further research and monitoring is warranted.

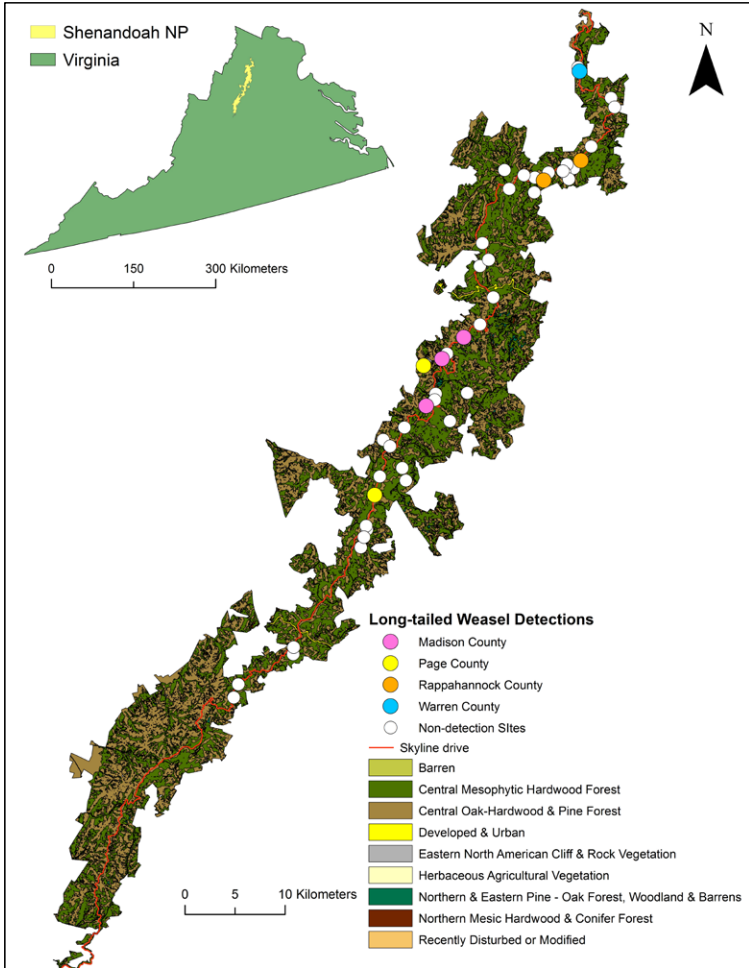


FIGURE 1: Detection sites for Long-tailed Weasels, classified by county, and non-detection sites in Shenandoah National Park, Virginia



FIGURE 2: Photographic capture of a Long-tailed Weasel in a closed canopy Oak-Hickory Forest intermixed with Mountain Laurel in Page County, Virginia, USA on 9 September 2015 at 0053h.

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## **Occupancy Rates and Detection Probabilities of Red-Backed Salamanders on the Virginia Fall Line**

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### **ABSTRACT**

To meet the conservation needs of declining amphibian populations, there is a need to assess monitoring techniques in various habitat types and seasons. I assessed detection rates and proportion of area occupied via transect monitoring for red-backed salamanders at a site along the Virginia Fall Line in Doswell, Virginia. I established 24 transects in a 3.2-ha area in both riparian and upland habitats. Objects providing natural cover along these transects were sampled 3 times a week in fall and spring over a two-year period. Models of occupancy and detection were developed and compared using Akaike's Information Criterion. Based on 113 captures, model selection indicated a low fixed initial occupancy of transects with seasonal changes in colonization and extinction. Detection probability was uniformly low, possibly contributing to model uncertainty in determining the best explanatory variables. I infer that the increased colonization of transect cover objects during fall and increased emigration from cover objects during spring is a result of changing moisture conditions and feeding opportunities. It is likely that occupancy and detection will vary substantially for survey sites based on habitat, season, or even by transect placement, and thus managers will need to estimate these parameters for any population monitoring program of red-backed salamanders.

### **INTRODUCTION**

In view of the worldwide decline of amphibian populations and the increased interest to conserve these populations, biologists have been highly interested in developing robust monitoring methods for specific populations, habitats, or sites of conservation interest (Dodd and Barichivich, 2007; Adams et al., 2013; Petitot et al., 2014). One problem in developing these methods is that, regardless of the sampling technique, there is a possibility that an observer will fail to detect an individual of the population of interest when they are present on the sampling site. Thus, the apparent absence of a taxon could mean that members of the population of interest are truly absent from the sampling site or that they are present but not detected. Because this problem has significant implications for whether management strategies are implemented, any robust monitoring method will require the estimation of detection probabilities, the probability (ranging between 0 – 1) that an individual will be detected by a survey procedure, given that is available to be found (Schmidt, 2003). Unfortunately, the detection probability is likely to vary extensively based on a suite of environmental

variables, species differences, population size differences, and even individual behavioral differences (Lancia et al., 1996; Dodd and Dorazio, 2004; Tanadini and Schmidt, 2011).

Monitoring is especially needed for a species integral to specific ecosystem functions (Davic and Welsh, 2004). Red-backed salamanders (*Plethodon cinereus*) are one such species, serving as a potentially useful candidate for long-term monitoring because they are fairly common within their range, often represent a high biomass in specific habitats, and may be tightly linked to the health of their environment (Welsh and Droege, 2001). Red-backed salamanders are likely to have low detection rates, however, largely as a function of their ecology. Bailey et al. (2004a), for example, have attempted to determine detection rates for the congener southern red-backed salamander (*Plethodon serratus*) using a Pollock's Robust Design for mark-recapture estimation, but found that because the majority of the population remained below ground, the likelihood of a red-backed salamander being available (above ground) to be found and then actually being detected could be as low as 4%. In addition, there was a tendency for individuals to be "trap-shy", and thus unlikely to be recaptured, biasing abundance estimates. These results led Bailey et al. (2004a) to suggest using count data to determine the proportion of an area occupied instead of estimating abundance. This reduces the sampling effort required while still allowing for the estimation of detection probabilities and occupancy rates (MacKenzie et al., 2002). This means that larger areas can be sampled with less intensity for the same amount of effort, which may be more functional for conservation management purposes than more intensive studies on smaller sites. Indeed, Bailey et al. (2004b) accomplished this for multiple species in an area in the Great Smoky Mountains. For researchers wishing to establish long-term monitoring at a particular field site, using the proportion of area occupied (PAO) survey seems a robust possibility.

Given their current conservation needs, it is desirable that long-term monitoring projects for amphibians, including the red-backed salamander, are initiated in multiple habitat types to gain a better picture of patterns in occupancy and detectability. Red-backed salamanders are particularly associated with well-drained mature montane forest with extensive leaf-litter and deep, pH-neutral soils, with plenty of cover objects (Burger, 1935; Petranka, 1998; Milanovich et al., 2010; McGhee and Killian, 2013). Many studies establishing monitoring methods are understandably in these types of habitats (Dodd and Dorazio, 2004; Hyde and Simons, 2005; Williams and Berkson, 2004). To establish a complete picture of occupancy for a species, however, a wide variety of habitats should be assessed, along with estimates of the amount of effort required to establish occupancy for these sites (Mackenzie and Royle, 2005). For example, the Randolph-Macon College Environmental Field Station occurs along the Virginia Fall Line, demarcating the eastern boundary between the piedmont and coastal region of the state. In hopes of testing the potential of a long-term monitoring protocol on this site, my objective was to assess occupancy and detection rates for a series of transects on a small subsection of the field station. The estimation of a detection rate is important to determine the likelihood of detecting an individual of a monitored species if present, and so it is helpful to translate the number of individual transect surveys that would be required to detect an individual that is actually present. To accomplish this I calculated the amount of sampling effort



that would be required to establish occupancy for a given transect (O'Connell et al., 2006).

#### STUDY SITE

Fourteen of Virginia's salamander species (approximately 28% of Virginia species) occur within the York River drainage (Mitchell and Reay, 1999). The Randolph-Macon College Environmental Field Station (EFS), owned by Martin Marietta Quarry, encompasses a small ridge in this drainage, next to a local rock quarry and bordering the Little River in Doswell, Virginia. The EFS is a 26.7 hectare (66 acres) property in northern Hanover County, Virginia, located about 8 miles from Ashland, Virginia (Randolph-Macon College, 2010). This site is located on the Fall Line, a sharp rise in elevation that runs through the state that acts as a geological border between the piedmont and coastal ecoregions of Virginia. It contains mature hardwood forest. Elevation ranges between 150 – 220 m.

#### METHODS

In August 2011, I established 24 permanent transects, each 25m in length. These transects were arranged in groups of four, radiating from the corners of a central silt fence enclosure being used for a related study (McGhee, 2013). Each array of 4 transects was established from a randomly located line moving perpendicular to the Little River through both a riparian and upland zone (elevation difference = ~50m). From randomly selected points along this line, I placed the center points of these arrays (a 25-m<sup>2</sup> silt fence enclosure) between 0 – 50 m away, only constraining the center of the transect arrays to be a minimum of 25 m away from each other. This resulted in 3 arrays of 4 transects in the upland zone, and 3 arrays of 4 transects in the riparian zone, over an area covering approximately 3.2 ha.

I sampled transects following Pollock's Robust Design (1982), wherein primary sampling periods are comprised of a series of secondary sampling occasions occurring over a short enough time period that a closed population can be assumed. An open population can be assumed across primary sampling periods (MacKenzie et al., 2003). Within an occupancy modeling framework, this allows for the estimation of 4 probabilities: initial occupancy of transect ( $\psi$ ), colonization of transect ( $\gamma$ ), extinction (or emigration) from transect ( $\epsilon$ ), and detection at transect ( $p$ ; MacKenzie et al., 2003). The colonization and extinction parameters allow for testing changes in occupancy over the time of the study. Primary samples were taken between 17 August – 4 December of 2011, 16 February – 23 May of 2012, 3 October – 6 December of 2012, and 26 February – 8 May 2013, with each separated by a 10-day period on average, and comprised of 3 secondary samples occurring over a 3 – 4 day period. A sample consisted of walking each 25m transect and searching under each natural cover object intersecting the transect. Salamanders found under natural cover objects were measured for total length (TL), snout-vent length (SVL), and identified to species. I assigned an age to red-backed salamanders (juvenile or adult) based on their SVL (adult SVL  $\geq$  34 mm; Petranka, 1998), and documented the color morphology of the individual (red-stripe on dorsum, or unstriped morph; Petranka, 1998). Only detections of red-backed salamanders were used

in our analysis. After measuring, salamanders were gently returned to their original position.

I developed a series of models to explain the number of times salamanders were detected in each transect over the study period. Each model serves as a mathematically specific hypothesis about how occupancy and detection function for the entire suite of transects. These models were then compared using Akaike's Information Criterion (AIC) in program PRESENCE (Bailey et al., 2004b), which compares competing models using the statistical likelihood for each model combined with a penalty term for the number of parameters in that model (Williams et al. 2002). To obtain specific models, model parameters ( $\psi$ ,  $\gamma$ ,  $\epsilon$ ,  $p$ ) were allowed to vary by year (2011 – 12, 2012 – 13), season (spring or fall), habitat (upland or riparian), array (each set of four transects), or by individual transect, or some combination thereof. In some models, parameters were also held constant in primary samples ( $\psi$ ,  $\gamma$ ,  $\epsilon$ ) or primary and secondary samples ( $p$ ). This resulted in 16 *a priori* models to consider as best describing red-backed salamander occurrence on site (Table 1). Finally, I calculated the number of surveys required to detect a red-backed salamander with 95% probability for a given occupied transect using Mackenzie and Royle's (2005) estimator  $1 - (1 - p)^K$ , where  $p$  is the detection probability estimated from the top-performing model(s) and  $K$  is the number of surveys undertaken.

## RESULTS

Over the course of two years I conducted 23 primary sampling periods (69 secondary samples) with 7 primary samples (21 secondary) in fall 2011, 5 primary samples (15 secondary) in spring 2012, 5 primary samples (15 secondary) in fall 2012, and 6 primary samples (18 secondary) in spring 2013. Each transect averaged ( $\pm$  standard error (SE))  $4.79 \pm 0.47$  cover objects. I detected red-backed salamanders a total of 113 times, with juveniles comprising 50% of detections. Unstriped morphs comprised 35% of total detections. Model selection indicated that those models showing a fixed initial occupancy followed by a seasonal change in colonization and extinction were most descriptive of the data, with combined model weight for  $\gamma$  (seasonal),  $\epsilon$  (seasonal) models totaling 99%. There was less clarity, however, regarding detection probabilities, with 3 models performing at a similar level ( $\Delta\text{AIC} \leq 2$ , Burnham and Anderson 2002). Each model assumes a constant initial occupancy with strongly seasonal colonization and emigration rates (Table 2). Each model varied, however, in its treatment of the detection rate. In model 1,  $p$  differed between upland ( $0.16 \pm 0.02$  SE) and riparian ( $0.10 \pm 0.02$  SE), and in model 2,  $p$  differed between transect arrays (A:  $0.20 \pm 0.04$  SE; B:  $0.16 \pm 0.04$  SE; C:  $0.09 \pm 0.03$  SE; D:  $0.11 \pm 0.03$  SE; E:  $0.05 \pm 0.03$  SE; F:  $0.08 \pm 0.03$  SE). Finally, in model 3,  $p$  is assumed to be constant ( $0.14 \pm 0.02$  SE). Using these estimates of  $p$ , the number of single transect surveys required to achieve a 95% confidence of detecting an individual given its occupancy varied from 13 – 57 surveys, with a weighted mean of  $26.24 \pm 3.94$  SE (Figure 1).

TABLE 1. Model selection results for estimating occupancy ( $\psi$ ), colonization ( $\gamma$ ), extinction ( $\epsilon$ ) and detection ( $p$ ) rates for red-backed salamanders captured at the Little River, Hanover County, Virginia, 2011 – 2013. For each model, estimated parameters vary according to a covariate shown in parentheses, where (.) means the parameter was held constant for the primary sample, and (..) means the parameter was held constant for both primary and secondary samples.  $\Delta$ AIC represents the difference between each model and the lowest AIC score model,  $w$ , represents the AIC model weight, and  $K$  represents the number of parameters in each model.

Model #	Model	$\Delta$ AIC	$w$	$K$
1	$\psi$ (.), $\gamma$ (season), $\epsilon$ (season), $p$ (habitat)	0.00	0.45	7
2	$\psi$ (.), $\gamma$ (season), $\epsilon$ (season), $p$ (array)	1.35	0.23	11
3	$\psi$ (.), $\gamma$ (season), $\epsilon$ (season), $p$ (..)	1.49	0.22	6
4	$\psi$ (.), $\gamma$ (season), $\epsilon$ (season), $p$ (seasonal)	3.41	0.08	7
5	$\psi$ (site), $\gamma$ (season), $\epsilon$ (season), $p$ (..)	7.75	0.01	11
6	$\psi$ (.), $\gamma$ (season, habitat), $\epsilon$ (season, habitat), $p$ (season, habitat)	9.88	<0.01	13
7	$\psi$ (.), $\gamma$ (season, year), $\epsilon$ (season, year), $p$ (season, year)	10.49	<0.01	13
8	$\psi$ (.), $\gamma$ (season), $\epsilon$ (seasonal), $p$ (declining)	11.69	<0.01	6
9	$\psi$ (.), $\gamma$ (season, array), $\epsilon$ (season, array), $p$ (season, array)	14.56	<0.01	25
10	$\psi$ (.), $\gamma$ (.), $\epsilon$ (.), $p$ (habitat)	16.43	<0.01	5
11	$\psi$ (habitat), $\gamma$ (habitat), $\epsilon$ (habitat), $p$ (.)	16.98	<0.01	7
12	$\psi$ (.), $\gamma$ (.), $\epsilon$ (.), $p$ (..)	18.05	<0.01	4
13	$\psi$ (.), $\gamma$ (habitat), $\epsilon$ (habitat), $p$ (habitat)	18.27	<0.01	7
14	$\psi$ (.), $\gamma$ (habitat), $\epsilon$ (habitat), $p$ (season)	18.30	<0.01	7
15	$\psi$ (.), $\gamma$ (season), $\epsilon$ (season), $p$ (transect)	27.17	<0.01	28
16	$\psi$ (habitat, array), $\gamma$ (habitat, array, transect), $\epsilon$ (habitat, array, transect), $p$ (habitat, array, transect, survey)	85.57	<0.01	144

TABLE 2. Parameter estimates for occupancy ( $\psi$ ), colonization ( $\gamma$ ), and emigration ( $\epsilon$ ) rates for red-backed salamanders captured at the Little River, Hanover County, Virginia, 2011 – 2013, based on the three most competitive models.

Model #	$\psi$ (SE)	$\gamma_{fall}$ (SE)	$\gamma_{spring}$ (SE)	$\epsilon_{fall}$ (SE)	$\epsilon_{spring}$ (SE)
1	0.08 (0.08)	0.23 (0.05)	0 (0)	0 (0)	0.21 (0.05)
2	0.09 (0.09)	0.28 (0.07)	0 (0)	0 (0)	0.20 (0.05)
3	0.08 (0.07)	0.21 (0.04)	0 (0)	0 (0)	0.21 (0.05)
Average	0.08 (0.08)	0.24 (0.07)	0 (0)	0 (0)	0.21 (0.07)

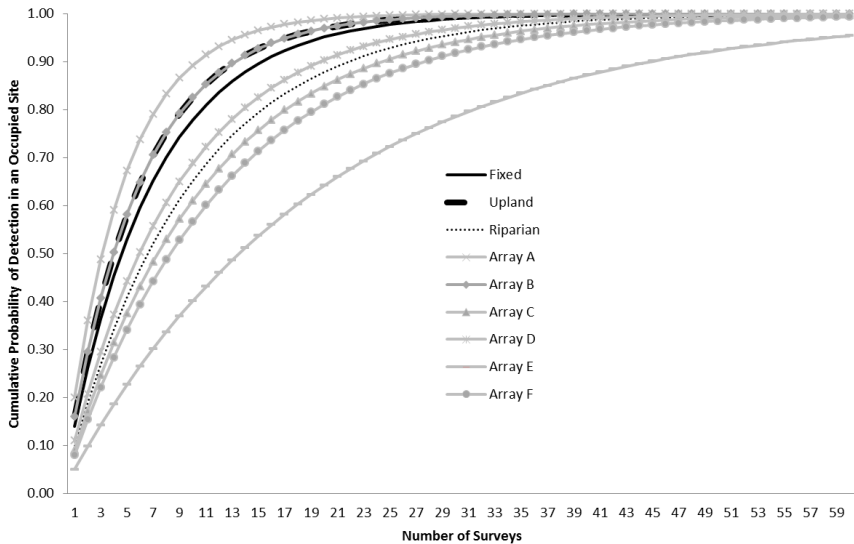


Figure 1. The relationship between the number of surveys completed for a given transect and the cumulative probability of detecting an individual assuming it is occupying a natural cover object in that transect. Each line is based on the detection probability estimated for the three top performing models for data collected on red-backed salamanders captured at the Little River, Hanover County, Virginia, 2011 – 2013. Model 1 detection rates differed by habitat (upland v. riparian, model 2 detection rates differed by transect array location (A – F), and model 3 detection rate was fixed for all transects. On average,  $26.24 \pm 3.94$  SE surveys would be required to detect an occupying individual with 95% confidence.

## DISCUSSION

Based on the best performing models, occupancy rates were low at the beginning of the study but improved through colonization during autumn, with no emigration from transects until spring when emigration rates rose with no new colonizations. Moore et al. (2001) also found a peak in red-backed salamander detections under cover objects in autumn. Males establish territories for feeding associated with cover objects, using them as moisture refuges during short dry periods rather than migrating below the soil surface where prey is presumably limiting (Jaeger, 1980; Kleeburger and Werner, 1982). The increase in detections and colonization of cover objects in autumn may be a response to improving moisture conditions or feeding opportunities, with the loss of those conditions in late spring likely resulting in a movement below ground (Blanchard, 1928; Taub, 1961).

Initial occupancy rates were consistent across models and relatively low in comparison to Bailey et al. (2004b) for the congener *Plethodon serratus* ( $\psi = 0.76 \pm 0.07$

SE) in the Great Smoky Mountain National Park (GSMNP). This highlights the need to estimate independent occupancy parameters for monitoring programs. Our fall line study site habitat differs from the montane forest habitat of GSMNP, and is likely much poorer habitat for red-backed salamanders. If multi-site monitoring programs can be established for a region of interest, a short-term intensive study to establish parameter estimates may offset some uncertainty in subsequent monitoring and management actions (Grant et al., 2013).

The model uncertainty in this study was associated with detection probabilities, which may primarily be a function of habitat, location of transects, or simply constant. Burnham and Anderson (2002) suggest that in situations where similarly competitive models differ by only 1 parameter, and have similar maximized log likelihoods, the most parsimonious model should be preferred. This would imply that the constant  $p$  model (model 3) may be preferred over the habitat model (model 1). My study site is rather flat, with upland and riparian sites differing by only about 50 m, and thus the constant  $p$  does not markedly differ between the upland and riparian estimates. The array model (model 2), however, differs from the habitat model by 3 parameters, incurring a substantial cost under an AIC rubric while still performing as well. The high performance of this model suggests that  $p$  differs not only by habitat, but also by the sites in which transects are placed. Arrays were not far away from each other, with all arrays within an area of 3.2 ha. This would imply that even highly localized conditions can affect the probability of detection, supporting Bailey et al. (2004b) in their inference that multiple factors are acting to vary detection probabilities. This creates a problem for designing sampling protocols because the detection probability is likely to vary substantially across survey sites. Determining occupancy will require substantial survey replication, particularly in areas that will have low detection probabilities. Unfortunately, those areas likely to suffer from both low occupancy and low detection rates may also be those in most need of active management and monitoring (Lesica and Allendorf, 1995; Channell, 2004; Hampe and Petit, 2005). Researchers monitoring salamander species near the edge of their ranges or close to poor quality habitat may need to allocate their surveys accordingly. Our results suggest this can be accomplished on average with 26 surveys, though possibly more may be required if site-specific differences are substantial.

#### LITERATURE CITED

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**Agriculture, Forestry, and Aquaculture**

VIRGINIA AGRICULTURE: LOOKING BACKWARD AND FORWARD. Sandra J. Adams, Virginia Department of Agriculture and Consumer Services. Agriculture, the number one private industry in Virginia, contributes \$52 billion annually to Virginia's economy and supports 415,000 jobs. Though Native Americans grew crops and instructed the Jamestown settlers in New World agricultural practices, farming in Virginia is dated to 1614, the year of the first tobacco exports to Europe. Virginia has made and continues to make incredible contributions to the development and modernization of agriculture. George Washington was a pioneer in crop rotation and his meticulous record-keeping led to many agricultural innovations. Thomas Jefferson's introduction of new agricultural products and new growing practices had an effect on American agriculture that endures to this day. Other famous agricultural products and innovations include Cyrus McCormick's development of the mechanical reaper; *Fragaria virginiana*, the first cultivar of the modern strawberry; country hams and Virginia style peanuts; *Crassostrea virginica*, Virginia's native oyster that was noted in the journals of Captain John Smith and today has made a dramatic comeback from near extinction; and famous animals such as Secretariat and Elevation the bull, the progenitor of 10 percent of the world's Holstein population. To remain viable in the future, Virginia agriculture must make a profit, care for the planet in new and different ways, utilize new technologies such as gene modification and gene editing, grow food in new places and educate the public about its production methods. Concerns for the future include fear of genetically modified organisms, a growing distrust of traditional production methods and attempts to over regulate them, public acceptance of new technologies, and the ways we will feed a world population of more than nine billion by the year 2050.

INJURY TO APPLES AND PEACHES AT HARVEST FROM FEEDING BY *HALYOMORPHA HALYS* (STÅL) (HEMIPTERA: PENTATOMIDAE) NYMPHS EARLY AND LATE IN THE SEASON. A. L. Acebes-Doria<sup>1</sup>, T. C. Leskey<sup>2</sup> & J. C. Bergh<sup>1</sup>, Alson H. Smith Jr<sup>1</sup>,

<sup>1</sup>Agricultural Research and Extension Center, Virginia Tech, Winchester, VA 22602 and <sup>2</sup>USDA-ARS, Appalachian Fruit Research Station, 2217 Wiltshire Rd, Kearneysville, WV, 25430. *Halyomorpha halys* adults and nymphs feed on tree fruits. Feeding injury from adults has been characterized but the injury from nymphs has not been

examined systematically. Since the four plant-feeding instars of *H. halys* (second through fifth) differ substantially in size, it is plausible that the effects of their feeding on fruit injury and injury expression may differ among them. We compared feeding injury at harvest from young nymphs (second plus third instars), older nymphs (fourth plus fifth instars), and adults that were caged on 'Smoothie Golden' apples and 'Redhaven' peaches in early June (peach and apple), late July (peach), and late August (apple). Individual apples and peaches were caged at fruit set and assigned to the following treatments (n = 28/treatment): 1) control (no *H. halys*), 2) young nymphs or 3) adults early in the season, and 4) young nymphs, 5) older nymphs or 6) adults later in the season. Fruit in each treatment were exposed to 3-4 young nymphs, two older nymphs or 1-2 adults placed in the cages for 96 h and evaluated for external and internal feeding injury within 36 h after harvest. No injury was recorded from unexposed peaches or apples. The percentage of injured fruit and number of injuries per fruit varied significantly among the exposed treatments. Early season feeding by young nymphs yielded the least injury to peaches and apples. In apples, the highest percentage of injured fruit and number of injuries per fruit were caused by late season feeding, by adults. In peaches, early season adult feeding produced the highest percentage of injured fruit and injuries per fruit. More internal than external injury was recorded on peach and no such difference was observed on apple. These findings have implications on *H. halys* management in fruit orchards. This study is based upon work that is supported by the National Institute of Food and Agriculture, U.S. Department of Agriculture, under award number #2011-51181-30937.

DETECTING ANTIBIOTIC RESIDUES IN AQUACULTURE USING A COMMERCIAL TEST KIT. M. David Crosby & Louis Landesman, Cooperative Extension, P.O. Box 9081, Virginia State University, Petersburg, VA 23806. Today's consumers are concerned with food safety. Consumers do not want food containing antibiotic residues. HACCP guidelines for fish processing list antibiotic and pesticide residues as a hazard for consumers. Premi®Test is an over-the-counter commercial test kit that is used to detect antibiotic residues in meats, poultry, eggs and milk. The test kit is fairly inexpensive costing about \$140 for 25 tests. The kit uses thermophilic bacteria (*Bacillus stearothermophilus* var. *calidolactis*) for detecting the presence of antibiotics. A pilot study was conducted to investigate the feasibility of using Premi®Test to detect antibiotics used in the aquaculture industry. Catfish and tilapia were fed antibiotic feed containing either Romet-30® or Aquaflor® which are used in the aquaculture industry. The test kit detected Romet-30® in the muscles of the catfish and tilapia but not Aquaflor®. The test kit detected both antibiotics in the feed.

Premi®Test showed some promise as a low cost detection system that can be used by small scale fish processors.

ELUCIDATING THE ANOREXIGENIC MECHANISM OF ALPHA-MELANOCYTE STIMULATING HORMONE. M. S. Delp, M. A. Cline & E. R. Gilbert, Department of Animal and Poultry Sciences, Virginia Tech, Blacksburg VA 24060. Alpha-melanocyte stimulating hormone ( $\alpha$ -MSH), a member of the melanocortin family, causes decreased food intake in both mammals and birds. However, little is known about the central mechanism mediating this response in non-mammalian species. Therefore, we started to elucidate the central mechanism of  $\alpha$ -MSH using 5-day post hatch chicks as models. Firstly, c-Fos immunohistochemistry was measured in key hypothalamic nuclei mediating appetite, and  $\alpha$ -MSH was associated with increased reactivity in the paraventricular nucleus (PVN), dorsomedial nucleus (DMN), lateral hypothalamus (LH), and arcuate nucleus (ARC). From the hypothalamus, samples of each nucleus were collected following injection of  $\alpha$ -MSH and the abundance of several appetite-associated mRNA was quantified. In the ARC, mRNA levels of neuropeptide Y (NPY), oxytocin receptor, and agouti-related peptide all increased. In the PVN, there was a reduction of NPY receptor sub-type 1 mRNA levels, while in the DMN, there was an increase of NPY and dopamine decarboxylase mRNA abundance. These results indicate the central mechanism of  $\alpha$ -MSH may be mediated through and elicit a response in multiple hypothalamic nuclei. (Supported by: Virginia Tech Phi Sigma Undergraduate Research Grant and The Virginia Academy of Science).

NUTRITIONAL QUALITY AND IN-VITRO PROTEIN DIGESTIBILITY AND SOLUBILITY OF CHICKPEA AS AFFECTED BY DIFFERENT PROCESSING METHODS. M. Obielodan, A. Cartier, K. Jordan, T. Hairston, A. Shannon, E. Sismour & Y. Xu, Agriculture Research Station, Virginia State University, Petersburg VA 23806. Chickpea is an important food legume and is a major ingredient in many human diets. The effect of different processing methods on chemical composition, amino acid profile, mineral content, anti-nutritional factors, protein solubility and *in-vitro* digestibility of chickpeas were investigated. All processing methods improved the amino acid profile, with the greatest increase caused by soaking with microwave cooking. Processing significantly reduced mineral, tannin and phytate contents among all treatments. Soaking with microwave cooking resulted in the largest reduction of tannins whereas soaking with pressure cooking resulted in the highest reduction of phytate. Soaking with microwave cooking also resulted in the highest increase in *in-vitro* digestibility. *In-vitro* protein digestibility was positively correlated with total amino acid content ( $r = 0.774$ ) and

total essential amino acid content ( $r = 0.838$ ), but was negatively correlated with total macroelements ( $r = -0.925$ ), tannins ( $r = -0.847$ ) and phytate ( $r = -0.818$ ).

EVALUATING A POTENTIAL AREA-WIDE IPM STRATEGY FOR MANAGING HEMLOCK WOOLLY ADELGID IN THE EASTERN UNITED STATES. Kenton Sumpter<sup>1</sup>, Scott Salom<sup>1</sup>, Carlyle Brewster<sup>1</sup>, Troy Anderson<sup>1</sup>, Albert Mayfield III<sup>2</sup> & Tom McAvoy<sup>1</sup>, <sup>1</sup>Virginia Polytechnic and State University and <sup>2</sup>USDA Southern Research Station. Use of the neonicotinoid insecticide, imidacloprid, has been found to be highly effective in suppressing hemlock woolly adelgid, *Adelges tsugae* Annand, (HWA). Similarly, *Laricobius nigrinus* Fender (Coleoptera: Derodontidae) has been found to be a likely candidate for biological control. Each control tactic has different objectives and outcomes. In an attempt to utilize the best of both approaches, a project was designed to develop a pest management strategy that utilizes both tactics concurrently within the same sites. The goal of this project is to assess the efficacy of a combined chemical – biological control strategy designed to reduce HWA populations and improve the health of hemlock forests as well as achieve recovery of *L. nigrinus*. The project is being conducted across three states; Kentucky, West Virginia and Tennessee, and began in 2010 with data having been collected annually through 2016. Data collected describe tree health, HWA population and recovery of *L. nigrinus* predators. Thus far, tree health has declined across all sites regardless of treatment type, and HWA population indices have been shown to be highly variable. *L. nigrinus* was initially recovered from its release plots (KY = 2010-2013, WV = 2011-2013) however, there have been no successful recoveries in 2014 and 2015 at any site.

EFFECT OF SEED PELLETING ON GERMINATION AND SURVIVAL OF SELECT SMALL-SEEDED SPECIES. Tiffany Patrick, Andrew Thomas, and Laban K. Rutto, Agricultural Research Station, Virginia State University, P.O. Box 9061, Petersburg, VA 23806. Crop species with exceedingly small seed present handling and singulation challenges during planting. They are also characterized by poor germination and seedling survival because of poor contact with growth media. These qualities force growers to sow large quantities of seed in order to achieve desired plant populations or to invest in labor intensive production techniques, e.g. use of greenhouse-raised transplants. One solution to this problem is seed pelleting, a process whereby mean seed diameter is significantly increased by coating individual seed with an inert, usually hydrophilic substrate to aid in handling and to facilitate mechanized planting. In this study, we evaluated germination in pelleted and non-pelleted seed from a select

group of small-seeded grains (finger and brown top millets) and vegetables (jute, amaranth, cat's whiskers). Seed pelleting was done using a Satec Concept ML2000 pellet mill (Satec Handelsges MBH, Elmshorn, Germany) with Seedworx Pellet Mix as the coating agent and 50% (v/v in water) Seedworx StixL-Neutral polymer as the binder (Aginnovation, Walnut Grove, CA). Germination tests were conducted in a bench-top incubator at 25 °C and seed was observed over a period of 10 days. Pelleting increased seed size by about 33-150% and a significant improvement in germination was observed for pelleted seed. Our results show that seed pelleting benefitted the dicots more than the grasses. Future work will compare survival and vigor in seedlings from coated and uncoated seed.

**Astronomy, Mathematics, and Physics**

IN SITU CHARACTERIZATION OF POLYMER AND DYE pH SENSING FILMS. D. M. Topasna, T. A. Emig, S. A. Cox, & G. A. Topasna, Department of Physics and Astronomy, Virginia Military Institute, Lexington, VA 24450. We describe the steps in creating the experimental set-up for in situ characterization of thin films made by ionic self-assembled monolayers technique. The films are fabricated by alternate immersion of transparent substrates in aqueous solutions of poly(allylamine hydrochloride) and Direct Yellow 4. The absorbance of films changes when the pH of the surrounding medium changes. These types of films have potential applications in the biomedical field and as optical pH sensors. The transparent films were immersed in solutions at various pH values and their absorbance was monitored. Long time (48 hours) measurements of absorbance also indicate photobleaching of the films.

THE SPECTRAL FUNCTION OF  $^{40}\text{Ar}$  THROUGH THE  $(e,e'p)$  REACTION. Donal Day, Department of Physics, University of Virginia, Charlottesville, VA 22904. Accelerator based neutrino-oscillation measurements, where neutrinos interact predominantly with nucleons bound in nuclei, demand an accurate description of the cross sections. Nuclear effects play a decisive role and are considered one of the main sources of systematic uncertainties. An effort is underway to develop theoretical models capable of a fully quantitative description of the neutrino-nucleus cross sections. An approach based on many body theory has proven successful in explaining electron-nucleus cross sections. The application of this approach to the analysis of neutrino interactions will require accurate models of the spectral functions for the nuclei in neutrino detectors. Of paramount importance is  $^{40}\text{Ar}$ , to be used in the Deep Underground Neutrino Experiment (DUNE, formerly LBNE). A  $^{40}\text{Ar}(e,e'p)$  experiment which will prove indispensable for the construction of the argon spectral function has been approved at Jefferson Lab. The  $(e,e'p)$  data, in addition, will advance theoretical developments, including the description of final-state interactions, unavoidable in neutrino experiments. The motivation, goals, experimental details and plans for the execution of this measurement will be discussed.

PHYSICAL MODELS OF TOROIDAL DIPOLE MOMENT (ANAPOLE). Amanuel Eshete, Armian Hanelli, Paige Andros, Denny Okudinani & Walerian Majewski, Dept. of Physics and SPS Chapter, Northern Virginia Community College, Annandale, VA 22003. We are

investigating two models of the third elementary dipole, known as the toroidal dipole moment (anapole). The magnetic model is a circumferentially and uniformly magnetized ring constructed of neodymium magnets. The electric model is a toroidal coil connected to a DC or AC voltage. DC-current electric and ideal magnetic toroids produce only an inner magnetic field, and interact directly with a curl of the external magnetic field, that is, in effect, with a conductive current density or with a displacement current. We studied the characteristics of permanent-magnet and electric toroids, and measured the effects of a curl of the external magnetic field (produced by linear current) or a time-dependent displacement current on the toroid. An effective toroidal dipole moment of the magnetic toroid was measured in interaction with the external current and compared with the calculated theoretical value. Rotating the magnetic toroid or passing an alternate current in the windings of electric toroid should make them act as electric dipole antennas and produce electric dipole quasi-static fields and radiation. These fields and radiations move as a wave, radially from the outer edge of the toroid. We are attempting to detect and measure these near-zone electromagnetic fields, as well as an integrated value of the external magnetic vector potential. A toroid's magnetic field should be also penetrating outside of the toroid if it is placed in an electromagnetic medium instead of a vacuum.

COSMIC RAY MUONS IN THE STANDARD MODEL OF FUNDAMENTAL PARTICLES. Hannah Glaser, Roberto Rivas, Angel Gutarra-Leon, [Cioli Barazandeh](#) & Walerian Majewski, Department of Physics and SPS Chapter, Northern Virginia Community College, Annandale, VA. Muons are one of the twelve fundamental particle types of matter, having the longest free-particle lifetime. It decays into three other leptons through an exchange of the weak vector bosons  $W^+$ / $W^-$ . Muons are present in the secondary cosmic ray showers in the atmosphere, and reach the sea level. Cosmic rays are a natural "poor man's accelerator" for community colleges. By detecting the delay time between arrival of the muon and an appearance of the decay electron in our single scintillation detector, we measured the muon's lifetime at rest in the material of our detector. After correcting it by the known ratio of positive and negative muons in the flux and taking into account the known rate of the negative muon capture in the material, we were able to extract from our data the lifetime of the free positive muon, identical to the lifetime of both muons in vacuum. It compares well with the established value. Using literature data on muon fluxes at different heights in the atmosphere, we estimated the relativistic time dilation of muons. From our lifetime measurement we were able to

calculate the ratio of  $g_w/M_w$  for the weak coupling constant  $g_w$  to the mass of the W-boson  $M_w$ . Using further Standard Model relations and an experimental value for  $M_w$ , we calculated the weak coupling constant, the electric charge of the muon, and the vacuum expectation value of the Higgs field. We also measured the sea-level flux of low-energy (below 160 MeV) muons, which are slow enough to be stopped in our detector. We also found the shapes of the energy spectra of low-energy muons and of their decay electrons. We have not found a systematic difference between day and night muon fluxes.

A COMPARISON OF ELECTROMAGNETIC LEVITATION WITH AN ELECTRODYNAMIC WHEEL ON DIFFERENT METAL CONDUCTORS. Angel J. Gutarra-Leon, Vincent Cordrey & Walerian Majewski, Department of Physics, Northern Virginia Community College, Annandale VA 22003. We built a high-density, externally driven magnetic wheel and tested a lift/drag formula derived initially for linear motions of magnets with respect to inductive tracks. We maximized the levitation efficiency by using Halbach array of dipole magnets, with the peak magnetic field strength of 0.4 Tesla and a working field of 0.18 Tesla at the measuring distance. Experiments were performed on a total of five different conductive plates or "tracks". We verified applicability of the theoretical lift/drag formula to this rotational system. The lift-off speeds were reached. We also developed a program to convert raw oscillatory data into the averaged force graphs. We are currently in the process of improving the stability of the system in order to get better measurements at higher to allow measurements at higher angular velocities. (Supported by: the Society of Physics Students and the Virginia Academy of Sciences)

EFFECT OF ANGLE DEPENDENCE ON THE ELECTRIC AND ENTROPIC CONTRIBUTIONS OF INFRARED POWER GENERATION. Justin M. Kaczmar<sup>1</sup>, Brian C. Utter<sup>2</sup>, Giovanna Scarel<sup>1</sup>, <sup>1</sup>Dept. of Physics and Astronomy, James Madison University, Harrisonburg VA 22802 and <sup>2</sup>Dept. of Physics and Astronomy, Bucknell University, Lewisburg PA 17837. In studying alternative methods for energy harvesting we use a power generator device to produce a voltage by striking it with infrared radiation. What we have been investigating is the effect of changing the angle at which we strike the device with in relation to the produced voltage. We observe an overall voltage rise with relation to the angle and attribute this to two components: an electric and entropic contribution in the time immediately after turning the infrared radiation on. By taking the total voltage by the two components and plotting it as a function of the angle at which they were taken at, we find a distinct effect on the total



voltage measured when performed at certain angles versus others. We specifically find that the electric contribution to the total sum becomes more pronounced as the angle increases while the entropic becomes less so.

EFFECT OF TRANSFORMATION TEMPERATURE ON NANO PARTICULATE SIZE. William B. Lambert & Rama Balasubramanian, Dept. of Mathematics, Computer Science, and Physics, Roanoke College, Salem VA 24153. Nanoparticles are structures ranging from 10 to 100 nanometers that can be used for a variety of applications spanning from nanotube substrates to smart coatings. Nanoparticles of hematite ( $\alpha$ -Fe<sub>2</sub>O<sub>3</sub>) and maghemite ( $\gamma$ -Fe<sub>2</sub>O<sub>3</sub>), used in magnetic storage media and as contrast agents in MRI, can be produced in a variety of ways such as weathering, sol-gel synthesis, and through solid state transformation. This study explores the properties of nanophase hematite formed from solid-state transformation of maghemite. In order to understand the dependence of particle size on transformation temperature, seven samples of hematite were synthesized from maghemite at temperatures ranging from 175-700°C. The hematite samples were characterized using X-Ray diffraction and the mean crystalline diameter (MCD) was calculated using Scherrer equation. As the transformation temperature increased from 175-325°C, XRD measurements showed the presence of both maghemite and hematite phases. The MCD of the samples ranged from approximately 9 nm to 16 nm. As the transformation temperature increased from 400-700°C, the samples were completely transformed to hematite and the MCD increased from approximately 18 nm to 78 nm. Between 175-325°C the particle size showed a linear dependence on transformation temperature. However, as the transformation temperature increased from 400-700°C the particle size followed a 2<sup>nd</sup> degree polynomial dependence. The transformation at lower temperatures is said to be topotactic while aggregation of hematite crystals was rapid at higher transformation temperatures. Detailed results from the study will be presented.

DEVELOPMENT AND VERIFICATION OF A NEW POST ROLL-UP MODEL FOR CHARACTERIZATION OF AIRCRAFT LIFT GENERATED WAKE VORTICES. Steven H. Lohrey, Dept. of Physics, Randolph-Macon College, Ashland VA. 23005. This presentation documents research findings for four post roll-up two-dimensional flow-field models for a counter-rotating pair of vortices generated by aircraft lift. The governing equations that define the velocity distributions for the four models considered are all derived from an identical physics based theoretical formulation. Vector potential methods were employed to derive the governing velocity

equations for each of the four models. Of the four models studied, three are well known in the literature and are currently used in a variety of wake turbulence applications; albeit they were not derived from vector field potentials. The fourth flow model is a new result that was developed during this research project. Basic parameters for each vortex model, such as peak tangential (Swirl) velocity, and circulation strength as a function of vortex core size, were examined. The new model was validated using data acquired in a NASA wind tunnel experiment using a 3% scale model of a Boeing 747 as the generator for the counter-rotating vortex pair flow field. Vortex induced forces and moment data were measured for three different well instrumented down-stream wing configurations immersed in the B747 wake flow field. The comparison between the calculated and measured vertical velocities showed very good results for the new Lohrey-Bowles model. The induced lift force coefficient and the induced rolling moment coefficient for the three different wing configurations were also calculated for this model and showed excellent agreement with the wind tunnel measurements.

**WHAT IS DATA SCIENCE?** David J. Marchette, Naval Surface Warfare Center, Dahlgren, VA, 22448. Data science, data analytics, big data: these terms are thrown around frequently these days. But what exactly is "Data Science"? In this talk I will try to answer this question by looking at the term through several lenses: as a process for data analysis; in relation to well-known disciplines such as statistics and computer science; a set of tools; and finally as it relates to science. I will give a very high-level view of the field, touching on these issues from various perspectives. I will discuss a few of the algorithms and tools that are used in the field (from a very idiosyncratic perspective) and talk very briefly about some applications.

**STEM TAKES FLIGHT.** Thomas C. Mosca III, Rappahannock Community College, Department of Mathematics, Warsaw, VA 22572. The Virginia Space Grant Consortium (VSGC) in partnership with the Virginia Community College System (VCCS), NASA Langley Research Center and NASA Wallops Flight Facility is offering the STEM Takes Flight initiative for Virginia's Community Colleges. STEM Takes Flight provides a suite of programs for Virginia community college students pursuing STEM majors and faculty in STEM disciplines statewide. Opportunities include \$5,000 mentored scholarships, paid onsite research experiences at NASA Langley and NASA Wallops, paid industry internships, new courses and a NASA

residential faculty professional development workshop. This year I participated in the faculty workshop, and the Antares ISS resupply mission simulation based on the Orbital ATK Cygnus Mission #2 launch. This presentation highlights the experience with hopes of encouraging participation in future opportunities.

**LASER MATTER INTERACTION.** Hai T. Nguyen, Department of Physics, University of Mary Washington, 1301 College Avenue, Fredericksburg, VA 22401. Single frequency lasers in conjunction with miniature Acousto-Optic Modulators (AOM) enables new kind of phase modulators for various experiments. We cool and trap Rubidium atoms to measure energy-dependent charge exchange cross sections in the  $\text{Cs}^+ + \text{Rb}(5s, 5p)$  system over a range of projectile energies. Also, we use AOM diffractions in interference experiments between two beams of light, in a Mach Zehnder interferometer. The frequency differences of the many different optical orders of diffraction are well known and are easily controlled *via* the AOM electronic circuits and phase modulation. We use it to study heterodyne detection which allows for high efficiency coherent optical memory with warm rubidium vapor experiments suitable for quantum information applications, therefore, reducing the need for complicated trapping and cooling. We developed this optical heterodyne detection technique for various applications including optical memory delay, storage and readout. Furthermore, the ability to manipulate, enhance, and control of up-conversion multicolor output of UCNPs is particularly important for their applications in multiplexed biological applications. The output brightness of upconverting nanoparticles has been limited by questions about energy transfer and relaxation within individual nanocrystals and unavoidable tradeoffs between brightness and size. The common denominator in these questions is the upconverting efficiency of each individual UCNPs. The aim is to increase our knowledge and ability to manipulate, enhance, and control of single UCNP  $\text{NaYF}_4$  co-doped with  $\text{Yb}^{3+}/\text{Er}^{3+}$  upconverting efficiency by trapping and exciting the single UCNP with one single laser at 915 nm.

**A ROTATING METRIC THAT INCLUDES THE ENERGY DENSITY OF THE GRAVITATIONAL FIELD.** Joseph D. Rudmin, Dept. of Integrated Science and Technology, James Madison University, Harrisonburg, VA, 22807. Several problems or concerns in general relativity are discussed regarding the use of an isotropic metric: Energy density of the fields, conformal flatness, rank-1 tensor derivatives for a rank-2 tensor field, reason and application of the equivalence principle,

and preservation of quantum unitarity. It is shown that conservation of energy with an isotropic metric yields a self-consistent model of general relativity and quantum mechanics. Since this talk is an instructional review rather than a presentation of results, publication of this abstract in the Virginia Journal of Science is not expected. The audience should be familiar with Maxwell's Equations and vector transformations.

**POLARIZATION OF THE OPEN CLUSTER NGC 7380.** G. A. Topasna, Dept. of Physics and Astronomy, Virginia Military Institute, Lexington, VA 24450. Polarization measurements were made of the open cluster NGC 7380 using the optical polarimeter on the 0.5 m telescope at the VMI observatory. The analysis shows that the ratio of total to selective extinction for the area centered on the cluster is  $3.05 \pm 0.09$ . A strong correlation exists between the wavelength of maximum polarization and the color index (B - V) and a linear correlation between the wavelength of maximum polarization and color excess but with outliers suggesting further study of these stars. The visual extinction is found to be  $1.7 \pm 0.1$  magnitudes and the cluster distance was determined to be  $2.5 \pm 0.4$  kpc.

**EXCITATION AND DETECTION OF UPCONVERTING NANOPARTICLES.** Pengcheng Zhang, Dept. of Physics, University of Mary Washington, Fredericksburg VA 22401. The up-conversion of nanoparticles has many applications in the fields of Biology and medical research, such as single molecule spectroscopy, colloidal dynamics, protein isolation, and controlled investigation of biological processes. In particular, it provides an alternative method for cancer treatment delivery. The advantages of using nanoparticles include higher sensitivity and the occurrence of quantum effects at the nano-scale. The up-conversion of nanoparticles has been experimented and well-studied with 980 nm lasers. However, due to its high water absorption, the 980 nm laser is not ideal for medical and surgical applications. Alternatively, many ongoing researches study the effectiveness of 915 nm lasers, or other lasers with relatively low water absorption. Particularly, we will look at an ongoing experiment at the University of Mary Washington Physics Department studying the excitation and detection of up-converting nanoparticle using both 915 nm and 980 nm lasers.

**Biology with Microbiology and Molecular Biology**

MOLECULAR BASIS OF EXOSOMAL FUNCTION DURING RIFT VALLEY FEVER VIRUS INFECTION. N. A. Ahsan<sup>1</sup>, G. Sampey<sup>1</sup>, B. Lepene<sup>2</sup>, R. Barclay<sup>1</sup>, S. Iordanskiy<sup>1</sup>, F. Kashanchi<sup>1</sup> & R. M. Hakami<sup>1</sup>, <sup>1</sup>George Mason University, Manassas, VA, and <sup>2</sup>Ceres Nanosciences, Inc., Manassas, VA. Exosomes are small “bioactive” extracellular vesicles that play a central role in intercellular communication and have garnered tremendous interest given the recent discovery of their critical role in a variety of diseases, including infectious diseases. Among these are immunomodulation, alteration of dissemination/infectivity during infection, and modulation of pathogenesis. However, their mechanisms of action remain largely unknown and their role during infections with biodefense agents remains unexplored. We have examined the role of host exosomes during infection with the Rift Valley Fever Virus (RVFV), a Category A priority Pathogen that carries the potential for both devastating public health and agricultural impacts. To assess the role of exosomes, clones of Vero cells that show resistance to RVFV infection and are unable to release functional virions were first generated. Exosomes from these clones contained exosomal markers such as CD63 and were able to activate the TLR3 pathway in recipient reporter cells. Interestingly, these exosomes contained viral RNA (signal for L, M, and S segments). Furthermore, exosomes derived from some of the resistant clones contained viral proteins such as N. Finally, treatment of immune recipient cells (T cells and monocytic cells) with some of the exosome preparations showed a drastic rate of apoptosis through PARP cleavage and caspase-3 activation. Collectively, our data suggest that exosomes from RVFV-infected cells alter the dynamics of the neighboring cells and may contribute to disease pathology.

S1P INHIBITS IL-1-INDUCED CCL5 EXPRESSION. Sabrina Andaluz, Debolina Biswas, Angela Gupta, Tomasz Kordula, Dept. of Biochemistry & Molecular Biology, VCU School of Medicine, Richmond, VA 23298. Both interleukin-1 (IL-1) and sphingosine-1-phosphate (S1P) are critical mediators of inflammation. However, it is unclear at the moment whether S1P promotes or inhibits IL-1-induced inflammatory responses. In preliminary studies we found that one of the five S1P cell surface receptors, S1PR2, may regulate inflammatory responses. To address this question, whether S1PR2 effects IL-1 signaling, we analyzed infiltration of macrophages into sites of sterile inflammation in wild type and S1PR2<sup>-/-</sup> mice. We employed a turpentine model of irritant-induced inflammation and found increase infiltration into the wound of S1PR2<sup>-/-</sup> mice. Accordingly, expression

of CCL5 chemokine, which attracts macrophages, was elevated in S1PR2<sup>-/-</sup> mice. We concluded that S1PR2 limits IL-1-induced expression CCL5, and recruitment of macrophages into sites of inflammation.

DETERMINATION OF THE IMPACT OF PYK1 DELETION ON INTERACTIONS OF *C. NEOFORMANS* WITH THE HOST IMMUNE SYSTEM. Yansirre Aviles & Elizabeth Rasmussen, Dept. of Biol., Liberty Univ., Lynchburg VA 24502. *Cryptococcus neoformans* is an important fungal pathogen of immune-compromised individuals that initially colonizes in the lungs and eventually migrates to the cerebral spinal fluid, eventually causing the death of about 625,000 people a year. Epidemiological evidence for the dormancy of cryptococcal infections exists, and our objective is to develop a model that allows for the effective study of said dormancy in such an important human pathogen. This study will be facilitated by quantifying cytokine expression in macrophages that have been exposed to pyruvate kinase mutants that fail to elicit inflammation in the lung. Initial results from Dr. Price's lab suggest that the removal of the pyruvate kinase gene in H99 wild type strains results in a lack of the immune system's ability to recognize the yeast. This dormancy phenomenon will be examined by analyzing the effects that alterations in carbon metabolism have on host immune cell cytokine production and cell signaling. *C. neoformans* wild type, *pyk1Δ*, *pyk1Δ PYK1*, and *hvk1Δ hvk2Δ* strains will be co-incubated with harvested primary BALB-C macrophages in conditions that simulate the interior of human lungs. Subsequently, the culture broth and macrophages themselves will be saved for ELISA analysis of cytokine production. To confirm compatibility between in-vitro studies of this culture data and actual in-vivo scenarios, live mice will be infected with both wild type and previously mentioned *pyk1Δ* mutant strains of *C. neoformans*. At set points post-infection, the spleens, lungs, and brains of the infected murine specimens will be harvested and used for cytokine and gene expression analysis.

MOLECULAR REGULATION OF TRKA TRAFFICKING IN SPACE AND TIME. Kelly A. Barford<sup>1</sup>, Christopher D. Deppmann<sup>2</sup> & Bettina Winckler<sup>1</sup>, <sup>1</sup>Department of Neuroscience, University of Virginia, Charlottesville VA 22908 and <sup>2</sup>Department of Biology, University of Virginia, Charlottesville VA 22908. Protein trafficking is involved in all aspects of neuronal function, including development, axon and dendrite growth, and synaptic function. Presumably due to cellular complexity, neurons have acquired special endosomal machinery to deal with protein trafficking. One such protein is neuron enriched endosomal protein of 21kDa (Neep21). Neep21 is involved in the trafficking of proteins involved in synaptic function and disease

including GluR2,  $\beta$ APP, and neurotensin receptor 1. Our lab has recently shown that Neep21 is involved in the axonal specification of the cell adhesion protein L1/NgCAM through transcytotic trafficking and avoidance of the lysosome. Another protein that undergoes both transcytosis and lysosomal evasion is the neurotrophin receptor TrkA. TrkA undergoes can undergo many different trafficking events both on its way to the axon and in a signaling endosome travelling retrogradely back to the soma. TrkA has the ability to undergo signaling transcytosis and to enter into the dendrites and affect synapse formation. However, the trafficking steps and molecules involved in the transport of TrkA have not been fully elucidated. Neep21's involvement in transcytosis and lysosomal avoidance make it an ideal candidate for involvement in TrkA signaling, and its placement in the somatodendritic region primes it to interact with TrkA during distinct spatiotemporal trafficking events. Indeed, Neep21 is a novel effector for the signaling endosome, and we are currently investigating the role of Neep21 in TrkA-dependent processes.

**DOMINANCE BEHAVIOR IN THE TUBE TEST, AND ITS RELATIONSHIP TO HOME CAGE BEHAVIOR IN MICE.** Hannah M. Belski, Meagan T. Darling & R. Parrish Waters, Dept. of Biology, University of Mary Washington. In previous studies, scientists have used mice for their versatility as a whole animal model in examining human conditions. In many of these studies, the mouse's behavior is the output and ultimately determines the result of the experiment. However, the mouse's natural ethology is rarely prioritized in interpreting these results. What this experiment aims to do is develop an ethologically valid model to more fully understand the mouse's behavior and, in turn, its response to stressful stimuli. Primarily, this study focuses on social stress; the most potent stressor animals encounter. To induce this social stress, we house five male CD-1 mice together in a cage in which close proximity elicits competition for resources. The design of this experiment consists of two components: behavioral and physiological. We performed several established tests of dominance, including a Tube Test for which we constructed an algorithm to generate a quantitative score of dominance, called a TDR (Total Dominance Ratio). To support these data, we determined fecal steroid hormone concentrations weekly and measured monoamines from several critical regions of the brain. We uncovered correlation between our TDR and other behavioral measures in several of the cages. When steroid hormones are compared to TDR, there is no significant correlation. However, emerging patterns of amygdalar dopamine and norepinephrine suggest depressive physiology in subordinate mice. Through both their behavioral and physiological measures, we hope to see consistent patterns that characterize dominant

or subordinate mice. Future directions of this project will include incorporating RFID technology as well as additional social stressors to model human pathologies.

**SKELETAL MUSCLE MAY INTERACT WITH VASCULATURE THROUGH O-GlcNAc TRANSFERASE (OGT).** Joel A. Brenny<sup>1</sup>, Emily R. Berguson<sup>1</sup>, David E. Gerrard<sup>2</sup>, Hao Shi<sup>2</sup> & Pei Zhang<sup>1</sup>, <sup>1</sup>Department of Biology and Chemistry, Liberty University, Lynchburg, VA, 24502 and <sup>2</sup>Department of Animal and Poultry Sciences, Virginia Polytechnic Institute and State University, Blacksburg, VA 24061. Cellular communication can occur through post-translational modification, the modification of proteins by enzymes such as O-linked N-Acetylglucosamine Transferase (OGT). OGT adds an N-acetylglucosamine (O-GlcNAc) moiety to a serine or threonine residue of the protein. O-GlcNAcylation is important because it can affect various functions of proteins. O-GlcNAcylation is also implicated in metabolic syndromes such as diabetes. At the center of metabolism, vasculature not only distributes nutrients and metabolites, but also actively interacts with surrounding tissue and remodels itself in response to physiologic and pathophysiologic stimuli. Many myokines, secreted by muscle cells, are involved in inflammation, and could be markers of vascular disease such as atherosclerosis. Using an OGT skeletal muscle knock-out murine model, we are examining the effects of OGT on vasculature with the goal of identifying basic mechanisms underlying interactions between skeletal muscle and vasculature. This study may shed new light on heterocellular metabolism and present new therapeutic potential for metabolic syndromes.

**ALTERED MITOCHONDRIAL DYNAMICS IN VENEZUELAN EQUINE ENCEPHALITIS VIRUS INFECTED CELLS.** Taryn Brooks-Faulconer, Moushimi Amaya, Forrest Keck, Charles Bailey & Aarthi Narayanan, National Center for Biodefense and Infectious Diseases, George Mason University, 10650 Pyramid Place, Manassas VA 20110. To establish productive infection, viruses profoundly alter both the intracellular environment and the cellular function. Mitochondria are critically important cellular organelles that generate energy and ensure cell survival. Mitochondria are also crucial for innate immunity response as they serve as the sentinels that sense infection and initiate host responses. For many viruses, the changes in mitochondrial dynamics were documented to occur early in infection. Venezuelan Equine Encephalitis Virus (VEEV) is a New World alphavirus that infects neuronal cells and produces an encephalitic phenotype. In this study, we demonstrate that VEEV infection results in mitochondrial alterations that include changes in the morphology and intracellular distribution of mitochondria, reduction in mitochondrial membrane potential and localization of their enzymatic components. In



particular, we report perinuclear accumulation of mitochondria in infected cells and partial co-localization of the viral capsid proteins with mitochondrial membranes. The pronounced changes to the mitochondria observed in VEEV infected cells probably play a role in the development of the virus-specific cytopathic effects. Our studies demonstrate that the mitochondria are critical intracellular platforms affected by alphavirus infections.

THE EFFECTS OF ROSMARINIC ACID ON EWING'S SARCOMA CELL VIABILITY. Samuel J. Clark & Rosemary Barra, Dept. of Biol., Univ. of Mary Washington, Fredericksburg VA. 22401. Botanical and herbal medications are among the most common complementary and alternative medications (CAM) used by cancer patients, both in the treatment of cancer and the management of cancer symptoms. However, information concerning the safety and efficacy of many CAM treatments has not been established. *Rosmarinus officinalis*, commonly known as rosemary, has been implicated as a possible cancer chemo preventive agent as well as a treatment due to its inherent antioxidant activity. It has been demonstrated in several cancer lines that rosemary extracts (RE) have a significant anti-proliferative effect through modifications on the cell cycle. RE's have not been tested on Ewing's sarcoma (ES) cell lines. This study aimed to evaluate the proliferative activity of RE's on an ES cell line. Also, one of RE's main active components, rosmarinic acid (RA) was evaluated. Both of these analyses used the MTT viability assay. Contrary to the effects on other cell lines, this study indicated a significant increase in cell proliferation. The Folin-Ciocalteu assay was used to determine the approximate phenol concentration within an RE. RE was applied to the system at concentrations of 30 µg/ml, 15 µg/ml, 7.5 µg/ml, and 3.5 µg/ml, an increase in cell viability by 119%, 140%, 157%, and 289%, respectively, was demonstrated. When the RA was applied to the system at concentrations of 30 µg/ml, 15 µg/ml, 7.5 µg/ml, and 3.5 µg/ml, an increase in cell viability by 120%, 175%, 246%, and 310% was recorded. These results suggest that RA is the major active component of the extract.

EFFECT OF SOCIAL STATUS ON BEHAVIOR AND NEUROENDOCRINE SYSTEMS IN MALE MICE. Meagan T. Darling<sup>1</sup>, Hanna M. Belski<sup>1</sup>, David H. Stahlman<sup>2</sup>, & R. Parrish Waters<sup>1</sup>, <sup>1</sup>Department of Biology and <sup>2</sup>Department of Psychological Sciences, University of Mary Washington, Fredericksburg VA 22401. Mice are social animals that form hierarchies, in which higher-ranking (dominant) animals display aggressive behavior toward lower-ranking (subordinate) animals. These social interactions are intensely stressful

and have profound effects on the physiological and behavioral state of both the dominant and subordinate individual. The effects of this social stress often resemble the symptoms of human stress-related pathology, including depression, PTSD, and addiction. Multiple laboratory paradigms utilize social stress in mice to model these disorders. However, many of these paradigms fall short of ethological and ecological validity: they use short-term and/or heavily weighted interactions between animals, which do not match the true nature of mouse social interactions, and are therefore less applicable to human pathology. Our model attempts to address this deficit. We assess the position of mice in dominance hierarchies and the stability of these hierarchies to analyze their effects on physiology and behavior. Our preliminary results suggest that aggressive home cage behaviors correlate to dominance status, that social cages are dynamic, and that social status impacts monoamine expression in the amygdala. This study was funded in part by the Irene Piscopo Rodgers '59 and James D. Rodgers Student Research Fellowship II and the UMW Summer Science Institute.

CHIMERIC PD1-EXPRESSING T CELLS AS A POTENTIAL TREATMENT FOR MULTIPLE TYPES OF CANCER. Kelsey Deal, Geoffrey Parriott, & Amorette Barber, Dept. of Biol. and Env. Sci., Longwood Univ., Farmville VA 23901. CD8 T cells are one of the immune system's best defenses against tumors. However, some tumors accumulate enough mutations to evade T-cell detection. On the other hand, most tumors express ligands for the Programmed Death receptor (PD1). Since PD1 ligands are expressed on most tumor types, and not many other cells, they are ideal targets for potential tumor therapies. One such therapy is the development of chimeric antigen receptors (CAR). CARs are modified receptors that use genetic engineering to replace the normal signaling domain with a different one. In our experiments we used a CAR with the PD1 receptor as the tumor-targeting domain attached to CD3 zeta activation and Dap10 costimulatory domains, called chimeric-PD1 receptor (chPD1). Previously, chPD1 expressing T cells were shown to effectively treat murine models of lymphoma and melanoma. In this study, we tested the anti-tumor efficacy of chPD1 T cells against murine kidney, pancreatic, liver, and colon cancers. Flow cytometry and RTPCR were used to determine that all tumor cells tested expressed the PD1 ligands. Then we measured tumor cytotoxicity of the chPD1 T cells compared to wild type T cells. For all tumor types tested, chPD1 T cells significantly increased killing of the tumor cells. Therefore, chPD1 T cells could be a novel therapeutic strategy to treat multiple types of cancer.

INFLUENCE OF ENVIRONMENTAL TEMPERATURE ON METABOLIC RATE IN AQUATIC ECTOTHERMS. J. C. Doran<sup>1</sup>, J. M. Doran<sup>1</sup>, S. Henkanaththegedara<sup>2</sup> & P. A. P. deHart<sup>1</sup>, <sup>1</sup>Dept. of Biol., Virginia Military Institute, Lexington VA 24450 and <sup>2</sup>Biol. & Environ. Sci., Longwood University, Farmville VA 23909. This research aimed to quantify the effects of environmental temperature on metabolic rate in aquatic ectotherms through the use of a model ectotherm, the crayfish. With rising global temp., environments are expected to rise in average temp., including aquatic environments. Due to the essential roles that aquatic ectotherms carry out in their ecosystems, it is crucial to discover how these organisms will be affected by environmental temp. changes. It is hypothesized that crayfish inhabiting warmer environments will reflect higher stress levels, indicated through increased metabolic rates. Crayfish of genus *Cambarus* were collected in streams within the Lexington, VA area. Three sets of ten crayfish were maintained for an average of 15 days in three separate aquaria held at 17, 21, and 25°C. After maintaining the crayfish, each organism was prepped and subsampled for stable isotope analysis (SIA). By observing metabolic rates from isotopic signatures, insight on the stress levels of each crayfish could be assumed. Values of  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  did not reflect the hypothesis. Both 17 and 25°C treatment groups reflected statistically more enriched signatures relative to the 21°C treatment in regards to  $\delta^{15}\text{N}$ . This would suggest that any deviation from an intermediate temp. would increase stress levels of ectotherms. However, these overall signatures are more likely to have occurred due to the short exposure time within treatment aquaria. Longer exposure will allow for the effects of varying environmental temp. to be reflected within the tissues of crayfish.

HABITAT MEDIATED DIFFERENCES IN THE TROPHIC NICHE OF ARACHNIDS AS CLARIFIED BY STABLE ISOTOPE ANALYSIS. J. M. Doran<sup>1</sup>, O. Howell<sup>2</sup>, J. C. Doran<sup>1</sup>, L.E. Hurd<sup>2</sup>, & P.A.P. deHart<sup>1</sup>, <sup>1</sup>Department of Biology, Virginia Military Institute, Lexington, VA 24450 <sup>2</sup>Biological Department, Washington & Lee University, Lexington, VA 24450. Our research aimed to determine the consequences that differing habitats has on arachnids' eating behaviors. In order to accomplish this, we utilized stable isotope analysis to quantify trophic levels on which each are operating. The objective for this project was to collect arachnids of varying species, including the harvestman of Order *Opiliones* and the orbweaver of Order *Araneae*, from within the Lexington, VA area from two different habitats; a woody habitat and a field habitat. Once collected, samples were prepared for stable isotope analysis (SIA) and taken to a stable isotope facility, for isotope analyses. Data was then be analyzed, examining trends in the ratios of heavy to light carbon and nitrogen isotopes across

treatment groups. Values of  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  indicated that field harvestmen operate at one trophic level higher than their counter parts in the forest habitat. However, the same was not witnessed for orbweavers, as both were operating on relatively equal trophic levels. Arachnids are a generalist predator that can influence species diversity, how they interact trophically is extremely important for the ecosystem as a whole. Determining how the habitat influences this trophic interaction has not been studied notably in the past. How and what each species eats in the two different habitats can reveal valuable information about how the environment influences each arachnid.

**INHIBITORY RECEPTORS PD-1 AND CTLA-4 DIFFERENTIALLY REGULATE NFKB ACTIVATION IN NKG2D- AND CD28-COSTIMULATED CD8 T CELLS.** Zachary Fasana & Amorette Barber, Dept. of Biol. and Env. Sci., Longwood Univ., Farmville VA 23901. Effector CD8 T cells are important for successful antitumor immune responses. The balance of activating and inhibitory receptors affects the potency of the CD8 T cell response by activating kinases and/or phosphatases. The optimal combination of receptors that induces effective antitumor immunity is still unknown. CD8 T cell activity is enhanced by activation of costimulatory receptors, including CD28 and NKG2D. Inhibitory PD1 and CTLA4 receptors are highly expressed by T cells and their ligands are expressed in the tumor microenvironment. While these activating and inhibitory receptors are all likely stimulated on T cells in the tumor, it is currently unknown how the CD28 and NKG2D receptor signaling is altered by concurrent activation of inhibitory receptors PD1 and CTLA4. Therefore, this study compared gene expression of NF $\kappa$ B pathway members involved in antitumor responses. Specifically, murine effector CD8 T cells were costimulated by activating NKG2D or CD28 receptors in combination with inhibitory PD1 or CTLA4 receptors and an RT-PCR array was used to measure expression of over 80 genes in the NF $\kappa$ B pathway. Compared to CD28 costimulation, CD8 T cells stimulated by NKG2D and CTLA4 or PD1 had fewer changes in gene expression. Thus, NKG2D is likely the more effective antitumor costimulatory receptor due to its resistance to be inhibited by PD1 and CTLA4 receptors.

**OPTIMIZATION OF ASSAY FOR IDENTIFICATION OF RNA-RNA INTERACTION.** A. Filatova<sup>1</sup>, A. Baranova<sup>1,2</sup> and M. Yu Skoblov<sup>1,3</sup>, <sup>1</sup>Centre for Medical Genetics, Federal Agency for Scientific Organizations, Moscow, 115478, Russian Federation, <sup>2</sup>School of Systems Biology, George Mason University, Manassas, VA, 20110, USA and <sup>3</sup>Moscow Institute of Physics and Technology, Moscow, 117303, Russian Federation. RNA-RNA interactions play an important

role in regulation of gene expression and maintenance of cell homeostasis. RNA-RNA duplex formation is a trigger for many cellular mechanisms which alter target RNA structure, half-life time, translational efficiency, and ability to form complexes with other targets. According to recent reports, the human genome contains more than 15,000 long non-coding RNAs (ncRNAs) genes. Many of these play an important role in diverse biological processes. ncRNA functions are often associated with their ability to form complexes with other RNA-molecules, both coding and non-coding. Thus, analysis of RNA partners of previously unexplored ncRNA allows us to investigate its possible function. One of the approaches to identify RNA-RNA interactions is RNA-RNA pull-down with short biotin-labeled ssDNA-oligonucleotides complementary to target RNA. This study is devoted to determine the most appropriate conditions for effective and specific detection of RNA partners. We made RNA-RNA pull-down experiments for high-level expressed and low-level expressed RNAs with different amount of probes on different hybridization conditions. We measured enrichment of target RNA, its partner, and reference RNA in pull-down samples relative to input samples by qPCR. Additionally, we evaluated the specificity of different protocols using non-specific probe controls.

CHARACTERIZATION OF CELL-TYPE SPECIFIC RESPONSES TO MISFOLDED PROTEIN STRESS IN *C. ELEGANS*. Claire Gormley, Kristen Hoffman, Rana Ihsan & Tim Bloss, Dept. of Biol. James Madison University, Harrisonburg, VA 22807. Cells experiencing misfolded protein stress can become debilitated and die, contributing to the onset of disease. Different cell types display varying sensitivities to this stress, with neurons being particularly susceptible to death. When a cell experiences misfolded protein stress in the endoplasmic reticulum (ER), the unfolded protein response (UPR) initiates cell-saving mechanisms that mitigate stress and, if the stress cannot be resolved, triggers cell death by apoptosis. The nascent polypeptide-associated complex (NAC) is a heterodimeric chaperone that mediates proper protein folding and localization during translation, and also triggers the UPR when the ER experiences misfolded protein stress. The role of the NAC in relation to the UPR is not well understood, nor is it known if this role is different in different cell types; our goal is to characterize the relationship of the NAC with the UPR in different cell types in the model organism *C. elegans*. We are characterizing how neurons respond to misfolded protein stress in the absence of the NAC by depleting the NAC via RNA interference and quantifying the number of neurons observed in the ventral nerve cord.

Generally, depletion of the NAC decreases the number of ventral nerve cord neurons while also leading to the mis-localization of the neurons that remain. In addition, we are characterizing the effects of depletion of the NAC in hypodermal cells, which are relatively more resistant to stress-induced death. Through these experiments, we hope to better understand how different cell types handle misfolded protein stress, and why some cell types are more likely to die in response to this stress while others live.

TAU AND BETA-AMYLOID INTERACTIONS IN ALZHEIMER'S DISEASE. Tyrrell C. Graham & Deborah A. O'Dell. Dept. Biol. Sci., Univ. Mary Washington, Fredericksburg, VA 22401 Characteristics of Alzheimer's disease include abnormal Tau protein phosphorylation and the presence of beta-amyloid plaques. The stimulus for changing Tau phosphorylation is unknown. The effect of the presence of beta-amyloid aggregates on the phosphorylation state of Tau was examined. We exposing cultured human cortical neurons to beta-amyloid aggregates for 48 hours and looked for the presence of p-231 Tau using immunohistochemistry. Microscopic examination of cells showed that cultured cells exposed to beta-amyloid aggregates had increased levels of p231-Tau compared to control cells. This suggests that the presence of beta-amyloid plaques stimulates the changes in the phosphorylation of Tau. This work was sponsored by an Undergraduate Research Grant to GH from UMW.

COMPARATIVE GENOMICS OF *STREPTOCOCCUS PARAUBERIS* IN FISH AND CATTLE. Ashley N. Haines<sup>1</sup>, Elvira N. Besong<sup>1</sup>, Kimaya R. Council<sup>1</sup>, Onaysha A. Lambert<sup>1</sup>, Miranda Ryan<sup>2</sup>, Dillion B. Matthews<sup>2</sup> & David T. Gauthier<sup>2</sup>, <sup>1</sup>Department of Biology, Norfolk State University, Norfolk, VA 23504 and <sup>2</sup>Dept. of Biological Sciences, Old Dominion University, Norfolk, VA 23529. *Streptococcus parauberis* is a gram-positive bacterium that infects fish and cattle, causing streptococcosis and mastitis respectively. We have sequenced, assembled and annotated 14 genomes, eight isolated from fish and six from non-piscine sources. In this study we investigated the selective pressures on the genomic resources of these isolates. We hypothesized that genes would be under different selective pressures, depending upon the host or environment in which they live. To demonstrate this, we created a phylogenetic tree of all available isolates based upon single nucleotide polymorphisms (SNPs). We then assessed whether the dN/dS ratio differed between branches on this tree and identified eight specific genes under positive selection. We also identified 45 genes unique to isolates infecting Chesapeake Bay striped bass (*Morone saxatilis*) and two genes not found in Chesapeake Bay

isolates. Identifying these genetic differences will help us understand how these bacteria adapt to different niches and become pathogens of new hosts.

RESPONSE OF IMMUNE CELLS TO NANODIAMONDS *IN VITRO*. Maisoun E. Bani Hani & Christopher Osgood, Dept. of Biol., Old Dominion University, Norfolk VA 23529. Nanodiamonds (NDs) are gaining more attraction for both imaging and theranostic applications. In addition to their unique physical and chemical properties, NDs are described as highly biocompatible with very low cytotoxicity in cultured cells, nematodes and mice. The intrinsic fluorescence of ND due to crystal defects in their lattice, usually known as nitrogen vacancies, made it possible to track ND both *in vivo* and *in vitro*. In order to achieve the safe use and application of NDs in biomedicine, it is essential to understand the effects of this material on the immune system at the cellular level. Our goal was to investigate the impact of ND on the immune system by studying their effects on innate immune cells viability, proliferation and function *in vitro*. Measuring the absorbance and the fluorescence from cells incubated with different concentrations of ND, the uptake by a mouse macrophage cell line increased with increasing the time of incubation and the concentration of NDs. Incubation of mouse cells with up to 100 µg/ml ND did not significantly reduce cell viability as assessed by MTS assay. In order to investigate the ability of ND to induce an immune response from mouse macrophages, we performed RT-qPCR for different immune markers and found increased expression of these genes which indicates an induced response in these cells. We also looked at the ability of these cells to respond to LPS after treatment with ND but did not find a significant difference. Our future work will be to perform a microarray assay to further investigate immune signaling pathways activated by NDs.

EFFECTS OF ANTIGEN-SPECIFIC AVIAN IGY ON PATHOGEN SHEDDING AND MURINE ANTIBODY RESPONSE IN *CITROBACTER RODENTIUM* INFECTION OF A MURINE MODEL. Nicole Hawkins, Robert Welch, & Randall Hubbard, Department of Biology and Chemistry, Liberty University, Lynchburg VA 24502. **Objective:** Assessment of whether administration of antigen-specific IgY would neutralize the pathogen *C. rodentium* in mice. **Method:** The following were performed: a 21-day infection of CF1 mice with DBS 101 (n=26) and a 10-day infection of C57BL/6 mice (n=6) with DBS 100. ELISA of serum and serial dilutions of pathogen load in stool were used to compare the responses of two treatment groups and a non-treated group to infection. **Results:** In both trials mice demonstrated lack of susceptibility to infection (50% of n=26 mice with stool assessed grew no pathogen). ELISA showed a decreased *C. rodentium*-specific IgG serum response level in treated CF1 mice but no similar

decrease in treated C57BL/6 mice. Serial dilutions of stool showed no significant advantage of treated groups versus non-treated in pathogen load reduction. **Conclusion:** A stronger pathogen and more susceptible model are needed to better assess therapeutic potential of IgY as well as additional trials.

EFFECTS OF SB100 ON RAGE RECEPTOR CONCENTRATION IN GLIOBLASTOMA CELLS. Brittany Hylander, Mary-Alison Lane & Deborah O'Dell, Dept. of Bio. Sci. Univ. Mary Washington, Fredericksburg, VA 22401 We studied the relationship between S100B protein and the RAGE receptor. It is known that at nanomolar concentrations, S100B produces neurotrophic effects on surrounding cells while micromolar levels lead S100B to induce neurotoxic effects. We hypothesized that if cells were exposed to a constant high level of S100B, this would lead to a change in RAGE expression. To test this hypothesis we examined the levels of RAGE protein in cultured glioblastoma cells that were exposed to high and low concentrations of S100B. After being exposed, some cells were homogenized while others were fixed. Homogenized cells were used to determine the concentration of RAGE receptors in the cells through an ELISA test using an antibody agonist of RAGE. The cells that were fixed were mounted onto slides and photographed to determine if there was an increase in RAGE receptors due to S100B concentration increase. The results of this experiment showed that levels of S100B do not seem to affect the amount of RAGE receptor, at least as measured by immunochemistry in glioblastoma cells. This work was supported by an Undergraduate Research Grant to BH and MAL from the University of Mary Washington.

EFFECTS OF ANTHOCYANINS ON ALCOHOL-INDUCED HEPATIC DAMAGE IN FETAL MICE. Chris D. Miller, Justin D. Paul & Roman J. Miller, Dept of Biol., Eastern Mennonite Univ., Harrisonburg VA 22802. Fetal Alcohol Syndrome (FAS) occurs when a mother chronically consumes alcohol during pregnancy. While the detrimental effects of FAS are well known, antioxidants, may have the potential to negate some alcohol-induced damage. To investigate this potential, three groups of mice were utilized: control group (CO) injected with saline, binge alcohol group (BA) injected with alcohol, and alcohol/anthocyanin group (AA) injected with both alcohol and anthocyanins. At 14 days gestation, fetuses were removed from pregnant mothers to determine gross level body weight, crown-rump length (CRL), total fetal area, and liver area parameters. For gross level parameters, BA fetuses were significantly smaller than CO and AA fetuses, based on ANOVA testing ( $p < 0.05$ ). In contrast CO versus AA fetuses did not significantly differ. When liver area was compared to total body area for each group, all of the groups were significantly



different from each other. On a cellular level based on microscopic observation and stereological determinations (liver nuclei, cytoplasm, and vessel/sinusoid volume density parameters), no statistically significant differences were found between the three groups. When liver function was biochemically assessed for total protein production and acid phosphatase activity, no statistically significant differences emerged between the groups. In summary, the data indicate that alcohol damage has an impact on a gross level, but not on liver cellular/biochemical level parameters. The addition of anthocyanins indicated that antioxidants negate some alcohol damage that was seen on gross level parameters. (Research sponsored in part by EMU's Daniel B. Suter Endowment in Biology.)

STUDYING REGULATION OF HUMAN GENES AFAP1-AS1, RIC8A, S100A13. I. A. Krivosheeva<sup>1</sup>, J. V. Vyakhireva<sup>1</sup>, A. V. Baranova<sup>1,2</sup>, M. Yu Skoblov<sup>1,3</sup>, <sup>1</sup>Centre for Medical Genetics, Federal Agency for Scientific Organizations, Moscow, 115478, Russian Federation, <sup>2</sup>School of Systems Biology, George Mason University, Manassas, VA, 20110, USA and <sup>3</sup>Moscow Institute of Physics and Technology, Moscow, 117303, Russian Federation. Regulation of gene expression is a necessary process for homeostasis of cells and whole organisms. It can be implemented by various mechanisms; e.g. sense-antisense interaction or modification of mRNA half-life time with different pathways such as miRNA-mediated or STAU-mediated decay. Here we study 3 genes which have natural antisense transcripts. RIC8A encodes guanidine-exchanging factor while S100A13 plays an important role in cell cycle regulation. AFAP1-AS1 is a NAT for the AFAP1 gene, which encodes proteins of unknown function that are associated with actin filaments of the cell. As a model to study regulation, we chose cell line HEK293N for all genes and K562 as a cell line with high level of AFAP1-AS1. We designed siRNA to these genes and made a knockdown. Knockdown of RIC8A decreased the level of its antisense partner, SIRT3, approximately 25 times relative to control. Knockdown of S100A13 didn't show any significant change in the level of CHTOP (NAT of S100A13). AFAP1-AS1 knockdown in K562 cells showed significant decrease of both partners. For overexpression of AFAP1-AS1 we created a vector based on pEYFP. It included regions of AFAP1-AS1 second exon with 2 overlapping regions with AFAP1. This construction was transfected to HEK293N. We showed significant overexpression, but no change in AFAP1 level. We also suggested that some mRNAs can interact with AFAP1-AS1 through Alu-repeat. This interaction may lead to degradation of both molecules by Stau-mediated decay (SMD). These partners must have an Alu-repeat in 3'-UTR, express in HEK293N and K562, and have an

alignment length of 130 bp. qPCR after AFAP1-AS1 knockdown showed significant increased level of 2 partners – PPIA and PAICS.

GENETICALLY ENGINEERING A PLASMID EXPRESSION VECTOR FOR NUCLEAR LOCALIZATION STUDIES, PART 1: ENGINEERING A 3GFP-3NLS PLASMID. Kristina Krumpos & Stephen Gallik, Dept. of Biol. Sci., University of Mary Washington, Fredericksburg, VA 22401. The long-term goal of this research project is to create a plasmid expression vector that can be used by future students to study the nuclear localization of proteins and the nuclear localization signal (NLS). Due to its relatively high molecular weight and its natural fluorescence, a fusion protein consisting of 3 copies of green fluorescent protein (GFP) linked to a single NLS is an ideal reporter protein for such studies. The specific objective of this study is to modify a commercially-available plasmid (pCMV/myc/nuc/GFP, ThermoFisher) that contains 1 copy of a GFP open reading frame (ORF) linked to 3 tandemly-arranged copies of an NLS, to create a new plasmid containing 3 copies of a GFP ORF linked to the 3 copies of the NLSs (3GFP\_3NLS). Once created, future studies would remove 2 of the NLSs to create the desired final 3GFP\_1NLS plasmid. To create the 3GFP\_3NLS plasmid, a 48 bp linker (spacer) was first inserted upstream of the parent GFP ORF. A second copy of the GFP ORF was then added upstream of the linker, creating an intermediate 2GFP\_3NLS plasmid. A 24 bp linker (spacer) was then added upstream of the second GFP ORF, followed by a third copy the GFP ORF, added upstream of the 24 bp linker, thus creating the 3GFP\_3NLS plasmid. Single pass DNA sequencing (ACGT, Inc) confirmed the successful insertions of the linkers and the GFP ORFs. The protein expressed by the 3GFP\_3NLS plasmid is expected to accumulate in the nucleus via the classic nuclear import process, to be confirmed in transfected HEK cells with fluorescence microscopy.

MOLECULAR MODELS OF AGING: COMPARATIVE ANALYSIS OF GENE SIGNATURES IN REPLICATIVE SENESENCE AND STRESS INDUCED PREMATURE SENESENCE. Kamil C. Kural & Ancha V. Baranova, School of Systems Biology, George Mason University, Manassas VA 20110. Senescence is defined as terminal phase of human cell populations. It can happen naturally termed as replicative senescence or form as a consequence of external challenges such as oxidative stress, radiation, activated oncoproteins and others termed as stress induced senescence. It is widely believed that the mechanisms at work for entering senescence provides a protection against cancer formation and suppresses tumor formation. Therefore, further identification is necessary to solve the mysteries behind

senescence. The goal is to find the differentially expressed genes in replicative and stress induced senescence in human fibroblast cells using geneXplain bioinformatics software platform. By doing so, we are hoping to highlight the important mechanisms underlying the causes between these two phenomena and identify the elements responsible for the process. Our approach was to classify the differentially expressed genes by their functions to predict the involvement of these genes in various cell cycle processes and different pathways involved in senescence. The list of genes which are exclusively up and down regulated in stress induced senescence and replicative senescence are compared at first step. These unique genes between two types of senescence are then used to perform promoter analysis to get potential transcription factor (TF) binding sites in combination with upstream analysis to predict master regulators that control the activity of these Transcription factors.

THE EFFECT OF TEMPERATURE SENSATION ON ACUTE TOLERANCE TO ALCOHOL IN *C. ELEGANS*. Maroua Lahrach, Laura Mathies & Jill Bettinger, Dept. of Pharmacology & Toxicology, Virginia Commonwealth University, Richmond, VA 23298. Alcohol is a widely used drug, and 17 million Americans have an alcohol use disorder, a pathological pattern of alcohol use. The acute mechanisms that contribute to alcohol's effects on human behavior are uncertain. We use *Caenorhabditis elegans* to understand the effects of alcohol as well as the development of acute functional tolerance (AFT) because *C. elegans* and mammals experience similar effects of alcohol at equivalent tissue concentrations, suggesting that there are similar drug targets in these animals. We tested how temperature sensitivity affects the worm's initial sensitivity and AFT levels. We observed that wild-type animals demonstrate different levels of AFT if they are reared at different cultivation temperatures and tested at 20°C. At 15 °C, animals show no significant AFT, while at 20°C, high AFT levels are exhibited. We hypothesized that animals reared at 15°C may have experienced heat shock while being tested at 20°C, and this disrupted AFT. To test this, we examined temperature response of hsf-1 animals. hsf-1 is a heat shock transcription factor that is used as one method of temperature sensation, and hsf-1 mutant animals are defective in temperature sensation. Results demonstrate that both initial sensitivity and AFT is temperature dependent, the wild types that were observed at both 20°C and 15°C differed in their adaptability to alcohol. Animal's reared at 15°C were unable to develop AFT compared to those reared at 20°C. Although we cannot conclude that temperature sensitivity contributes to different AFT levels, we can predict that alcohol may

have an effect on many lurking variables including temperature change. More tests should be conducted to gain a better understanding on the significance gene *hsf-1* has on the development of AFT; Absence of this gene might lead to improvement in AFT levels and also providing evidence that sensation of temperature is one of many factors that affects AFT.

MOTIF ENRICHMENT ANALYSIS FOR DIFFERENTIALLY EXPRESSED GENES IN ALZHEIMER'S DISEASE IDENTIFIES SREBF2 AS POSSIBLE TRANSCRIPTIONAL REGULATOR. John T. Lawson, Rachel C. Bordelon, Bria E. Johnston & Gary D. Isaacs, Department of Biology and Chemistry, Liberty University, Lynchburg VA 24515. Alzheimer's disease (AD) is a complex neurodegenerative disorder that affects over five million Americans every year. A previous study generated a list of genes found to increase or decrease in expression in hippocampus from an AD mouse model compared to normal. It is possible that differential expression in AD could be due to altered functionality or availability of important transcription factors (TFs). To identify TFs that may regulate the genes differentially expressed in AD, proximal promoters (-1900 to +100 relative to the transcription start site of the most 5' promoter of each gene) were analyzed using the Analysis of Motif Enrichment program, which is part of the MEME Suite toolset, for relative enrichment of certain TF motifs compared to the promoters of random, unchanging hippocampal genes. Seven statistically significant ( $\alpha = 0.05$ ) TF motifs were found to be enriched in the upregulated gene list, including the motif for SREBF2, which was associated with 148 of the upregulated genes. Two of these upregulated genes, *Gfap* and *Trem2*, were selected for further study because both show significant change in expression in AD compared to normal, have been linked in literature with AD pathology, and have never before been shown to be regulated by SREBF2. Electrophoretic mobility shift assays (EMSAs) can be used to validate these putative DNA-TF binding interactions *in vitro*, potentially helping to elucidate mechanisms behind some of the differential expression in AD.

THE EFFECTS OF THE TREATMENT OF VARIOUS LIPID SPECIES ON MITOCHONDRIAL AND CELLULAR FUNCTIONS OF HEPATOCYTES. Peter M. Masschelin<sup>1,3</sup>, Katie Gwilliam<sup>2,3</sup>, Aybike Birerdinc<sup>1,3</sup>, Rohini Mehta<sup>3</sup> & Ancha Baranova<sup>1,3</sup>, <sup>1</sup>School of Systems Biology, George Mason University,<sup>2</sup> College of Science, George Mason University, <sup>3</sup>Center for Integrated Research, Inova Health Systems, Falls Church VA. Non-Alcoholic Fatty Liver Disease (NAFLD) is a chronic, progressive liver disease characterized by fatty infiltration of the hepatocytes and inflammation. Elucidation of the molecular mechanisms behind this disease can help our understanding

of how the disease progresses and potential treatments. Treatment of hepatocellular carcinoma cells, HepG2, with different lipid species serves as a model system to understand molecular pathways involved in lipid accumulation in hepatocytes. Mitochondria plays central role in energy homeostasis. Thus, mitochondrial dysfunction will be measured at several levels along with assessment of intracellular lipid accumulation, cellular apoptosis and proliferation, epigenetic modifications and change in mitochondrial mass. The goal is to understand mitochondrial dynamics upon exposure to a lipid and glucose rich environment to help determine the cause of NAFLD.

THE INVOLVEMENT OF A *DROSOPHILA* MITOCHONDRIAL FISSION GENE IN OXIDATIVE STRESS RESISTANCE. Grayson Mast, Charise Garber, and Jeff Copeland, Department of Biology, Eastern Mennonite University, Harrisonburg, VA. While oxidative damage is known to play an important role in the aging process, the molecular mechanisms are still poorly known. To better understand the cellular response to oxidative stress, we screened the X chromosome in *Drosophila melanogaster* to find mutants resistant to elevated oxygen levels. A loss-of-function mutant in the mitochondrial fission gene *CG7772* showed increased resistance to hyperoxia, but not to paraquat, another reactive oxygen species generator, nor starvation. *CG7772* mutants also had a normal life span. Since mitochondrial fusion and fission is essential for spermatogenesis, and *CG7772* is highly expressed in *Drosophila* testes, *CG7772* mutants showed defects in male fertility.

CHARACTERIZATION BY DELETION OF GENES RELATED TO CARBON METABOLISM IN CRYPTOCOCCUS NEOFORMANS. Kirk Nickish<sup>1</sup>, Joshua Herts<sup>1</sup>, John R. Perfect<sup>2</sup>, & Michael S. Price<sup>1,2</sup>, <sup>1</sup>Dept. of Biology and Chemistry, Liberty University and <sup>2</sup>Dept. of Medicine, Duke University Medical Center. The mechanism by which *C. neoformans* recovers glucose metabolism is currently unknown. The goal of the presented project is to identify genes that show increased activity in the rescue mutant compared to the *pyk1* mutant. Three genes in *C. neoformans* putatively related to recovery of glucose metabolism in *pyk1* mutants will be knocked out and characterized. The characterization process will include testing for various auxotrophies and ability to grow on different carbon sources. Several markers of virulence in *C. neoformans*, such as melanin production, will be evaluated in the new strain. Two of the knockout constructs have been produced successfully. Both biolistics and electroporation have been used to insert the chimeric DNA into the cells. Negative controls have exhibited growth after treatment by electroporation, although neither

the positive nor experimental samples have produced viable colonies. This failure indicates that the procedure reported by Lin X. et al. may be incomplete, as the cells have been shown to be viable after treatment and the pJAF1 plasmid includes the selectable marker.

EXAMINING THE EFFECTS OF ALDOSTERONE ON THE EXPRESSION OF THE LNCRNA GAS5 IN IMCD3 AND MPKCCD CELLS. B. Nolan<sup>1</sup>, M. L. Gumz<sup>2</sup> & D. L. Zies<sup>1</sup>, <sup>1</sup>Department of Biological Sciences, University of Mary Washington and <sup>2</sup>Department of Medicine, Division of Nephrology, Hypertension & Renal Transplantation, University of Florida College of Medicine. Hypertension is the number one risk factor for premature death worldwide, and yet many of the cases have no obvious underlying cause. Understanding the normal regulation of blood pressure may help determine these underlying causes and reduce hypertension rates and deaths. Aldosterone is a steroid hormone that binds to the mineralocorticoid receptor (MR) and acts as a primary blood pressure regulator. This research will explore the relationship between the long non-coding RNA growth arrested specific 5 (Gas5) gene and aldosterone. It was recently shown that Gas5 acts as a decoy for the glucocorticoid receptor and reduces its ability to activate target gene transcription in the presence of its steroid hormone, cortisol. Additional data suggests that Gas5 may do the same for aldosterone and MR. Preliminary data from our collaborator, Dr. Michelle Gumz, shows that aldosterone treatment of kidney cell lines increases the expression of Gas5 at 4 hours after treatment, but not at 24 hours. We hypothesize that aldosterone first acts to increase the expression of Gas5, but as Gas5 accumulates it will act as a decoy for aldosterone bound MR and thereby reduce its own expression. To test this hypothesis, mouse inner medullary collecting duct (IMCD3) cells and minipig kidney cortical collecting duct (mpkCCD) cells were grown in conditions that mimic their physiological state. The cells were treated with Aldosterone for 0, 4, 8, and 24 hours. After treatment total RNA was isolated and converted into cDNA. Quantitative real-time PCR (qRT-PCR) was performed to measure the expression of Gas5, Sgk1, and actin. Our initial results suggest that aldosterone does not regulate Gas5 in IMCD3 cells at any time point but does have an effect on Gas5 expression in mpkCCD cells. Current work is aimed at replicating experiments to generate data necessary to determine statistical significance. Progress on these experiments will be reported. Further study will be necessary to understand the relevance of differences in gene regulation between these two kidney cell lines.

INTIMIN IS LIKELY USED TO CAUSE DISEASE DURING COMPETITION WITH COMMENSAL *ESCHERICHIA COLI*. Dominique J. Richburg, Dept. of Biol. and Chem., Liberty University, Lynchburg VA 24515. The intimin gene in the Locus of Enterocyte Effacement (LEE) pathogenicity island is the primary attachment mechanism in *Citrobacter rodentium*. Intimin is a bacterial adhesin that attaches to the epithelial wall of the intestine. An intimin deletion mutant bacteria is used to study colonization and pathogenesis in the mouse gastrointestinal tract. Additionally, *Citrobacter rodentium* is an attaching/effacing pathogen, and a useful murine model in understanding Enterohemorrhagic *Escherichia coli* (EHEC) infection in humans. *E. coli* and *C. rodentium* cause gastroenteritis in humans and mice, respectively. *C. rodentium* is a murine pathogen commonly used to model gastrointestinal disease. Results have shown intimin and *C. rodentium* useful in causing disease during competition with commensal *E. coli*. By studying the mechanisms and genes involved in pathogenic adhesion in *C. rodentium*, it will be easier to find out a cure or treatment for illness cause by the before mentioned *E. coli* strains such as Crohn's disease, ulcerative colitis and colonic tumorigenesis. (supported by: The Virginia Academy of Science).

THE ROLE OF ATRX DURING DEVELOPMENT IN *XENOPUS LAEVIS*. N.D. Taliaferro, S.E. Wahl & A.J.G. Dickinson, Dept. of Biology, Virginia Commonwealth University, Richmond VA 23284. Alpha thalassemia syndrome X-linked (*ATR-X*) is a rare recessive disease affecting multiple organ systems of the body. Symptoms include developmental delay, gastrointestinal defects, and alpha thalassemia. Facial deformities are common and can include microcephaly, a protruding tongue with drool, and improper positioning of the teeth of the upper jaw in relation to those of the lower jaw. This disease is caused by a defect of the ATRX gene, which lies on the X-chromosome. ATRX is a nuclear protein that localizes to nuclear compartments called PML bodies and pericentromeric heterochromatin, where it interacts with a component of heterochromatin, HP1. It is suggested, ATRX helps regulate the activity of other genes through a process known as chromatin remodeling, as well as regulate HB1 and HB2, which are necessary for hemoglobin production. To determine the role of ATRX in orofacial development, we examined expression of ATRX during development in the African clawed frog (*Xenopus laevis*). PCR, using primers we designed, was used to characterize ATRX mRNA. A commercially available ATRX antibody was used to investigate protein expression during development. Results demonstrate that ATRX is expressed at the mRNA level during

development, ATRX expression changes as development progresses, and ATRX protein expression increases during development. Additional time points and samples are needed to further characterize the expression of ATRX at the mRNA and protein levels.

IDENTIFYING PHENOTYPES IN OVEREXPRESSION OF PUTATIVE KINASES IN CRYPTOCOCCUS NEOFORMANS. Nicolas Terreri & Joshua Sellwood, Dept. of Biology and Chemistry, Liberty University, 1971 University Blvd, Lynchburg VA, 24502. *Cryptococcus neoformans* persists in the central nervous system via the utilization of carbon sources mainly from sugars like glucose. In a prior study assessing the role of pyruvate kinase in the central nervous system persistence by *C. neoformans*, we observed the delayed appearance of *pyk1Δ* colonies on glucose-containing medium. When the *C. neoformans* grew on glucose containing media, another possible kinase which activates only when the *pyk1Δ* suppressor mutant was deleted, was brought into question as the possible substitute for the *pyk1* gene. The colonies appeared as one of three different morphotypes: filamentous, pseudohyphal, or yeast. Increased filamentation and haploid fruiting has been observed with overexpression of *STE12* in *C. neoformans*.<sup>2</sup> This observation leads us to believe that this gene coding for the kinase is located in the MAT locus of chromosome 5 in the *C. neoformans* Serotype A strain, H99. Due to the filamentous nature of many of the *pyk1Δ* suppressor mutants, We hypothesize that a partial genome duplication may be responsible for suppression of the *pyk1Δ* mutant glucose utilization phenotype, and that the likely area of duplication centers around the MAT locus on Chromosome 5. A possible genetic basis for the expression of a different kinase's growth phenotypes will be assessed using novel molecular approaches. Different putative genes, found using various microarray data, will be amplified and transformed into *Cryptococcus neoformans pyk1Δ* gene mutants. Overexpression of the putative rescue genes in *C. neoformans* may show the same phenotypes of the original *pyk1Δ* mutant phenotypes of delayed growth on glucose containing media and a complete loss of virulence in the host.

EARLY NEUTROPHIL RECRUITMENT BY CHEMOKINE-RELEASING NANOPARTICLES IMPROVES BACTERIAL CLEARANCE AND SURVIVAL OF ANTHRAX-CHALLENGED MICE. Allison L. Teunis<sup>1,2</sup>, Taissia G. Popova<sup>1</sup> & Serguei G. Popov<sup>2</sup>, <sup>1</sup>Center for Applied Proteomics and Molecular Medicine, Department of Molecular Biology, School of Systems Biology, George Mason University, <sup>2</sup>National Center for Biodefense and Infectious Diseases, School of Systems Biology, George Mason University. Nanomaterials capable of directing immune cell recruitment hold promise as novel tools for basic research and therapeutic applications. In this study,



hydrogel nanoparticles chemically coupled with Cibacron Blue affinity bait were used as carriers for the directed release of chemokines, human CXCL8 and mouse CCL3, to enhance migration of neutrophils and to improve outcome of anthrax infection in a mouse model. Mice were given a prophylactic dose of nanoparticles and challenged into footpads a few hours later with *Bacillus anthracis* Sterne 34F2 spores. Released chemokines induced a massive influx of neutrophils to the site of spore inoculation and regional lymph nodes resulting in reduced bacterial burden, decreased inflammatory response and up to 70% survival of mice over 13 days ( $p < 0.0001$ ). All untreated mice died within 4 days with a strong inflammation of footpads. Stimulation of neutrophil chemotaxis with nanoparticle-released chemokines may be considered as a novel strategy to treat anthrax. This work was supported by the grant 1R21AI117425-01 from the National Institutes of Health, USA.

GENETICALLY ENGINEERING A PLASMID EXPRESSION VECTOR FOR NUCLEAR LOCALIZATION STUDIES, PART 2: ENGINEERING A 2 GFP-0NLS AND A 3GFP-0NLS PLASMID. Rachel Thomas & Stephen Gallik, Dept. of Biol. Sci., University of Mary Washington, Fredericksburg, VA 22401. The long-term goal of this research project is to create a plasmid expression vector that can be used by future students to study the nuclear localization of proteins and the nuclear localization signal (NLS). Due to its relatively high molecular weight and its natural fluorescence, a fusion protein consisting of 3 copies of green fluorescent protein (GFP) linked to a single NLS is an ideal reporter protein for such studies. In part 1 of this project, plasmids containing 2 and 3 copies of a GFP open reading frame (ORF) linked to 3 tandemly-arranged copies of an NLS (2GFP\_3NLS and 3GFP\_3NLS, respectively) were created from a commercial plasmid. The specific objective of part 2 of this project is to remove the 3 NLSs from the 2GFP\_3NLS and 3GFP\_3NLS plasmids, to create plasmids containing 2 GFP ORFs and 3 GFP ORFs, respectively, but lacking the NLSs (2GFP\_0NLS and 3GFP\_0NLS). Once created, a future study would insert a single NLS to create a 2GFP\_1NLS plasmid and the desired final 3GFP\_1NLS plasmid. To create the 2GFP\_0NLS and 3GFP\_0NLS plasmids, a *NotI* cut site was, in each case, inserted downstream of the 3 NLSs, sandwiching the NLSs between the new *NotI* cut site and an original *NotI* cut site located just upstream of the 3 NLSs. The 3 NLSs were then removed with *NotI* restriction digestion followed by ligation. DNA sequencing confirmed the successful creation of the 2GFP\_0NLS, but not the 3GFP\_0NLS. Slow diffusion of the fusion protein expressed by the 2GFP\_0NLS plasmid in transfected HEK cells will be demonstrated by fluorescence microscopy.

### Biomedical and General Engineering

IMPROVED PREDICTIVE CAPABILITY OF A COMPUTATIONAL FOOT/ANKLE MODEL USING ARTIFICIAL NEURAL NETWORKS. Ruchi D. Chande & Jennifer S. Wayne, Department of Biomedical Engineering and Department of Orthopaedic Surgery, Virginia Commonwealth University. Computational models are a valuable means of investigating the biomechanics of human joints. These models' non-invasive, cost-effective nature allows for the simulation of multiple experimental iterations with relative efficiency. Given their usefulness, it is imperative to provide these models with appropriate inputs to obtain meaningful predictions. To adequately represent the joint within a computational space, cadaveric studies are referenced to inform soft tissue properties; however, some tissues are not well-described in the literature. As a result, this work sought to apply artificial neural networks (ANNs) to optimize soft tissue properties, specifically ligament stiffness, for the greater purpose of improving the predictive ability of an existing patient-specific computational foot/ankle model of Adult Acquired Flatfoot Deformity (AAFD). Both feedforward (FFN) and radial basis function (RBFN) networks were trained with known kinematic-stiffness data prior to providing final ligament stiffness predictions. The predictions from each network were then supplied to the existing AAFD model and its resulting kinematic measures, specifically navicular and 1st cuneiform heights and talo-1st metatarsal and talo-navicular angles, were compared to the patient radiograph. While the FFN performed better than the RBFN, both networks' predictions resulted in an improvement of the computational model's performance as three of the four measures of interest moved closer to those kinematic values measured on the patient radiograph.

HYPER-ELASTIC THIN-FILM-NITINOL CHARACTERIZATION FOR NEUROVASCULAR FLOW-DIVERTERS. Y. Chen<sup>1</sup>, C. Howe<sup>2</sup>, Y. Lee<sup>2</sup>, S. Cheon<sup>3</sup>, W.-H. Yeo<sup>2</sup>, Y. Chun<sup>1</sup>, <sup>1</sup>Department of Industrial Engineering, University of Pittsburgh, Pittsburgh, PA 15261 and <sup>2</sup>Department of Mechanical and Nuclear Engineering, Virginia Commonwealth University, VA 23284 and <sup>3</sup>Division of Mechanical & Automotive Engineering, Kongju National University, Republic of Korea. A cerebral aneurysm can occur when a small section of a neurovascular blood vessel is weakened, which allows blood to flow into ballooned section, or sac. Recent advancement in aneurysm treatment includes a new device called a 'flow-diverter'. A flow-diverter can safely reduce blood flow into the aneurysm sac. In previous study, it has been found that a flow-diverter based on thin-film-nitinol (TFN) works very effectively, however there have been no studies on the mechanical safety of the TFN flow-diverter in irregular,

curved blood vessels. Here, we study the mechanical behaviors and structural safety of a micro-structured TFN membrane. Through computational and experimental study, we establish the fundamental characteristics of the stretching and bending mechanics of the structure. The results show a good agreement between computational and experimental behavior of the TFN with negligible strain change in up to 180° in bending and 500% in radial stretching. These results show ideal characteristics for the use in highly curved neurovascular blood vessels. *In vitro* experimental test qualitatively demonstrates the mechanical flexibility of the flow-diverter with multi-modal bending. *In vivo* micro X-ray and histopathology study demonstrate that the TFN can have conformal deployment in the curved blood vessel of a swine model without any significant complications or abnormalities.

TRANSDERMAL MICRONEEDLE PATCH DRUG DELIVERY. Dong Sup Lee & Hong Yeo, Dept. of Mechanical & Nuclear Engineering., Virginia Commonwealth Univ., Richmond VA 23220. Transdermal drug delivery (TDD) is known for enhanced delivery effectiveness of drug compared to the oral intake, which has limitation of diminished drug concentration in the body. TDD system with penetration enhancers allow efficient transport of drug through the skin(stratum corneum) into the blood vessels. However, pain is involved with the use of penetration enhancers. Therefore, major concern was to minimize the pain involved while piercing through the skin. Recently developed methods using silicon microneedles introduced effective tissue penetration with minimal pain. Different microfabrication techniques has developed to manufacture various shapes of microneedle to optimize its design; a hollow-type needle with pen and pyramidal/concave shapes are preferable to other designs because of its capability of controlling drug amount and rate. Silicon material, however, has potential toxicity in the clinical use due to its fragility and harmful effects once it gets into the bloodstream. To resolve detrimental effects on human body, we developed a soft, conformal, skin patch with polymer microneedles in an array fashion that is biocompatible and has potential to deliver drug with pain-less injection. Microneedle will be incorporated with a thin elastomeric membrane to ensure conformal contact to the skin for continuous long-term drug delivery application. Our microfabrication method involves molding technique, which can recycle mold for multiple production of microneedle at a relatively low cost. Ultimately, the microneedle will be integrated with biological sensors and microchips in order to diagnose and release drug with automated system.

WIRELESS, STRETCHABLE INTRAORAL ELECTRONIC SYSTEM FOR PH MONITORING. Yongkuk Lee & Woon-Hong Yeo, Dept. of Mechanical & Nuclear Engineering, Virginia Commonwealth

University, Richmond, VA 23284. The development of thin, soft, stretchable wireless electronics is highly desired due to their potentials in healthcare applications. Even though many types of diminutive wireless and wearable devices have been developed, most of them still use rigid and planar platforms that limit the direct integration with soft tissues and often cause discomfort in use. Here, we introduce a wireless, stretchable intraoral electronic system for pH monitoring, which has the capability of stable, long-range Bluetooth telemetry within a low-profile, soft circuit platform. The key concept involves assemblies of hard chip-scale components and stretchable meander interconnects, together in a thin, elastomeric enclosure, to provide pH sensing via Bluetooth wireless telemetry. Experimental and computational studies establish the fundamental aspects of the bending and stretching mechanics of the intraoral electronic system. In order to enable the Bluetooth communication, antenna design is carefully optimized to maximize the range of Bluetooth signals. The system maintains Bluetooth signals even in deformation along curved surfaces. The whole system of the intraoral electronics is encapsulated with a silicone elastomer which is known to be tissue-compatible and nontoxic. For detecting pH levels of oral fluid intake, the system is laminated on a dental retainer that has curved, contoured surfaces. The functionality of the wireless intraoral electronics is demonstrated with various pH calibration solutions via wireless data recording.

DEVELOPMENT AND CHARACTERIZATION OF PROTEIN NANOPARTICLES DERIVED FROM PORCINE LUNG EXTRACELLULAR MATRIX. P. A. Link, R. A. Pouliot, M. Valentine, & R. L. Heise, Department of Biomedical Engineering, Virginia Commonwealth University, Richmond, VA 23284-2006. The objective of this work was to develop and characterize a naturally derived drug delivery vehicle for pulmonary applications. Using chemical detergents, we decellularized en bloc porcine lung tissue. We then lyophilized and cryomilled remaining scaffold into powder. Acetic Acid proved to be a good solvent to retain the digested powder in solution. The resulting solution maintained a charge and was then electrosprayed onto aluminum foil. Sterile particulate was formed through washing the particles with ethanol. The particles were suspended in sterile water and lyophilized again. Using dynamic light scattering the average diameter measured 334 nm. The zeta potential was -11.9 mV. Preliminary data shows increased cellular proliferation a in lung epithelial cell line, A549, when using 2 mg of nanoparticles per ml of media. We also found that the formed particles do not activate macrophages. Future work will examine stabilizing the nanoparticles for commercial drug delivery. This work was funded by NSF CMMI 1351162.

IN VITRO VALIDATION OF A COMPUTATIONAL MODEL OF FIBRONECTIN ASSEMBLY. Devin B Mair, Thomas Petet, Lewis E. Scott, Seth H. Weinberg & Christopher A. Lemmon, Department of Biomedical Engineering, Virginia Commonwealth University, Richmond, VA. **Introduction:** The extracellular matrix is an assembly of proteins that surround cells and serves as the cell substrate *in vivo*. A primary component of newly synthesized ECM is fibronectin (FN), which is critical for embryonic development and wound healing. Despite years of research, the mechanism of FN assembly is still not completely understood. We hypothesize that FN assembly occurs through the revelation of buried FN-FN binding sites within *any* of the 15 Type III FN domains, and that these binding sites are exposed in a stretch-dependent manner, facilitating FN molecule binding via a beta-strand addition mechanism. **Methods:** *In-vitro*: Cells were plated on coverslips and allowed to assemble fibrils for a set amount of time. Custom MATLAB code was developed to analyze the properties of the individual fibrils assembled. *In-silico*: We developed a computational model of cell-FN-substrate biomechanical-chemical interactions to test our FN assembly hypothesis. In the model, FN-III domains are represented by Hookean springs with distinct stiffnesses, such that a FN dimer is represented by 30 springs in series. Integrin binding/unbinding is represented by a stochastic first-order reversible chemical, with force-dependent off-rate. FN-FN binding is represented by a stochastic irreversible reaction, in which binding site exposure depends on FN-III domain stretch. Model results are validated using the *in vitro* results. **Results:** Experiments and simulations both illustrate that FN fibril stretched and relaxed length and thickness approach steady state values after approximately 24 hours. Simulations predict that the ratio of stretched-to-relaxed length is maximally four, consistent with experimental results. *In-silico* predictions of substrate force reach steady state levels after approximately 24 hours. The fraction of attached integrin fibril-cell surface connections is roughly constant throughout simulations. **Conclusion:** Experimental FN fibril and the computational model predictions all illustrate three unique features: 1) FN fibrils are highly elastic, but have a maximal 4-fold elongation of their resting length; 2) FN fibril length and force are not strongly correlated. 3) FN fibrils exhibit discrete stable lengths, suggesting local minima of force balance within growing fibrils. This fibril stability has not previously been identified.

EXPLORING THE APPLICATION OF BIO-MIMICRY FOR SUSTAINABLE DESIGN. Katie S. McCullar<sup>1</sup>, Preston C. Rhodes<sup>2</sup>, Austin Underhill<sup>2</sup> & J. Nagel<sup>2</sup>, <sup>1</sup>JMU Biology, <sup>3</sup>JMU Integrated Science and Technology, <sup>2</sup>JMU Engineering, Harrisonburg VA, 22801. Bio-inspired design, or biomimicry, is an approach to innovation that takes nature's time tested patterns, forms, functions, processes, and materials

and uses them to develop engineering solutions. As design challenges increase in complexity, the resulting solutions are also increasing in complexity. This translates to an exponential increase in system components, data, and the time for verification and validation. Biological systems are equally complex, however, they have evolved into elegant systems that have multi-use components, perform “up front” processing to reduce data streams, and are optimized for particular environments. Adopting inspiring features, characteristics, or strategies of biological systems can significantly impact additive manufacturing as well as improve their adaptability. We propose to investigate biological system complexity and integration at multiple levels of abstraction and then translate the insight gained to address manufacturing system sustainability. Currently many products contain multiple materials to provide different functions within the product. These multi-material products are often difficult if not impossible for consumers to recycle without immense amounts of added work. Using additive manufacturing to implement complex, bio inspired designs allows the product to provide multiple functions using a single material that can be easily recycled. Key contributions of this research include approaches for additive manufacturing strategies such as material utilization that align with a product’s life cycle, thus increasing the recyclability of the product.

**REAL-TIME CLASSIFICATION OF ELECTROOCULOGRAPHY SIGNALS.** Saswat Mishra & W-H Yeo, Dept. of Mechanical and Nuclear Engineering, Virginia Commonwealth University, Richmond VA, 23284-2006. Electrooculography(EOG) signals are used in important applications such as, driver fatigue recognition, activity recognition, and interfacing with disabled users. The impact of these signals is powerful in assisting disabled users. The classification algorithm presented is able to detect and classify an EOG signal with more than 90% accuracy. A graphical user interface is demonstrated with a real-time classification algorithm. The classification methodology incorporates centering the signals using a peak detection method which is then transferred over to the machine learning algorithm. The machine learning algorithm identifies the motion of the signal from a database of stored signals. The database is created by the user of the program and added on to an array of previously stored values, referred to as the trained dataset. The trained dataset consists of five features, Wavelet Energy, Definite Integral, Amplitude, Amplitude Velocity, and Average Signal. The testing dataset consists of the same five features which are assessed real-time. The compilation of the peak detection algorithm, machine learning interface, and the features allows this classification program to be highly accurate.

USING ORGANOPHOSPHATE HYDROLASE AND POLYANILINE FABRIC TO DETECT AND DEGRADE NERVE AGENTS. E. Newsome, C. Tang, and S.S. Fong, Dept. of Chemical & Life Science Engineering, Virginia Commonwealth University, Richmond, Virginia 23284. Chemical warfare is a reality that puts civilian and military personnel at serious risk. The goal of this project is to develop a functionalized fabric that can detect and neutralize nerve agents. The design will utilize an organophosphate hydrolase (OPH) enzyme that has been designed with a molecular tag for cloning and isolation. The OPH will be attached to polyaniline and in the presence of water, select nerve agents (e.g. sarin, tabun) can be neutralized resulting in a localized change in pH that can be detected colorimetrically. Progress to date includes design of a tagged OPH and isolation of the tagged protein. Ongoing work is focusing on immobilization on polyaniline fibers and functional characterization of the immobilized enzyme using proxy chemicals (non-toxic analogues to target nerve agents).

AN ACTIVE IMMOBILIZATION SYSTEM FOR HEAD AND NECK EXTERNAL BEAM RADIOTHERAPY USING A 6D TABLE AND RADIOFREQUENCY LOCALIZATION. M. Ostyn<sup>1,2</sup>, S. Kim<sup>1</sup>, W. H. Yeo<sup>2</sup>, T. Dwyer<sup>2</sup>, R. Cruikshank<sup>2</sup>, M. Rosario<sup>2</sup>, & D. Martinez<sup>2</sup>, <sup>1</sup>Radiation Oncology, Medical Physics Graduate Program, School of Medicine, Virginia Commonwealth University, Richmond, VA 23298, <sup>2</sup>Department of Mechanical and Nuclear Engineering, School of Engineering, Virginia Commonwealth University, Richmond, VA 23284. One of the most widely used tools used for cancer treatment is external beam radiotherapy. Aside from acute exposure, the greatest risks involved in radiotherapy are incidental radiation dosage to healthy tissue and lack of adequate coverage to the target disease; patient motion and improper patient setup exacerbate both of these problems. Here we present an active immobilization system to reduce these risks in head and neck treatments. The system includes a mechanical device for actively correcting the position of a patient's head, and a radiofrequency localization system designed to track patient motion. A prototype of the mechanical device is presented. The localization system comprises of skin-wearable RF beacons and an external tracking system. We develop an analytical model to estimate the angulation accuracy of the proposed tracking system using Monte Carlo. The results indicate that such a system requires an angular resolution of about 0.04° to achieve the millimeter-level localization accuracy desired in the clinical environment. This study was funded by the Mark A. Sternheimer Award and School of Engineering, Virginia Commonwealth University.

A COMPUTATIONAL MODEL OF LUNG FIBROBLAST MIGRATION WITH IN VITRO VALIDATION. James A. Ratti<sup>1</sup>, Angela M. Reynolds<sup>2,3</sup> & Rebecca L. Heise<sup>1,3</sup>, <sup>1</sup>Pulmonary Mechanobiology Lab, Dept. of Biomedical Engineering and <sup>2</sup>Dept. of Mathematics & Applied Mathematics, VCU; <sup>3</sup>Johnson Center for Critical Care & Pulmonary Research, VCU Medical Center. Chronic Obstructive Pulmonary Disease (COPD) is currently the 3<sup>rd</sup> leading age-adjusted cause of death in the United States. The primary cause of this disease is known to be tobacco smoke, yet it is unclear which cellular pathways produce these symptoms. Fibroblasts are known for their roles in tissue inflammation and remodeling, and these functions have been found to be inhibited in COPD patients. To evaluate how lung fibroblast populations from COPD patients differ from healthy ones, we developed an agent-based model of lung fibroblasts during wound healing using the NetLogo platform. This model separates the healing response in terms of the migration, proliferation, death and senescence rates of these cells, and accounts for the effects of serum deprivation and cigarette smoke condensate exposure. Simulations were performed in NetLogo to select biologically suitable parameter sets for each cell type. Model results were validated using data gathered from *in vitro* experiments consisting of scratch-migration assays and MTT assays. This model is the first step in creating a computational tool that will allow us to explore the role of these fibroblast functions on the overall disease progression and evaluate responses to therapeutics for COPD.

IMMUNOSUPPRESSIVE MODALITIES OF TUMOR-STROMA INTERACTIONS IN A THREE-DIMENSIONAL IN VITRO CO-CULTURE BREAST TUMOR MODEL. Lewis E Scott, Dept. of Biomed. Eng., Virginia Commonwealth Univ., Richmond, VA 23220. Tumors repurpose extrinsic and intrinsic modalities of evading immunosurveillance, both of which inhibit aberrant immune responses. The latter is exacted through expression of cell surface receptors, such as programmed death ligand 1 (PD-L1), which have inhibitory effects to cytotoxic tumor immunity. Extrinsic control mechanisms are less understood, though regulatory T cells (Treg) appear to play a dominant role in suppression of helper and cytotoxic T cells within the tumor microenvironment. Due to the role tumor associated fibroblasts (TAF) have been shown to play in tumor progression, we propose that TAF induce a Treg subpopulation via transforming growth factor beta (TGF- $\beta$ ) to suppress anti-tumor immunity. In this model, PD-L1 expression prevents expansion of cytotoxic T cells (CD8+) whereas TAF induction of Treg inhibits helper and cytotoxic T cell expansion, thus presenting a bimodal immunosuppressive mechanism of immune evasion. To test this, we co-cultured PD-L1-expressing malignant mammary epithelial cells with activated, naïve T lymphocytes in Matrigel. Next, we



introduced adipose stem cells (bASC) to the co-culture and measure changes in the population of Treg. In the presence of malignant epithelial cells, bASC secrete inflammatory cytokines associated with T cell recruitment as well as Treg differentiation, notably is TGF- $\beta$ . In future work, we will inhibit PD-L1 and perform cell viability assays to determine the effectiveness of CD8<sup>+</sup> T cell-induced tumor apoptosis. Additional TGF- $\beta$  blocking is expected to down regulate immunosuppression, increase CD8<sup>+</sup> activity and consequently lead to tumor suppression.

CONTROL OF A PROSTHETIC HAND WITH SKIN-LIKE ELECTRONICS. Michael J. Teller & Woon Hong Yeo, Dept. of Mech. Engineering, Virginia Commonwealth Univ., Richmond VA. 23284. Recent advances in manufacturing expand various fabrication opportunities for creating complex features such as human organs and body parts. Here we utilize the 3D printing method that fabricates a realistic prosthetic hand based on the ABS plastic. Electromechanical components including a series of servos finalize the assembly of an electronic prosthetic hand. A portable wireless system with skin-mounted electrodes allows the high-quality recording of surface electromyograms (EMG), produced by the movement of the skeletal muscles. Data acquisition software including signal filtering and classification algorithms collects raw EMG data from the forearm and convert them to digital signals for a continuous control of the prosthetic hand. The development of the prototype device and control system enable EMG from wide ranging areas of the body. The measurements have quality sufficient for advanced forms of human-machine interfaces.

AUTOMATIC CALCULATION OF FEMORAL VERSION USING PROXIMAL FEMUR LANDMARKS. Nathan J Veilleux & Jennifer S Wayne, Department of Biomed. Eng., Virginia Commonwealth Univ., Richmond VA. 23220 The success of total hip replacement surgery depends on proper anteversion of the femoral stem. Femoral version is defined as the angle between the femoral neck and condyles in a horizontal plane. An excessively retroverted or anteverted femoral stem will lead to impingement and stem loosening, shortening the life of the hip implant. However, there is no accurate way for surgeons to measure femoral version, since the femoral condyles aren't visible during the surgery. This means that a new technique for estimating version that only uses the proximal femur (the region visible during surgery) needs to be developed. CT scan data for 80 entire femurs and 215 proximal femurs has been provided by the VCU Department of Radiology. Each CT scan has been converted into a 3D model in the program Mimics. The point cloud from each model has been extracted and imported into Matlab, where a program has been developed that is able to

automatically calculate femoral version using the entire femur. The program is currently being adapted to perform calculations of femoral version using only the proximal femur. The consistency of version calculations using only the proximal femur will be evaluated with respect to those made using the entire femur with the ultimate goal of creating a tool that can be used to assist orthopaedic surgeons in aligning the stem of a femur implant during total hip arthroplasty.

THE EFFECT OF MTBI ON READING PERFORMANCE BASED ON EYE MOVEMENT MEASUREMENT AND ANALYSIS. Zoe Villamar & Paul A. Wetzel, Department of Biomedical Engineering, Virginia Commonwealth University, Richmond, VA 23220. Mild traumatic brain injuries (mTBIs) can cause cognitive impairment and oculomotor dysfunction. The subjects of this study are military personnel who have sustained an mTBI from a blast or motor vehicle accident in a combat zone. While reading, a person mainly uses versional eye movements, particularly saccades. Saccades are voluntary jumps between phases of fixations. The reading task requires a certain level of cognitive ability compared to a simple tracking task and it also requires the use of the saccadic system. Using an eye movement tracking system, this study aims to objectively determine how eye movements are affected by mTBI when performing a reading task. Eye movements were measured using the EyeLink II. This headgear-supported eye tracking system collects data at 500 Hz. The data were analyzed for the number of saccades, saccade duration, saccade velocity, saccade acceleration, position accuracy, and fixation stability. The number of regressions and blinks were also assessed. It was found that those who had mTBI had difficulty with the reading task compared to the normal control group. The mTBI group had significantly more regressions while reading, demonstrating the cognitive impairment that the subjects sustained due to the mTBI. The mTBI group also showed an overall negative effect on the saccadic system compared to the control group. This work shows how eye movement tracking on a reading task can be used to determine the effects of mTBI on the oculomotor system as well as the effects on a cognitive task.

NOVEL SMALL AIRWAY MODEL USING ELECTROSPUN DECELLULARIZED LUNG EXTRACELLULAR MATRIX. B. M. Young, B. P. Allen, B. A. Blakeny, R. A. Pouliot, R. L. Heise, Department of Biomedical Engineering, Virginia Commonwealth University, VA 23284. This research included the development of an *in vitro* small airway model using electrospun decellularized pig lung for the study of smooth muscle cellular interactions with surrounding extracellular matrix (ECM). With currently no relevant or controllable *in vivo* or *in vitro* model to investigate diseases and normal interactions of small airway components, the development of a physiologically

relevant *in vitro* model with comparable cell attachment, signaling, and organization to natural tissue is necessary to develop new treatments for airway disease. The addition of DPLECM significantly changed the PLLA scaffold mechanically, biologically, and physically to bring it closer to the characteristics of the human lung. DPLECM scaffolds exhibited a significant decrease in the elastic modulus compared with PLLA alone. Histological staining and SDS-PAGE showed that after scaffold fabrication, essential proteins or protein fragments in natural ECM are still present after processing. Human bronchial smooth muscle cells (HBSMCs) seeded onto PLECM scaffolds formed multiple layers of cells compared to scaffolds composed solely of PLLA. Phenotype of smooth muscle is better maintained when DPLECM is incorporated into the scaffold shown by enhanced contractile protein expression and increased collagen production for normal smooth muscle remodeling of the scaffold. In summary, this research demonstrates that a PLLA/DPLECM composite electrospun mat is a promising tool to produce an *in vitro* model with the potential to uncover unknown characteristics of bronchiole smooth muscle behavior in diseased or normal states. This study was funded by NSF Career Award.

### Botany

ACTIVE LEARNING THROUGH COURSE-EMBEDDED RESEARCH IN PLANT BIOTECHNOLOGY. Michael H. Renfroe, Department of Biology, James Madison University, Harrisonburg VA 22807. An upper-level elective biology course was revised to use course-embedded research as an instructional approach. Goals were to develop student skills in searching primary literature, experimental design, development of protocols, medium and stock preparation, mastery of laboratory techniques, quantitative skills and technical writing. Additional goals in the metacognitive realm included students working independently, improving decision-making, and taking more responsibility. Students designed experiments, conducted research, made observations, and analyzed and presented the data, including a poster presentation at a research symposium. Assessment indicated that improvements were made in all goals. Student frustrations included a perceived lack of structure because they were not given protocols but had to develop them. However students gained confidence in developing protocols and working more independently. Assessment also indicated that students had mastery of course content without conventional lectures, but from “just-in-time” classroom teaching and reading primary literature. Courses with embedded research have a number of challenges including student initial discomfort, but the rewards for the students include a greater self-reliance and greater confidence of course content and methodology with an increased understanding of the process of science.

ECOLOGICAL INTERACTIONS IN THE FACE OF CLIMATE CHANGE: A HISTORICAL CASE EXAMPLE FROM THE FORESTS OF NORTH AMERICA (*MELAMPYRUM LINEARE*; OROBANCHACEAE). Maryam Sedaghatpour, Karoline A. Oldham & Andrea Weeks, Department of Biology, George Mason University, Fairfax VA 22030. *Melampyrum lineare* Desr. is a hemiparasitic annual flowering plant native to the northern latitudes of North America. It obtains water and nutrients by penetrating the roots of its host species, which include maple trees, pine trees, and members of Ericaceae. Due to this dependency, *M. lineare* is limited to the geographical range of its hosts. Molecular phylogeographic investigation was begun to uncover patterns of this species’ genetic diversity as a complement to our recent morphological revision of its four taxonomic varieties and to test the hypothesis that *M. lineare* found historical refuge in the southern Appalachian region. Molecular data were collected from 29 eastern US populations ranging from Georgia to Maine and 20 Canadian populations in Alberta and British Columbia. Four nuclear microsatellite markers (MsO66<sup>P</sup>, MsO70<sup>M</sup>, MsG2, and MsB58) showed allelic variation and four chloroplast gene regions showed inter- and

intra-population variability (*trnS-trnG*, *psbA-trnH*, *trnT-trnL*, and *rps16-trnQ*). Analysis of *trnS-trnG* indicated nine haplotypes are shared among 170 individuals in 49 populations. Two haplotypes are present in Canada, and all nine are present in southeastern US populations. Further analysis of microsatellite and chloroplast gene regions data will refine our ability to test patterns of *M. lineare*'s historical range expansion and to better describe its biological diversity.

RESPONSE OF ATLANTIC WHITE CEDAR RADIAL GROWTH TO VARYING HYDROLOGIC CONDITIONS IN THE GREAT DISMAL SWAMP. Julie M. Slater, Abigail H. Weaver & Robert B. Atkinson, Department of Organismal and Environmental Biology, Christopher Newport University, Newport News VA 23606. Atlantic white cedar (*Chamaecyparis thyoides* (L.) B.S.P.) swamps are globally threatened ecosystems that occur in freshwater peatlands in a narrow belt along the Atlantic coast of the United States. Dendrochronological analysis of dead and living Atlantic white cedar (AWC) has the potential to uncover information about historical hydrologic conditions in these swamps and to inform future management and restoration plans. Combining thesis data collected by students in our lab (Merry 2005, and Patterson 2011), this study explores use of basal area increment (BAI) calculations to learn about the age-related growth trend of AWC, the responses of AWC to historic hydrologic changes, and differing growing conditions at Alligator River National Wildlife Refuge and Great Dismal Swamp National Wildlife Refuge (GDSNWR). Our findings suggest that BAI may not be age-dependent after the first 15 years of growth in AWC, making it a potential predictor of environmental conditions. AWC at GDSNWR (the relatively dry site) grew faster and appeared to experience growth release around 1940-1945 and 1955-1965, which may correspond to more aggressive efforts to drain the swamp. BAI is a promising tool for evaluating historic hydrologic conditions at AWC swamps, especially due to its ability to capture growth responses of AWC to past events independent of trees' age.

EFFECT OF STORAGE ON ANTIOXIDANTS IN CULINARY HERBS. T. Warner Lowry, Peter T. Ko & Michael H. Renfroe, Department of Biology, James Madison University, Harrisonburg VA 22807. Antioxidants are compounds present in foods and beverages that have many beneficial effects to human health. Herbs and spices have long played important roles in food preservation and improvement of food palatability. We investigated the presence of antioxidants in several common culinary herbs: rosemary, organic rosemary, sage, thyme, and basil. We also investigated how concentrations of hydrophilic and lipophilic antioxidants changed over storage times of up to 25 months. We found significant differences in antioxidant

concentrations from batch to batch of fresh herbs. Thyme holds antioxidant content well while rosemary, sage and basil tend to hold steady over the short term, but then drop over long storage. Organic rosemary has a higher initial decline in antioxidant content, but stabilizes between 19 and 25 months. Even at 25 months of storage at room temperature, these herbs can still provide a nutritional source of antioxidants.

CHALLENGES IN PROPAGATING THE PINELANDS NERVERAY, *TETRAGONOTHECA HELIANTHOIDES* L. Richard Curzon & Philip M. Sheridan. Meadowview Biological Research Station, 8390 Fredericksburg Tnpk., Woodford, VA 22580. *Tetragonotheca helianthoides* is an extremely rare plant in Virginia with one extant occurrence and ranked S1 by the Virginia Dept. of Conservation and Recreation. *Tetragonotheca helianthoides* is part of a rare plant reintroduction program at our Joseph Pines Preserve in a longleaf pine ecosystem restoration project. Therefore, the seed ecology of pinelands nerveray needed to be understood for successful propagation and reintroduction of this rare species. Due to the limited number of indigenous seeds available for research, adaptive research was employed to determine an effective way to produce seedlings for restoration purposes. We found that *Tetragonotheca* seedlings were highly susceptible to fungal pathogens, and the thick seed coat inhibited germination. We devised a seed sterilization and embryo extraction technique that allowed us to get seedlings into the second year of growth, including winter dormancy. *Tetragonotheca* continues to be a challenging plant to raise, and the many hurdles we face in its cultivation may explain its rarity in the wild.

AN ECOLOGICAL CHARACTERIZATION OF TERRESTRIAL COMMUNITIES AT THE SHENANDOAH UNIVERSITY RIVER CAMPUS AT COOL SPRING BATTLEFIELD, CLARKE COUNTY, VIRGINIA. Sydney J. Vonada<sup>1</sup>, Woodward S. Bousquet<sup>1</sup> & Gary P. Fleming<sup>2</sup>, <sup>1</sup>Environmental Studies Program, Shenandoah University, Winchester, VA 22601, and <sup>2</sup>Virginia Natural Heritage Program, Richmond, VA 23219. In April 2013, Shenandoah University acquired its River Campus at Cool Spring Battlefield. The 195-acre site, a former golf course, is situated along the Shenandoah River at the foot of the Blue Ridge Mountains in Clarke County, Virginia. Our study constituted the first documentation of the property's natural communities. Using the relevé (Braun-Blanquet) method, seven communities were described by their vegetation and physical characteristics. Natural communities belonging to five ecological community types were identified: mountain floodplain forest, acidic oak/hickory forest, rocky bars and shores, central Appalachian low-elevation boulderfield forest, and mountain swamp forest. The plots

examined contained a total of 221 vascular plant species. We documented 11 new county records, including the natives *Rumex verticillatus*, *Tilia americana* var. *heterophylla* and *Quercus shumardii*, and the invasive introduced species *Torillia japonica*. Although the number of introduced species ranged from 15 to 33 percent of the species totals in the communities studied, their dominance was much lower, ranging from only 2 to 22 percent cover. Our research provides a baseline for evaluating the intended protection of natural communities and reduction of invasive species at the River Campus. This project was funded in part through a student fellowship from the Virginia Foundation for Independent Colleges (VFIC).

DEVELOPING A TREE RING MODEL TO EVALUATE HISTORIC WATER LEVELS IN THE GREAT DISMAL SWAMP. Abigail H. Weaver, Julie Slater & Robert Atkinson, Department of Organismal and Environmental Biology, Christopher Newport University, Newport News VA 23606. Atlantic white cedar (AWC) stands are an endangered type of wetland ecosystem due to logging and disturbed hydrologic conditions. Alligator River National Wildlife Refuge (AR) and the Great Dismal Swamp National Wildlife Refuge (GDS) are about 100 km apart and contain mature AWC stands but have different historic hydrologic conditions. Historically, AR has had higher water tables than GDS, which has been shown to reduce AWC growth as shown by narrower tree rings. The purpose of the current study was to determine if tree ring widths differ between AR (wet) and GDS (drier) AWC stands using Palmer drought severity index (PDSI) as a surrogate for historic climate conditions including precipitation and temperature. AWC ring widths were positively correlated with PDSI from the associated current year in GDS, suggesting that tree growth increased in wetter, warmer conditions. Ring width was negatively correlated with current year PDSI in AR where tree growth increased with drier, cooler conditions. At both sites, previous year PDSI was negatively correlated with ring widths, possibly resulting from drier, warmer conditions that led to increased tree growth in both sites the following year. (Correlation coefficients ranged from -0.35 to 0.29.) These results suggest that AWC trees respond differently to historic climate conditions based on the site-specific hydrologic conditions, and that there may be water levels that are optimal for AWC growth. These findings may help to inform future restoration plans for these sites and other AWC stands throughout the country.

ANATOMY AND MORPHOLOGY OF THE ALLOMORPHIC FLOWERS OF *ACALYPHA SETOSA* (EUPHORBIACEAE). Sarah Kwon & W. John Hayden, Department of Biology, University of Richmond, Richmond, VA 23173. *Acalypha setosa* is an herbaceous annual widely distributed in the Neotropics. These weedy plants

routinely produce three distinct types of flowers: staminate flowers in short axillary spikes, ordinary pistillate flowers borne in terminal spikes, and allomorphic pistillate flowers that form on distal extremities of pistillate spikes. All three flower types were studied with light and scanning electron microscopy. We interpret allomorphic flowers of *A. setosa* to consist of one enlarged carpel that takes the form of a ridge-like crest flanked by a pair of crown-like concave cylinders plus two rudimentary carpels found at one end of the ridge-like crest; we interpret filiform processes emerging from the junction of the grossly dissimilar carpels to be styles and stigmas. The enlarged allomorphic carpel bears a single ovule with its micropyle oriented towards the style base, and containing readily identifiable integuments and nucellus (megasporeangium).

**BIG DATA FOR VIRGINIA PLANT TAXONOMY.** Andrea Weeks, Department of Biology and Ted R. Bradley Herbarium, George Mason University, Fairfax, VA 22030. All plant taxonomic references that we enjoy today, such as the *Flora of Virginia*, arise out of practices that have remained essentially unchanged from the days of Linnaeus. Herbarium specimens are collected, consulted, compared, measured and annotated in person, in turns, by botanists. However, the plant taxonomic world is flattening in the 21st century due to new technologies and an ethos of highly collaborative, open-access collections-based research. What opportunities does this bring for improving floras? What may be its challenges? How can you become involved and contribute to this brave new world? Activities of the NSF-sponsored collaborative research grant entitled, "The key to the cabinets: building and sustaining a research database for a global biodiversity hotspot," liberate the power of big data for Virginia botany.

**THE FLORA OF VIRGINIA PROJECT: A 2015-2016 PROGRESS UPDATE.** Marion B. Lobstein, Northern Virginia Community College. The Foundation of the Flora of Virginia Project continues to make solid progress on development of the *Flora of Virginia* mobile application (app). The *Flora of Virginia* app is at the point in which a prototype will be produced by the developer for field testing during the 2017 growing season. Volunteers have completed parsing (i.e., taken specific information such as flower color from species descriptions) for all 3200 species in the *Flora of Virginia*. Roughly 60% of the species will be included in the prototype. Fundraising efforts, including grant requests, have had positive results with the Mary Morton Parsons Foundation challenge grant awarded for a 1:1 match up to \$40,000. Over \$20,000 of this grant has been matched to date. This grant is active through the end of 2016. A recent \$10,000 grant from Dominion Power has also been awarded. Another \$30,000 still needs to be raised to complete the production of the mobile version (for mobile phones and for tablets) of



the app. A future version of this app for computers, both Macintosh and PCs, is being planned.

GENES BEHIND THE FLORAL FLOURISH. April N. Wynn, Department of Biological Sciences, University of Mary Washington, Fredericksburg VA 22407. Flowers are often the most recognizable and colorful reproductive features of angiosperms. The formation and developmental patterning of flowers are determined by several classes of genes, originally termed the ABC Model, and expanded to include class D and E genes. These classes of homeotic genes control the formation of the floral tissues (sepals, petals, stamens, and carpels) and contribute to the diversity of floral phenotypes. Layered on top of the expression of the homeotic genes of the ABC Model is their genetic regulation by transcription factors that control the temporal and spatial expression of the homeotic genes. Perturbations or mutations in either the homeotic genes or transcription factors result in floral variation between species, within species or between plants grown in differing environments. My research examines the genetic regulatory networks comprised of transcription factors that contribute specifically to proper carpel and ovule formation in *Arabidopsis thaliana*. Two critical transcription factors for carpel and ovule formation are *SEUSS* and *AINTEGUMENTA*; however, their direct function and location within the genetic regulatory network controlling carpel formation has yet to be fully characterized. Using previous transcriptomics studies, sets of downstream candidate genes have been identified, including *PERIANTHIA* and *EARLY RESPONSE TO DEHYDRATION 10*, and are being functionally characterized for their contribution to carpel and floral development and environmental sensitization of the formation of these tissues.

ANALYSIS OF THE EFFECT OF HYDROLOGIC CONDITIONS ON EARLY GROWTH IN ATLANTIC WHITE CEDAR (*CHAMAECYPARIS THYOIDES*) IN PEATLANDS IN SOUTHEASTERN VIRGINIA: IMPLICATIONS FOR MANAGEMENT. Christina G. Mirda, Julie Slater & Robert B. Atkinson, Department of Organismal and Environmental Biology, Christopher Newport University, Newport News, VA 23606. Early growth of Atlantic white cedar [AWC, *Chamaecyparis thyoides* (L.) B.S.P.] is routinely measured during stand reestablishment; however, few summaries that evaluate important site conditions such as hydrology exist. Many of the studies reported that survival is most strongly related to soil inundation and that indicators of soil saturation are better predictors of stem diameter growth and height growth of AWC. The purpose of this research was to review studies and analyze the relationship between hydrology and early growth (height and stem diameter) in order to inform future AWC planting strategies. Existing

research that investigated hydrologic conditions and two measures of growth (stem diameter and height) of AWC seedlings was compiled and evaluated. Other parameters affecting growth were also noted. Our study found that indicators of soil saturation are the most important factors affecting early growth in AWC, but other influences include competition, shade, and soil quality. We recommend that future AWC studies follow a standard method for measuring mean water table depth to allow for expanded collaboration and data comparison. In order to help reestablish AWC, additional studies should also focus on examining growth relationships under the mean water table depth range of -50 cm to -30 cm, the likely range for optimum AWC seedling growth.

SELECTION IMPOSED BY POLLINATION MODE MAY INFLUENCE EVOLUTION OF POLLEN MORPHOLOGY. Rebecca P. Humphrey<sup>1</sup> & Alison Ossip-Klein<sup>2</sup>, <sup>1</sup>Hollins University, Roanoke, VA 24020 and <sup>2</sup>Indiana University, Bloomington, IN 47405. Natural selection has likely exerted significant influence over the evolution of reproductive structures such as the angiosperm pollen grain, yet the evolutionary implications of the interactions between pollen grains and their environment are not fully resolved. Across the flowering plants, variation in external pollen grain morphology often relates to pollination mode, suggesting that the conditions of pollen transmission exert specific selective pressures on features of pollen grains. Within some species, individuals produce multiple pollen-grain types, each with a different number of apertures (pollen heteromorphism), and fertilization success of individual grain type varies with aperture number and local environment. Through phylogenetic comparative methods, we examine correlated evolution between pollen morphology (grain size and aperture number) and pollination mode within the angiosperm genus *Thalictrum* (Ranunculaceae). Additionally, we calculate phylogenetic heritabilities of pollen traits. We find a strong phylogenetic signal for pollination mode. Pollen grain size shows high phylogenetic heritability and lower variation within wind-pollinated species. Insect-pollinated species have a higher calculated optimum number of pollen-grain apertures, which may relate to pollen competition.

A MORPHOMETRIC ANALYSIS OF INFRASPECIFIC VARIATION WITHIN *ELEOCHARIS TENUIS* (CYPERACEAE). Lane D. Gibbons & Conley K. McMullen, Department of Biology, James Madison University, Harrisonburg, VA 22807. North America contains a large portion of the most problematic and least resolved array of diversity in the genus *Eleocharis* (Cyperaceae). Of noteworthy significance are lasting taxonomic uncertainties involving plants referable to *Eleocharis tenuis* (var. *tenuis*, var. *verrucosa*, and var. *pseudoptera*) of the *E. tenuis*

species complex (subg. *Eleocharis*, sect. *Eleocharis*, ser. *Eleocharis*, subser. *Truncatae* Svenson). Morphology of *E. tenuis* is traditionally recognized as being intermediate among taxa along a broad geographic gradient. However, paired cytological and morphometric data indicate that the varieties of *E. tenuis* are well separated by five morphological characters, that these characters do not intergrade between taxonomic entities, and that cytological data are unique to and correlate with currently recognized entities. We propose that the current rank applied to *E. tenuis* var. *verrucosa* does not appropriately reflect the evolutionary importance of this entity, and we raise it as a species distinct from *E. tenuis* var. *tenuis* and *E. tenuis* var. *pseudoptera*.

CYATHIUM ANATOMY AND MORPHOLOGY IN *EUPHORBIA SPATHULATA* (EUPHORBIACEAE). Christian Rabot & W. John Hayden, Department of Biology, University of Richmond, Richmond, VA 23173. *Euphorbia spathulata* is an herbaceous annual widespread throughout North America. We undertook structural and developmental studies of its cyathia in order to investigate a possible mechanism for their self-pollination while still enclosed by subtending cyathophylls. Cyathia studied consist of a gland-bearing involucre enclosing five staminate flowers (each a single stamen), and a central 3-carpellate pistillate flower. In early cyathia still enclosed by cyathophylls, only the pistillate flower is exerted from the involucre and its short styles are erect. Subsequently, while still enclosed by cyathophylls, styles elongate and reorient to a deflexed position, bringing stigmas near or in direct contact with open anthers held at or slightly above the involucre rim; pollen grains were observed on stigmas at this stage. Later, when the cyathophylls have spread apart, exposing the cyathium to the external environment, styles and stigmas are once again ascendant. Our observations suggest that each cyathium of *E. spathulata* combines the potential for early anthesis self-pollination and late anthesis cross-pollination.

INITIAL STUDIES ON THE REPRODUCTIVE BIOLOGY OF *BUCKLEYA DISTICHOPHYLLA* (SANTALACEAE). Conley K. McMullen<sup>1</sup>, Ryan Huish<sup>2</sup> & Melissa Manow<sup>3</sup>, <sup>1</sup>Department of Biology, James Madison University, Harrisonburg, VA 22807, <sup>2</sup>Department of Natural Sciences, The University of Virginia's College at Wise, Wise, VA 24293, and <sup>3</sup>Department of Biology, Hollins University, Roanoke, VA 24019. *Buckleya distichophylla* (Santalaceae), commonly known as piratebush, is a rare dioecious and hemiparasitic shrub endemic to the Appalachian Mountains of Virginia, North Carolina, and Tennessee. Previous studies on piratebush suggest sexual reproductive deficiency as a possible explanation for its rare and scattered distribution. To investigate the reproductive biology of piratebush, we examined sex ratio and flowering phenology in the densest population of piratebush

known (Poor Mountain, southwest Virginia). Sex ratio data were collected through field surveys along ecological transects. Results show a male-biased sex ratio (61:39) of flowering individuals, with 15% nonflowering. Size data confirm significantly smaller size for nonflowering individuals, suggesting combined characteristics of size and nonflowering as an indicator of juvenility. Floral phenology data were recorded from 23 males and 20 females in varied representative elevation and aspect gradients. Flower abundance was male-dominant by >24:1. Floral phenology showed individual variation, with males beginning and ending approximately 1 week before females, but considerable synchrony for the bulk of flower production for about 2 weeks during the first half of May. The widely male-dominant sex ratio and floral abundance, as well as variation in flower production by females, underscore the importance of ongoing investigations into the reproductive and regenerative health of this rare species.

EVALUATING RELATIONSHIPS BETWEEN WOOLGRASS (*SCIRPUS CYPERINUS* (L.) KUNTH) TISSUE ELEMENTAL CONCENTRATIONS AND GROWTH OF SAPLINGS PLANTED IN CREATED WETLANDS OF VIRGINIA. Autumn K. Tilghman & Robert B. Atkinson, Department of Organismal and Environmental Biology, Christopher Newport University, Newport News VA 23606. Nutrient (C, N, and P) concentrations in created wetlands can be lower than those in naturally occurring wetlands and may reduce tree biomass in forested wetland mitigation sites. The purpose of this study was to determine the relationship between nutrient concentrations in plant tissue of *Scirpus cyperinus* (L.) Kunth, the dominant herbaceous species at our study sites, and annual sapling morphometric change (height, basal stem diameter, and canopy) among seven species and three planting types in three created wetlands in Loudoun County, VA. Aboveground vegetation was clipped at ground level in 0.25-m<sup>2</sup> plots adjacent to each planted sapling. Samples were returned to lab, dried, and analyzed for C and TKN using an elemental analyzer and P using an ashing/acid extraction technique. Tissue nutrient content averaged 43.26±1.40 for %C, 5.07±0.88 for %TKN, and 0.202±0.026 for %P. Nitrogen was positively correlated to sapling height ( $r=0.32$ ,  $p=0.02$ ) while there was no correlation of P to height ( $p>0.05$ ). Both N:P ( $r=0.31$ ,  $p=0.02$ ) and C:N ( $r=-0.29$ ,  $p=0.04$ ) were significantly correlated with sapling height. These findings indicate that N may be one of the factors limiting tree growth in our study sites. Extensive soil nutrient analyses at these sites failed to detect nutrient limitations, which suggests that *S. cyperinus* tissue nutrient concentrations may be a more sensitive measure that could guide nutrient amendment decisions to enhance tree establishment in created or restored wetlands.

NITROGEN TISSUE CONTENT IN *JUNCUS EFFUSUS* AS A PREDICTOR OF SAPLING GROWTH IN CREATED WETLANDS OF VIRGINIA. Stephen W. Bendele, Autumn K. Tilghman & Robert B. Atkinson, Department of Organismal and Environmental Biology, Christopher Newport University, Newport News VA 23606. *Juncus effusus* (L.) is an emergent facultative wetland plant species found worldwide in fresh or brackish wetlands. Previous studies of created wetlands found plant tissue nutrient concentration to be more sensitive than soil nutrient concentrations as a predictor of aboveground primary production. The purpose of this study was to evaluate whether the concentration of nitrogen in aboveground vegetative tissue of *J. effusus* could predict sapling morphology. Basal stem diameter, height, canopy cover, and *J. effusus* tissue nitrogen were collected from a three 7-year-old created non-tidal wetlands located in Loudoun County, VA. Tissue nitrogen concentration of *J. effusus* was not related to basal stem diameter ( $r=0.19$ ,  $p>0.1$ ), height ( $r=-0.06$ ,  $p>0.1$ ), or canopy coverage ( $r=-0.06$ ,  $p>0.1$ ). The weak relationship between *J. effusus* tissue nitrogen and tree morphological parameters could be due to the confounding effects of competition, hydrology, and micro- or macro-nutrient limitations.

THE EFFECT OF PHOSPHORUS CONTENT IN *JUNCUS EFFUSUS* ON GROWTH OF SAPLINGS PLANTED IN CREATED WETLANDS OF VIRGINIA. Hannah R. Leich, Autumn K. Tilghman & Robert B. Atkinson, Department of Organismal and Environmental Biology, Christopher Newport University, Newport News VA 23606. The nutrient (C, N, and P) concentrations can be lower in created wetlands than in naturally occurring wetlands, which may reduce the biomass of trees in forested wetland mitigation sites. This work was completed to determine the relationship between the P content in plant tissue of *Juncus effusus*, which was a dominant herbaceous species at our study sites, and the annual sapling morphometric change (basal stem diameter, canopy, and height) among seven species and three planting types in three created wetlands in Loudoun County, VA. Aboveground vegetation was clipped at ground level in 0.25-m<sup>2</sup> plots adjacent to each planted sapling. Samples were brought to the lab, dried, ground, and analyzed for P using an ashing/acid extraction technique. There was no correlation found of P to the basal stem diameter, canopy, or height of the saplings. This is likely because P acts along with other nutrients (C and N) and alone is not enough to predict tree growth.

USING *SCIRPUS CYPERINUS* ELEMENTAL ANALYSIS AS A PREDICTOR OF SAPLING GROWTH IN CREATED WETLANDS. Andrew B. Simmons, Autumn K. Tilghman & Robert B. Atkinson, Christopher Newport University, Newport News VA 23606. Created

wetlands have been a focus of study in response to Clean Water Act requirements, but factors affecting sapling growth are poorly understood. The purpose of this study is to determine if tissue nutrient (C, N, P) concentration of surrounding dominant vegetation (*Scirpus cyperinus*) is a predictor of annual sapling morphometric change (height, canopy, and basal stem diameter) and to illustrate nutrient distribution in a created wetland in Loudoun County, Virginia. Aboveground vegetation was collected at the end of the 2015 growing season. Samples were dried and analyzed for C, N, and P by elemental analyzer or ashing/acid extraction technique. Percent N was weakly related to sapling height ( $r=0.32$ ,  $p<0.02$ ). Comparing the portions of the site having above average sapling growth and nutrients with the site map showed somewhat random distribution, but some clustering in the central low-lying areas of the created wetland was observed. These findings suggest that nitrogen may be one of the factors limiting tree growth in created wetlands, and that visualizing distribution may enhance nutrient amendment decisions in similar created or restored wetlands.

## Chemistry

PHOTOSWITCHABLE ARYLAZOPYRAZOLE LIGANDS: SYNTHESIS AND COORDINATION CHEM. Keset Y. Ghebreyessus and Janay I. Little, Department of Chemistry and Biochemistry Hampton University, Hampton, VA 23668. Novel palladium(II) and platinum(II) coordination complexes incorporating pyridine and benzothiazole functionalized photo-switchable arylazopyrazole ligands were synthesized and characterized. The photo-physical properties of the ligands and their corresponding metal complexes have been investigated. The effect of the nature of the ligands and type of metals on the rate of the *cis-to-trans* photo-isomerization process has also been explored. The ligands and the metal complexes exhibit strong absorption bands in the UV region, and weak bands in the visible region. Upon irradiation with UV-light ( $\lambda = 365$  nm) these compounds clearly undergo efficient and almost quantitative *trans-to-cis* photo-isomerization of the azopyrazole unit followed by the reverse *cis-to-trans* isomerization upon standing under normal light. The results indicate that the percent conversion of the *trans-to-cis* isomerization is highly dependent on the substitution pattern on the pyrazole ring, which also has a significant influence on the electronic properties of the compounds. (Supported by: the Hampton University Faculty Research Fund, NSF CREST Center and PREM)

PROGRESS TOWARD A "GREEN" REDUCING AGENT. Charles M. Bump, Department of Chemistry and Biochemistry, Hampton University 23668. Solutions of freshly brewed green tea have been successfully used in the synthesis of gold and silver nanoparticles. During that process, the tea reduces metal ions ( $\text{Au}^{3+}$ ,  $\text{Ag}^{+}$ ) to their elemental state and caps those atoms with a large organic molecule that prevents the aggregation and precipitation of colloidal metal. Among the more conventional reducing agents used in the synthesis of nanoparticles is  $\text{NaBH}_4$ . That same reducing agent is widely used for the conversion of aldehydes and ketones to alcohols. We are beginning an investigation on expanding the use of green tea for the reduction of aldehydes and ketones. Some of the difficulties to be addressed include the poor solubility of ketones in aqueous media (e.g. tea), determining the length of time required for the reduction to take place, and the isolation of the reduction product.

MICROWAVE - ASSISTED SYNTHESIS AND CHARACTERIZATION OF METALLIC NANOMATERIALS Peter N. Njoki, Department of Chemistry & Biochemistry, Hampton University, Hampton VA 23668. Laboratory microwaves are becoming common tools in research and teaching laboratory. Compared to

conventional means of heating, microwaves provide shorter reaction times, better reproducibility, and enhanced reaction control. The key component of microwave-assisted heating is that nanoparticles synthesis can be completed within minutes thereby saving time and energy. Nanomaterials possess novel properties as the size of particles decreases to nanoscale. To understand and exploit these novel properties, we need to develop effective strategies to synthesize nanoparticles with controllable size, shape, and composition. In this presentation, we focus on synthesis of gold and silver nanoparticles due to their unique optical properties and ease of fabrication. This presentation describes results of a microwave-assisted irradiation method aimed at developing the ability to control the size and composition of gold and silver nanoparticles. We will also discuss applications of these nanoparticles in fuel cell and forensic analysis. An array of analytical techniques such as transmission electron microscopy and UV-Vis spectrophotometry were used for the characterizations.

ENGAGING INQUIRY-BASED STUDENT LEARNING USING COMPUTATIONAL CHEMISTRY IPAD APPS IN ORGANIC CHEMISTRY LABORATORIES. Michelle K. Waddell, Charles Bump, Godson Nwokogu & Edmund Ndip, Dept. of Chem. and Biochem., Hampton Univ., Hampton VA. 23668. Inquiry-based classrooms encourage students to explore and understand their world using scientific methods. Spartan has been used to integrate computational chemistry into undergraduate chemistry curriculum. Computers and modeling software were purchased for use in chemistry courses. However, calculations using Gaussian and Argus Lab software to minimize energies of organic molecules routinely ran for several hours and are not feasible for use in the organic laboratory sections. The free website 4 Mol D was adopted for its quick efficient calculations. This project compares organic chemistry students' comprehension of inquiry-based learning of computational chemistry 3D models of various experiments using 4 Mol D and the iPad mini iSpartan app. Student outcomes were assessed in retrospective web-based surveys administered on-line through Blackboard™ 9.0 to access comprehension of the material, level of success in achieving learning outcomes and technical ability to utilize the molecular modeling applications. Results indicated that students were more comfortable using the iPad minis due to its ease of use and reliability of access. Student data collected from iPad minis and 4 Mol D was comparable to one another.

GC/MS QUANTIFICATION OF THE BIOFUEL POTENTIAL OF ALGAE. Grant A. McClure & Todd Allen, Dept. of Biol. and Chem., Liberty Univ., Lynchburg VA. 24502. Microalgae has the potential to be used as an alternative to petroleum-based feedstock necessary for

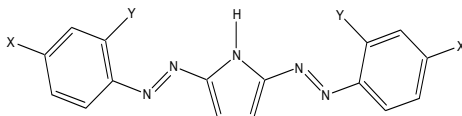


transportation fuels. Several companies are researching cost-effective ways to optimize algae growth and harvesting techniques in an effort to convert the lipid fraction of the algae biomass into various types of fuels. The ability to accurately quantify the lipid content of algae is essential to evaluating the fuel potential. A validated GC/MSD (Gas Chromatography coupled with Mass Spectrometric Detection) method was previously developed for the quantitative analysis of the biofuel potential in algal samples provided in lyophilized and filtered mediums. Companies are exploring new oil extraction techniques to maximize the harvest efficiency of the oil from the algae. The new oil extract required additional steps be taken in the beginning stages of the sample preparation to accommodate the unique matrix. After the additional steps for the new matrix, all subsequent steps merge with the other matrices preparation method. Results will be presented.

**MICROWAVE - ASSISTED SYNTHESIS AND CHARACTERIZATION OF METALLIC NANOMATERIALS** Peter N. Njoki, Department of Chemistry & Biochemistry, Hampton University, Hampton VA 23668. Laboratory microwaves are becoming common tools in research and teaching laboratory. Compared to conventional means of heating, microwaves provide shorter reaction times, better reproducibility, and enhanced reaction control. The key component of microwave-assisted heating is that nanoparticles synthesis can be completed within minutes thereby saving time and energy. Nanomaterials possess novel properties as the size of particles decreases to nanoscale. To understand and exploit these novel properties, we need to develop effective strategies to synthesize nanoparticles with controllable size, shape, and composition. In this presentation, we focus on synthesis of gold and silver nanoparticles due to their unique optical properties and ease of fabrication. This presentation describes results of a microwave-assisted irradiation method aimed at developing the ability to control the size and composition of gold and silver nanoparticles. We will also discuss applications of these nanoparticles in fuel cell and forensic analysis. An array of analytical techniques such as transmission electron microscopy and UV-Vis spectrophotometry were used for the characterizations.

**EFFECTS OF SUBSTITUENTS AND BRIDGE LENGTH ON NLO PROPERTIES OF A SERIES OF BIS AZO DYES.** Edmund Moses N. Ndip, David S. Barnes & Binal Patel. Department of Chemistry and Biochemistry, Hampton University, Hampton, VA 23668. In the present study, the effect of variations in donor – acceptor pairs on frequency dependent nonlinear optical properties has been investigated. Semi-empirical calculations of the frequency-dependent linear polarizability,  $\alpha$ , the second,  $\beta$ - and third,  $\gamma$ -order hyperpolarizabilities at the fundamental (1028 nm) and other wavelengths were achieved by applying the *ab initio* time-dependent coupled perturbed Hartree -Fock

(TDHF) method at the Restricted Hartree- Fock (RHF) level using routines contained in MOPAC2012 program for various model systems shown below (1-10). The D- $\pi$ -D structures were found to have lower bandgaps (6.585 – 7.004 eV) as well as significantly lower second-order hyperpolarizability values compared to values for A- $\pi$ -A, A- $\pi$ -D, or D- $\pi$ -A structures. This work was funded in part by Hampton University's NSF CREST (ACLaSS) and HRD 1238838.



Compound #	X	X	Y	Y	Type
1	H	H	H	H	
2	CO <sub>2</sub> H	CO <sub>2</sub> H	H	H	A- $\pi$ -A
3	NH <sub>2</sub>	NH <sub>2</sub>	H	H	D- $\pi$ -D
4	CO <sub>2</sub> H	NH <sub>2</sub>	H	H	A- $\pi$ -D
5	OH	OH	H	H	D- $\pi$ -D
6	CO <sub>2</sub> H	OH	H	H	A- $\pi$ -D
7	NH <sub>2</sub>	OH	H	H	D- $\pi$ -D
8	H	H	OH	OH	No type
9	NPH <sub>2</sub>	NPH <sub>2</sub>	H	H	D- $\pi$ -D
10	N-Carbazoyl	N-Carbazoyl	H	H	

CHEMISTRY AND SPECTROSCOPY IN KBR MATRICES, T.C. DeVore, Dept of Chem. and Biochem., James Madison University, Harrisonburg VA 22807. Fifty years ago, several methods for trapping ions in KBr crystals were discovered. The IR spectrum of these ions could then be investigated and chemical reactions involving these ions could be investigated by heating the crystal. One way to use this method in the chemistry labs is to investigate the spectra of isoelectronic series of molecules. One example of this presented is the changes in the bond strengths for 16 electron linear molecules BO<sub>2</sub><sup>-</sup>,

CO<sub>2</sub>, and NCO<sup>-</sup>. The IR spectrum of BO<sub>2</sub><sup>-</sup> shows a clear isotope effect and can also be used to measure the isotopic abundance of boron. A similar exercise can be done with the isoelectronic tetrahedral molecules/ ions BH<sub>4</sub><sup>-</sup>, CH<sub>4</sub>, and NH<sub>4</sub><sup>+</sup>. The thermal decomposition of the oxalate ion in KBr produced by allowing an aqueous solution containing 1% sodium oxalate- 99% KBr to evaporate to dryness. Heating the solid to 775 K produced 4 new IR bands that may arise from COCO<sub>3</sub><sup>2-</sup> which has been predicted to be an intermediate in this decomposition.

CHARACTERIZATION OF ALGAL PIGMENTS USING ABSORBANCE AND FLUORESCENCE. C. M. Fleming, Z. J. Schreiber & T. M. Allen, Department of Biology and Chemistry, Liberty University, Lynchburg VA 24502. The effectiveness of identifying different types of chlorophyll within microalgae is vital in predicting not only the lipid content, but also understanding why chlorophyll is contaminating the hexane layer during the sample preparation process. This contamination of chlorophyll and other proteins inhibits the extracted lipids from immediately being used for biofuels. A comparison of absorbance and fluorescence in both the organic and aqueous layers were measured to analyze the ratio between extracted and non-extracted pigments found in the organic layer.

## Computer Science

DISCOVERING RASPBERRY PI. Bruce C. Chittenden, Department of Computer Science, Hampton University, 100 E. Queen Street, Hampton, Virginia 23668. The Raspberry Pi is a low cost, credit-card sized single-board computer that plugs into a HDMI monitor and uses a standard USB keyboard and mouse. The design is based around a **Broadcom BCM2835 SoC**, which includes an ARM1176JZF-S 700 MHz processor, VideoCore IV GPU, and 512 Megabytes of RAM. The design does not include a built-in hard disk or solid-state drive, instead relying on an SDHC card for booting and long-term storage. Currently there are several versions on Linux that runs on the Raspberry Pi. The most popular is Raspbian which is based on a version of Debian Linux. Several Programming Languages are also available for the Raspberry Pi including C, C++, FORTRAN, Java, Python, Scratch. Last summer I worked with a student from Hampton University on an Externship from the National Center for Atmospheric Research, NCAR. Over the summer we created a cluster using four Raspberry Pi computers and a Western Digital Router. We ported Message Passing Interface mpich2 to interconnect the Raspberry Pi computers. This configuration is now referred to as Raspberry Pi Bramble. With the experience I gained over the summer I introduced Raspberry Pi into CSC 301 - Operating Systems. There were four assignments using the Raspberry Pi. The first assignment was to download a Raspbian Image from raspbian.org and get the Raspberry Pi up and running and become familiar with Linux running of the Raspberry Pi. The second assignment was to download source code for the Linux Raspbian kernel from github.com and build a kernel from source code. The third assignment was to develop a System Call and add it to the Linux kernel that they had just built. And the fourth assignment was to write a device driver for the Linux Operating System. This gave the students some real hands-on Operating System development experience and the chance to work closely with the hardware. In all I would say that the class was a real success. This summer I plan on another Externship with a student from Hampton University at NCAR and we are going to build a Cloud Computing environment using the Raspberry Pi Bramble.

DYNAMICAL SYSTEM MODELING. Yen-Hung Hu, Department of Computer Science, Norfolk State University, Norfolk VA 23504. A dynamical system is a mathematical model which combines an abstract state space and a dynamical rule. In a system, we could describe the dynamical state at any time of this system and to specify the future trend of all states by giving only the present values of those states. In brief, dynamical system is an evolution rule that defines a trajectory as a function of time on a set of states. The evolution rule can be deterministic or stochastic. If the system is deterministic, every state in

the system will have a unique consequent state. Otherwise, it is stochastic. Network traffic used to be one feasible source for network administrators and researchers to study potential network malicious activities. However, due to the growing use of open source software and the asynchronous and anonymous nature of the Internet, identity of a malicious connection could be easily forged to increase the difficulty and complexity in analyzing abnormal characteristics of the malicious connection and cause a false negative. To resolve this issue, this paper proposes a dynamical systems approach to monitor network activities. We assume that every state of a network characteristic in a certain period of time would be deterministic and will present dynamical behaviors since network resources associated with this characteristic are bounded. We have described our dynamical approach in detail and included several examples to demonstrate it. Meanwhile, performance study of the approach is studied as well.

USING OPEN SOURCE VULNERABILITY ANALYSIS TOOLS TO ASSESS CODING SECURITY AND QUALITY. Yen-Hung Hu, Department of Computer Science, Norfolk State University, Norfolk VA 23504. Security and quality are two vital attributes of any software application no matter how infinitesimal it might be. Software is created using a programming language, so when a programming language has a security issue it affects its product, which is the software. Tackling a problem by its source is one of the most trusted models used in problem solving approaches. However, since the complexity of a software, it is inefficient and costly to manually examine the source codes of the software. In this paper, we want to ensure that all undergraduate Java learners write codes based on the security and quality guidelines expected in the industry right from the day they start learning the first program in Java. Therefore, a tool that can automatically identify security and quality vulnerabilities of any Java code and create a report for fixing them will be crucial. In the research, sample codes getting from several Java books used in teaching Java concepts for undergraduate courses were used as the case study. These sample codes were tested using an open source tool named findBugs which was developed based on security and quality guidelines. The tool determines the vulnerability level in any Java source code passed as an input to it then it analyzes the source code and generates a report indicating the threat level and suggestions based on the vulnerabilities in the code. The results of this paper will be published and those vulnerabilities in the sample codes along with suggestions for fixing them will be included as well.

INTELLIGENT PARKING SYSTEM (IPS) – LEVERAGING THE INTERNET OF THINGS. Syed R. Rizvi, Susan Zehra & Stephan Olariu, Dept. of Computer Science, Old Dominion University, Norfolk, VA 23529. The Intelligent Parking System (IPS) is proposed in an Internet of Things (IoT) environment for drivers by exploiting the ubiquitous sensing and transmitting nature of these systems for Areas of Interest (AoI). IPS is an end-to-end software infrastructure that automates the procedures of obtaining spatio-temporal parking-related data from IoT at public and private parking facilities, processes these spatio-temporal parking-related data to promote IoT synergy (data correction, data resolution enhancement, and registration), provides an analysis framework for software agents for making parking reservations. The IPS leverages a number of cutting-edge computing technologies, frameworks, and novel algorithms. Utilizing IoT provides significant benefits to both the parking facilities as well as the driving community. First, the quantity and quality of spatio-temporal parking-related data from IoT allows for rapid determination of available parking spots in an AoI. Second, the sensing and connectivity of IoT can facilitate parking enforcement of cars that are violating parking restrictions. Lastly, the vast amount of data obtained from IPS can be leveraged for active and focused AoI monitoring in order to build optimal parking pricing policies. The automation capability of IPS will enable efficient processing of a vast amount of data, allowing multiple AoI to be simultaneously monitored, ultimately providing decreased waste of time and fuel for drivers looking for parking, lower traffic congestion, and better utilization of public and private parking facilities.

## Education

CLIMATE CONNECTIONS: YOUR ACTIONS MATTER. Eugene G. Maurakis & Richard C. Conti, SMV, 2500 W. Broad St., Richmond, VA 23220. Objectives are to educate the general public about the science of climate change on global and local scales; current and potential future impacts of climate change on Virginia and its communities; community resiliency and why it is important; how they can contribute to the resiliency of their own community by taking personal steps to be prepared for weather events and health threats related to climate change; and, convert awareness and understanding into personal action to increase readiness and resiliency in homes, schools, and communities. Communication methods used to convey climate change and resiliency information are: a NOAA Science on a Sphere®, the museum's digital Dome theater, a statewide digital media series (24 audio and 12 video productions/year); social media; lecture series; resiliency-themed programming (Art Lab, Challenge Lab, EcoLab), extreme event challenge workshops; and community preparedness event and resiliency checklist and certification programs. A front-end evaluation was conducted to survey general audience understanding of the difference between climate and weather, climate change impacts, and resilience. Funded by NOAA Award NA15SEC0080009 and Virginia Environmental Endowment.

THE RELATIONSHIP BETWEEN SCIENCE CLASSROOM FACILITY CONDITIONS AND NINTH GRADE STUDENTS' ATTITUDE TOWARD SCIENCE. Kurt Y. Michael & Angela Y. Ford, School of Education, Liberty Univ., Lynchburg, VA 24515. The purpose of this correlational study was to identify the relationship between high school science teachers' perceptions of their school science environment and their ninth grade students' attitudes toward science. Archival data was used from the High School Longitudinal Study of 2009 conducted by the National Center for Educational Statistics. A sample of 11,523 cases was extracted from the national data set. Three research questions looked at teachers' perceptions of the instructional equipment, demonstration equipment, and the condition of the school building in relationship to students' *enjoyment*, *boredom* and *value* of their science class. A series of multiple linear regressions was used to analyze the data. All three questions were statistically significant at ( $p < .01$ ). The best predictors of high school students' *enjoyment* of their science class were demonstration equipment ( $p < .001$ ) and facilities ( $p < .001$ ). The best predictors of high school students' *boredom* of their science class were demonstration equipment ( $p < .001$ ) and facilities ( $p < .001$ ). The best predictors of high school students'

PROMOTION OF INQUIRY-BASED LEARNING USING CULTURAL HERITAGE MATERIALS AND OTHER LOCALLY ACCESSIBLE RESOURCES: A PROFESSIONAL DEVELOPMENT ACTIVITY FOR PRECOLLEGE STEM TEACHERS. I.T. Urasa<sup>1</sup>, V. T. Ward<sup>2</sup>, V. Carroll<sup>3</sup>, A. Adibi<sup>4</sup>, D. Gibson<sup>5</sup> & P. Gueye<sup>6</sup>, <sup>1</sup>Dept. of Chemistry & Biochemistry, <sup>2</sup>University Museum, <sup>3</sup>Preservation Dept., <sup>4</sup>Dept. of Biological Sciences, <sup>5</sup>Dept. of Marine & Environmental Science, <sup>6</sup>Dept. of Physics, Hampton Univ., Hampton, VA 23668. This pilot study was guided by the State of Virginia Standards of Learning (SOL). Selected teachers from three Hampton City Schools designed and tested inquiry-based laboratory activities utilizing resources from Hampton University's inventory of cultural heritage and historical materials; the Chesapeake Bay watershed; and Hampton Univ. faculty research programs. The project culminated in a two-week summer institute. Findings from the pilot project: (1) practicing science teachers need professional development opportunities to acquire new skills that will allow them to teach content and subject matter using the inquiry method and sustained learning experience; (2) teachers are responsive to new curriculum guidelines and other mandates coming from the school districts; however, there are areas within those mandates that restrict the teachers' ability to implement new ideas (for example, SOL tests, resource and time limitations, etc.); and (3) precollege students adapt easily and quickly to new expectations if they are challenged. This study was supported by a grant from the National Science Foundation (NSF).



## Entomology

FRIENDS WITH BENEFITS; NATURAL ENEMIES AND POLLINATORS IN VIRGINIA CUCURBIT PRODUCTION. James M. Wilson & Thomas P. Kuhar, Department of Entomology, Virginia Tech, Blacksburg, Virginia 24061. Cucurbit production in Virginia provides the commonwealth with melons, pumpkins, summer squashes, and cucumbers, all of which are dependent upon adequate pollination for fruit set and development. Growers liberally apply fungicides in Virginia, and often will tank-mix broad-spectrum insecticides that are known to have negative effects on both natural enemy populations and pollinators regardless of pest pressure. Here we examine the potential role of narrow-spectrum insecticides and their interactions with the squash bug egg parasitoid, *Gryon pennsylvanicum* (Hymenoptera: Scelionidae). *G. pennsylvanicum* has been identified around the commonwealth and is capable of high levels of parasitization on eggs of the squash bug, *Anasa tristis* (Hemiptera: Coreidae) a pest of many cucurbit crops. Bioassays were conducted utilizing field collected squash bug egg masses with four narrow-spectrum insecticides and a broad-spectrum insecticide (used as a baseline). Squash bug egg masses were dipped in formulations of label-rate insecticides, and squash bug nymphs and parasitoid wasp adults were allowed to hatch. In 2015, there were no significant differences between treatments after a  $\chi^2$  goodness of fit test, in which the control replicate data were used as the “expected” values of background parasitization levels. A shift away from broad-spectrum insecticide use may reduce the risk of releasing secondary pest populations. Adapting effective narrow-spectrum insecticide use may help to mitigate the negative impacts of insecticides on pollinators and the natural enemy complex in cucurbit crop systems.

FLEA BEETLE SPECIES AND THEIR IMPACT ON CABBAGE AND EGGPLANT IN VIRGINIA. James A. C. Mason & Thomas P. Kuhar, Department of Entomology, Virginia Tech, Blacksburg VA 24061. Flea beetles (Coleoptera: Chrysomelidae), *Epitrix* spp. and *Phyllotreta* spp., are important economic pests of vegetable crops in North America, particularly eggplant and cabbage. An experiment was conducted in Whitethorne, Virginia on both “classic” eggplant and “Bravo” cabbage in 2015 to determine the impact of flea beetle feeding injury on crop yield. Beetle density and defoliation were assessed weekly for about one month by counting the number of beetles present on 10 plants per plot, and using a percent defoliation scale counting 10 randomly selected leaves per plot: 1= no defoliation; 2= 1-20% defoliation; 3= 21-40% defoliation; 4= 41-60% defoliation; 5= >60% defoliation. After one month, all plots were treated with a broad spectrum insecticide combination to eliminate any further insect injury.

Crop yield was assessed on eggplant by counting the number of eggplant harvested per plot, and cabbage by taking the total weight of all 16 cabbage heads per plot. Individual plants were monitored using the same defoliation scale by assigning an overall defoliation rating 1-5 to the entire plant, 10 plants were used for each category of defoliation. Yield of these individual plants was also assessed by counting the number of eggplant per plant, and the individual weights of the cabbage heads. The predominant flea beetle species found on eggplant was *Epitrix fuscula* and on cabbage were *Phyllotreta striolata* and *Phyllotreta cruciferae*. There was a significant negative relationship between defoliation rating and yield in both crops on a per plot basis and on an individual plant basis.

ATTRACT-AND-KILL TECHNOLOGY FOR HARLEQUIN BUG, *MURGANTIA HISTRIONICA* (HAHN), IS OPTIMIZED WITH NEW INSIGHTS ON BEHAVIORAL RESPONSE TO COLOR AND SEMIOCHEMICALS A. S. DiMeglio<sup>1</sup>, T. P. Kuhar<sup>1</sup> & D. C. Weber<sup>2</sup>, <sup>1</sup>Department of Entomology, Virginia Tech, Blacksburg, VA 24061 and <sup>2</sup>USDA - ARS, Beltsville, MD, 20705. To develop an effective attract-and-kill trap for harlequin bug (HB), *Murgantia histrionica* Hahn, an economically important pest of brassicaceous crops in the United States, we conducted a series field and lab studies. In a lab color-choice study, we found dark green to be most attractive for nymphs and adults, and yellow and white the least attractive. We compared green and yellow panel traps in the field, both baited with the aggregation pheromone of HB, murgantiol, and showed that green panels caught significantly more HB and fewer non-target lady beetles than did the baited yellow panels. Investigating the effect of trap type with the addition of semiochemical attractants in the field, we found that a pyrethroid-treated ramp, square panel, and pyramidal trap all intercepted HB effectively in the field, with the pyramidal trap killing the most HB. Also, the combination of murgantiol with a low emission of benzyl isothiocyanate was more attractive than murgantiol alone for all trap types. To integrate this information into an effective attract-and-kill strategy, we explored the potential of using pyrethroid treated screen as a killing agent for the trap. We determined the LT<sub>50</sub> of HB nymphs on PermaNet®, a deltamethrin-treated screen, and showed this product to be promising as trap construction material for managing HB.

SESQUITERPENOID PHEROMONE BIOSYNTHETIC PATHWAYS IN STINK BUGS (PENTATOMIDAE). Jason Lancaster<sup>1</sup>, Ashot Khimian<sup>2</sup>, Dawn Gundersen-Rindal<sup>2</sup>, Tom Kuhar<sup>3</sup> & Dorothea Tholl<sup>1</sup>, <sup>1</sup>Department of Biological Sciences, Virginia Tech, Blacksburg, VA 24061, <sup>2</sup>USDA-ARS, Beltsville, MD, 20705 and <sup>3</sup>Department of Entomology, Virginia Tech, Blacksburg, VA 24061. The stink bugs (Pentatomidae) harlequin bug (*Murgantia histrionica*), brown

marmorated stink bug (*Halyomorpha halys*), and southern green stink bug (*Nezara viridula*) are significant agricultural pests both in the United States and globally. The aggregation pheromones produced by these insects are known and their structures have been determined, however their use in pest management strategies is limited due to laborious synthetic chemistry and lack of knowledge of *de novo* biosynthetic pathways. This project aims to identify and functionally characterize farnesyl diphosphate synthase and sesquiterpene synthase genes in harlequin bug, brown marmorated stink bug and southern green stink bug. We hypothesize that Pentatomidae produce sesquiterpene aggregation pheromones *de novo* via a biosynthetic pathway utilizing a bisabolyl carbocation. We have cloned and expressed a gene from both harlequin bug and southern green stink bug whose encoding amino acid sequences are similar to other insect farnesyl diphosphate synthases but enzymatic assays produce primarily sesquiterpenes. We believe these genes are involved in production of the stink bug's aggregation pheromone and are currently investigating a similar gene in brown marmorated stink bug. This project will advance our understanding of the genetic mechanisms of terpene production in Pentatomidae and provide resources for novel, genetic based solutions.

OLD FOES, NEW LANDS: USING GCMS AND PCR TO EXAMINE PREDATOR-PREY RELATIONSHIPS IN THE INVASIVE POISON HEMLOCK PLANT. C. D. Alencar<sup>1</sup>, K. F. Haynes<sup>2</sup>, J. J. Obrycki<sup>2</sup>, K. J. Athey<sup>2</sup>, & J. D. Harwood<sup>2</sup>, <sup>1</sup>Biology Department, University of Virginia, Charlottesville VA, 22904 and <sup>2</sup>Entomology Department, University of Kentucky, Lexington KY, 40506. Poison hemlock, *Conium maculatum* (Apiaceae), is a long-established invasive plant of North America with a unique toxic chemistry. Previous research on this plant has focused on identifying potential biological control agents or describing the plant's suite of alkaloids. However, none have examined the role of higher trophic levels in the food web surrounding poison hemlock or the possibility that its unique chemistry imparts cascading effects. Generalist predators and food web interactions are an important component of studies investigating long-term invasion effects, as plant or animal introductions can alter ecosystem functioning. In this study, plant resources and predators foraging in poison hemlock were sampled at the foliar and ground levels. Predator-resource linkages were quantified using molecular gut-content and chemical analyses. Foliar *Harmonia axyridis* (Coccinellidae) contained aphid DNA and plant chemicals, while *Harpalus pensylvanicus* (Carabidae) only contained alkaloids, suggesting that ground predators obtain plant chemicals via alternative food resources. Feeding trials between *H. axyridis* and their potentially toxic prey, *Hyadaphis foeniculi* (Aphididae), showed quickened development in the exotic predator through immature stages when consuming aphids from poison hemlock compared to similar

alternative diets. This study revealed that three Eurasian species may be facilitating one another, illustrating the importance of continued examination of invasive species interactions.

NOVEL MODES OF PATHOGEN TRANSMISSION FROM MEDICALLY IMPORTANT ARTHROPOD VECTORS. Hameeda Sultana, Center for Molecular Medicine, Department of Biological Sciences, Old Dominion University, Norfolk, VA 23529. Molecular determinants and mechanisms of arthropod-borne flavivirus transmission to the vertebrate host are poorly understood. Research in my laboratory has shown that both tick and mosquito-borne flaviviruses use exosomes, the small membranous extracellular vesicles for transmission from arthropods to human host. Our studies have revealed that arthropod derived exosomes are important means of communication and transmission between the vector and the vertebrate host. We have found that Langkat virus (LGTV), a flavivirus member closely related to tick-borne encephalitis virus and mosquito-borne dengue viruses, are transmitted from vector to the vertebrate host through exosomes as novel modes of transmission. The exosomes containing LGTV and dengue viruses were viable, secured and highly virulent in all tests such as re-infection kinetics, trans-migration and viral plaque formation assays, suggesting exosomes as favorable modes of transmission. Both matured virions and replicative forms of arthropod-borne flaviviruses were found to be using exosomes for transmission. Our data also showed that arthropod derived exosomes facilitate infection of human cells that eventually produce exosomes loaded with flaviviruses. These transmission strategies used by flaviviruses to exit arthropods and infect human host were envisioned as best approaches to develop transmission-blocking vaccines against molecules or determinants that facilitate pathogen transmission.

PATHOGEN-VECTOR-HOST INTERACTIONS: NEW LESSONS LEARNED FROM THIS OLD CONNECTION. Girish Neelakanta, Center for Molecular Medicine, Department of Biological Sciences, Old Dominion University, Norfolk, VA 23529. In the United States, *Ixodes scapularis* ticks transmit various pathogens to humans that include *Anaplasma phagocytophilum*, the agent of Human anaplasmosis. Ticks ingest *A. phagocytophilum* while feeding on an infected animal. Upon entering, *A. phagocytophilum* establishes itself in the salivary glands and is then transstadially maintained through different developmental stages of these ticks. Our previous studies have elucidated that *A. phagocytophilum* manipulates arthropod cell signaling to survive in its vector. In the view for the development of an anti-vector vaccine as an effective means to block transmission of *A. phagocytophilum* from these ticks, understanding the role of conserved arthropod molecules in vector biology and interactions with pathogens

remains important. This study provides evidence for the role of some of the conserved arthropod molecules in tick-*A. phagocytophilum* interactions. RNAi knockdown studies and *in vitro* cell culture experiments elucidate the role of important arthropod conserved molecules in *A. phagocytophilum* transmission and survival in these ticks. Collectively, this study defines a new pathway not only to understand vector biology but may also lead to the development of better strategies to block transmission of *A. phagocytophilum* and perhaps other Rickettsial species from these ticks.

IMPACT ASSESSMENT OF *LARICOBIVS NIGRINUS* (COLEOPTERA: DERODONTIDAE), A PREDATOR OF HEMLOCK WOOLLY ADEGLID. A. Heminger<sup>1</sup>, A. Mayfield<sup>2</sup>, J. Elkinton<sup>3</sup>, G. J. Wiggins<sup>4</sup>, J. F. Grant<sup>4</sup>, J. Lombardo<sup>5</sup>, T. McAvoy<sup>1</sup>, A. Tait<sup>6</sup>, B. Mudder<sup>2</sup> & S. Salom<sup>1</sup>, <sup>1</sup>Virginia Polytechnic Institute and State University, Blacksburg, VA, 24061 <sup>2</sup>USDA - Forest Service, Asheville, NC 28804 <sup>3</sup>University of Massachusetts, Amherst, MA 01003 <sup>4</sup>University of Tennessee, Knoxville, TN 37996, <sup>5</sup>Dartmouth College, Hanover, NH 03755 <sup>6</sup>North Carolina State University, Asheville, NC. *Laricobius nigrinus* is a predator of hemlock woolly adelgid (HWA). HWA is a serious pest of eastern and Carolina hemlocks causing hemlock dieback and death. HWA is being controlled through several different methods including through the use of predators such as *L. nigrinus*. In the fall, we set up nine field sites in six states from New Jersey to Georgia. Three treatments were set up at each of the sites, cage (excluded *L. nigrinus*), no cage which allows for predation, and open cage to assess cage microclimate. The first sample was taken in October/November, the second in February/ March, and the third in March/April. The first sample documented HWA/cm, the second HWA mortality and signs of *L. nigrinus*, and the third to assess presence of *L. nigrinus*. The second year of the study was conducted at the same field sites. Results show that larval impacts appear greater than adult only; plots further south showed slightly higher rates of *L. nigrinus* activity, and *L. nigrinus* predation was seen at six out of the nine sites and *L. nigrinus* larvae was recovered from five off the nine in year 1. The study was funded by USDA Forest Service.

STRUCTURE ACCESS AND SETTLING SITE PREFERENCES OF OVERWINTERING BROWN MARMORATED STINK BUGS (*HALYOMORPHA HALYS*). B. D. Chambers<sup>1,2</sup>, T. P. Kuhar<sup>2</sup>, A. R. Pearce<sup>1</sup>, T. C. Leskey<sup>3</sup> & G. Reichard<sup>1</sup>, <sup>1</sup>Department of Building Construction, Virginia Tech, Blacksburg, VA 24061 <sup>2</sup>Department of Entomology, Virginia Tech, Blacksburg, VA 24061 <sup>3</sup>USDA-ARS, Kearneysville, WV 25430. The brown marmorated stink bug is an Asian invasive species that is spreading across the United States. In the

years since its introduction, it has become an important agricultural pest. It has also become a major nuisance in houses, where it often spends winters. This research examined some aspects of the entry and settling of these insects as they seek winter harborage in homes. One part tested movement directions of *H. halys* walking on building exteriors in the fall. A tendency towards upward movement was observed. A test of bugs disturbed from diapause in spring also suggested a tendency towards upward movement. To determine the limits on sizes of gaps in walls through which *H. halys* can pass, heat was used to encourage bugs to move through holes of various sizes. Passage was limited by rigid width and vertical compressibility. Males did not pass 3 mm tall or 7 mm wide holes, and females mostly did not pass 4 mm tall or 8 mm wide holes. The effect of the dead bugs often left over from previous years was examined with several tests. Non-tactile tests suggest the possibility that bugs may avoid clusters of dead bugs, but tactile tests suggested a willingness to aggregate with the dead.

INTERACTIONS OF HOST PLANT CHEMISTRY AND LEAF COLOR ON CABBAGE WHITE BUTTERFLY, *PIERIS RAPAE*. Wallis L. Hudson, Claire M. Ingram & Mary E. Lehman, Department of Biological and Environmental Sciences, Longwood University, Farmville VA 23909. *Pieris rapae* is known to detect the chemical content of plants that would be best suited for offspring development. Host plant chemistry and leaf color were manipulated to determine the main and interactive effects on ovipositional choices of *P. rapae*. Cabbage leaf disks were sprayed with deionized water (control) or 1.0 mM *p*-coumaric acid (PCO) or sinapic acid. The effects of these host chemicals and leaf color were first assessed individually and then in an interaction experiment. Females and cabbage leaf disks were placed in mesh enclosures and eggs were counted after a 24- hour period. When tested individually, PCO significantly stimulated oviposition and sinapic acid acted as a significant deterrent. In the interaction experiments, the main effects of both chemicals were not significant, but the main effect of leaf color was highly significant, with more eggs consistently laid on green than on white leaf disks. A significant interaction effect was only seen with sinapic acid. This study provides evidence that leaf color is an important determinant of ovipositional choices, an effect that may be strong enough to counteract or override the possible secondary cues of phenolic acid chemicals in host plants.

## Environmental Science

ECOLOGICAL WATERSHED MONITORING IN THE HEADWATERS OF THE NORTH FORK OF THE SHENANDOAH RIVER USING MACROINVERTEBRATE AND TURTLE SURVEYS. Diana Mendoza, Sam Stoner, Ryan Keiner, Douglas S. Graber Neufeld & James M. Yoder, Department of Biology, Eastern Mennonite University, Harrisonburg Virginia 22802. This study is part of an ongoing collaborative project developed by Eastern Mennonite University and various partners to address watershed health in the primarily agricultural community of Bergton, Virginia. Short-term goals of this project are to collect baseline data of watershed health at future restoration sites in the German River and Crab Run watersheds using stream macroinvertebrate and wood turtle population surveys. Virginia Stream Condition Index (VSCI) values from samples collected in the fall and spring of 2014-2016 indicated primarily good to excellent conditions (>65) within both watersheds, although marginal (<65) and stressed (< 59) VSCI values were seen at two sites, particularly at the first proposed restoration site. Fall VSCI values were generally lower overall as well, likely due to lower stream levels leading in the summer and early fall, negatively affecting the macro community in this reach. Wood turtles are an excellent indicator of long-term overall watershed health and are species of concern in the state of Virginia. Biannual surveys in 2014-2016 indicated an established but relatively small population (25 individuals) within the watersheds, likely due to lack of quality in stream habitat. Only 1 out of 25 turtles were juvenile, indicating reduced reproductive success of the population. Monitoring will continue as restoration efforts at multiple sites are completed over the next 2 to 5 years. This study is supported by a grant from the National Fish and Wildlife Foundation.

NUTRIENT LOADING, SEDIMENTATION AND OVERALL WATERSHED HEALTHASSESSMENT IN THE HEADWATERS OF THE NORTH FORK OF THE SHENANDOAH RIVER. Jesse H. Reist, Tyler Brennehan, James Yoder & Doug Graber-Neufeld, Dept. of Biol., Eastern Mennonite University, Harrisonburg VA 22802. A two year integrated water quality study was conducted in Bergton, Virginia to assess chemical parameters of stream health in headwaters of the North Fork of the Shenandoah River. The Shenandoah River, consisting of mountain headwater streams, feeds the Chesapeake Bay – keeping its water healthy is of importance for downstream marine life and commercial activity. Nine chemical parameters were assessed, including turbidity, nitrate, phosphate, ammonium, dissolved oxygen, coliform bacteria, temperature, pH, and conductivity in two sub-watersheds (Crab Run and German River) feeding the North Fork of the Shenandoah River. Comprised of 88% forested cover, the

watersheds are categorized as primarily forested in the upper reaches, with agriculture dominating the remaining 12% of the land use. Results were collected periodically during bi-monthly sampling, as well as during storm events (5-6 per year) and showed that primary parameters of concern were high summer temperatures ( $>20^{\circ}\text{C}$ ), sediment and nutrient loading, and elevated bacteria colony counts. Total sediment discharge for the watershed was estimated to be 1.4 million kg annually, and total phosphate and nitrate loading were estimated to be 365538 and 17326 kg/year, respectively. Impairment of all parameters of concern were higher in the Crab Run sub-watershed than in the German River sub-watershed. This study is supported by a grant from the National Fish and Wildlife Federation.

ENVIRONMENTAL FATE OF PIRLIMYCIN, A COMMONLY USED ANTIBIOTIC FOR DAIRY PRODUCTION. H. T. V. Le, K. M. Dayley & K. Xia, Department of Crop & Soil Environmental Sciences, Virginia Tech, Blacksburg VA 24060. A large amount of antibiotics have been used in livestock industry, which is of growing environmental concern since 40 to 95% of antibiotics administered to animals are excreted in feces and urine and enter the environment via manure land application. Released antibiotics not only are bioactive but also have potentials to increase antibiotic resistance in the environment. In this study, the environmental fate of pirlimycin (PLY), a commonly used lincosamide antibiotic in dairy production, was investigated. Since sorption of antibiotics into soil components can reduce their mobility to surrounding environment, the sorption isotherms of PLY to a clay soil and a sandy soil were studied using the batch equilibrium experiment. Rainfall simulation study was conducted to monitor movement of PLY in fields receiving dairy manure amendment via surface application and subsurface injection. The sorption of PLY to the clay and sandy soils followed Langmuir and Freundlich sorption isotherms, respectively. Sorption coefficients ( $K_d$ ) values were 124 and 67  $\text{L kg}^{-1}$  for the clay and sandy soils, respectively, indicating PLY has higher mobility in sandy soils comparing to clay soils. Manure subsurface injection concentrated PLY in the injection slits with limited horizontal diffusion beyond 15 cm from the slit, while surface application evenly distributed PLY throughout the soil surface. However, rainfall after manure application increased vertical and horizontal movement of PLY from the injection slits, resulting in detection of PLY in up to 20 cm soil depth at a distance of 40 cm from the slits. Similarly, rainfall increased PLY levels in 5-20 cm depth of the surface application plots. Mass balance study showed that after the rainfall, 5% of PLY was carried in runoff for surface application plots, while only 0.18% of PLY was detected in the runoff for the subsurface injection plots. Comparing to surface application, higher percentage of applied PLY appeared in the deeper profile of the injection slits after rainfall. This study suggested



that subsurface manure injection in sandy soil might result in enhanced downward movement of PLY due to its limit sorption to sandy soils compared to clay soils.

**WRECK ISLAND, VIRGINIA SHORELINE CHANGES SINCE 1999.** James D. Haluska, Department of Ocean, Earth, and Atmospheric Sciences, Center for Coastal Physical Oceanography, Old Dominion University, Norfolk, VA. 23529. The Virginia barrier islands stretch from Assateague Island in the north to Fisherman Island in the south. These islands are subject to Atlantic extra-tropical storm and hurricane influence and sea level rise. Wreck Island changes were determined from satellite and aircraft images using ArcGIS software and the USGS Digital Shoreline Analysis System (DSAS). Transects every 300 meters alongshore were calculated for the island using DSAS. The resulting shoreline locations from the remote sensing image dates were used to construct time series of shoreline movement for each transect location. From 1999 to 2004, the island's shoreline was relatively stable. In 2004, a significant input of sand, possibly due to increased tropical storm activity, widened the island. Since 2004, the rate of shore loss at all locations has been much lower than other islands in the barrier island group. Significant shoreline gain coincides with exceptional events such as several hurricanes in a short period of time (12 to 18 months) in 2004-2005 and the El Nino of 2009-2010. Rates of shoreline change are a maximum gain of up to 54 meters/year at the southern end and a loss of up to 29 meters/year at the northern end. The gain/loss makes the island appear to be rotating counter-clockwise. The island has also shifted southward with an apparent increase in this shift post-2004.

**VIRGINIA = FUTURE OF DROUGHT?** Richard S. Groover, Former member of the Governor's Climate Change and Resiliency Update Commission, Mechanicsville, VA 23116. This presentation covers evidence supporting that critical drought may occur in some portions of Virginia in this century. The most affected areas may be in south central and central Virginia, northern Virginia and several other smaller regions. Numerous agencies in the Commonwealth of Virginia predict deleterious impacts from drought. As state population increases over the next 30 years, there will be 32% increase in water demand, raising questions if Virginia's freshwater supply will be enough, especially as increased evaporation due to climate change occurs and as water demand increases. The projected surface water demand may increase by 86%. Groundwater levels in SE Virginia are critically low, yet expected demand for water from aquifers is expected to increase by 14%. Eighty-two percent of current riparian removal of surface waters

are unregulated, and a reevaluation of water withdrawal regulation is needed. If persistent drought occurs and withdrawal increases, negative abiotic conditions would occur for aquatic system's biota. The Virginia Department of Environmental Quality recommends increased water storage; thus three new large state-owned reservoirs are recommended in this presentation.

PRESENCE OF *BORRELIA*, *RICKETTSIA*, AND *EHRlichia* IN FIELD-COLLECTED TICKS ON CANDLERS MOUNTAIN, VA. Lara E. Colombo & Heather E. Stanley, Department of Biology, Liberty University, Lynchburg VA, 24515. Tick-borne disease is found throughout the world. Tick collection is required for disease surveillance and depends on a comprehensive understanding of ticks' habitats, life cycles, and diseases they may carry. A tick field collection was begun in March of 2015 on Candler's Mountain in Lynchburg, Virginia to begin investigation of the tick population at that site and to obtain ticks for testing for three disease-causing agents. CO<sub>2</sub> traps were utilized in the capture of the ticks. To create the most efficient trap, the tapes used on the traps were experimentally tested with a force transducer in the lab. The goal was to use traps that would capture the greatest number of ticks. The results from the experiment identified colored lab tape and duct tape as the most efficient tapes to use for the CO<sub>2</sub> traps. From March 2015 through June 2015, three deer ticks (*Ixodes scapularis*) and 59 lone star ticks (*Amblyomma americanum*) were collected. Analysis will be done on the captured ticks, looking for disease-causing agents. The individual tick DNA will be extracted, purified, isolated, amplified, and run on gel electrophoresis. The agents tested for will include those that cause Lyme disease, Rocky Mountain spotted fever, and Ehrlichiosis in humans. This is a long-term ecological project. Tick collection has resumed in March 2016 to continue analysis of the tick population on Candler's Mountain.

AN EVALUATION OF SHORELINE STABILIZATION EFFORTS IN BACK BAY, VIRGINIA. Reece K. O'Donnell & Heather D. Harwell, Dept. of Environmental and Organismal Biology, Christopher Newport University, Newport News, VA 23606. Five shorelines at False Cape State Park and Back Bay National Wildlife Refuge in Virginia Beach, VA will be stabilized as part of a larger effort to design a regional network of effective adaptation projects to address the broad array of climate change impacts faced by human and wildlife populations. The purpose of this study is to evaluate the effectiveness of the living shorelines created to mitigate shoreline erosion. Analysis of vegetation communities pre- and post-construction and monthly erosion rate measurements will be performed to determine the effectiveness of each created living shoreline. We expect that the stabilization of these sites

using a living shoreline will drastically reduce erosion rates compared to reference sites, and will bring rise to a sustainable, native vegetation community that is different from the original composition of the site. This work will test the efficacy of living shoreline stabilization as a viable method of wetland restoration in Back Bay, and will contribute to the growing body of literature that supports this methodology.

A MILLENNIAL RECORD OF MARSH ACCRETION IN THE TIDAL REACHES OF THE POTOMAC RIVER. Megan K Clevenger<sup>1</sup>, Neil E. Tibert<sup>1</sup>, J.B. Hubeny<sup>2</sup>, Tom Cronin<sup>3</sup>, Tammy Prescott<sup>1</sup> & R.C. Lyle<sup>1</sup>, <sup>1</sup>University of Mary Washington, <sup>2</sup>Salem State University, <sup>3</sup>United States Geological Survey. Tide gauge records in the Chesapeake Bay region, eastern US, indicate that relative sea-level rise over the last century is due to a combination of melting land ice, ocean thermal expansion, and subsidence, mainly due to glacio-isostatic adjustment. However, there has been little research to establish a millennial record of marsh accretion to quantify pre-20th century rates of Late Holocene sea level rise in the proximal-central estuary of the Potomac River downstream of Washington DC. Our primary objective is to establish a baseline physical, biological, and geochronological record of marsh deposits spanning the past three millennia. Three marsh cores were collected from Mattox Creek, Rosiers Creek, and Wilkerson Creek; tidal creeks adjacent to the Potomac River, Virginia. The cores range in thickness from 5.0-6.7 meters in length and comprise two primary lithofacies of basal grey clay and an upper organic-rich peat and clay. The grey clay lithology ranges in Total Organic Matter (TOM) from 4-20% and has highly variable magnetic susceptibility intensity peaks. In contrast, the alternating organic rich peat and clay ranges in TOM from 14-82% and has negligible variability with respect to magnetic susceptibility values. Microfossils extracted from the cores include an association of marsh and estuarine foraminifera that include *Ammonoastuta inepta*, *Miliammina fusca*, *Trochammina inflata*, *Jadammina macrescens*, and *Ammobaculites*. Results of AMS<sup>14</sup>C of woody matter, peat, and skeletal calcite yield a basal age of the cores as 3540 ybp at 5.01 meters below the marsh surface.

HEALTH ASSESMENT OF MASSAPONAX CREEK AND THE NI RIVER IN SPOTSYLVANIA COUNTY, VA IN 2015-2016. Cameron Stewart, Jessalyn Cockrell, Elizabeth Weast & Michael L. Bass, Department of Earth & Environmental Sciences, University of Mary Washington, Fredericksburg, VA 22401-5300. This research is a continuation of work starting in 2012 to compare the health of Massaponax Creek and the Ni River, in Spotsylvania County, VA, by benthic macroinvertebrate sampling and water quality analysis. Massaponax Creek flows through areas stabilizing after development.

The Ni River flows through a more rural area, though there is planned development of Ni Village community surrounding the river. Total Suspended Solids (TSS) and Total Dissolved Solids (TDS) were determined by vacuum filtration and Fecal Coliform levels by MF technique. TSS were greater in the Ni River and TDS were greater in Massaponax Creek. Fecal Coliform concentrations were higher in Massaponax Creek in 2015. Massaponax Creek also had a higher average DO concentration and Conductivity. Aquatic insects were separated by order to calculate %EPT. The %EPT of both streams was just under 80%. Results from 2012 found greater variety of the EPT orders in the Ni River, whereas 2015 showed similar variation in both of the streams. Analysis of samples showed average Phosphate, Nitrate, Hardness and Alkalinity were higher in Massaponax Creek. In conclusion, Massaponax Creek appears to be recovering after the stabilization. Further research is necessary to determine if the pattern of recovery found in Massaponax Creek would be the result for the Ni River if the surrounding area were to be developed.

## Medical Science

HIGH DIETARY SALT AND FRUCTOSE INCREASE NFAT5 EXPRESSION IN THE KIDNEY AND LIVER OF SPRAGUE DAWLEY RATS. B. Herman<sup>1</sup>, K. Ferguson<sup>1</sup>, J. Halterman<sup>1, 2</sup> & J. Fernandez<sup>2</sup>, <sup>1</sup>Dept. of Biology and <sup>2</sup>MA in Biomedicine Program, Eastern Mennonite University. In the current diet of an average American, there is an increasing amount of both salt and high fructose corn syrup. Individuals with a higher sensitivity to salt or fructose can develop hypertension and metabolic syndrome. This study aimed to determine how the consumption of a high salt diet or a high fructose diet altered tissue-specific expression of the NFAT5 gene. A total of 50 Sprague Dawley rats were put either on a control diet of 0.25% NaCl and 6% fructose, a 4% NaCl diet, 8% NaCl diet, or 64% fructose diet over the course of 8 weeks. After 8 weeks, 16 different body tissue samples were harvested. RNA was then purified from the samples, reverse transcription was used to convert the purified RNA into DNA, and DNA samples were then run under real-time PCR in order to measure expression of the NFAT5 gene. The results showed an increase in NFAT5 expression in the kidney medulla under a 4% NaCl diet and in the kidney cortex under a 64% fructose diet. The results also displayed an increase in NFAT5 expression in the liver under a 64% fructose diet and 8% NaCl diet. From this study it can be concluded that NFAT5 is differentially expressed in different tissues in response to various diets. This study was funded by The Thomas F. and Kate Miller Jeffress Memorial Trust, Bank of America, Trustee.

ROLE OF THE GASTROINTESTINAL MICROBIOME IN OPIOID TOLERANCE. R.A. Mischel, M. Kang, W. Dewey, & H.I. Akbarali, Dept. of Pharmacology & Toxicology, Virginia Commonwealth University, Richmond, VA 23298. Gastrointestinal microbial dysbiosis is known to alter physiologic homeostasis and contribute to pathogenesis. Though morphine and other narcotics are the most widely prescribed therapy for moderate to severe pain, they have been noted to alter microbial composition and promote bacterial translocation to other tissues. Translocated microbes may then modulate local cell signaling and gene expression. One of the most immediately vulnerable compartments following bacterial dissemination is the intestinal wall, containing many terminal processes of extrinsic primary afferent neurons (EPANs) from dorsal root ganglia (DRG). These neurons play an integral role in analgesic tolerance, a major limiting factor of clinical narcotic use. Despite this, the impact of intestinal microbiota on the development of tolerance in these cells has not been well characterized. To this end, we investigated how bacterial depletion via broad-spectrum antibiotic treatment (ABX) impacts nociceptive tolerance with chronic morphine use in mice. We found

that ABX was effective in preventing tolerance in both the tail-immersion and acetic acid stretch assays of behavioral nociception. These findings were recapitulated on a single-cell level in neurons isolated from DRGs; namely, ABX prevented tolerance development to morphine-induced reductions of excitability, as measured by increases of threshold potential in whole-cell current clamp recordings. These findings suggest that gastrointestinal flora play an important role in modulating the pharmacodynamic properties of morphine in mice, and may be a useful target of therapy in man.

**CORRELATION OF MOLECULAR MARKERS WITH QUANTIFIED FIBROSIS LEVELS OF NON-NAFLD, NAFLD, AND NASH LIVER SAMPLES.** S. Stoddard<sup>1,2</sup>, Z. D. Goodman<sup>3</sup>, A. Birerdinc<sup>2</sup> & A. Baranova<sup>1,2</sup>, <sup>1</sup>School of Systems Biology, College of Science, George Mason University Fairfax, VA, <sup>2</sup>Center for the Study of Chronic Metabolic Diseases, George Mason University, Falls Church, VA, <sup>3</sup>Inova Health System, Falls Church, VA. Global prevalence of nonalcoholic fatty liver disease (NAFLD) is currently at 25%, and is expected to increase as a major public health concern due to the current worldwide obesity epidemic. The spectrum of NAFLD includes nonalcoholic steatohepatitis (NASH) and steatohepatic hepatocellular carcinoma (SH-HCC). Not all patients with NAFLD progress to NASH, and the diagnosis and grading of NAFLD/NASH is dependent on the “gold standard” of invasive liver biopsy. Molecular mechanisms behind the development and progression of this disease are poorly understood, and this study attempts to correlate serum biomarkers and adipocyte gene expression with quantified fibrotic liver changes in NAFLD patients. Liver biopsies from obese non-NAFLD, NAFLD, and NASH patients were analyzed via computerized morphometry to quantify levels of steatosis and fibrosis. Preliminary results of immunoassays on serum samples indicate a negative correlation of INF- $\gamma$  ( $p < 0.05$ ), IL-4 ( $p < 0.05$ ), and G-CSF ( $p < 0.01$ ) with percent collagen of liver biopsies. qPCR of adipose tissue will also be performed for further understanding of this tissue as a driver of the disease process. Correlation of serum biomarker levels with amount of fibrosis as determined by computerized morphometry will be analyzed to assess potential biomarkers for use as a diagnostic and/or prognostic tool.

**UNDERSTANDING OMICS PROFILING EXPRESSION BY USING DISTANCE-BASED ANALYSIS AND NETWORK BIOLOGY.** T. Cui, J. R. Hamre & A. V. Baranova, School of Systems Biology, George Mason University, Manassas VA. 20110. With large amount of Omics data being generated every day, it's important to link the omics profiling expression to patients who have varying degrees of disorder. In this study, we propose a novel approach integrating distanced-based

analysis and network biology to analyze transcriptomics and proteomics data. We used several datasets including mRNA-Seq, miRNA-Seq and proteomics datasets with different disease states from GEO, TCGA and our collaborator at NIH. Samples from each group were clustered close to the attractor that defines their corresponding space center by global distances. The invasive breast carcinoma mRNA-Seq dataset from TCGA showed a substantial separation between normal/cancer samples. Additionally, the comparison between colon adenocarcinoma miRNA-Seq and mRNA-Seq plot showed a better separation between different disease states from the former, which suggests the holistic miRNA landscape has superior predictive power and can be used as a better diagnostic and prediction tool for reflecting pathophysiological states of human tumors. Lastly, we applied our model to a HDL proteomics dataset that is from 101 patients who have varying degrees of cardiovascular disease. We were able to find four key proteins that may be responsible for the disease severity. In conclusion, using the measurement of Pearson's holistic distances together with network biology analysis, we demonstrated that the omics profiling can be used to reflect sample's regulatory landscapes of overall gene/protein expression signatures, and help us understand the role of diseased-associated genes/proteins in the complex system.

NFAT5 IS DIFFERENTIALLY REGULATED IN THE BRAIN AND BLADDER OF RATS FED A 4% NACL, 8% NACL, AND 64% FRUCTOSE DIET. K. M. Ferguson, J. V. B. Fernandez & J. A. Halterman, Dept. of Biology, Eastern Mennonite University, Harrisonburg VA 22802. This research investigates the effects of a 4% NaCl, 8% NaCl, 64% fructose, and control diet on the expression of Nuclear Factor of Activated T-Cells 5 (NFAT5) in rat tissues. In human health, diets low in salt lower the risk of cardiovascular disorders and diets low in fructose lower the risk of metabolic disorders. However, "low in salt/fructose" is a relative term because studies have demonstrated individual differential responses to the same diets. In this study, the effects of salt/fructose diets on the expression of NFAT5 were examined in the bladder and brain of Sprague Dawley rats. This was done by purifying and quantifying RNA, creating cDNA, and quantifying NFAT5 expression by real-time PCR. The bladder tissue showed decreased expression of NFAT5 following consumption of an 8% salt and 64% fructose diet. The brain tissue showed NFAT5 expression is increased following consumption of a 4% salt diet. These results suggest that NFAT5 is differentially regulated in various tissues of the body in response to dietary changes. Further inquiry into the expression of NFAT5 in other tissues is needed in order to obtain a comprehensive understanding of NFAT5 regulation in the body.

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EXPLORING PHARMACOLOGICAL AND BEHAVIORAL MECHANISMS INVOLVED IN ALCOHOL DEPENDENCE DURING ADOLESCENCE. Rabha M. Younis<sup>1,2</sup> & Imad Damaj<sup>2</sup>, <sup>1</sup>Dept. of Microbiology & Genetics, <sup>2</sup>Dept. of Pharmacology & Toxicology, Virginia Commonwealth University, Richmond, VA, 23219. Alcoholism is a serious illness that is marked by uncontrollable drinking and physical dependence to alcohol. Long-term alcoholism has been linked to many health concerns such as cirrhosis of the liver and cardiovascular disease. Alcohol is one of the most commonly used drugs among adolescent populations. Given that adolescence is a unique developmental stage during which alcohol has long-term effects on future drug-taking behavior; it is essential to understand how early exposure to alcohol during adolescent may affect the abuse liability of the drug later in life. Our studies focus on identifying behavioral mechanisms involved in alcohol dependence during adolescence by using well-established mouse models of alcohol drinking. We hypothesize that exposure to alcohol during early adolescence will increase alcohol intake later in adulthood. We investigated the impact of alcohol drinking in male and female early adolescent C57BL/6J mice using the Drinking in the Dark (DID) model. Our results showed that exposure to alcohol during early adolescence enhanced ethanol intake later in adulthood in the DID paradigm. Our data illustrates that enhanced alcohol intake are affected by the duration and age of exposure. In addition, we conducted behavioral studies to elucidate the mechanisms underlining the relationship between adolescent ethanol exposures and enhance alcohol intake in adult mice. Our results showed that ethanol exposure during adolescence altered the aversive state and enhanced the rewarding properties of ethanol later in adulthood.

A FORMAL EXAMINATION OF TIMING OF HEART SOUNDS RELATIVE TO ECG EVENTS IN COLLEGE STUDENTS. Harold J. Grau & Michaela Miller, Dept. of Molecular Biol. & Chem., Christopher Newport Univ., Newport News, VA 23606. During each cardiac cycle, the first heart sound (S1) is produced after the ventricle begins contracting (enters systole), the pressure from which causes the atrioventricular valve to slam shut. Once the ventricle begins relaxing (ends systole), the semilunar valves close, creating the second heart sound (S2). We recorded electrocardiograms (ECGs) and heart sounds from 29 female (n=17) and male (n=12) college students (all between the ages of 20 – 22 years of age) to see if there were any patterns to variations in the timing of these heart sounds relative to the beginning of systole (the peak R wave on the ECG) and end of systole (peak T wave on the ECG). We also looked at other parameters such as heart



rate, height, weight, and Basal Metabolic Index (BMI) to see if any correlations occurred. There were no significant differences between males and females (although there were differences in the distribution of both heart sound delays). The timing of the heart sounds did not correlate with each other, nor with heart rate, but did show a significant (S1:  $P < 0.01$ , S2:  $P < 0.05$ ) correlation to the BMI. Heart rate and BMI did not correlate, but heart rate did correlate inversely ( $P < 0.05$ ) with the interval between S1 and S2, which was also inversely correlated significantly with the S1 delay ( $P < 0.01$ ) and with heart rate ( $P < 0.05$ ). A longer S1 delay means that the ventricle takes longer to reach the valve-closure pressure; a longer S2 delay indicates a longer period for the ventricular pressure to fall below aortic pressure. The fact that these are positively correlated with BMI suggests a possible health link that merits further investigation.

EFFECTS OF METHYLENE BLUE ON THE ONSET AND PROGRESSION OF BEHAVIORAL DEFICITS IN A TRANSGENIC MOUSE MODEL OF ALZHEIMER'S DISEASE. H. J. Grau, S. E. Fink, Q. E. Pace, B. C. Genovese, N. Khan, E. Croushore, K. Whitcomb, R. A. Schendzielos, D. A. Mitrano & L. S. Webb, Dept. of Molecular Biol. & Chem., Christopher Newport Univ., Newport News, VA 23606. Alzheimer Disease (AD) is a degenerative form of dementia that is associated with the accumulation of neurofibrillary tangles (tau protein) and amyloid= $\beta$  (AB) plaque formation. Methylene blue (MB) has been FDA approved for reducing protein aggregations in AD and other diseases. In this study, we used a triple transgenic mouse model of AD (3xTg-AD) to assess any effects of MB on the formation of these protein tangles and deposits development (reported elsewhere) and on spatial learning and memory tasks (reported here), by comparing mice given weekly intraperitoneal injections of MB with those given saline injections (controls). We used the Morris Water Maze (MWM) to test for spatial learning and memory on mice at 3, 4.5, 6, 7.5, 9, 12, 15, and 18 months of age; mice were given 5 days of training (with platform), and then tested (no platform) on day 6. The completed study will have 12 mice for each treatment at each age end-point; the data reported here include results from about half of that total. While the results to date are not conclusive, mice at 6 and 7.5 months of age did perform better at the MWM training (spatial learning) than their saline counterparts. On the test day, which assesses spatial memory, MB treated mice did better at 3, 6, and 15 months of age. Differences between the groups should become more evident once the data are complete.

NEUROPATHOLOGICAL EFFECTS OF METHYLENE BLUE ON THE ONSET AND PROGRESSION OF ALZHEIMER'S DISEASE IN A TRANSGENIC MOUSE MODEL. R. A. Schendzielos<sup>1</sup>, S. E.

Fink<sup>1,2</sup>, Q. E. Pace<sup>1</sup>, N. Kahn<sup>2</sup>, B. C. Genovese<sup>1</sup>, E. Croushore<sup>2</sup>, K. Witcomb<sup>1</sup>, D. Mitrano<sup>1,2</sup>, L. S. Webb<sup>1,2</sup>, & H. J. Grau<sup>1,2</sup>, <sup>1</sup>Dept. of Molecular Biology & Chemistry and <sup>2</sup>Program in Neuroscience, Christopher Newport University, Newport News VA 23606. Alzheimer's disease (AD) is a neurodegenerative disease that has been shown to cause neurological changes in the brain, including the development of both amyloid-beta (A $\beta$ ) plaques and neurofibrillary (tau) tangles, as well as impaired cognitive functioning. Past studies have shown that Methylene blue (MB), an FDA approved compound, can reduce the formation of protein aggregates in AD and other diseases. In this study, the 3xTg-AD mouse model, which contains the human transgenes PS1<sub>m146V</sub>, APP<sub>Swe</sub>, and Tau<sub>p301L</sub>, was used to further explore the effect that MB has on the development of AD, specifically on the accumulation of the A $\beta$  plaques and tau tangles. Weekly intraperitoneal injections of either MB (10 mg/kg) or 0.9% saline were given, starting at 4 weeks and continuing until either 3, 4.5, 6, 7.5, 9, 12, or 15 months of age. At these endpoints, the mice were transcardially perfused with 4% paraformaldehyde/0.1% glutaraldehyde, and the brains were removed and fixed for 72 hours in 4% paraformaldehyde and then cut with a vibrating microtome at 60 $\mu$ m. The tissue samples were stained with the clone 6E10 mouse monoclonal antibody for A $\beta$  plaques and with the p-tau rabbit antibody for the tau tangles. Preliminary qualitative observations of the tissues suggest that the MB-treated mouse brains had less A $\beta$  and tau accumulation; future work will incorporate quantitative analyses.

ROLE OF VAV2 IN PODOCYTE INFLAMMASOME ACTIVATION AND GLOMERULAR INJURY DURING HYPERHOMOCYSTEINEMIA. S. M. Conley<sup>1</sup>, Z. Chen<sup>1</sup>, M. Xia<sup>1</sup>, T. W. Gehr<sup>2</sup>, K. M. Boini<sup>1,2</sup> & P.L. Li<sup>1</sup>, <sup>1</sup>Dept. of Pharmacology & Toxicology, <sup>2</sup>Division of Nephrology, Internal Medicine, Virginia Commonwealth University, School of Medicine, Richmond, VA. Recently, our lab has reported that Vav2, a member of the guanine nucleotide exchange factor (GEF) family contributes to the activation of NADPH oxidase (NOX) in a membrane lipid signaling platform in response to elevated levels of homocysteine (Hcys). However, it remains unknown whether Vav2-mediated NOX activation is able to trigger the NLRP3 inflammasome in podocytes and thereby lead to podocyte dysfunction and glomerular injury associated with hyperhomocysteinemia (hHcys). In our experiments, murine podocytes were pretreated with either a Rac-1 inhibitor, NSC23766 or Vav2 activator, uridine triphosphate (UTP) and then stimulated with Hcys for 24 hours. Confocal microscopic analysis showed that treatment with UTP increased the colocalization of inflammasome proteins NLRP3 with ASC or with caspase-1, suggesting inflammasome formation. However, pretreatment of podocytes with NSC23766 blocked Hcys-

induced inflammasome formation. Similar to Hcys, Vav2 activator, UTP increased caspase-1 activation and consequent IL-1 $\beta$  production in podocytes. However, attenuated caspase-1 activation and lower IL-1 $\beta$  levels were observed when podocytes were treated with NSC23766. Our results suggest that Vav2 is a key signaling molecule in mediating Hcys-induced podocyte inflammasome formation and activation and consequent podocyte dysfunction and glomerular injury.

NICOTINE PREVENTS CHEMOTHERAPY-INDUCED PERIPHERAL NEUROPATHY *IN VIVO*, AND FAILS TO STIMULATE THE GROWTH OF LUNG CANCER CELLS OR INTERFERE WITH THE EFFECTIVENESS OF CHEMOTHERAPY *IN VITRO*. S. L. Kyte<sup>1</sup>, W. Toma<sup>1</sup>, M. I. Damaj<sup>1</sup>, X. Fang<sup>2</sup> & D.A. Gewirtz<sup>1</sup>, <sup>1</sup>Dept. of Pharmacology & Toxicology and <sup>2</sup>Dept. of Biochemistry & Molecular Biology, Virginia Commonwealth University, Richmond, VA 23298. Chemotherapy has played a significant role in the treatment and survival of cancer patients. However, its use can lead to long-term symptoms of drug toxicity, including chemotherapy-induced peripheral neuropathy (CIPN), a result of peripheral nerve fiber dysfunction or degeneration. Paclitaxel (Taxol), a taxane commonly used to treat breast, lung, and ovarian cancers, has been found to cause CIPN in 59 to 78% of patients. There is currently no effective preventative or therapeutic treatment for this side effect. Our studies revealed that the nicotinic acetylcholine receptor (nAChR) agonist, nicotine, is capable of reversing and preventing the development of paclitaxel-induced CIPN *in vivo*, and does not interfere with the cytotoxic properties of paclitaxel *in vitro*. The use of von Frey filaments revealed that nicotine dose-dependently reverses and prevents paclitaxel-induced mechanical allodynia. The *in vitro* studies showed that nicotine fails to significantly stimulate growth of A549, H460, Lewis lung carcinoma, or human explant lung cancer cells. Most importantly, paclitaxel-induced H460 growth inhibition was not significantly attenuated by nicotine. Moreover, nicotine failed to alter the sub-G1 DNA content of paclitaxel-treated A549 cells. These findings suggest that nAChRs may be promising drug targets for the prevention and treatment of CIPN.

DOWNREGULATION OF MYELIN GENE EXPRESSION IN THE ENTORHINAL CORTEX OF FINGOLIMOD TREATED MICE. Jessica L. Jurmain & Michael F. Miles, Dept. of Pharmacology & Toxicology, Virginia Commonwealth Univ., Richmond, Virginia, 23298. Studies in human and mouse models of alcoholism suggest a role for myelin in the development of alcohol use disorders (AUDs) and their associated pathologies. Myelin genes are downregulated in the frontal cortex of alcohol dependent patients and in mouse models of AUD. Studies in C57BL/6J and DBA/2J mice show correlations

between basal myelin gene expression and behavioral responses to acute ethanol exposure. Fingolimod (FTY720) is an FDA approved drug for the treatment of relapsing multiple sclerosis. Literature reports on FTY720 suggest a direct role for its receptor in demyelination and remyelination. The significant overlap of genes regulated in hippocampus of FTY720-treated immune-deficient mice with an ethanol responsive gene set from our laboratory included several myelin genes. This may suggest a common mechanism between the effects of FTY720 and chronic ethanol treatment on myelin gene expression that may be useful in elucidating a treatment for the myelin-related pathologies associated with ethanol use. Using qRT-PCR we examined the effects of oral FTY720 on myelin gene expression in ethanol-naïve mice. There were no significant changes in myelin gene expression in medial prefrontal cortex, caudate putamen, or nucleus accumbens. Two myelin genes, *Mbp* and *Plp*, were significantly downregulated in the entorhinal cortex of FTY720 treated mice, and this downregulation was strongly correlated with the downregulation of *NFκB* and *Tlr4* expression. This suggests that FTY720 may aid in elucidating a mechanism for demyelination associated with chronic ethanol use.

MISSENSE MUTATIONS IN GONADOTROPIN-RELEASING HORMONE RECEPTOR GENE IN PATIENTS WITH NORMOSMIC IDIOPATHIC HYPOGONADOTROPIC HYPOGONADISM. N. V. Zernov<sup>1</sup>, M. Y. Skoblov<sup>1,2</sup>, A. V. Baranova<sup>1,2,3,4</sup> & K. Y. Boyarsky<sup>5</sup>, <sup>1</sup>Federal State Budgetary Institution Research Centre for Medical Genetics, Moscow, 115478, Russia, <sup>2</sup>The Moscow Institute of Physics & Technology, Dolgoprudny, Moscow Region, 141700, Russia, <sup>3</sup>Center for the Study of Chronic Metabolic Diseases, School of Systems Biology, College of Science, George Mason University, Manassas, VA, USA. <sup>4</sup>Atlas Biomed Group, Moscow, 123317, Russia and <sup>5</sup>Center of Human Reproduction "Genesis", Department of Obstetrics and Gynecology, State Pediatric Medical University, St.-Petersburg, 194100, Russia. Isolated hypogonadotropic hypogonadism (IHH) is a rare genetic disease occurring in about 1-10 cases per 100,000 births. In only 40% of these patients the sense of smell is unchanged. This normosmic IHH (nIHH) is due to a failure of gonadotropin-releasing hormone (GnRH) pulsatile secretion in hypothalamus or its action in pituitary. In nIHH patients, mutations are identified in genes *GnRH1*, *GnRHR*, *KISS1*, *KISS1R*, *TAC3* and *TAC3R*. We present our observations of two non-consanguineous Russian female patients diagnosed with nIHH. Prior to referral to fertility clinic, each patient underwent about 10 years of hormone replacement therapy. Treatment was successful in both patients who delivered phenotypically healthy offspring. Direct sequencing of *GnRHR* gene identified homozygous mutation c.416G>A in one patient and compound heterozygous c.

416G>A/c.806C>T in another patient. To our knowledge, this is the first observation of successful pregnancy of patients lacking GnRHR function.

HAS THE ERADICATION OF THE POLIO VIRUS CONTRIBUTED TO THE RISE IN SOLID TUMOR CASES IN RECENT YEARS? K. Gwilliam<sup>1</sup>, A. Baranova<sup>1,2</sup> & A. Birerdinc<sup>1,2</sup>, <sup>1</sup>Center for Study of Chronic Metabolic Disease, College of Science, George Mason University, Fairfax, VA 22030 and <sup>2</sup>Betty and Guy Beatty Center for Integrated Research, INOVA Health System, Falls Church, VA 22042. A genetically altered version of the polio virus is being used in a Phase I study to treat glioblastoma multiforme (GBM) at the Preston Robert Tisch Brain Tumor Center at Duke University by Dr. Gromeier and his team. The genetically engineered poliovirus, PVS-RIPO, infects cells that have poliovirus receptors yet is disabled from replicating in normal, healthy cells. This raises the question of why and how polio virus can target glioblastoma cells and its evolutionary origins. This study uses publicly available databases, including the World Health Organization, to assess the administration of the inactivated and oral polio vaccine and the correlation incidence of polio and solid tumors reported on a global scale. Out of 69 countries studied, from five WHO regions, most displayed a decline in the incidence of poliomyelitis from 1988 onward. Many of the 69 countries also showed an increased number of cancer cases between 1983 to 2007. A multivariate analysis will be performed to determine if there is a correlation between the eradication of polio in the human population and the rise in solid tumors. Outcomes could reveal the polio virus' ability to regulate solid tumors as well as possible influences that vaccination may have had on this regulation. This information could assist in better administering the polio virus for the treatment of solid tumors, such as GBM, as well as provide valuable mechanistic and evolutionary insight into the co-evolution of the polio virus and humans.

EPIGENETICS IN THE ETIOLOGY OF CHRONIC DISEASES. K. Y. Jeong, A. Birerdinc & A. Baranova, Dept. of Biology, George Mason University, Fairfax VA 22030 and Betty and Guy Beatty Center for Integrated Research, Inova Health System, Falls Church VA 22042. Several recent lines of research have shown that in addition to a genetically predisposed profile, certain epigenetic factors play an important role in both the development and progression of certain chronic diseases such as NAFLD. Particularly for diseases under the umbrella of Metabolic Syndrome, it has been demonstrated that epigenetic as well as genetic factors play a crucial role in both the presence and severity of these comorbidities. This article focused on incorporating the epigenetic regulations found with the etiological

pathways of chronic diseases. A systematic review was performed to assess the work done to date. The two main investigated areas inflicted by Metabolic Syndrome include the heart and the liver. Results found that the accumulation of visceral fat increases expression of lipase genes in the liver, leads to greater FFA mobilization and results in hyperlipidemia linked with Steatosis and cardiovascular diseases. Recruitment of cytokines released by adipocytes may be linked to increased DNA methylation of glucokinase gene and hypomethylation of hepatic cell cycle inhibitor *Cdkn1a*, which led to decreased and increased expression, respectively, and contribute to NAFLD. The subsequent review paper will aim to summarize and consolidate the relationship between the epigenetic and genetic contributions to chronic diseases under the umbrella of Metabolic Syndrome.

LIPID PROFILING IN CARDIOVASCULAR RISKS. S. Srivangipuram<sup>1,3</sup>, T. Cui<sup>2</sup>, J. Hamre<sup>2</sup>, B. Veytsman<sup>2</sup>, A. Birerdinc<sup>2,3</sup> & A. Baranova<sup>1,2,3</sup>, <sup>1</sup>College of Science, George Mason Univ., Fairfax VA 22030, <sup>2</sup>School of Systems Biology, George Mason Univ., Manassas 20110, <sup>3</sup>Center for Study of Chronic Metabolic Diseases, George Mason Univ., Falls Church., VA., Inova Health System, Falls Church., VA 22042. Different classes of High-density lipoproteins transport cholesterol in blood and their inverse correlation with cardiovascular disease (CVD) is well known. It is believed that HDL facilitates the removal of excess cholesterol from peripheral tissues and delivers it to the liver for excretion. Mass spectrometry was employed to identify 187 proteins and the amount of given protein detected in blood was collected from 101 patients who have varying levels of cardiovascular disease as measured by CT angiography. The HDL proteomics data was displayed as a weighted spectrum count. The statistical software “R” was used to perform descriptive and inferential statistics on the data. To see if the proteins are expressed differently in diseased samples, a preliminary analysis was conducted by comparing the median distance of the counts to the normal space center. Since the mild severity group was closer to the normal, these two groups were combined for distance based analysis. There was not a gravitation of the counts towards a specific severity. A Wilcoxon signed-rank test was used to test for significant differences in protein expression among the different severity groups ( $\alpha=0.05$ ). There was no significant difference in protein expression between normal to moderate group, normal to severe, and moderate to severe. The results suggest for further analysis by separating the HDL binding proteins to see if they contribute to disease severity.

FIBRONECTIN FIBRIL-ASSOCIATED GROWTH FACTORS IN BREAST CANCER MICROENVIRONMENTS. Pascal Shukuru & Lynne Elmore, Dept. of Pathology, Virginia Commonwealth University,

Richmond VA 23298. Fibronectin (FN) is expressed in many breast cancers (BC) and implicated in tumor progression. FN forms fibrils, which can bind >40 soluble growth factors, many with pro-oncogenic properties. Data indicate that breast adipose-derived mesenchymal stem cells (bMSCs) promote BC cell growth and invasion as well as the development of a FN-rich extracellular matrix. These resident stem cells also express numerous FN binding growth factors, including TGF- $\beta$ 1, and assembly FN fibrils, which bind latent TGF- $\beta$ 1 binding protein 1 (LTBP1), an activator of TGF- $\beta$ 1. These experimental data prompted us to investigate whether TGF- $\beta$ 1 and/or LTBP1 co-localize with FN fibrils in clinical specimens of BC. Tissue microarrays were stained with Masson's Trichrome to identify fibrosis. Immunohistochemistry was performed to assess protein expression levels and localization of TGF- $\beta$ 1 and LTBP-1. Many BC specimens exhibited abundant FN-rich fibrotic stroma. TGF- $\beta$ 1 co-localized with FN fibrils in the extracellular matrix of many BC tissues, while LTBP1 was expressed at variable levels in BC and stromal cells. These data provide a foundation for testing whether co-localization of FN binding growth factors (or mediator of these growth factors) and FN fibrils associate with severity of disease.

ABUSE-RELATED EFFECTS OF GABA<sub>A</sub> RECEPTOR POSITIVE ALLOSTERIC MODULATORS IN AN ASSAY OF INTRACRANIAL SELF-STIMULATION IN RATS. K.L. Schwientek<sup>1</sup>, G. Li<sup>2</sup>, M. M. Poe<sup>2</sup>, J. M. Cook<sup>2</sup>, M.L. Banks<sup>1</sup> & S.S. Negus<sup>1</sup>, <sup>1</sup>Dept. of Pharmacology & Toxicology, Virginia Commonwealth University, Richmond, VA, 23298 and <sup>2</sup>Dept. of Chemistry & Biochemistry, University of Wisconsin-Milwaukee, Milwaukee, WI, 53211. GABA<sub>A</sub> receptor positive allosteric modulators (GABA<sub>A</sub> PAMs) are used clinically but also have abuse liability. Novel GABA<sub>A</sub> PAMs have been developed that vary in efficacy at, and selectively for, GABA<sub>A</sub> receptor subtypes that contain  $\alpha$ 1,  $\alpha$ 2 or  $\alpha$ 3 subunits. Intracranial self-stimulation (ICSS) is one preclinical procedure that has been used to evaluate abuse potential of drugs. This study compared effects on ICSS produced by diazepam (high-efficacy and relatively non-selective), zolpidem (high-efficacy and selective for GABA<sub>A</sub> receptors containing an  $\alpha$ 1 subunit), and the compounds JY-XHe-053, XHe-II-053 and HZ-166 (intermediate-efficacy with putative selectivity for GABA<sub>A</sub> receptors that contain  $\alpha$ 2/ $\alpha$ 3 subunits). Adult, male Sprague-Dawley rats (n=17) were trained in an ICSS procedure. Diazepam (0.1-10 mg/kg) and zolpidem (0.032-3.2 mg/kg) produced transient abuse-related effects at low doses. JY-XHe-053 (3.2-32 mg/kg) and HZ-166 (3.2-32 mg/kg) produced significant but weaker and less reliable effects, and XHe-II-053 (3.2-32 mg/kg) had no effect. These results are consistent with other evidence for abuse potential of diazepam and zolpidem and also suggest that

high efficacy and/or selectivity at  $\alpha 1$  GABA<sub>A</sub> receptor subtypes contributes to abuse-related effects of GABA<sub>A</sub> PAMs. Supported by NIH grants R01-NS070715, R01-MH096463, and VCU School of Medicine.

ANTI-INFLAMMATORY DIDOX AS A TREATMENT IN A NEW PROGRESSIVE MODEL OF EAE. D. J. Adkins<sup>1</sup>, K. C. Clark<sup>2</sup>, M. Joslyn<sup>2</sup>, G. H. DeVries<sup>2</sup> & J. L. Dupree<sup>2</sup>, <sup>1</sup>Thomas Nelson Community College, Hampton VA, 23666 and <sup>2</sup>Dept. of Anatomy & Neurobiology, Virginia Commonwealth University, 23284. Multiple sclerosis is an autoimmune inflammatory disease that presents with motor, sensory and cognitive impairment. In this study we used a mouse model of MS, known as experimental autoimmune encephalomyelitis (EAE), to investigate the efficacy of a novel anti-inflammatory drug as a potential treatment for progressive MS. We induced a progressive form of EAE in four mice which were monitored daily as they developed the disease. At peak disease stage, two animals were administered the novel drug, known as didox, for five consecutive days. Based on previous observations from our lab using a chronic model of EAE, we hypothesized that 1) the mice at peak disease would lose specific axonal domains known as axon initial segments (AIS) in the brain and nodes of Ranvier in the spinal cord; 2) that microglia, the resident immune cells of the central nervous system, would make contact with AIS and nodes of Ranvier; and 3) that the didox treated mice would exhibit recovery of their AIS and nodes of Ranvier. Consistent with our hypothesis, we found that both AIS and nodes of Ranvier were reduced and microglia contacted ~20% of all AIS and all nodes of Ranvier. Lastly, we found a partial recovery of AIS and nodes of Ranvier in the didox treated mice. We conclude that in this progressive EAE model, didox treatment may facilitate the reclustering of proteins essential for AIS and node of Ranvier function.

NETWORK ANALYSIS OF CHRONIC ETHANOL RESPONSIVE GENE EXPRESSION IN PREFRONTAL CORTEX REVEALS CONSERVED CHANGES ACROSS MOUSE AND MACAQUE. M.L. Smith<sup>1</sup>, J.W. Bogenpohl<sup>1</sup>, C. Helms<sup>2</sup>, M.F. Lopez<sup>3</sup>, K.A. Grant<sup>2</sup>, H.C. Becker<sup>3</sup> and M.F. Miles<sup>1</sup>, <sup>1</sup>Dept. of Pharmacology & Toxicology, Virginia Commonwealth Univ., Richmond, VA 23298, <sup>2</sup>Dept. of Behavioral Neuroscience, Oregon Health & Science Univ., Portland, OR 97239, and <sup>3</sup>Dept. of Psychiatry, Medical University of South Carolina, Charleston, SC 29425. Alcohol use disorder (AUD) is a significant public health problem. Characteristic features include craving, withdrawal, and increased consumption. Here we use two animal models explore gene expression responses in the prefrontal cortex (PFC). Ethanol's effect on PFC is of interest due to its role in executive function. C57BL/6J mice were exposed to ethanol by chronic



intermittent ethanol (CIE) with 2 bottle choice drinking. Rhesus macaques were exposed to ethanol using schedule induced polydipsia (SIP). Gene expression was measured using Affymetrix microarrays, and expression data was analyzed using Weighted Gene Correlated Network Analysis (WGCNA). With WGCNA, groups of genes showing significantly correlated expression in both mice and monkeys were identified. Gene Ontology analysis revealed that modules represented known biological processes including neurotransmission, myelination, mitochondrial respiration, and regulation of gene expression. These results indicate a conserved gene expression response to chronic ethanol exposure in mice and monkeys. These consensus modules, therefore, reveal biological themes in the conserved response to chronic ethanol exposure across species, and may represent therapeutic targets to modulate the behavioral features of AUD such as escalating ethanol consumption.

PACLITAXEL-INDUCED NEUROPATHY AND MECHANICAL ALLODYNIA DO NOT CORRELATE WITH BEHAVIORAL DEPRESSION IN RATS. Luke P. Legakis & S. Stevens Negus, Dept. of Pharmacology & Toxicology, Virginia Commonwealth University, Richmond, VA. Paclitaxel is a cancer chemotherapy drug with adverse effects that include chemotherapy-induced peripheral neuropathy (CIPN), neuropathic pain, and depression of mood and behavior. These adverse effects can limit the clinical use of paclitaxel and reduce patient well-being for decades. Preclinical research on expression and treatment of paclitaxel-induced neuropathic pain has relied almost exclusively on hypersensitive reflex-withdrawal responses to mechanical or thermal stimuli as the primary measure of "pain." It is unknown if paclitaxel can also produce signs of pain-depressed behavior in animals. The objective of this study was to evaluate paclitaxel effects on rates of positively reinforced operant responding in rats as a measure of functional impairment and behavioral depression. The specific aim was to test the hypothesis that regimens of paclitaxel treatment sufficient to produce neuropathy and mechanical allodynia would also depress rates of positively reinforced operant responding in assays of intracranial self-stimulation (ICSS) and food-maintained responding. Paclitaxel decreased rates of both ICSS and food-maintained responding in some rats, but the magnitude of depression was not statistically significant in analysis of group data. Moreover, in analysis of individual data, the magnitude of depression in rates of operant responding did not correlate with either IENF loss or decreases in mechanical sensitivity threshold. These results suggest that neuropathy and mechanical allodynia do not cause behavioral depression and may have different mechanisms than behavioral depression.

ETHANOL REGULATION OF NDRG1 AND THE EFFECTS OF PFC MODULATION OF NDRG1 ON DRINKING BEHAVIOR. G. M. Harris, A. D. van der Vaart, S. O. Park & M. F. Miles, Dept. of Pharmacology & Toxicology, Virginia Commonwealth University, Richmond, VA 23298. Multiple molecular, pathological and neuroimaging studies show perturbation of myelin or myelin gene expression in alcoholics. Prior microarray studies in our laboratory found down-regulation of myelin-related genes in prefrontal cortex (PFC) of human alcoholic post mortem tissue as well as regulation of myelin genes by acute ethanol exposure in mouse PFC. A measure of acute sensitivity to ethanol that is frequently used in animal behavioral studies is the Loss of Righting Reflex (LORR) duration. Our laboratory has implicated N-myc down-regulated gene 1 (*NdrG1*) as a potential candidate gene that modulates ethanol-induced changes in myelin-related gene expression and acute sensitivity to ethanol. Analysis of PFC expression data found that *NdrG1* expression was positively correlated with ethanol intake across the BXD panel of mice and demonstrated that the basal levels of *NdrG1* mRNA expression in the PFC across seven different strains of mice was inversely correlated with LORR duration time. PFC-specific knockdown of *NdrG1* mRNA by stereotactic injection of lentivirus expressing *NdrG1*-shRNA in B6 mice, caused increased ethanol LORR duration and decreased preference for ethanol. While CNS *NdrG1* is thought to be expressed mainly in oligodendrocytes, we have observed it co-localized within neurons in the PFC. A detailed characterization of *NdrG1* regulation at the mRNA and protein expression level following acute or chronic ethanol is currently underway. Viral methods are also being developed to up-regulate *NdrG1* expression in oligodendrocytes and pyramidal neurons within the PFC.

EFFECTS OF CHRONIC AMPHETAMINE ON BEHAVIORAL AND NEUROCHEMICAL EFFECTS OF COCAINE IN RATS. Amy R. Johnson & S. Stevens Negus, Dept. of Pharmacology & Toxicology, Virginia Commonwealth Univ., Richmond, VA. Amphetamine maintenance decreases cocaine use in preclinical studies and clinical trials. The mechanisms underlying the anti-cocaine effects of amphetamine are not well understood. This study evaluated abuse-related effects of cocaine on intracranial self-stimulation (ICSS) and on nucleus accumbens dopamine and serotonin (NAc DA and 5HT) levels. We hypothesized that amphetamine maintenance would decrease both cocaine-induced ICSS facilitation and enhancement of NAc DA. Male Sprague-Dawley rats were used for all studies. For ICSS, electrodes were implanted in the medial forebrain bundle, and responding on a lever produced pulses of electrical brain stimulation in a frequency-rate ICSS procedure. Effects of cumulative cocaine doses (1-10 mg/kg IP) were determined before and after 7-day treatment with saline, 0.1 or

0.32 mg/kg/hr amphetamine delivered by a subcutaneous osmotic minipump. For microdialysis, rats were implanted with cannulae targeting the NAc, and dialysates were analyzed for concentrations of DA and 5HT before and after 10 mg/kg IP cocaine. Cocaine facilitated ICSS and increased NAc levels of both DA and 5HT. Amphetamine maintenance facilitated ICSS throughout treatment and eliminated cocaine-induced ICSS facilitation. Amphetamine maintenance also increased basal DA concentration and eliminated cocaine-stimulated increases in NAc DA without affecting basal 5HT concentration. These results suggest that amphetamine maintenance decreases abuse-related behavioral effects of cocaine by decreasing cocaine-induced increases in mesolimbic DA. Supported by R01DA026946.

IMMUNOLOGICAL LANDSCAPE OF NSCLC: OPPORTUNITIES FOR INTERVENTION? Se W. Jeong & Timothy N.J. Bullock, Dept. of Pathology, University of Virginia, Charlottesville, VA 22908. Lung cancer is the leading cause of annual cancer related mortality in the United States. More than 150,000 people in the US will die from lung cancer this year which will lead to roughly as many deaths as breast, prostate, colon, and pancreatic cancers combined. Lung cancer is also of global concern as tobacco use in developing countries has risen significantly. Stage II and III non-small cell lung cancer (NSCLC) patients often have a 5-year disease free survival of less than 50% even after surgical resection and post-operative chemotherapy and a dismal 5% overall survival for patients with metastatic disease. Even with the advent of PD-1/PDL-1 checkpoint blockade immunotherapies, only about 20% of patients are responsive to the treatment. This limited scope of success may be due to our lack of understanding the immune landscape of NSCLCs. Therefore, we set out to characterize the immune composition from surgical lung resections via flow cytometry. We found that NSCLCs have a diverse set of checkpoint inhibitor and co-stimulatory molecules expressed by T cells, including PD-1, TIM-3, and TIGIT. Interestingly, even though PD-1 was elevated compared to a normal donor, the expression level was lower than either breast or melanoma tumor samples. In addition, regulatory T cell populations have a profound presence in these lung tumors. These findings suggest potential molecules as targets of interest for immunotherapy but also the importance of recognizing the diversity of immune populations that exist within a tumor.

EFFECTS OF TRAUMATIC BRAIN INJURY ON OXYCODONE REINSTATEMENT AND PHYSICAL DEPENDENCE. Neil B. Varshneya & Katherine L. Nicholson, Dept. of Pharmacology & Toxicology, Virginia Commonwealth University, Richmond, VA 23298. Epidemiological data indicate that patients who experience a traumatic brain injury (TBI) have an elevated risk of developing a substance use

disorder (SUD), however the underlying neurobiological connections remain unclear. We investigated the effects of TBI on the abuse-related effects of oxycodone in preclinical models. Our evaluation utilized a lateral fluid percussion injury of moderate severity in adult male Sprague-Dawley rats. In the first aim, we tested the hypothesis that moderate TBI increases the risk for relapse to an opioid use disorder as measured by reinstatement of lever-pressing behavior following extinction in an intravenous oxycodone self-administration procedure. In the second aim, we tested the hypothesis that moderate TBI increases physiological dependence to oxycodone as measured by decreases in food-reinforced lever-pressing behavior and increases in other withdrawal behaviors in both precipitated withdrawal and spontaneous withdrawal. In reinstatement tests, non-injured subjects reinstated responding under oxycodone-associated cue- and oxycodone prime-induced conditions, however, brain-injured subjects did not reinstate lever-pressing behavior under any conditions. In dependence tests, brain-injured subjects showed no significant differences from non-injured subjects in mean withdrawal scores or food-reinforced lever-pressing behavior. Overall, these data suggest that brain-injured patients with no significant pre-morbid history of opioid abuse have lesser risk of relapse to opioid use disorders. Supported by Department of Defense, W81XWH-11-1-0374.

**ETHANOL REVERSAL OF OPIOID TOLERANCE IN MICE.**  
Joanna C. Jacob & William L. Dewey, Dept. of Pharmacology & Toxicology, Virginia Commonwealth University, Richmond, VA 23298. Prescription opioids are generously prescribed due to their highly effective antinociceptive properties, however their use is limited by a high abuse potential due to euphoric effects. Chronic opioid use, whether for clinical or recreational purposes, is often coupled with the use of other substances, such as ethanol. Dangerous drug interactions are known to occur with opioids and ethanol, yet the mechanisms are not understood. This led to the investigation and hypothesis that ethanol reverses tolerance to at least some opioid effects, such as analgesia and respiratory depression, ultimately leading to overdose and death. We found a significant and dose-dependent reversal of morphine-induced analgesic tolerance by ethanol, as measured by warm-water tail withdrawal in Swiss Webster mice. Similar studies were carried out for oxycodone and hydrocodone, where analgesic tolerance was developed via chronic s.c. injections of an ED<sub>80</sub> dose, and then altered by a single i.p. injection of 1g/kg ethanol. The ED<sub>50</sub> for oxycodone was significantly shifted from 0.9 mg/kg (0.72 – 1.12) to 1.70 mg/kg (1.42 – 2.03) after repeated administration and returned to 1.02 mg/kg (0.77-1.37) after 1 g/kg ethanol administration. Similarly, the ED<sub>50</sub> for hydrocodone was shifted from 3.92 mg/kg (3.26 – 4.71) to 9.01 mg/kg (6.44 – 12.62) and returned to 4.73 mg/kg (3.51 – 6.38) after 1 g/kg

ethanol administration. Together these data support the hypothesis that ethanol reverses analgesic tolerance to opioid drugs in addition to morphine, and could be working through the same mechanisms.

PROLIFERATIVE RECOVERY AND REVERSIBILITY OF THERAPY-INDUCED SENESENCE IN NON-SMALL CELL LUNG CANCER. Tareq Saleh & David A. Gewirtz, Virginia Commonwealth Univ., Dept. of Pharmacology & Toxicology, Richmond, VA. Lung cancer is the leading cause of cancer-related death in both men and women in the United States. Most lung cancer cases are diagnosed in advanced, inoperable stages and are treated with chemoradiation; while chemoradiation is effective in suppressing tumor progression, recurrence following treatment is not infrequent. The involvement of autophagy, senescence and apoptosis in the actions of etoposide, one of the primary drugs utilized in the treatment of non-small cell lung cancer (NSCLC), was studied in H460 NSCLC cells. Exposure to etoposide resulted in growth arrest accompanied by the induction of senescence, but minimal apoptosis. Growth arrest was transient in that proliferative recovery was evident by day 7 post exposure. Quantification of senescence over time based on C<sub>12</sub>FDG staining and flow cytometry demonstrated that the reversal of growth arrest coincided with a decline in the extent of senescence. To more precisely define the source of the recovered cells, senescent and non-senescent but growth arrested cells were separated by flow cytometry based on their relative  $\beta$ -galactosidase expression and replated. Both cell populations demonstrated the ability to re-emerge from the growth-arrested state and recover proliferative capacity. These observations suggest that senescence is ultimately a transient process in that at least a subpopulation of tumor cells can and will recover proliferative capacity. We propose that the reversibility of therapy-induced senescence (TIS) might be developed as a model for studies of tumor dormancy and disease recurrence.

MOLECULAR MODELLING AND VIRTUAL SCREENING OF *PLASMODIUM FALCIPARUM* GLYCOGEN SYNTHASE KINASE 3 (*pf*GSK-3) FOR ATP NON-COMPETITIVE INHIBITORS. S. Obeng, S. A. Zaidi, P. D. Mosier & Y. Zhang, Dept. of Medicinal Chemistry, Virginia Commonwealth University, Richmond VA 23298-0540. The purpose of this study was to screen for new lead compounds that are ATP non-competitive *pf*GSK-3 selective inhibitors to treat malaria. Since there is no crystal structure of *pf*GSK-3 available, a blast search of the PDB database was carried out. The human glycogen synthase kinase 3 $\beta$  (*h*GSK-3 $\beta$ ) (PDB: 4ACC) was identified as the enzyme with the highest homology to *pf*GSK-3 with a sequence identity and similarity of 42.9% and 60.4% respectively. Using *h*GSK-3 $\beta$  as the template, 100 models of *pf*GSK-3 were constructed using MOD9.14.

The best model was selected by docking a known *pf*GSK-3 ATP non-competitive inhibitor (manzamine) into a binding pocket comprising residues Arg 96, Arg 180, Lys 205, and Tyr 216. The best model obtained was used to carry out a virtual screening of the NCI and ZINC-sigma aldrich libraries using UNITY. The hits obtained were docked into the *pf*GSK-3 homology model using GOLD52 and the scoring function CHEMPLP. Three compounds with CHEMPLP scores of 111.07, 97.81, and 95.47 were identified as lead compounds.

NOVEL PHOTOAFFINITY PROBES FOR  $\alpha$ -N-TERMINAL WRITERS. B. D. Mackie<sup>1</sup>, S. L. Richardson<sup>2</sup> & R. Huang<sup>1</sup>, <sup>1</sup>Dept. of Medicinal Chemistry and <sup>2</sup>Dept. of Chemistry, Virginia Commonwealth University, Richmond VA 23219. Protein  $\alpha$ -N-terminus undergoes a variety of modifications including methylation, acetylation, myristoylation, and palmitoylation. It has been hypothesized that there is dynamic interplay among those modifications, such as methylation and acetylation. Methylation has recently demonstrated its important role in regulating protein-DNA interactions, mitotic division and DNA damage repair. Acetylation is involved in protein degradation, localization, and complex formation. To explore these dynamic modifications, we developed photoaffinity probes to profile enzymes that are responsible for  $\alpha$ -N-terminal modifications. We have successfully synthesized a photoaffinity probe which contains three main components: a recognition element, a photocrosslinker, and a fluorescent tag. The recognition element is crucial for selectivity, the photocrosslinker is needed to covalently bond the probe to the target and the tag is necessary for fluorescence imaging. The photoaffinity probe's recognition element was derived from the N-terminus of Retinoblastoma 1 (RB1), a substrate of N-terminal methyltransferase 1 (NTMT1). Our results suggest that our probe exhibits in a dose dependent, time dependent and competitive manner. Photoaffinity labeling was competitively inhibited when NTMT1 was incubated with the probe and varying concentrations of RB1-10. The RB1 probe also selectively labeled NTMT1 when NTMT1 was spiked into a nuclear extract cell line, verifying specificity of the probe for NTMT1. Lastly, the probe was enzymatically methylated by NTMT1, giving further validation that labeling is driven by recognition.

IMPROVING THE SIDE EFFECT PROFILE OF ANTICOAGULANTS USING THROMBIN ALLOSTERISM. D. K. Afosah<sup>1,2</sup>, S. Verespy<sup>2,3</sup>, R. Karuturi<sup>1,2</sup>, R.S. Boothello<sup>2</sup> & U. R. Desai<sup>1,2</sup>, <sup>1</sup>Dept. of Medicinal Chemistry and <sup>2</sup>Institute for Structural Biology, Drug Discovery and Development, Virginia Commonwealth University, Richmond VA 23219, and <sup>3</sup>Dept. of Chemistry, Virginia Commonwealth University, Richmond VA 23284. Thrombin is a serine protease that occupies a central position in the coagulation cascade. Its

key feature is the conversion of fibrinogen to fibrin, a key component of blood clots. Currently, all thrombin inhibitors on the market inhibit the protease fully resulting in increased risk of bleeding with their use. We reasoned that it should be possible to develop partial inhibitors of thrombin that allow reasonable proteolytic activity even at saturating concentrations of the inhibitor. Based on our earlier work with sulfated benzofuran dimers, which showed 75% inhibition at saturation concentrations, we designed advanced analogs using computational virtual screening. These analogs were synthesized and their biological profile studied using biochemical assays. The results show that a distinct group of analogs display inhibition efficacies of ~50-60% at saturation in chromogenic substrate assay, whereas others exhibit efficacies of  $\geq 80\%$ . Similar results were observed in the fibrinogen assay suggesting that submaximal inhibition is maintained with thrombin's *in vivo* substrate. The results indicate that thrombin's high plasticity can be exploited to realize a clinically relevant homeostatic inhibitor that resolves bleeding risk.

INTERPLAY BETWEEN ARTEMIS AND TDP1 IN SENSITIVITY TO RADIOMIMETIC AGENTS. A. Kawale, K. Akopiants & L. F. Povirk, Dept. of Pharmacology & Toxicology, Virginia Commonwealth University, Richmond, VA 23298. DNA double-strand breaks (DSBs) containing unligatable termini are potent cytotoxic lesions leading to growth arrest or cell death. Artemis, which is associated with the Non-Homologous End Joining (NHEJ) pathway, is the major end processing nuclease that resolves unligatable termini, especially the 3' blocks, by nucleolytic trimming. Tyrosyl-DNA Phosphodiesterase 1 (TDP1) is an enzyme which is biochemically competent in 3'-phosphoglycolate processing. The purpose of this study is to investigate if TDP1 is an end-processing enzyme involved in the NHEJ pathway. A cell line with combined deficiency in Artemis and TDP1 was generated by infecting Artemis<sup>-/-</sup> single mutants with a lentivirus expressing a TDP1 shRNA. Positive clones were screened for maximum TDP1 knockdown which was found to be around 14X. Clonogenic survival assays carried out on shTDP1 & Artemis<sup>-/-</sup> single mutants and the Artemis<sup>-/-</sup>.shTDP1 double mutants showed similar sensitivity to NCS and Calicheamicin. Thus, surprisingly, these experiments suggest that TDP1 functions are epistatic with Artemis in the NHEJ pathway for repair of DNA double-strand breaks.

HYBRID MOLECULES AS TOOLS TO STUDY CLASSICAL 5-HT<sub>2A</sub> RECEPTOR LIGANDS. U. H. Shah<sup>1</sup>, S. A. Gaitonde<sup>1</sup>, J. L. Moreno<sup>2</sup>, J. Gonzalez-Maeso<sup>2</sup>, M. Dukat<sup>1</sup> & R. A. Glennon<sup>1</sup>, <sup>1</sup>Dept. of Medicinal Chemistry, and <sup>2</sup>Dept. of Physiology & Biophysics, Virginia Commonwealth University, Richmond VA 23298. Serotonin-2A (5-HT<sub>2A</sub>) receptor antagonists have therapeutic applications in

schizophrenia and belong to diverse chemical classes. Current pharmacophore models for 5-HT<sub>2A</sub> receptor antagonists suggest that these agents might have multiple binding modes. Reported pharmacophores include *two* aromatic/hydrophobic regions and a protonated amine. Hybrid molecules (Ket/Ris and Ris/Ket) of two known 5-HT<sub>2A</sub> antagonists, ketanserin (Ket) and risperidone (Ris), were synthesized by our laboratory to study their binding modes. Binding data showed that Ket/Ris ( $K_i = 0.96$  nM) binds with 19-, 5- and 13- fold higher affinity than Ket, Ris and Ris/Ket, respectively. The high affinities of Ris and Ket/Ris can be attributed to an additional bifurcated interaction as shown in homology models of 5-HT<sub>2A</sub> receptors, which is not possible with the benzoyl ring in Ket and Ris/Ket. We synthesized deconstructed analogs of Ris and Ket to determine which portions contribute to 5-HT<sub>2A</sub> receptor affinity and antagonism. 4-(4-Fluorobenzoyl) piperidine and FBIP, deconstructed analogs of Ket and Ris, respectively, were found to be 5-HT<sub>2A</sub> receptor antagonists. Therefore, we have successfully identified a new pharmacophore for 5-HT<sub>2A</sub> receptor antagonists that consists of *one* aromatic region, hydrogen bond acceptors, and a basic protonated amine. FBIM ( $K_i$  ca 12 nM), a deconstructed analog of Ris ( $K_i = 5$  nM), was identified as a new high-affinity 5-HT<sub>2A</sub> receptor antagonist. Published 5-HT<sub>2A</sub> receptor antagonist models will now need to be completely revised.

THE TALE OF THREE TESTS: PARADOXICAL MODULATION OF OXYCODONE-INDUCED ANTINOCICEPTION BY LORCASERIN. Kumiko M. Lippold & William L. Dewey, Dept. of Pharmacology & Toxicology, Virginia Commonwealth Univ., Richmond, VA, 23298. Prescription opioids are important therapeutic agents for the treatment of pain that with chronic use, produce tolerance, dependence, and in many cases, addiction. There is a need to develop new approaches for minimizing the risk of opioid abuse and reducing overdose-related deaths. The underlying neurobiological mechanisms of these conditions suggest a modulatory role of the serotonergic system in the pharmacological effects of opiates. Lorcaserin is a selective agonist, at the serotonergic 5-HT<sub>2C</sub> receptor, approved by the FDA for the treatment of obesity but recently investigated as a potential treatment for drug abuse and dependence. Preclinical studies have demonstrated the efficacy of lorcaserin to reduce the abuse-related effects and dependence behaviors of opiates. We evaluated lorcaserin in the acute effects of oxycodone using tests of nociception and observed paradoxical modulation of the antinociceptive effects of oxycodone, in a divergent manner, in models of spinally-mediated and supraspinally-mediated thermal nociception. Lorcaserin potentiated the antinociception produced by oxycodone in the tail immersion test (a spinally-mediated reflex) and in the hot plate assay (a test of supraspinally-mediated pain), there was an attenuation



of the antinociceptive effects of oxycodone. Interestingly, lorcaserin also suppressed stretching activity in mice that received an intraperitoneal injection of acetic acid but when administered prior to oxycodone, no significant effects were observed. These data suggest a contrasting role of the 5-HT<sub>2C</sub> receptor in the effects elicited by oxycodone in the brain, spinal cord, and viscera.

### Natural History & Biodiversity

HIGH ABUNDANCE AND LOW DIVERSITY OF FOSSIL TETRAPOD VERTEBRATES IN THE SOLITE QUARRY (VIRGINIA-NORTH CAROLINA, UPPER TRIASSIC, 225 MILLION YEARS AGO). Alexander K. Hastings & Christina J. Byrd, Virginia Museum of Natural History, 21 Starling Avenue, Martinsville, VA 24112. The Solite Quarry, which spans the Virginia-North Carolina border, contains a Late Triassic (ca. 225 million-year-old) shale deposit with thousands of fossil plants, insects, and vertebrates. Among the plant fossils preserved, cycad fronds and stems of conifers are common. Numerous fossil insects and other arthropods have been collected from a three cm thick unit, including over 2,400 individual specimens, with many more to be catalogued. The dominance of aquatic insects suggests a shallow lake basin, but the phenomenal preservation and black sediments suggest a deeper, anaerobic setting. In either case, the site represents a relatively low-energy freshwater lake environment. 484 vertebrate fossils have been collected thus far. The rarest vertebrate species is the gliding reptile *Mecistotrachelos apeoros*, which was likely non-aquatic. Fish account for at least 140 of the vertebrates, including coelacanths, semionotiforms, and palaeonisciforms. The remaining 340 vertebrate specimens belong to the aquatic reptile *Tanytrachelos ahynis*. Based on this sample, over 70% of the vertebrates and over 98% of the tetrapods belonged to a single species. The high abundance of insects and low abundance of predatory competition may explain why the *Tanytrachelos* are so common at Solite. Despite a high level of sampling, there appears to be very low levels of tetrapod alpha diversity during the Triassic of this region.

TESTING THE CEPHALIC ADHESIVE ORGAN HYPOTHESIS IN CYPRINID PROTOLARVAE. George E. Maurakis, College of Science, Virginia Tech, Blacksburg VA 24061. The objective of this research is to test the hypothesis that protolarvae of the phytophilous species, *Hybognathus hankinsoni*, *Notemigonus crysoleucas*, *Carassius auratus*, and *Cyprinus carpio* (Cyprinidae), contain cephalic adhesive glands. The hypothesis is if newly hatched larvae of phytophilous species attach to aquatic vegetation, then they have adhesive glands. SEM examination of 11 areas around the head (including dorsal, lateral, and ventral) of each specimen indicated there were no adhesive organs on the control species (*S. corporalis*), or test species (*H. hankinsoni*, *N. crysoleucas*, *C. auratus*, and *C. carpio*). The hypothesis that if newly hatched protolarvae of the phytophilous species attach to aquatic vegetation, then they have adhesive organs, is rejected. Both SEM and light microscopy indicated the absence of adhesive organs on all areas of heads of all control and test protolarvae. The control species (*S.*

*corporalis*) only had epithelial pores with no epidermal mucus. Although test species did not have adhesive organs, all of them had both epithelial pores and epidermal mucus. This mucus is probably responsible for the adhesion of protolarvae to aquatic vegetation, a hypothesis that could be tested in the future. Light microscopy indicated that the control species (*S. thoreauianus*) had a two cell-thick epidermis, whereas the epidermis of *N. crysoleucas* was about three to four cell layers thick. The three to four cell layer thick epidermis of *N. crysoleucas* is significant as some cells were goblet cells opening outside of the epidermis. Goblet cells have been reported to contain mucus (mucopolysaccharides), which can serve as an adhesive substance.

EVOLVING ISOLATION MECHANISMS AMONG HOST-FOODPLANT SOURCES OF A PARASITIC WASP SPECIES. Justin P. Bredlau & Karen M. Kester, Integrative Life Sciences & Department of Biology, Virginia Commonwealth University, Richmond VA 23284. Parasitic wasps are highly diverse and play a major role in suppression of herbivorous pest populations. Recent research has demonstrated that previously identified species of some parasitic wasps are actually complexes of cryptic species resulting from adaptations to specific hosts or host foodplants. *Cotesia congregata* (Braconidae) is reported to attack at least 15 species of sphingid caterpillars, most of which are plant family specialists. We expanded on our earlier finding that wasps from *Manduca sexta* on tobacco ("MsT") and *Ceratomia catalpae* ("CcC") represent distinct genetic lineages with both pre- and post-zygotic barriers to reproduction by testing for post-zygotic barriers to reproduction among wasps from additional host-foodplant complexes. Wasps were collected from five host sources in two subfamilies of Sphingidae: MsT, CcC, and *Sphinx kalmiae* on privet ("SkP") (Sphinginae), and *Darapsa myron* and *Eumorphia pandorus* from wild grape and Virginia creeper ("DmV" and "EpV") (Macroglossinae). Reciprocal hybrid crosses were established between MsT and CcC wasps with each of these additional sources to test for the production of viable fertile hybrids. All reciprocal crosses produced hybrid females. Most hybrid females from CcC♂xMsT♀, CcC♂xEpV♀, SkP♂xMsT♀, and DmV♂xMsT♀ failed to produce F<sub>2</sub> offspring, whereas the reciprocal crosses produced viable offspring. Dissections of hybrid females revealed that sterile wasps lacked mature ovaries. The pattern of asymmetric hybrid dysgenesis indicates that *C. congregata* is diverged into at least two reproductively isolated groups.

COMPARATIVE THERMAL PERFORMANCE IN A CATERPILLAR-PARASITOID-HYPERPARASITOID TRI-TROPHIC SYSTEM. Kanchan Anand Joshi<sup>1</sup>, Salvatore J. Agosta<sup>2</sup> & Karen M. Kester<sup>1</sup>, <sup>1</sup>Department of Biology and <sup>2</sup>Center for Environmental

Studies, Virginia Commonwealth University, Richmond VA, 23284. Among the predicted impacts associated with global climate change, the effects of warming on organismal performance are of special interest because the rates of all physiological processes are temperature-dependent. Ectotherms such as insects, which are the most abundant and speciose animal taxon, are likely to be most affected due to their limited ability to control body temperature. Further, responses are likely to vary among species, which may have significant consequences for the dynamics of species interactions, such as those between hosts and parasites. In this study, we measured tolerance to extreme high temperatures, i.e., critical thermal maximum ( $CT_{max}$ ) to test differences among component species in a tri-trophic system including a caterpillar, *Manduca sexta*, a parasitoid wasp, *Cotesia congregata*, and a hyperparasitoid in the genus *Spilochalcis*. Critical thermal tolerance ( $CT_{max}$ ) varied significantly among the component species. The parasitoid wasp had the lowest  $CT_{max}$  and the hyperparasitoid had the highest  $CT_{max}$ . Both unparasitized and parasitized caterpillars had  $CT_{max}$  values that were intermediate between the parasitoid and hyperparasitoid. Our results demonstrate that species involved in multi-trophic interactions can vary in their tolerances to high temperatures and suggest that climate change may result in disruption of these interactions.

THE CENTER FOR BIODIVERSITY AT JOSEPH PINES PRESERVE. Philip M. Sheridan, Meadowview Biological Research Station, 8390 Fredericksburg Tnpk., Woodford, VA 22580. The Center for Biodiversity is a 1.5 acre parcel with a 3000 s.f. building adjacent to Meadowview's 232 acre Joseph Pines Preserve. The Center supports the conservation, protection, and restoration of the endangered longleaf pine/pitcher plant ecosystem in Virginia. The Center allows Meadowview staff to train students and the general public about the need and value of rare plant and animal conservation, support ongoing scientific research and restoration efforts at the Joseph Pines Preserve, and demonstrate how a sustainable lifestyle can support habitat restoration. The Center property is part of a conservation plan to acquire over 2000 contiguous acres and provide one of the largest and best managed examples of a longleaf pine ecosystem in a multi-state area. This property represents the northern limit of the known range of the longleaf pine ecosystem. Habitat restoration has included mechanical clearing, chemical site treatments, prescribed fire, and controlled reintroductions of 18 indigenous rare plant taxa (including one federally endangered species). Habitat is also provided for one federally endangered bird species (red-cockaded woodpecker), one state threatened bird species (Bachman's sparrow), and one endangered fish species (black-banded sunfish).

PRESCRIBED FIRE INCREASES PLANT SPECIES RICHNESS IN RESTORED VIRGINIA LONGLEAF PINE HABITATS. Philip M. Sheridan<sup>1</sup> & Alex Petzke<sup>2</sup>. <sup>1</sup>Meadowview Biological Research Station, 8390 Fredericksburg Tnpk., Woodford, VA 22580. <sup>2</sup>Department of Environmental and Forest Biology, State University of New York College of Environmental Science and Forestry, Syracuse, NY. Longleaf pine forests are known for high plant species diversity. A number of research studies in the southeastern U.S. support the hypothesis that high plant species diversity in longleaf pine forests is enhanced by disturbance provided by frequent fire. We were interested in determining whether this hypothesis was supported in longleaf pine restoration sites in Virginia, when Virginia longleaf pine habitats might rival plant diversity found in southeastern U.S. longleaf pine habitats, and if herbicide had a negative effect on plant diversity. We collected data on two longleaf pine nature preserves in Sussex and Prince George County, VA (Cherry Orchard Bog Preserve and Joseph Pines Preserve) and found that plant diversity increased over time with prescribed fire. We predict that Virginia longleaf pine forests, managed with regular prescribed fire, could achieve 50 species/m<sup>2</sup> within 48 years and rival diversity found in their southeastern counterparts. We also found that herbicide did NOT have a negative effect on plant diversity.

RESTORING GROUNDWATER HYDROLOGY IN A VIRGINIA PITCHER PLANT SEEPAGE WETLAND. Marissa Merhout & Philip M. Sheridan. Meadowview Biological Research Station, 8390 Fredericksburg Tnpk., Woodford, VA 22580. Pitcher plant habitats in southeastern Virginia are typically located on 0 order headwater seepage wetlands with marine deposits of sandy to sandy loam soils. Frequent fire is important in keeping pitcher plant habitats open and preventing the encroachment of competing vegetation. We initiated a longleaf pine/pitcher plant ecosystem habitat restoration program at our Joseph Pines Preserve in Sussex County, VA that included an aggressive assault on competing woody plant species. We observed an increase in groundwater in our seepage bogs after treatment of woody plant competitors and conducted graduate and intern research to measure the effects of woody plant control on groundwater hydrology at our preserve. We found that conversion of a dense mixed oak/pine forest to longleaf pine savanna resulted in a 25% reduction in evapotranspiration, or a retention of almost 4 million gallons of water/year on a 24 acre watershed. We also found that seepage bog pore water was potable and met state certified lab criteria as drinking water with a t.d.s of 9. We found that rainfall enters the ground water column within two days of a rain event. These results indicate that Virginia pitcher plant wetland hydrology is very sensitive to woody plant invasion, that pitcher plant seepage water is very clean, and that surface activities could quickly contaminate the aquifer.

GENETIC CONNECTIVITY OF RACOONS (*PROCYON LOTOR*) IN A NATURALLY FRAGMENTED COASTAL LANDSCAPE: EVIDENCE FROM MITOCHONDRIAL AND MICROSATELLITE MARKERS. N. D. Moncrief<sup>1</sup>, J. H. Roberts<sup>2</sup>, E. M. Hallerman<sup>3</sup>, R. A. Van Den Bussche<sup>4</sup> & R. D. Dueser<sup>5</sup>, <sup>1</sup>VA Museum of Natural History, Martinsville, VA 24112, <sup>2</sup>Dept. of Biol., Georgia Southern Univ., Statesboro, GA 30458; <sup>3</sup>Dept. of Fish and Wildlife Conservation, Va. Polytechnic Inst. & State Univ., Blacksburg, VA 24061; <sup>4</sup>Dept. of Integrative Biol., Oklahoma State Univ., Stillwater, OK 74078 and <sup>5</sup>Dept. of Wildland Resources, Utah State Univ., Logan, UT 84322. We used mitochondrial (mtDNA) and nuclear DNA markers to identify past and present dispersal corridors of raccoons (*Procyon lotor*) on the Virginia barrier islands and adjacent Delmarva Peninsula mainland. We found complex patterns of spatial population structure and migration rates in this system. Results of this study suggest that the metapopulation structure of raccoons on the Virginia barrier islands is highly dynamic, with most movement of raccoons occurring among groups of islands that are inter-connected by marsh and relatively shallow, narrow, open-water channels. These results are consistent with our direct observations of overwater movement by raccoons within this system. These genetic data also support predictions from our models based on cost-distance analysis of landscape resistance to movement by raccoons among islands and the mainland.

MORPHOMETRIC DIVERGENCE AND FUNCTIONAL SIMILARITY IN *SCIURUS VULGARIS* (EURASIAN RED SQUIRREL) AND *SCIURUS CAROLINENSIS* (EASTERN GRAY SQUIRREL). J. S. Scheibe, Dept. of Biology, Southeast Missouri State Univ., Cape Girardeau, MO 63701 & N. D. Moncrief, Va. Museum of Natural History, Martinsville, VA 24112. We used geometric morphometric techniques to explore and compare the shapes of dentaries and skulls in 4 species of tree squirrels: Eurasian red squirrel (*Sciurus vulgaris*), eastern gray squirrel (*S. carolinensis*), eastern fox squirrel (*S. niger*), and western gray squirrel (*S. griseus*). These species were chosen because of current competitive interactions amongst the species, and because of their phylogenetic affinities. A canonical variates analysis of Procrustes shape coordinates revealed significant shape differences between the skulls and dentaries of *S. carolinensis* and *S. vulgaris*. We compared biomechanical properties of the dentaries for the 4 species, and used discriminant functions analysis to discriminate between the species in a jaw-function space. Here, there was extensive functional overlap between *S. carolinensis* and *S. vulgaris*, but not between *S. carolinensis* and *S. niger*. Although the skulls and dentaries of *S. carolinensis* and *S. vulgaris* differ morphologically, they are functionally similar.

AN EVALUATION OF THE EXTINCTION RISK OF THE FISH ORDER SCORPAENIFORMES IN THE OCEANIA REGION. J. Deal<sup>1</sup>, H. Motomura<sup>2</sup>, G. Ralph<sup>3,4</sup>, K. Carpenter<sup>3,4</sup>, and H. Harwell<sup>1</sup>, <sup>1</sup>Dept. of Organismal and Environmental Biology, Christopher Newport University, <sup>2</sup>Kagoshima University Museum, <sup>3</sup>Old Dominion University, <sup>4</sup>Marine Biodiversity Unit, Global Species Programme, International Union for Conservation of Nature. The remote region of Oceania is home to high species diversity and endemism, yet the conservation status of many of these species remains unknown. This project is part of a large, collaborative effort to complete the first comprehensive assessment of the relative extinction risk of all marine bony fishes of the Oceania region. Here, we present results for three families of the fish order *Scorpaeniformes*. Species-specific information regarding taxonomy, distribution, population status, habitat, ecology, potential threats, and current conservation measures was compiled from available literature for each member of the families Dactylopteridae, Platycephalidae, and Scorpaenidae occurring within the region. These data were verified and supplemented by leading scientific experts at a Red List Assessment Workshop held in Suva, Fiji, in March 2015. Applying Red List Criteria, each species was assigned a Red List Category of relative extinction risk. Species-specific digital distribution maps were compiled to identify geographic areas of high species richness and potential areas of concern. Eighty-six per cent of the species included were listed as Least Concern and 12% were listed as Data Deficient; however, two species endemic to the Hawaiian Islands (*Caracanthus typicus* and *Sebastapistes coniora*) were listed as Near Threatened. This information will help guide future marine conservation and fisheries management efforts within the region.

SPATIAL AND TEMPORAL PATTERNS OF VEHICLE COLLISION-INDUCED WILDLIFE MORTALITY IN CENTRAL VIRGINIA. J. Gibson, G. Cole, E. Robertson & S. Henkanaththegedara, Department of Biological & Environmental Sciences, Longwood University, Farmville, VA 23909. Roads could pose significant ecological impacts on environment and wildlife communities. Wildlife mortality due to vehicle collisions is one of the leading causes for population declines, population fragmentation and potentially, local extirpations. The impact of vehicle collisions on wildlife mortality in Virginia is poorly documented. We assessed the influence of road type (2-lane and 1-lane), seasons (spring and fall) and adjacent habitats (forest, agriculture and developed) on average animal mortality rate. Highest mortality rates were reported for gray squirrel (30%) followed by Virginia opossum (16%) and white-tailed deer (12%). A significantly higher rate of average mortality was recorded for 1-lane highway (40 total) compared to 2-lane highway (22 total). Although we observed more mortalities in spring 2016 (36) compared

to fall 2015 (26), we failed to detect any seasonal effects. There was more wildlife mortality reported in road segments adjacent to forests (37) followed by agriculture (16) and developed (4). However, we failed to detect any significant habitat effects on average mortality rate. Understanding ecological implications of collision-induced wildlife mortality is important in implementing conservation measures.

POTENTIAL IMPACTS OF A CHANGING CLIMATE ON WINTERING BIRD POPULATIONS OF CENTRAL PIEDMONT VIRGINIA. E. Salamon, C. Labosier & S. Henkanaththegedara, Department of Biological & Environmental Sciences, Longwood University, Farmville, VA 23909. Global climate change is significantly altering the structure and functioning of many ecosystems, and temporal and spatial patterns of wildlife populations. Previous studies have shown a decline in common wintering bird populations in the Eastern United States. We assessed long-term trends of local climate and wintering bird species together with any correlations between them. Bird population sizes for 76 wintering bird species were collected from Christmas Bird Count (CBC) database and 53 species with significant trends were included in this analysis. Bird data were collected from Darlington Heights, Lynchburg, Warren, Lake Anna and Gordonsville CBC count circles. Climate data (18 variables) were collected from National Oceanic and Atmospheric Administration's (NOAA) Climatic Data Center (NCDC). Long-term climate trends, bird population trends and correlations between climate variation and bird population sizes were analyzed using linear regression models. Significant trends for number of extreme minimum temperature days for November ( $P < 0.01$ ) and number of days in month with maximum temperature less than or equal to  $0\text{ }^{\circ}\text{C}$  for December ( $P < 0.01$ ) suggest a warming winter. We observed significant population declines for several bird species (field sparrow, song sparrow, fox sparrow, wood duck, winter wren and downy woodpecker) correlated with above weather changes.

ECTOSYMBIOTIC RELATIONSHIPS BETWEEN THE APPALACHIAN BROOK CRAYFISH (*CAMBARUS BARTONII*) AND THE BRANCHIOBELLIDAN, *CAMBARINCOLA INGENS* INVOLVING DISSOLVED OXYGEN UPTAKE AND GILL BACTERIA. Thomas P. Holman, Joseph E. Davis & Kyle J. Harris, Department of Biology and Chemistry, Liberty University, Lynchburg VA 24515. Ectosymbiotic relationships between crayfish (*Cambarus bartonii*) and the segmented worms, branchiobdellidans (*Cambarincola ingens*), have shown a cleaning symbiosis that includes the removal of bacteria from the crayfish gill filaments. Eleven *C. bartonii* were randomly divided into two groups. The control group (N=5) had no worms and the experimental group (N=6) had four worms each. The



crayfish were monitored in individual 10 gallon aquaria over twenty weeks. It was expected that the presence of worms would decrease the amount of bacteria in the gill chamber, thus allowing an increase in both growth rates and dissolved oxygen (DO) uptake. A gill chamber bacterial analysis and characterization showed that the logged mean number of colony forming units/gram crayfish ( $\log_{10}$  CFUs/g) was greater for the experimental group ( $3.80 \log_{10}$  CFUs/g) than the control group ( $2.84 \log_{10}$  CFUs/g). The observed percent change in blotted wet mass (BWM) over eight weeks showed that the control group grew 26.2% more than the experimental group. However, this difference in BWM percent change could be due to the variation of initial BWM between control and experimental groups. No significant difference was found in the mean DO consumption between control ( $0.094 \text{ mg/l/g*2hr}$ ) and experimental ( $0.090 \text{ mg/l/g*2hr}$ ) groups.

MESOCOSM EXPERIMENTS REVEAL NEGATIVE IMPACTS OF INVASIVE RED-SWAMP CRAYFISH ON NATIVE PIEDMONT CRAYFISH. D. Conner, C. Perry, P. Hale, J. Wilson & S. Henkanaththegedara, Department of Biological & Environmental Sciences, Longwood University, Farmville, VA 23909. Previous laboratory experiments showed that invasive red-swamp crayfish (*Procambarus clarkii*) have mixed effects on native Piedmont crayfish (*Cambarus* sp. C). Invasive crayfish had higher levels of aggression towards natives and they outcompeted native crayfish for food. However, there were no significant differences of survival between native and invasive crayfish under sympatry for 120 hours. Although laboratory experiments are informative, the results may be limited by the simplicity of the experimental setup. Therefore it is critical to generate more realistic data to better inform conservation practitioners. We conducted preliminary mesocosm experiments under semi-natural conditions using 360 gallon tanks. We stocked invasive and native crayfish in allopatry and sympatry, and ran the experiments for 21 days. Native crayfish survival was 58% under sympatry, when allopatric survival rate was 94%. Invasive crayfish had high survival rates under both allopatry (100%) and sympatry (92%). There were significant differences of invasive and native crayfish growth under sympatry and allopatry ( $P < 0.05$ ). Invasive crayfish biomass grew rapidly under both allopatry ( $4.43 \pm 2.3 \text{ g}$ ) and sympatry ( $1.98 \pm 0.75 \text{ g}$ ). However, invasive crayfish grew slowly in allopatry ( $0.62 \pm 0.19 \text{ g}$ ) compared to a biomass reduction ( $0.83 \pm 0.76 \text{ g}$ ) under sympatry with invasive crayfish. This suggests significant negative impacts of invasive red-swamp crayfish on native Piedmont crayfish growth and survival.

HABITAT USAGE AND CONSPECIFIC INTERACTIONS OF THE HARVESTMAN *CYNORTA MARGINALIS* IN A COSTA RICAN RAIN FOREST. Nathaniel Schaus, Tatyana Zvonareva, Ashley Shriver, Cynthia Richardson, Sarah Locke, Maynard H. Schaus, and Victor R. Townsend, Jr., Department of Biology, Virginia Wesleyan College. This study investigated habitat use and intraspecific interactions among adult *Cynorta marginalis*, an arboreal harvestman, at La Selva Biological Station, Costa Rica. In the field, we repeatedly sampled 15 transects (40 m in length) in the morning and evening, resulting in the capture and marking (with paint) of 146 males and 112 females. Only three individuals (all females) were recaptured, indicating that the population size at this site was relatively large. Heavy rains significantly reduced the surface activity of adults, with 0.9 individuals per transect observed during heavy rain vs. 3.6 individuals per transect during light or no rain. Harvestmen were most commonly observed using leaves as perches (65% of captures), but also used tree trunks (31%), and were rarely observed on branches or in the leaf litter. Adults were most commonly found alone on perches (85%), however, we observed several harvestmen in male-female pairs (8%), same sex pairs (4%) and heterosexual groups (7%). Although we did not observe reproductive or aggressive behaviors in the field, our observations of intraspecific interactions under laboratory conditions revealed that at least some individuals (15%) actively attempted to copulate. Aggression was not observed in intersexual trials or in interactions between females and was only rarely observed between males (1 out of 20 trials). This study was funded by a VWC Summer Faculty Development Grant (MHS) and a Virginia Foundation of Independent Colleges Mednick Fellowship (VRT).

HARVESTMAN SIZE IMPACTS THE REALIZED TROPHIC LEVEL IN A RAIN FOREST FOOD WEB. Maynard H. Schaus & Victor R. Townsend, Jr., Department of Biology, Virginia Wesleyan College. Harvestmen (Arachnida, Opiliones) are frequently thought to be omnivorous, potentially ingesting invertebrates, plant tissues, detritus, and/or fungi. However, for most harvestmen species, quantitative diet data are lacking. We quantified the diets of several harvestman species at La Selva Biological Station, Costa Rica, using multiple stable isotope analysis. For cosmetid harvestmen, there was a significant increase in  $\delta^{15}\text{N}$  with body size. The diet of a cosmetid nymph was  $\sim 1$  trophic level lower than that observed in adult harvestmen (*Cynorta marginalis*, *Eucynorta tenuipes*, *Cynortellana oculata*, and the sclerosomatid *Prionostemma sp.*). The trophic level of the largest species (*Eupoecilaema magnum*) was  $\sim 1/3$  trophic level higher than that of other adult harvestmen. This indicates that the diet of harvestmen can vary ontogenetically, and that larger species can subdue larger prey items, which are more likely to occur at a higher

trophic level. Our results also indicate that this technique may provide valuable insight into the diet of harvestmen and can complement other analyses of diet. This study was funded by a VWC Summer Faculty Development Grant (MHS) and a Virginia Foundation of Independent Colleges Mednick Fellowship (VRT).

### Psychology

DISCRIMINATIVE STIMULUS PROPERTIES OF KETAMINE IN C57BL/6 MICE. T.J. Braxton<sup>1</sup>, T.J. Donahue<sup>2</sup>, K.A. Webster<sup>2</sup> & J.H. Porter<sup>1,2</sup>, Depts. of <sup>1</sup>Biology and <sup>2</sup>Psychology, Virginia Commonwealth University, Richmond, Virginia, USA. Ketamine is a noncompetitive NMDA receptor antagonist that is classified as a dissociative anesthetic, hallucinogen, and psychotomimetic. Recent research in humans has shown that it produces rapid antidepressant effects (~4 hours) unlike conventional antidepressant drugs that take weeks to be effective. The present study examined the discriminative stimulus properties of 10 mg/kg ketamine in mice using a two-lever drug discrimination assay that measures the subjective cue of a drug, which is mediated by activity at specific neurotransmitter receptors. In this operant task, mice were rewarded for selecting the condition-appropriate lever during test sessions – i.e. they were trained to discriminate ketamine from vehicle. All of the mice met the training criteria (10 responses per minute; drug-lever appropriate responding of  $\geq 80\%$ ). Tested drugs included: ketamine (NMDA antagonist), PCP (NMDA antagonist), MK-801 (NMDA antagonist), Memantine (NMDA antagonist), Imipramine (Tricyclic antidepressant), and Fluoxetine (SSRI antidepressant). Only the NMDA antagonists produced ketamine-appropriate responding (i.e. shared discriminative stimulus properties with ketamine via NMDA antagonism). Thus, the discriminative stimulus properties of ketamine do not appear to be related to its antidepressant effects that are evident in humans and that have been shown in other preclinical, animal assays that measure antidepressant-like behaviors specifically.

EXPLORING ETHNIC IDENTITY AND PEER NORMS: A KEY TO UNDERSTANDING DIFFERENCES AND SIMILARITIES IN EATING PATHOLOGY BETWEEN AFRICAN AMERICAN AND EUROPEAN AMERICAN COLLEGE WOMEN. L.M. Howard, B.N. Haislip & K.E. Heron, Virginia Consortium Program in Clinical Psychology and Old Dominion University, Norfolk VA 23529. Research suggests African American (AA) women experience less body dissatisfaction and eating pathology than European American (EA) women. This disparity may be accounted for by differing ethnic identification (feelings of belonging to culture) or perceived peer norms (degree one's peers value thinness) between AA and EA women. AA ( $n=25$ ) and EA ( $n=28$ ) female undergraduates completed online measures of ethnic identity, perceived peer norms, thin ideal internalization, body dissatisfaction, and eating behaviors. AA women experience higher ethnic identification than EA women ( $p=.001$ ), but

not perceived peer norms, although means were in the expected direction (AA reporting peers value thinness less). Perceived peers norms ( $ps < .001$ ), but not ethnic identity, was associated with greater disordered eating, thin ideal internalization, and body dissatisfaction for all women. This preliminary evidence suggests perceived peer norms are associated with less disordered eating and body dissatisfaction. Future research with larger samples is needed to further investigate whether ethnic identity and perceived peer norms are particularly protective for AA women. Norm based treatments, such as peer led interventions, might help reduce rates of disordered eating on college campuses for both AA and EA women.

ASSOCIATION BETWEEN TRAUMATIC LIFE EVENTS AND SOCIAL AND PSYCHOLOGICAL FUNCTIONING IN COLLEGE STUDENTS. R.I. MacIntyre<sup>1</sup>, H. Hamrick<sup>2</sup>, K.E. Heron<sup>1,2</sup>, S.B. Scott<sup>3</sup> & V. Juth<sup>4</sup>, <sup>1</sup>Virginia Consortium Program in Clinical Psychology, <sup>2</sup>Psychology, Old Dominion University, <sup>3</sup>Aging Studies, University of South Florida, and <sup>4</sup>Health Sciences, University of California, Irvine. Young people will have experienced a number of potentially traumatizing life events by the time they reach college. The aims of this study were 1) to examine the prevalence of traumatic life events in a diverse college sample and 2) to evaluate if number of event types, recency, and severity of adverse life events influence current social and psychological functioning. Undergraduate students ( $N=742$ ,  $Mage=23.4$ ) completed web-based measures of adverse life events, stress, depression, somatic symptoms, and social vigilance. Results addressing Aim 1 showed participants reported an average of 5.93 different types of adverse life events; average number of event types differed by race ( $p < .001$ ), but not gender ( $p = .08$ ). Event types by gender and race were examined and were largely consistent with previous findings. Aim 2 results showed event type total, recency, and severity were positively associated with stress ( $p < .05$ ), depression ( $p < .05$ ), and somatic symptoms ( $p < .05$ ); social vigilance was only associated with event type total ( $p < .001$ ). These findings provide information regarding the prevalence of traumatic life events in a diverse college sample and suggest recent and severe events impact student mental health while experiencing a greater number of different adverse events place students at risk for both mental health issues and social vigilance.

### Statistics

SERUM ALBUMIN AS A ZINC CARRIER. Katarzyna B. Handing<sup>1,2</sup>, I. G. Shabalin<sup>1,2</sup>, M. Chruszcz<sup>3</sup> & W. Minor<sup>1,2</sup>, <sup>1</sup>Department of Molecular Physiology and Biological Physics, University of Virginia, Charlottesville, VA 22908, <sup>2</sup>New York Structural Genomics Research Consortium (NYSGR), <sup>3</sup>Department of Chemistry and Biochemistry, University of South Carolina, Columbia, SC 29208. Serum albumin (SA) is the most abundant protein in mammalian blood plasma (~600 $\mu$ M), making it the main determinant of plasma osmotic pressure. SA is able to bind and transport a wide variety of molecules including fatty acids, hormones, drugs and metal ions. SA is an important physiological transporter of several essential metal ions and assists in maintaining levels of free metal ions in blood. The aim of our study was to characterize zinc binding sites on SA in mammalian species. We have solved the crystal structures of human (HSA) and horse serum albumin (ESA) in complex with zinc. The presence of metals in the structures has been confirmed experimentally by X-ray anomalous scattering. Our data confirms that metal-binding site A is the primary binding site for Zn<sup>2+</sup> and reveals the residues responsible for zinc coordination and the geometry of the binding sites. We see that site A is conserved between mammals. Additionally, pHs below physiological levels affect albumin affinity for zinc by protonation of zinc-coordinating histidine, which we show adopts different conformations at different pHs. We find several secondary zinc binding sites that are of biological interest, since site A may be disrupted by fatty acid and/or other metal binding. These findings expand our knowledge of metal transport and distribution in mammals. A better understanding of metal binding to SA is critical for the understanding of metal homeostasis and its contribution to disease states.

EXPLAINING THE TEN-YEAR INCREASING TREND IN KIDNEYS RECOVERED FOR TRANSPLANTATION BUT DISCARDED. Victoria C. Garcia<sup>1,2</sup>, B. J. Carrico<sup>2</sup> & D. E. Stewart<sup>2</sup>, <sup>1</sup>Dept of Biostatistics, Virginia Commonwealth University, Richmond, VA 23198, <sup>2</sup>United Network for Organ Sharing, Richmond, VA 23198. Though the proportion of kidneys recovered for transplantation but discarded (discard rate, DR) have remained relatively stable from 2009 to present, the DR steadily increased from 1999 to 2009, from 13% to 19%. An analysis was performed to determine if this increase could be explained by a rise in the recovery of kidneys from a broader, more expansive donor pool, as opposed to more risk-averse practices among transplant hospitals. Data on all deceased donors with a kidney recovered for transplantation from 1999 to 2009 were analyzed, with

donor characteristics collected by the United Network for Organ Sharing as the Organ Procurement and Transplant Network. From Oct. 25, 1999 to 2009, 65,292 deceased donors had 129,734 kidneys recovered for transplant, and 21,306 of them were discarded. After accounting for Kidney Donor Risk Index (KDRI) characteristics, the time trend was no longer statistically significant, suggesting changes in the characteristics of recovered kidneys explained the ten-year DR increase. Yet, also adjusting for biopsy-related factors reversed the predicted DR change over time, suggesting that, if the donor characteristics and the proportion of kidneys biopsied had remained stable, the DR would have actually improved slightly. However, after including the clinical decision of whether or not to pump a kidney, the residual time effect reversed again, suggesting that more pumping of kidneys may be a way to increase the available supply of kidneys for transplantation.

CHARACTERIZING THE VAGINAL MICROBIOME BASED ON A LARGE CROSS-SECTIONAL STUDY. Paul J. Brooks, David J. Edwards & Victoria V. Pokhilko, Department of Statistical Sciences and Operations Research, VA Commonwealth University. We conducted an analysis of 16S rRNA surveys of the vaginal microbiome based on samples from over 6,000 women. Subjects also provided medical, lifestyle, and demographic information. We investigate differences in microbiome composition by demographic factors, history of pregnancy complications, history of sexually-transmitted infections (STIs), the diagnosis at the time of sample collection, and pregnancy status. Vaginal microbiome profiles are typically dominated by a single bacterium, leading to a classification of samples into groups that we call *vagitypes*. Vagitype classifications facilitate the discovery of relationships between microbiome profile and clinical data. The presence or absence of *Lactobacillus* species and a diagnosis of bacterial vaginosis have been shown to play an important role in the reproductive health of a woman. Our analysis provides information about these patterns and suggests roles for other bacteria in health and dysbiosis.

ANALYZING HONG KONG AIR POLLUTION USING DIMENSION REDUCTION. H. Moradi Rekabdarkolae, Edward L. Boone & Qin Wang, Department of Statistical Sciences and Operations Research, Virginia Commonwealth University, Richmond, VA, 23284. Dimension reduction and variable selection play important roles in high dimensional data analysis. Minimum Average Variance Estimation (MAVE) is an efficient approach among many others. However, because of using least squares criterion, MAVE is not robust to outliers or errors with heavy tailed distributions. In this paper, we propose a

robust extension of MAVE which can adapt to different error distributions. Our proposed estimate is shown to have the same convergence rate as the original MAVE. Furthermore, we combine the proposed method with adaptive LASSO to select informative variables. This new approach is illustrated through simulation studies and a data analysis on air quality of Hong Kong.

A DESIGNED EXPERIMENT APPROACH FOR THE TUNING OF OPTIMIZATION SOFTWARE. Toni P. Sorrell, J. Paul Brooks & David Edwards, Department of Statistical Sciences and Operations Research, Virginia Commonwealth University, Richmond, VA 23284. The tuning of optimization software is of key interested to researchers solving mixed integer programming (MIP) problems because the efficiency of the optimization software is can be greatly impacted by the solver's parameter settings and the structure of the MIP. Design of experiment methodology can provide insights into choosing good optimization solver's parameter settings for MIPs. A designed experiment approach is used to fit a model that would suggest settings of the parameters that provided the greatest impact on the primal integral, which is the comparison metric being used. Primarily, this research focuses on using classes of MIPs to not only obtain good parameter settings for a practitioner to use on future instances of the same class of MIPs, but to also gain understanding of why the settings work well for that class of MIPs. These experiments are conducted on three classes of MIP problems: survivable fixed telecommunication network designs, a formulation of Support Vector Machines (SVM) with Ramp Loss, and Coding Theory graphs. We compared our method to four other methods. Three methods come from the Selection Tool for Optimization Parameters (STOP), and they are random, greedy heuristic, and pairwise coverage. The last is CPLEX's and Gurobi's own tuning, which we consider a black box approach. An exhaustive search for the best parameter settings on a limited number of parameters provides a reference for the effectiveness of the methods used as a proof a concept for further experiments involving a large number of parameters.

DETECTING TINY SIGNALS IN MASSIVE DATA FROM HIGH-ENERGEY PHYSICS. Karen Kafadar, Department of Statistics, University of Virginia, Charlottesville, VA 22904. Experiments in high-energy physics provide terabytes of data, from which critical information about the state of matter, governed by the theory outlined in the "Standard Model", must be extracted. Opportunities abound for increased efficiencies in approaches to the data, from the design of experiments, to the collection of data, and finally to analysis and inference. Due to the massive amounts of data, from various sources



(different experiments from different collaborations, experiment-based simulations, etc.), new ways of analyzing the data to answer questions of interest are devised. This talk describes the framework for these experiments and illustrates methods for analyzing massive data sets from such experiments (with some mention of data sets from genomics and the Internet).

**Structural Biology, Biochemistry & Biophysics**

RE-EXAMINATION OF THE STRUCTURE AND ELASTICITY OF THE TITIN IG65-70 SEGMENT. Rachel A. Policke, Chris E. Berndsen & Nathan T. Wright, Department of Chem. and Biochem., James Madison Univ., Harrisonburg VA. 22807. Previously published crystal structure data of 6 tandem domains of titin, Ig65-70, suggest that the region is rigid in structure with discrete hinge points in a “carpenter’s ruler” model. However, this model does not agree with the more recent, modified wormlike model (mWLM), in which the linkers between semi-rigid Ig domains are hinge points capable of bending. In order to reconcile the differences between these two models, we re-analyzed the crystal structure data. We found that the apparent rigid regions of I6 could be explained through crystal packing interactions rather than intermolecular interactions. Thus, while domain-domain and domain-linker interactions may contribute minimally to titin elasticity, most of this elasticity must be accomplished through intradomain stretching and an entropic spring-like motion.

HIGH RESOLUTION STRUCTURE OF TITIN ZIG10. Allyn Letourneau & Nathan T. Wright, Dept. of Chemistry and Biochemistry, James Madison University, 800 South Main St, Harrisonburg, VA 22807. Titin domains ZIg9/10 bind to obscurin domains Ig58/59 during myofibrillogenesis. Mutations in this region lead to hypertrophic cardiomyopathy (HCM) in humans. While the cellular consequences of this interaction are well characterized, the molecular determinants governing this structure are unknown. Previous work from our lab has solved the high-resolution structure of the obscurin domains of the complex. Here, we describe the purification and complete structure characterization of titin domain ZIg10. (Supported by Jeffress Memorial Fund, Research Corporation Cottrell College Grant, NSF-REU (CHE-1461175)).

COMPARING THE TEMPORAL DYNAMICS OF CASPASE ACTIVITY DURING THE INTRINSIC AND EXTRINSIC PATHWAYS OF APOPTOSIS. S. Morris, P. Stuckey & R. Reif, Department of Chemistry, University of Mary Washington, 1301 College Ave., Fredericksburg VA 22401. Apoptosis, a process in which a cell systematically triggers its own death in response to DNA damage or external stimuli, is widely utilized in the body in both aging and cancer prevention. There are several known pathways that execute apoptosis utilizing a family of enzymes called caspases, with the intrinsic and extrinsic pathways undergoing separate initiation mechanisms and caspases, before converging to a conserved cascade. The goal of this experiment was to elucidate and compare the temporal dynamics of apoptosis with respect to the onset and duration of caspase

activity for both the intrinsic and extrinsic pathways. This was done through a novel combination of microfluidics and fluorescence microscopy. The intrinsic pathway showed consistent caspase activation around 3.5 hours after induction, with a consistent duration of two hours. The extrinsic pathway showed varying onset times with an average around 4 hours post induction, and a duration that exceeded the 6 hour window of this experiment. By timing the major events of apoptosis through both pathways, the overall apoptotic process can be more clearly understood.

OPTIMIZATION OF RNA SELEX PARAMETERS. K. Kerns, S. Clark & R. Reif, Department of Chemistry, University of Mary Washington, 1301 College Ave., Fredericksburg VA 22401. Systematic Evolution of Ligands by Exponential Enrichment, or SELEX, is a method of selectively purifying and amplifying stereospecific, high affinity RNA aptamers (oligonucleotides) for certain target ligands. First, a random DNA sequence is obtained, purified, amplified, and reverse transcribed into RNA. Then, the target is exposed to this random sequence RNA, and the RNA that binds to the target is extracted and amplified. This procedure is repeated for every successive step of SELEX isolating RNA with the highest affinity for the target. Because there are many different methods for conducting PCR, RNA transcription, reverse transcription, and RNA and DNA purification, it is essential to develop a standard, efficient method by which the SELEX experiment can be conducted. This experiment focuses on the optimization of the RNA transcription process as well as the filtration and purification of the aptamer-protein complex through a nitrocellulose membrane; focusing specifically on the extraction and amplification of the aptamer sequence. This is designed to create an abbreviated and efficient procedure which future researchers can follow to expedite the RNA SELEX process.

SEQUENCE VERSUS STRUCTURE: A LOOK AT HOW THE GENETIC CODE DIRECTS PROTEIN FOLDING. John T. Bedford, Jason C. Collins, Brittney L. Ruedlinger & Lesley H. Greene, Department of Chemistry and Biochemistry, Old Dominion University, Norfolk VA 23529-0126. The immunoglobulin-binding domain of protein G (GB1) is a relatively small protein consisting of 56 amino acid residues. GB1 is, although thermostable at high temperatures, classified as a mesophilic protein. A structural alignment was created using the DaliLite server. One of the proteins that was identified was the small archaeal modifier protein 1 (SAMP1) from the extremophile *Haloferax volcanii*. A long-range interaction network was constructed for each protein and a betweenness centrality measure was applied. Several residues were identified as having high betweenness centrality in each protein. When comparing these residues with the alignment three

of them correspond in location. We propose that these residues are important in the formation and/or stability of the ubiquitin-like  $\beta$ -grasp fold found in both proteins. The results of computational studies will be presented in the context of structure, stability, conservation, and folding.

**MECHANISTIC INSIGHTS INTO UBC13-CATALYZED UBIQUITINATION.** Aaron G. Davis<sup>1</sup>, Walker M. Jones<sup>1</sup>, Serban Zamfir<sup>2</sup> & Isaiah Sumner<sup>1</sup>, <sup>1</sup>Department of Chemistry and Biochemistry, James Madison University, Harrisonburg VA 22807 and <sup>2</sup>Department of Chemistry, Virginia Commonwealth University, Richmond VA 23284. Ubc13 is an E2 enzyme that catalyzes lysine ubiquitination, a type of protein post-translation modification. Ubiquitinating a protein can signal for its degradation, affect its activity and also plays a role in DNA repair and inflammatory response. Defects in this process are linked to different disorders including cancer and Parkinson's and Alzheimer's diseases. The accepted mechanism for Ubc13-catalyzed ubiquitination is a stepwise mechanism that proceeds through an oxyanion intermediate. This intermediate is hypothesized to be stabilized by a nearby asparagine residue, which is known as the "oxyanion hole." However, the validity of the accepted mechanism has recently come into question. In our study, we use a combination of simulation techniques including classical molecular dynamics, *ab initio* molecular dynamics and hybrid Quantum Mechanics/Molecular Mechanics to examine this hypothetical mechanism. Our calculations indicate that several different intermediates are possible and that water may stabilize the intermediate, whereas the asparagine serves to stabilize a random coil near the active site.

**STRUCTURAL AND FUNCTIONAL BASIS OF THE LYSOSOMAL PROTEIN PHAFIN2.** Tuo-Xian Tang, Amy Jo, Iulia Lazar, Richey Davis & Daniel G. S. Capelluto. Department of Biological Sciences, Biocomplexity Institute, Virginia Tech, Blacksburg VA 24061. The lysosomal protein Phafin2 is one of the fourteen members of the Phafin protein family, which all contain a N-terminal PH (pleckstrin homology) domain followed by a C-terminal FYVE (Fab 1, YOTB, Vac 1, and EEA 1) domain. Phafin2 was found to interact with the serine/threonine kinase Akt and forms the Akt-Phafin2 complex, which plays an important role in the induction of autophagy. It was demonstrated that after the induction of autophagy, Akt-Phafin2 complex accumulates on the lysosome via the interaction of Phafin2 with lysosomal phosphatidylinositol 3-phosphate (PtdIns(3)P). Our study focuses on the elucidation of the structural and functional properties of Phafin2 using NMR and other biophysical techniques, and its interaction with PtdIns(3)P. Phafin2 has been purified to homogeneity and exhibits an elongated monomeric  $\alpha/\beta$  structure of

about 6.5 nm with modest thermal stability. NMR data also indicates that the protein presents substantial random coil regions in its structure. Using surface plasmon resonance, we show that Phafin2 interacted with PtdIns(3)P-enriched liposomes with high affinity. By studying the structural properties of Phafin2, we can better understand its molecular interactions at high resolution.

STRUCTURAL BASIS OF LIGAND RECOGNITION BY THE ENDOSOMAL ADAPTOR PROTEIN TOM1. Wen Xiong, Xiaolin Zhao, Michael Hodge, Phillip Choi & Daniel G. S. Capelluto, Department of Biological Sciences, Biocomplexity Institute, Virginia Tech, Blacksburg, VA 24061. Tom1 (target of Myb 1) plays a role in membrane trafficking by serving as an endosomal sorting complex required for transport (ESCRT) component, or at least be associated with the ESCRT machinery. A model has been proposed in our recent study in which Tom1 interaction with the Toll-interacting protein (Tollip) induces the unfolded TBD domain of Tollip to fold, and then this folding modulates lipid binding of Tollip, mediating its dissociation from phosphatidylinositol-3-phosphate (PI(3)P) and committing Tollip to cargo trafficking. However, there are still some underlying questions about this model that need to be addressed as follows: **(i)** how does the Tom1 distinguish between structurally distinct polyubiquitin chains which represent functionally distinct intracellular signals; **(ii)** what the structural basis of Tom1 VHS interactions. Recently, Tom1 VHS domain has been shown to serve as a new PI(5)P effector at signaling endosomes through its VHS domain delaying epidermal growth factor receptor degradation in a bacterial infection model; Tom1 VHS domain also binds ubiquitin and form a complex with Tollip through the Tom1 GAT domain for receptor degradation; therefore, we hypothesize that the ubiquitin and PI(5)P compete each other for Tom1 VHS binding. Addressing these questions will unveil the role of Tom1 in endosomal protein trafficking and the integration of membrane trafficking with cellular processes.

ESTIMATING EFFICIENCY IN RNA-MIRNA BINDING PREDICTION SOFTWARE. O. M. Plotnikova<sup>1</sup>, A. Baranova<sup>2,3</sup>, D. A. Zubtsov<sup>1</sup> & M. Y. Skoblov<sup>1,2</sup>. <sup>1</sup>Moscow Institute of Physics and Technology, Moscow, 117303, Russia, <sup>2</sup>Centre for Medical Genetics, Federal Agency for Scientific Organizations, Moscow, 115478, Russia, <sup>3</sup>School of Systems Biology, George Mason University, Manassas, VA, 20110, USA. miRNA play a key roles in regulation of gene expression as guide molecules in the RNA-induced silencing complex (RISC). There are now more than 2,500 identified human's miRNAs, but the question about the mechanism of interaction between mRNA and miRNA is still open. A theory of canonical interactions is considered that the 2-8nt seed region at the 5' end of miRNA interacts with 3'UTR

end of mRNA according to the complementary Watson-Crick pairing rule. There are five widely used predictive algorithms for searching miRNA-mRNA interactions: TargetScan, Pictar2, PITA, RNA22, and miRanda. Recently, CLASH method was designed for revealing miRNA-mRNA binding sites in HEK293 cell line. Thus we have the opportunity to assess the effectiveness of all five algorithms. We developed a multiparameter analysis of prediction and function of software including estimation of sensitivity and positive predictive value. Analysis of what region of mRNA interacted with miRNA revealed different types of “canonical”, “noncanonical”, and “energy class” interactions. We conducted a qualitative comparison of predicted miRNA and miRNA from the experimental data-set. The activity of binding mRNA was analyzed and compared experimental data with the complete level of expression of mRNA in HEK 293 using the FANTOM5 project data. According to our analysis, we have established a low level program ability to predict real miRNA-mRNA interactions.

EXPLORING NOVEL FLAVIN-DEPENDENT CHEMISTRY: THE MECHANISM OF OLEATE HYDRATASE FROM *ELIZABETHKINGIA MENINGOSEPTICA*. Madeline Marcus, Kim Harich, Julia S. Martin del Campo & Pablo Sobrado, Department of Biochemistry, Virginia Tech, Blacksburg, VA 24060. Oleate hydratase (OhyA; E.C.4.2.1.53) from *Elizabethkingia meningitis*, is a flavin-dependent enzyme that catalyzes the conversion of oleic acid to 10-hydrostearic acid. OhyA has become the focus of studies due to its ability to introduce hydroxyl groups without wasted energy on cofactor recycling. By converting oleic acid to (R)-10-hydroxystearic acid, oleate hydratase provides a product that has potential use as a surfactant, a lubricant, in cosmetic applications, and a possible initiation to greater polymer chemistry. The hydration of unsaturated fatty acids is believed to be a detoxification mechanism and a survival strategy. The reaction catalyzed by OhyA does not involve a net redox change, however the enzyme requires the flavin cofactor in the reduced form for activity. Our hypothesis is that the flavin cofactor in OhyA functions as an acid, which represents a novel role in nature. OhyA was expressed as a recombinant protein in *Escherichia coli* and purified with FAD bound (Figure 1). Overall purification resulted in 68.3 mg of purified OhyA per 1 liter of growth media, with FAD incorporation of 49 %. Activity assays were performed in the presence of oleic acid and the coenzymes: NADH, NADPH under both anaerobic and aerobic conditions. OhyA was active only under anaerobic and reduced conditions. We present the stopped-flow kinetic characterization of OhyA and propose a role for the reduced FAD in this non-redox reaction. (supported in part by NSF-CHEM 1506206.)

**94<sup>th</sup> Annual Meeting *Best Student Presentation Awards***

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**ASTRONOMY, MATH and PHYSICS with MATERIAL SCIENCE***Best Student Oral Presentation and CARPENTER AWARD:*

**Steven H. Lohrey**, Dept. of Physics, Randolph-Macon College  
Development and verification of a new post roll-up model for  
characterization of aircraft lift generated wake vortices

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**BIOLOGY with MICROBIOLOGY and MOLECULAR  
BIOLOGY***Best Graduate Student Oral Presentation and REMSBURG AWARD:*

**Kelly Barford**, Dept. of Neuroscience, University of Virginia  
Molecular regulation of TrkA trafficking in space and time

*Best Undergraduate Student Oral Presentation and REMSBURG  
AWARD:*

**Hannah Belski**, Dept. of Biology, University of Mary  
Washington  
Dominance behavior in the tube test, and its relationship to home  
cage behavior in mice

*Best Graduate Student Poster Presentation:*

**Allison L. Teunis**, Center for Applied Proteomics and National  
Center for Biodefense & Infectious Diseases, Dept. of Molecular  
Microbiology, School of Systems Biology, George Mason  
University  
Early neutrophil recruitment by chemokine-releasing  
nanoparticles improves bacterial clearance and survival of anthrax  
spore-challenged mice

*Best Undergraduate Student Poster Presentation:*

**Kelsey Deal** and **Geoffrey Parriott**, Dept. of Biological & Environmental Sciences, Longwood University  
Chimeric PD1-expressing T cells as a potential treatment for multiple types of cancer

*Honorable Mention – Student Poster Presentation:*

**Kristina Krumpos**, Dept. of Biological Sciences, University of Mary Washington  
Genetically engineering a plasmid expression vector for nuclear localization studies, Part 1: Engineering a 3GFP-3NLS plasmid

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**BIOMEDICAL and GENERAL ENGINEERING***Best Student Oral Presentation:*

**Ruche Chande**, Dept. of Biomedical Engineering and Dept. of Orthopaedic Surgery, Virginia Commonwealth University  
Improved predictive capability of a computational foot/ankle model using artificial neural networks

*Honorable Mention – Student Oral Presentations:*

**Patrick A. Link**, Dept. of Biomedical Engineering, Virginia Commonwealth University  
Development and characterization of protein nanoparticles derived from lung extracellular matrix

**Mark Ostyn**, Dept. of Mechanical & Nuclear Engineering and Dept. of Radiation Oncology, Virginia Commonwealth University  
An active immobilization system for head and neck external beam radiotherapy using a 6D table and radiofrequency localization

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**BOTANY**

*Best Student Oral Presentation and BAKER AWARD:*

**Sarah Kwon**, Dept. of Biology, University of Richmond  
Anatomy and morphology of the allomorphic flowers of *Acalypha setosa* (Euphorbiaceae)

*Best Student Oral Presentation and HARVILL AWARD:*

**Sydney J. Vonada**, Environmental Studies Program, Shenandoah University  
An ecological characterization of terrestrial communities at Shenandoah University's River Campus at Cool Spring Battlefield, Clarke County Virginia

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**ENTOMOLOGY**

*Best Student Oral Presentation:*

**James M. Wilson**, Dept. of Entomology, Virginia Tech  
Friends with benefits: Natural enemies and pollinators in Virginia cucurbit production

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**ENVIRONMENTAL SCIENCE**

*Best Student Oral Presentation:*

**James Haluska**, Dept. of Ocean, Earth & Atmospheric Sciences, Old Dominion University  
Shore and location changes to Wreck Island, Virginia since 1999

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**MEDICAL SCIENCES***Best Student Oral Presentation:*

**Braden Herman**, Dept. of Biology, Eastern Mennonite University  
Design and SAR study of small molecule CtBP inhibitors

*Best Student Poster Presentation:*

**Sarah L. Kyte**, Dept. of Pharmacology and Toxicology, Virginia Commonwealth University  
Nicotine prevents chemotherapy-induced peripheral neuropathy in vivo and fails to stimulate the growth of lung cancer cells or interfere with the effectiveness of chemotherapy *in vitro*

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**NATURAL HISTORY and BIODIVERSITY***Best Student Oral Presentation and WOOLCOTT AWARD:*

**Justin Bredlau**, Integrative Life Sciences and Dept. of Biology, Virginia Commonwealth University  
Evolving isolation mechanisms among host-foodplant sources of a parasitic wasp species

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**PSYCHOLOGY***Best Student Poster Presentation and RAY KIRBY AWARD:*

**Rachel I. MacIntyre**, VA Consortium Program in Clinical Psychology, Old Dominion University  
Association between traumatic life events and social and psychological functioning in college students

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## STATISTICS

*Best Student Oral Presentation:*

**Hossein M. Reabdarkolaee**, Dept. of Statistical Sciences & Operations Research, Virginia Commonwealth University  
Analyzing Hong Kong air pollution using dimension reduction

*Honorable Mention – Student Oral Presentation:*

**Victoria C. Garcia**, Dept of Biostatistics, Virginia Commonwealth University, and United Network for Organ Sharing  
Explaining the ten-year increasing trend in kidneys recovered for transplantation but discarded

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**STRUCTURAL BIOLOGY, BIOCHEMISTRY and BIOPHYSICS***Best Student Oral Presentation:*

**Wen Xiong**, Protein Signaling Domains Laboratory, Dept. of Biological Sciences, Biocomplexity Institute, Virginia Tech  
Structural basis of ligand recognition by the endosomal adaptor protein Tom1

*Best Student Poster Presentation:*

**Kimberly Kerns**, Dept. of Chemistry, University of Mary Washington  
Optimization of SELEX parameters for RNA aptamer selection

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