

ABSTRACTS OF PAPERS PRESENTED AT THE 2006 COLLEGIATE MEETINGS

EASTERN REGION

PELLISSIPPI STATE TECHNICAL COMMUNITY COLLEGE
KNOXVILLE, TENNESSEE

THE FLORA OF THE HIWASSEE/OCOEE RIVERS STATE PARK. *William Nation and Allen D. Moore, Tennessee Wesleyan College, Athens, Tennessee.* Vegetation scientists attempt to understand the degree of species inter-dependence within communities, how the distribution of communities depends upon past and present environmental factors, and what the role of communities is in such ecosystem activities as energy transfer, nutrient cycling, and succession. However, communities must first be measured and summarized in some effective way before these questions can be addressed. The ongoing attempt to inventory the world's vegetation is based on a small sample of the total vegetation cover because of limitations in people, time, and resources. These samples must be taken carefully to ensure the resulting estimates will be accurate and useful. In this study, we have used the Releve Method to determine species abundance and community structure of the Hiwassee-Ocoee Rivers State Park.

A SURVEY OF THE TREES IN A MIXED-HARDWOOD FOREST IN HARALSON COUNTY, GEORGIA. *Robert H. Floyd, Maryville College, Maryville, Tennessee.* Trees were surveyed on a 40 ha tract in Haralson County, Georgia. The county is located in west Georgia at the border between the Ridge and Valley and Piedmont and contains trees common in both. A series of 100 m transects were established in 4 habitats, 3 Oak forests and one Bottomland Hardwood forest. Trees were sampled using point-quarters at 10 m intervals along every transect. Twenty-two different species from 12 families were encountered. Importance Values (IV) were calculated for every species both within each habitat and for the tract as a whole. Southern Red Oak (*Quercus falcata*) had the highest IV overall and in two of the Oak forests. White Oak (*Quercus alba*) and Sweet Gum (*Liquidambar styraciflua*) had the highest IV in the third Oak forest and the Bottomland Hardwood forest respectively.

TURTLE ACTIVITY ON WOLFTEVER CREEK AND WILKERSON BRANCH FROM APRIL TO NOVEMBER, 2004. *Kristi Weis, Southern Adventist University, Collegedale, Tennessee.* Turtle activity was studied from April to November 2004 on Wilkerson Branch and Wolftever Creek, two medium-sized streams that feed into the Tennessee River in Southeastern Tennessee. The turtles captured in hoop traps were numbered and data was taken for each. The anticipation was to find out what turtle species is prevalent on the smaller streams in Eastern Tennessee and to find out more about the turtles' movement. Forty turtles were captured between the two creeks with five recaptures. *Chelydra serpentina* constituted 85% of turtles

captured. Due to this large percentage, the detailed analysis of the data focused on this species. June had the most captures, with May coming in second. The only noticeable difference between male and female movement was in June. When a turtle was placed back in the trap as bait, new turtles were captured only 20% of the time.

TURTLE MOVEMENT IN TWO TENNESSEE CREEKS. *Bryce Anderson, Southern Adventist University, Collegedale, Tennessee.* A study was done during the summer of 2005 to observe *Chelydra serpentina* movement in two Tennessee creeks. The observation looked for movement trends in time of year, gender and also temperature. Data were collected from Wolftever Creek and Wilkerson Branch. Trapping and radio tracking were two methods used. Hoop traps with netting and either bait or chicken wire wings were used to guide turtles into the traps. Tracking was done with a GPS (global positioning system) and a radio receiver to pick up signals from transmitters that were placed on the carapace of a turtle. Trapping data showed increased movement in May and June and a great decrease in August. However, tracking showed that movement remained high during August. No trends separated gender and only temperature showed effects in early spring. These results support the conclusion that movement trends are based on time of year.

ARTHROPOD BIODIVERSITY OF RED OAK CANOPIES IN THE MARYVILLE COLLEGE WOODS. *Jenna Wade, Maryville College, Maryville, Tennessee.* Large quantities of insects reside in forest canopies. Most studies have been completed in tropical rainforests, with little attention given to temperate forests. In this study, the canopies and understories of four Southern Red Oaks located in the Maryville College Woods in Maryville, Tennessee were sampled over a period of 8 weeks using a composite flight-interception trap. A total of 2,142 arthropods were collected from 11 Orders and 65 Families. Shannon's and Simpson's diversity indices indicated minimal difference between each sampling site. However, Sorenson's quantitative index measuring community similarity revealed more distinct differences. The least similar communities were the ridgetop sample tree canopies versus the floodplain canopies, which shared 49% of the Families found. The most similar communities were all of the canopies compared to the understories, sharing 67% of the Families found.

CHELONIAN POPULATIONS OF TWO WATERWAYS IN THE SOUTHERN TENNESSEE VALLEY. *Tyler Shelton, Southern Adventist University, Collegedale, Tennessee.* Waterways in the Tennessee Valley (TV) provide a rich wetland habitat for many organisms and have a major role in human water needs. In an effort to further understand the biological diversity of the Tennessee watershed, turtle populations of two minor waterways, Wolftever Creek and N. Chickamauga Creek, were surveyed over the period of early March through late September

in 2003. The turtle populations living along TV waterways play an important role in the regulation of their ecosystem as much of their diet is diverse, ranging from small insects and macroalgae to birds and small mammals. A large portion of the turtles captured were found to be of the species *Chelydra serpentina* and *Trachemys scripta*. A strong correlation between capture rates and reproductive activity was discovered. The results of this survey are then discussed in correlation with certain environmental factors of the two waterways, such as the locality of the Chickamauga Dam and perspective water flow rates.

RECONSTRUCTING THE PAST: AN ANALYSIS OF MIOCENE AGED VERTEBRATE FOSSILS IN EAST TENNESSEE. *Patricia J. Cross, King College, Bristol, Tennessee.* A biological analysis of the fossil vertebrate faunas of the Southern Appalachian region. The newly discovered fossil vertebrates deposited within a former sinkhole in East Tennessee provide a rare example of late Miocene fauna. These discoveries reveal the hidden biological diversity once present approximately seven million years ago. The Gray Fossil Site is among the most diverse fossil communities in the United States, enriched with fossils ranging from rhino, to the newly discovered species of panda and badger, and has the largest concentration of fossil tapirs ever recorded. Through the analysis of data collected during excavation, the Gray Fossil Site presents an untold story of the Southern Appalachian region and reveals a hidden diversity unseen for several million years.

POPULATION STATUS OF EASTERN HELLBENDERS IN SOUTHEAST TENNESSEE. *Kevin McGrath, Rebecca Norton, and Michael Freake, Lee University, Cleveland, Tennessee.* Eastern hellbenders *Cryptobranchus alleganiensis* (Cryptobranchidae) are the largest salamander in the United States, and recent studies indicate they are declining over most of their range. We are mapping the current distribution of hellbenders in the Cherokee National Forest and Great Smoky Mountains National Park in Tennessee and North Carolina, which represent much of the highest quality habitat for hellbenders in the southern Appalachians. When a population is identified, we are assessing the age structure and population viability, and investigating potential environmental stressors. We have identified just two rivers in which all size classes are represented, including gilled larvae (indicating current reproduction and recruitment). Preliminary tissue analyses revealed the presence of DDT metabolites and mercury residues; however the levels are very low, and unlikely to be biologically significant. We found four additional rivers containing hellbenders. However population densities appear relatively low and as yet there is no evidence of current reproduction. Our data confirm that hellbenders have declined across their historical range in southeast Tennessee. Likely causes include habitat fragmentation, siltation and dam construction.

CORRELATION OF OPOSSUM LITTER SIZE TO MATERNAL BODY CONDITION AND ENVIRONMENTAL VARIATION. *Karan Sharma, Lee University, Cleveland, Tennessee.* Individual fitness is related to number of offspring produced, which may be influenced by energy balance relating to the mother's body size, intra- and interspecific competition and environmental factors that vary over space and time. We tested hypotheses on these relationships in *Didelphis virginiana*, a key mesopredator in which litter size is easily assessed, by operating

two trapping grids near Memphis, Tennessee over three summers (12,000 trap-nights). Forty six female opossums were captured with pouch young. Mean number of young per litter was 8.8. No significant relationships were found between number of pouch young per litter and trap grid, year, the mother's mass or length, or predator numbers on a grid and the body condition index of the female. Wide variation in number of young may indicate, however, that differences in genetics, health, or other factors affecting individual females influenced litter size.

DIVERSITY AND ABUNDANCE OF SALAMANDERS FOUND IN SPICEWOODS BRANCHES, GREAT SMOKY MOUNTAINS NATIONAL PARK. *Sara Brickley Ashcraft, Pellissippi State Technical Community College, Knoxville, Tennessee.* On a monthly basis, students from Pellissippi State have monitored stream quality and salamander diversity from Spicewoods Branches in the Great Smoky Mountains National Park. Data collected include water temperature, pH, turbidity, as well as species type, length, and weight. Data have been analyzed to examine change in the diversity over time, prevailing species, prevalence of larvae versus adults, and changes in size for the most common species found. This information is being used to create a database showing seasonal changes in the salamander population.

GLYCOSYLATED HEMOGLOBIN LEVEL AS AN INDICATOR FOR DEPRESSION AMONG HISPANIC DIABETICS. *Cheryl Tilley, East Tennessee State University, Johnson City, Tennessee.* Diabetes mellitus is a progressive disease that is rapidly becoming a major health concern among the Hispanic population. Hispanic diabetics have been identified as having an increased incidence of depression. Improving identification of depression in this subgroup is crucial. The objective was to determine whether elevated glycosylated hemoglobin levels could be used as an indicator for depression among Hispanic individuals with diabetes. This retrospective chart audit identified 14% of patients with a diagnosis of diabetes and depression based on ICD-9 codes. An independent *t*-test was used to determine differences between groups. There was no significant difference between patients diagnosed with depression and diabetes and patients without depression. Further research is needed with larger samples to understand the relationship between depression and diabetes in the Hispanic population.

PREDICTIONS ON THE LIKELIHOOD OF H5N1 INFLUENZA MUTATION TO FACILITATE HUMAN TO HUMAN TRANSMISSION. *Kelienne M. Verdier, Pellissippi State Technical Community College, Knoxville, Tennessee.* H5N1 avian influenza has emerged as a global concern, successfully transmitting among fowl and, more recently, from fowl to humans. However, H5N1 has not yet developed the ability to transmit readily from human to human. The hemagglutinin protein, which varies among influenza strains, is essential in host cell binding and infectivity. To date, only H1, H2 and H3 influenza strains readily transmit from human to human. A literature search was conducted to determine which amino acids are essential for binding to avian vs. human sialic acid receptors. Thirty hemagglutinin protein sequences from H2, H3 and H5 influenza strains, infecting either avian or human hosts, were obtained from NCBI and compared. Based on sequence comparisons at key sites, and the error prone viral replication

system, predictions concerning the likelihood of human to human transmission of avian H5N1 influenza were made.

EXPLORING POTENTIAL MECHANISMS FOR THE CONFORMATIONAL CHANGE LEADING TO INFECTIVITY IN INFLUENZA HEMAGGLUTININ. *Kara D. Whitlock, Pellissippi State Technical Community College, Knoxville, Tennessee.* Recent concerns about a potential pandemic of bird flu have drawn attention to influenza viruses. In particular, the mechanism by which the virus infects the host cell is a critical concern. The glycoprotein hemagglutinin binds to sialic acid receptors on the cell surface, and the virus is then endocytized. Once inside the cell, pH changes in the endosome result in a major conformational change in the hemagglutinin molecule, resulting in membrane fusion and release of viral RNA into the host cell. Several possibilities for the mechanism by which the conformational change occurs have been explored using computer modeling of protein structure in Rasmol.

OCCURRENCE OF METHACILLIN RESISTANT *STAPHYLOCOCCUS AUREUS* IN CLASSROOM AIR. *Andrew Moren, Southern Adventist University, Collegedale, Tennessee.* Methicillin-resistant *Staphylococcus aureus* (MRSA) is a primary pathogen and significant source of nosocomial and community acquired infections. In recent years, community-acquired MRSA infections have been increasing. The occurrence of airborne *S. aureus* was unknown, and air was sampled in classrooms during class time for the presence of airborne *S. aureus*. A PBI Air Sampler was used to collect airborne bacteria on mannitol salt agar plates, and mannitol fermentation positive colonies were tested for methacillin resistance. The results show the presence of MRSA in classroom air.

OCCURRENCE OF FUNGAL SPORES ON SOUTHERN ADVENTIST UNIVERSITY CAMPUS. *Lauren Hayes, Southern Adventist University, Collegedale, Tennessee.* Airborne fungal spores are prevalent in Southeast Tennessee, and the purpose of this study was to determine the prevalence of airborne mold spores on the campus of Southern Adventist University. Samples were collected weekly from September to November 2005 using a PBI Air Sampler, which deposited airborne fungal spores on Sabouraud Dextrose Agar (SDA) and Malt Extract Agar (MEA) petri plates. The number of fungal spores collected, as determined by colony counts, was positively correlated to both location and average daytime temperature. Those locations that were moist and shaded had the highest colony counts, and the total number of colonies declined as the average daytime temperature lowered with the onset of winter.

CHILDHOOD REFLECTIONS OF ADULT MALE INCARCERATED CHILD SEXUAL ABUSERS. *Linda H. Garrett, East Tennessee State University, Johnson City, Tennessee.* Child sexual abuse has existed since earliest recorded history. It is believed that one in three females and one in five males are sexually abused before their 18th birthday, and many researchers believe this is a gross underestimation of the problem. Child sexual abuse has been studied extensively from the perspective of the victim. Child sexual abusers have been studied over the last few decades but with inconsistent definitions and methods applied among studies. This qualitative study explored the childhood reflections of 8 incarcerated child sexual abusers in a southern

Appalachian prison. One-on-one in-depth interviews were conducted at the prison with the 8 male participants. After multiple readings of the transcripts, analysis was completed and the stories emerged. The investigator used van Manen's descriptive-interpretive process. This process involved guided existential reflections based on spatiality, corporeality, temporality, and relationality. The existentials were used as an aid to understand how incarcerated adult male child sexual abusers experienced childhood. Questions were asked using the existentials in order to better understand childhood experiences in this underserved and often forgotten population. Data were managed using NVivo qualitative data analysis software. Textural themes were organized into essential structural themes which were abstracted into the essential categorical themes of failure to root, what you see is what you learn, these are the moments of your life, and stupid is as stupid does. All participants demonstrated failure to root as evidenced by their inability to recall their childhood homes or their play life during childhood. Results indicated that participants developed a sense of self resulting from external perceptions which left them with a disembodied concept of self. A disembodied concept of self was demonstrated through the experiences of both failure to root and what you see is what you learn. These are the moments of your life is the abstraction of the participants' method of coping with the abuses they suffered from different family members. The resulting personal view of lived body is represented by the abstraction of stupid is as stupid does. Results are discussed and related to nursing practice, education, and research.

THE EXPERIENCE OF PERINATAL CARE AT A BIRTHING CENTER: A QUALITATIVE PILOT STUDY. *Amber T. Pewitt, East Tennessee State University, Johnson City, Tennessee.* Perinatal health care providers face a crisis as rates of malpractice and litigation rise. Perhaps one solution lies in focusing on patient satisfaction with the birth experience. Defining a satisfactory birth experience is ingrained in this solution. This study aimed to uncover women's experiences of nurse-midwife directed care at a freestanding birthing center. Qualitative description and phenomenology guided the design. The sample included seven primiparous women. Data were collected through interviews, and qualitative methods guided data analysis. Three themes emerged: 1) Empowerment, 2) Sense of Motherhood and 3) Establishing and Strengthening Relationships. These themes portray positive effects participants attributed to care received at the birthing center. Findings revealed that caring providers result in positive psychosocial outcomes and increased patient satisfaction.

THE JOVE SYSTEM RADIO TELESCOPE: MONITORING OF THE GALACTIC CENTER, JUPITER AND SOL. *Tyler Moore, Roane State Community College, Harriman, Tennessee.* A Jovian Radio Spectrum Observational Portal with the use of Radio-Skypipe for the purpose of conducting field surveys of inter and extra-solar abnormalities: The reception of signals from the Jovian magnetic fields at a 15 meter wavelength, in addition to signals from the Galactic Center, Sol and other sources; the digital readout and interpretation through the use of Radio-Skypipe; the process of gathering the data and the visual interpretation are all discussed. Conjointly discussed topics will include: proposed future projects, construction of further equipment, additional experiments and methods of using the system in an educational environment.

A SOLAR STUDY AT 20.1 MHZ. **Katie Sloop**, *Roane State Community College, Harriman, Tennessee*. The energy output of the sun encompasses the entire electromagnetic spectrum from radio waves to gamma rays. This study was undertaken to understand what solar phenomena are associated with the sun's low frequency radio emissions seen at 20.1 MHz. Two automatic monitoring systems, one at the Tamke-Allan Observatory near Rockwood, Tennessee and one located in Oak Ridge were utilized to survey solar radio output. Solar physical data including the presence of sunspots, their surface area and magnetic complexity, X-ray flares, coronal mass ejections, and other solar events was retrieved daily from internet accessible data bases. Numerous radio signals were directly correlated with X-ray flares and coronal mass ejections. Statistical results (regression analyses and one-way ANOVAs) indicated a significant relationship exists between the daily duration of radio signals, the total sunspot area and the spot's magnetic complexity. Furthermore, increases in X-ray flare output are related to increasing sunspot area and complexity.

DEMONSTRATION OF A KARL FISCHER TITRATION. **Kelly Ailey**, *Walters State Community College, Morristown, Tennessee*. An Aquametry I apparatus, a coulometric device produced by Labindustries, was used to determine the percentage of water in student prepared cyclohexene.

THE FISCHER ESTERIFICATION WITH A DEAN-STARK TRAP. **Joshua A. Arrington**, *Walters State Community College, Morristown, Tennessee*. The Fischer esterification with a Dean-Stark trap demonstrates the production of an ester. This reaction is an acid-catalyzed process involving an alcohol and a carboxylic acid. The process is an application of the LeChatelier principle. This demonstrates that if one component of a mixture at dynamic equilibrium is removed, the reaction will tend to shift in that direction. The water, which is produced from the reaction, "settles out" and fills the side-arm adapter of the Dean-Stark trap. As the reaction progresses at reflux, a higher yield of ester is synthesized.

LOW PRESSURE CATALYTIC HYDROGENATION. **Laura Brudecki**, *Walters State Community College, Morristown, Tennessee*. A system for low pressure catalytic hydrogenation was demonstrated. A side-arm flask was used to hydrogenate *endo*-norbornene-*cis*-5,6-dicarboxylic acid over palladium on carbon.

DEMONSTRATION OF A MORTON FLASK WITH A MECHANICAL STIRRER. **Jennifer Butler**, *Walters State Community College, Morristown, Tennessee*. A Morton flask equipped with a mechanical stirrer was used to demonstrate efficient agitation of a multiphase system.

DEMONSTRATION OF A SOXHLET EXTRACTION APPARATUS. **Matthew R. Craven**, *Walters State Community College, Morristown, Tennessee*. Diacetone alcohol, 4-hydroxy-4-methyl-2-pentanone, was made by refluxing acetone over calcium hydroxide in a Soxhlet extraction apparatus. Equilibrium strongly favors the reactants over the products. However, using a Soxhlet extractor allows one to take advantage of Le Chatelier's principle to produce a small amount of product on each pass through the thimble.

DEMONSTRATION OF HIGH PERFORMANCE THIN LAYER CHROMATOGRAPHY. **Jenny Dalton**, *Walters State*

Community College, Morristown, Tennessee. Circular, radial, and linear types of normal phase high performance thin layer chromatography were demonstrated using a development system from Analtech.

DEMONSTRATION OF TRACE PHOTOCHEMICAL EQUIPMENT. **Barbara Johnson**, *Walters State Community College, Morristown, Tennessee*. Photochemical synthesis of benzopinacol was demonstrated by photolysis of benzophenone in 2-propanol. The rate of reaction was compared with rates from photolysis experiments using solar irradiation.

DEMONSTRATION OF THE ABBE REFRACTOMETER. **Tyler Jones**, *Walters State Community College, Morristown, Tennessee*. Use of the Abbe Refractometer was demonstrated. The Abbe Refractometer measures the extent light is bent as it passes through a liquid. The Abbe Refractometer can help identify or confirm the identity of a substance, and can help determine the purity of a substance by comparing the refractive index to standards.

DEMONSTRATION OF PRODUCT DRYING WITH A CHEM-DRY INSTRUMENT. **Amanda Keener**, *Walters State Community College, Morristown, Tennessee*. The Chem-Dry apparatus from Laboratory Devices is used to quick dry a small laboratory sample in a set-up much simpler to use than the Abderhalden apparatus. The sample, under vacuum, is heated by adjusting the voltage to an oven which contains the sample chamber. At reduced pressure the sample is quickly dried.

DEMONSTRATION OF PRODUCT DRYING WITH AN ABDERHALDEN APPARATUS. **Jamie-Lee B. Marques**, *Walters State Community College, Morristown, Tennessee*. The Abderhalden apparatus is used to quick dry a small laboratory sample. The sample, under vacuum, is heated by refluxing a solvent over a chamber holding the sample. At reduced pressure the sample is quickly dried.

CONTINUOUS LIQUID-LIQUID EXTRACTION WITH HEAVIER THAN WATER SOLVENTS. **Ryan Sutton**, *Walters State Community College, Morristown, Tennessee*. The process of extraction is based on the relative solubility of a solid in two immiscible liquids. Solubility is the chemical property that defines the maximum mass of a solid that will dissolve in a particular solvent. Two immiscible solvents will form two phases; the phase that has the greater density will appear at the bottom. This is the solvent that has a density greater than water. NoDoz will be the free agent which contains caffeine. Dichloromethane is used as the extracting solvent, because it is immiscible in water and has a greater solubility for caffeine.

DEMONSTRATION OF LARGE SCALE FRACTIONAL DISTILLATION. **Zachary A. Taylor**, *Walters State Community College, Morristown, Tennessee*. Using a large-scale fractional distillation apparatus, two organic solvents, which have boiling points within 25 degrees of one another, were separated. The two organic solvents chosen for the demonstration are methyl isobutyl ketone, which has a boiling point of 117.4 C, and toluene, which has a boiling point of 109.6-111.6 C.

DEMONSTRATION OF SOLID PHASE EXTRACTION. **Steven Michael Watson Jr**, *Walters State Community College,*

Morristown Tennessee. Solid phase extraction is a method that uses a solid phase and a liquid phase to isolate one compound in a solution. This is the preferred extraction method because it is inexpensive and yields accurate results. It is also typically easier and faster than others due to its small sample size.

SHORT PATH VACUUM DISTILLATION FOR N,N-DIETHYL-META-TOLUAMIDE. *Craig W. Wojciechowski, Walters State Community College, Morristown, Tennessee.* The purification of N,N-diethyl-meta-toluamide (DEET) was demonstrated by conducting a short path vacuum distillation. Experimental parameters were presented.

MIDDLE REGION

TENNESSEE STATE UNIVERSITY
NASHVILLE, TENNESSEE

TELOMERASE ACTIVATION IN HUMAN PAPILLOMAVIRUS-ASSOCIATED AND UNASSOCIATED CERVICAL CANCER CELLS. *Deanna Gardenhire and Jennifer Thomas, Belmont University, Nashville, Tennessee.* Human papillomavirus (HPV) is linked to invasive cervical cancer in its high-risk form. The primary mechanism for tumor progression involves the binding of viral oncoproteins E6 and E7 to cell cycle regulators p53 and Rb, respectively. However, another potential role of HPV, specifically through its viral protein E6, in cancer progression is in the upregulation of telomerase. This reverse transcriptase adds base pairs to chromosomal telomeres, allowing for continued DNA replication and cell division that can eventually result in malignancies. The role of telomerase expression in HPV-associated cervical cancers has been explored, although the mechanisms for controlling this expression are yet to be fully understood. This project compares the level of expression of hTERT, the catalytic subunit of telomerase, in different laboratory-cultured cell lines, including HPV16-expressing (CaSki) cells, HPV18-expressing (HeLa) cells, HPV31-expressing (LKP) cells, and HPV-negative (C33A) cervical cancer cells. Results indicate upregulation of hTERT in HPV16- and 18-expressing cell lines, but not in HPV31-expressing or HPV-negative cell lines. This difference in activation of telomerase could be linked to one or more specific viral products of HPV16 and HPV18 that are altered or missing in HPV31-infected cells or in HPV-negative cells.

INVESTIGATION OF MUSCULAR PROTEINS OF CAENORHABDITIS ELEGANS. *Chasity Ducksworth, Natoya Hopkins, Yvonne Myles, Todd Gary, and E. Lewis Myles, Tennessee State University, Nashville, Tennessee.* Sydney Brenner suggested (in the early 60s) that nematodes would serve as a good model organism to perform molecular studies. The nematode worm *Caenorhabditis elegans* would become that experimental model with which to begin a comprehensive study of animal development and the underlying principles of nervous system function in simple animals. *Caenorhabditis elegans* is a translucent organism found in the soil. It is approximately 1 mm in length and lives for approximately two weeks. This animal model offers several experimental advantages. It is very easy to grow in the laboratory and gives birth to a large number of progeny (approximately 300) by self-fertilization. Our lab is studying gene expression in extreme environments such as Space

exploration. To do these kinds of studies, it is necessary to preserve the macromolecules for examination. RNA later is a compound used to preserve RNA when proper refrigeration is not available. We found that RNA later preserved RNA longer than 2 weeks at room temperature.

STUDY OF THE EFFECT OF LANTHANUM CATIONS ON THE ENZYMATIC ACTIVITY OF THREE DIFFERENT HYALURONIDASES. *Esther Udoji and Koen Verduyck, Tennessee State University, Nashville, Tennessee.* Using gel permeation chromatography (GPC) equipped with photodiode array (PDA) detection, we investigated the effect of the presence of lanthanum (La) cations on the enzymatic activity of three different types of hyaluronidase: mammalian, testicular hyaluronidase and bacterial hyaluronan (HA) lyase produced by *Streptococcus pneumoniae* or *Streptomyces hyalurolyticus*. Bacterial HA lyases hydrolyze their substrate through an elimination reaction yielding reaction products with unsaturated monosaccharide units at their non-reducing end. The emergence of these unsaturated reaction products can be studied by monitoring the enzymatic reaction through UV spectrophotometry at 232 nm. By combining GPC with UV spectrophotometry we were able to relate the emergence of these unsaturated reaction products to the decline in the average molecular mass of HA and evaluate the balance of endolytic and exolytic enzymatic activity of these bacterial enzymes.

REGULATION OF EPITHELIAL TIGHT JUNCTIONS BY TYROSINE PHOSPHORYLATION. *Jeralyn Powell, R. K. Rao, and E. Lewis Myles, Tennessee State University Nashville, Tennessee (JP, ELM) and University of Tennessee, Memphis, Memphis, Tennessee (RKR).* Epithelial tight junctions form a barrier against the transport of allergens, pathogens, and toxins into intestinal tissue. Tight junctions are formed by interactions between many proteins such as occludin, ZO1, ZO2, and ZO3. Previous studies have shown disruption of the junction by inflammatory mediators, such as oxidative stress, when tyrosine phosphorylation occurs. This study was designed to determine if tyrosine phosphorylation modulated the binding of signaling proteins to occludin. The protein, GST-occludin, was purified and phosphorylated. The optimal binding conditions were selected using ERK1 with Binding buffer 1, an Immunoprecipitation Buffer, in the presence of BSA. These conditions were used for binding of proteins to occludin. Results show a reduced binding to occludin when tyrosine is phosphorylated. ERK1 and PP2A ζ binding of ERK2 and PKC bound equally to both nonphosphorylated and tyrosine phosphorylated occludin. These results suggest that tyrosine phosphorylation affects the binding of threonine kinases to occludin.

ADAPTATION OF RESIDENCE TIME DISTRIBUTION-BIODEGRADATION TO MEASURE TRICHLORODETHYLENE BIODEGRADATION. *Jameka Johnson and Lashun King, Tennessee State University, Nashville, Tennessee.* Karst aquifers have been recognized as one of the most challenging geological media in terms of groundwater modeling. Numerical models based on Darcy's Law often are unable to accurately characterize contaminant flow through the heterogeneous fractures and dissolution features. This field study was conducted to determine if a numerical model incorporating residence time distribution (RTD) coupled to a first-order rate of biodegradation (k') could be used to measure trichloroethylene (TCE) removal in a single karst-well injection system. This study involved injecting a

conservative tracer, as well as lactic acid, to enhance anaerobic biodegradation of TCE. The conservative tracer and TCE concentrations were monitored for several weeks. The RTD was calculated using the declining tracer-concentration curve through time, and the biodegradation rate was derived from the TCE data. The RTD-biodegradation formula was used to measure the enhanced biodegradation of TCE in the karst aquifer. Results have shown close agreement between the RTD-biodegradation model prediction and the measured concentration. This model has confirmed that this method can be used to quantify enhanced TCE biodegradation in a single, karst, injection well.

SYNTHESIS AND REACTIONS OF FLUORINATED CYCLIC S-TRANS-VINYLOGOUS ACIDS AND AMIDES: PALLADIUM-CATALYZED COUPLING OF FLUORINATED CYCLIC S-TRANS-ENAMINONE WITH ARYL AND HETEROAROMATIC HALIDES. *Olugbeminiyi O. Fadeyi and Cosmas Okoro, Tennessee State University, Nashville, Tennessee.* Cyclic vinylogous acids and amides are useful intermediates in organic synthesis as synthon for the construction of biologically active compounds, functionally interesting heterocyclic compounds and efficient building block synthesis of natural products. Cyclic, s-trans-enaminones have been utilized for preparation of biologically active compounds, such as antitumor agents, antibacterial, anticonvulsant, dopamine autoreceptor agonists, and acetyl cholinesterase inhibitors. Also N-aryl and N-heterocyclic-enaminones have been reported to display antidepressant and anticonvulsant activities. Palladium-catalyzed coupling of C-N bond now provides important tools for organic synthesis using aryl and heteroaromatic halides. Fluorinated compounds exhibit specific and unique properties. Thus, the introduction of a trifluoromethyl group into cyclic compounds especially at a strategic position of drug molecules has become an important aspect of pharmaceutical research, owing to the unique physical and biological properties of fluorine. Our research group has established a procedure for the synthesis of hitherto unreported cyclic enaminone containing a trifluoromethyl group on a six-membered ring system. The primary objective of this study was to demonstrate the synthetic utility of the above two compounds for the synthesis of fluorinated enaminone ester derivatives by two methods: 1) palladium catalyzed coupling with aryl and heteroaromatic halides, and 2) one pot condensation reaction of fluorinated cyclic vinylogous acid with aromatic and heteroaromatic halides. Preliminary data on the synthesis of substituted-phenylamino-6-trifluoromethyl-2-oxocyclohex-3-en-1-ones will be presented.

ANTICARCINOGENIC EFFECT OF MILK THISTLE ON BREAST CANCER CELLS. *LaKeisha Woods and E. Lewis Myles, Tennessee State University, Nashville, Tennessee.* Breast cancer is the most common type of cancer among women in this country. Women with breast cancer have many treatment options. Some women with breast cancer use complementary and alternative medicine (CAM). Cancer patients take these CAM supplements in hope they might boost their overall health and fight cancer. Phytochemicals are non-nutritive plant chemicals that have protective or disease preventive properties and are not required by the human body for sustaining life. Milk Thistle is an approved herb for medicinal use that's composed of the active substance silymarin. Its main use is Adjunctive treatment in chronic inflammatory liver disease. A number of studies, suggest that Milk Thistle is potentially beneficial in treating or preventing various cancers, and some chemicals from Milk Thistle may

increase the effectiveness of current anticancer drugs. The objectives of this experiment were to determine if Milk Thistle extract has any anticarcinogenic effect on Breast cancer cells, evaluate which extract (aqueous or crude) inhibits the growth of cancer cells, and to perform Western Blot Analysis to demonstrate which of the two extracts or both may have an apoptotic effect on Cyclin D1 during the cell cycle. The Killing Curve, Procedures revealed no relationship between the control DMSO and experimental groups, aqueous and crude, for the BT-549. As for the MCF-10, there was a relationship between the crude extract. However, as for the Aqueous, it only began to kill the cells at higher concentrations. Western Blot Analysis revealed that with prolonged exposure, the Cyclin D1 decreases with the MCF-10 cells. Unfortunately for the BT-549 cells, there was an increase of Cyclin D1 for the short and long exposure to Milk Thistle. As for the Killing Curve Analysis, results indicate that Milk Thistle does have apoptotic effects. However, the Western Blot Analysis reveals something different and as of yet we are not sure of their correlation.

GEOGRAPHIC INFORMATION SYSTEMS AND REMOTE SENSING IN SUPPORT OF URBAN FOREST PRESERVATION AND ENVIRONMENTAL EDUCATION. *Christopher Norwood and David A. Padgett, Tennessee State University, Nashville, Tennessee.* Radnor Lake, Tennessee's first state natural area was established in 1971 and is located in the Nashville/Davidson County Metropolitan Area. Radnor Lake by law is defined as a "fragile forest" and "protected area", and the removal of trees, plants, and fishing is prohibited. Over the past two decades, there has been a consistent increase in residential development, and additional subdivisions with one home per acre densities are planned. The Friends of Radnor Lake coupled with other non-profit organizations are currently working to prevent further development. This project was designed to support the Friends of Radnor Lake and the Radnor Lake State Natural Area staff to conserve this unique ecosystem. A collaborative effort including the faculty advisor, one undergraduate student, two Friends of Radnor Lake volunteers, and six area local teachers was initiated to elevate and educate the community on the importance and ecological value of Radnor Lake. Geographical information systems (GIS), global positioning systems (GPS), and remote sensing technology will be used to conduct the study. The GIS workstation located at the park's environmental education center will be used to apply Multispec GIS/remote sensing software to produce a canopy and land cover map of the area. A teacher training workshop will be developed to introduce and familiarize Metro school teachers with the national GLOBE (Global Learning and Observations to Benefit the Environment) Land Cover Protocol system. Finally six local K-12 teachers will be trained and certified in the GLOBE Land Protocol and two Friends of Radnor Lake volunteers will be trained in GIS and remote sensing applications in urban forestry. Fund-raising efforts will be supported by the maps produced. An undergraduate research assistant will be trained in GIS, GPS, and remote sensing techniques in the analysis of urban forests. The application of CITYGREEN GIS software will be applied to quantitatively assess the ecological importance of the Radnor Lake watershed's urban forest.

EXAMINATION OF MUSCULAR ACTIVITY AFTER HYPERGRAVITY IN CAENORHABDITIS ELEGANS. *Natoya Hopkins, Chasity Ducksworth, Yvonne Myles, Todd Gary, and E. Lewis Myles, Tennessee State University, Nashville, Tennessee.* In

the early 1960s, Sydney Brenner suggested that nematodes would serve as a good model organism to perform molecular studies. *Caenorhabditis elegans* was that organism. *Caenorhabditis elegans* is a eukaryotic nematode that grows to approximately 1 mm in length and has a life cycle of only 2–3 weeks. Amongst its many experimental advantages, *C. elegans*, by way of self-fertilization, can give birth to a large progeny (approximately 300). This organism has allowed scientists to begin studies of animal development and the principles of nervous system function in simple animals. Our lab focuses on muscle activity in extreme environments such as Space exploration. To complete these kinds of studies, it is necessary to determine the effects on muscular movement under normal and hypergravity situations. Attempting to derive a computer based program capable of determining minute distances, it is our intent to chart the muscular activity of these organisms after exposure to such conditions.

THE ANTICANCER EFFECTS OF SOME WEST AFRICAN MEDICINAL PLANTS. *Saudat T. Adamson, Olugbeminiyi O. Fadeyi, Elbert L. Myles, and Cosmas Okoro, Tennessee State University, Nashville, Tennessee.* According to the World Health Organization, despite the influence of modern medicine, about 79% of the world's population still relies heavily on the use of Traditional medicine. Traditional medicine incorporates a variety of plants that are administered based on known medicinal properties. This increases the likelihood that one or more active components may also be effective in inhibiting cancer. Six plants from Nigeria that are frequently used locally for the treatment of various diseases have been selected to examine their action on the breast cancer cell line BT549 and the immortalized normal breast cell line MCF-10. The crude extracts and the Hexane, Dichloromethane, Ethyl Acetate and aqueous fractions were tested for anticancer activity. All six plants have shown appreciable potency on the BT549 cell line.

ISOLATION AND CHARACTERIZATION OF THE ACTIVE COMPONENTS OF SETARIA MEGAPHYLLA. *Olugbeminiyi Fadeyi, Carolyn Cummings, Saudat Adamson, Cosmas O. Okoro, and E. Lewis Myles, Tennessee State University, Nashville, Tennessee.* Over 60% of pharmaceutical drugs currently in use have their origin from natural products. *Setaria megaphylla* is a tropical plant from West Africa that has been used for herbal medicines, but several effects have not been documented. The crude extract has exhibited anticancer activity; therefore the purpose of this research was to isolate, purify and characterize the active components to determine the chemical constituent of *Setaria megaphylla*. The aqueous-methanol fraction was extracted with different solvent systems, followed by compound isolation using thin layer chromatography and counter current chromatography. GC-MS analysis of isolated compounds will be presented.

WESTERN REGION

THE UNIVERSITY OF TENNESSEE HEALTH SCIENCE CENTER
MEMPHIS, TENNESSEE

DETECTION OF OXYTOCIN RECEPTOR MRNA IN HUMAN AMNION DERIVED WISH CELLS USING RT-PCR. *Elizabeth Young, Raquel Furlong, and Sarah Lundin-Schiller, Austin Peay State University, Clarksville, Tennessee.* Oxytocin is

a peptide that has the ability to stimulate smooth muscle contraction in targeted tissues. In order for oxytocin to have its effect, the target tissue must express a specific membrane protein known as the oxytocin receptor. Oxytocin receptor expression increases in the myometrium and intrauterine tissues toward the end of gestation. The purpose of this project was to establish methods for examining the regulation of oxytocin receptor mRNA in human amnion derived WISH cells. Oxytocin receptor mRNA expression was detected using reverse transcriptase-polymerase chain reaction (RT-PCR), which amplified the mRNA extracted from the cells by making numerous DNA copies. Before extracting the mRNA, the cells were left untreated or treated with estradiol. Gel electrophoresis was conducted on the PCR products to determine if the DNA produced in RT-PCR was created from oxytocin receptor mRNA. Initial results have shown cells treated with 10 nM estradiol produced the oxytocin receptor mRNA. Further experimentation is planned to determine if other biochemical substances, such as progesterone and corticotrophin releasing hormone, are capable of causing WISH cells to produce oxytocin receptor mRNA.

CONSERVATION AND ECOLOGY OF JAGUARS IN PRIVATE RANCHES IN THE PANTANAL. *Jennifer Bernard, Claudia Ferro, and Leandro Silveira, Christian Brothers University, Memphis, Tennessee (JB) and Jaguar Conservation Fund, The Pantanal, Brazil (CF, LS).* This study focuses on the conservation and ecology of the jaguar in private ranches in the Pantanal, the largest wetland in the world and home to the second largest jaguar population. Seasonal flooding maintains the habitats of wildlife in this region. However, cattle ranching in this area brings domestic and wildlife into close proximity, and has led to the hunting of jaguars to protect livestock. The ecological study works to determine baseline jaguar population sizes, diet, and predation of livestock. The 11 private ranches involved in the conservation strategy receive compensation for cattle that were confirmed to be killed by jaguars. Between May 3 and July 25, 2005, 191 hours were worked in the field, over about 715 km, 36 jaguar or puma signs were recorded, and 2 jaguars were captured and radio-collared. From January to May 2005, 18 cattle carcasses were claimed as jaguar predation. The compensation program in the Pantanal also offers health and educational programs for the employees of the ranches. The healthcare specialists present at this program included a pediatrician, general doctor, dentist, and gynecologist. On June 4, 2005, 39 people attended the social program at Fazenda Rio Negro. The dentist examined all children and young adults, and 75 percent of the women went to the gynecologist. The conservation program in the Pantanal has been successful in the past and will continue to gain support as more people learn about the jaguar. (Supported by NIH MHIRT award 5T37TW000123-03)

AN ASSESSMENT OF INDIVIDUAL ANNUAL GROWTH RATE, SPREAD RATE, AND DENSITY OF ASIMINA TRILOBA IN OVERTON PARK (MEMPHIS, TENNESSEE). *Christina M. Champion, Teresa M. Bell, and David H. Kesler, Rhodes College, Memphis, Tennessee.* Our study took place in Overton Park, a 175 acre old-growth forest in an urban setting (Memphis, Tennessee). Once a rare tree species in the park, *Asimina triloba* (pawpaw), has risen in the past 30 years to become the most important tree in the forest (importance value of 57.8). Using increment cores, tree size, measured as diameter

at breast height (DBH), was related to age by $y = -0.0003x^3 + 0.0209x^2 + 0.2517x$ ($n = 47$; $R^2 = 0.97$) or a diameter growth rate of ca. 0.59 cm/year. This is the first description of pawpaw age-size relationship of which we are aware. Data from four transects in different areas of the Park revealed a patch spread rate (mean \pm SEM) of 2.6 ± 0.96 meters per year ($n = 4$) and a density of 4300 ± 2100 stems per ha ($n = 5$). It is well-documented that *A. triloba* does not have a high fruit set, but that it can produce clonally. Future research will focus on the genetic similarity of stems within and among patches.

MEADOW VOLES MAY HAVE THE CAPACITY FOR EPISODIC-LIKE MEMORY. *Amy M. Combs, Andrew A. Pierce, Javier delBarco Trillo, and Michael H. Ferkin, University of Memphis, Memphis, Tennessee.* Episodic memory involves the ability to recall the "what", "where", and "when" of a single past event. Although episodic memory is found in humans, recent work suggests that animals also may possess episodic-like memory. We tested the hypothesis that male meadow voles, *Microtus pennsylvanicus*, possess the capacity for episodic-like memory with relation to mate selection in two experiments. In the first experiment, male voles were allowed to explore an arena that contained two chambers. One chamber contained a pregnant female (24 h prepartum). The other chamber contained a sexually mature female that was neither pregnant nor lactating, a reference female (REF). Twenty-four hours after the exposure, the males were placed in the same arena, which was empty and clean. At this time, the pregnant female would have delivered her pups and entered postpartum estrus (PPE); PPE females are more attractive to males and mate more readily as compared to REF. Males spent significantly more time investigating the chamber that originally housed the pregnant female (now a PPE female) than the chamber that originally housed the REF female. The second experiment was similar to the first, except that males explored an arena that contained a chamber with a PPE female and the other chamber contained a REF female. Twenty-four hours later the PPE females were no longer in postpartum estrus, and the males were placed into the same, but clean and empty arena. Lactating females and REF females are similar to one another in the readiness to mate with males. These results suggest that male meadow voles have a capacity for episodic-like memory. For male voles, an episodic-like memory for the reproductive condition of females may allow them to remember the location of females in heightened states of sexual receptivity.

CONTINUOUS CONNEXIN 26 MRNA EXPRESSION IN GT1-7 CELLS. *Kyle R. Covington, Keri L. Mahaffey, and Gilbert R. Pitts, Austin Peay State University, Clarksville, Tennessee.* A small number of GnRH neurons, which are widely scattered throughout the preoptic area of the hypothalamus, comprise the final common pathway for the regulation of reproduction. Successful reproduction requires that GnRH neurons synchronously secrete pulses of GnRH. GnRH neurons may maintain synchronicity by using gap junctions for intercellular communication. Previous studies have shown that GnRH-secreting cells express connexin 26 and need to be cultured for at least five days before GnRH release is synchronized. Our hypothesis is that GnRH cells express connexin 26 immediately after passaging. Immortalized, GnRH-secreting GT1-7 cells were cultured for one to seven days and total RNA was harvested. Harvested mRNA was amplified using RT-PCR with primers specific for the connexin 26 gene. Connexin 26 mRNA was observed on all

days of culture. The concentration of cells was 1.0×10^6 , 2.4×10^6 , 1.0×10^7 , and 1.5×10^7 cells/plate on days one, three, five, and seven respectively. The confluence of GT1-7 neurons was 174.5, 428.2, 1871.8, and 2649.6 cells/mm² on days one, three, five, and seven, respectively. These results demonstrate that connexin 26 is expressed immediately after passaging. Therefore, events other than connexin 26 mRNA expression need to occur to allow synchronization of GnRH secretion. These events may include the assembly of the connexins into connexons and the alignment of hemichannels between adjacent neurons.

THE ROLE OF LIGHT AND OXYGEN IN CHAOBORUS PUNCTIPENNIS (INSECTA: DIPTERA) DIEL VERTICAL MIGRATION. *Mark Stratton, Rhodes College, Memphis, Tennessee.* Aquatic phantom midge larvae (*Chaoborus punctipennis*) exhibit diel vertical migration (DVM) in response to fish visual predation, remaining in deeper waters during the day and ascending nocturnally to feed. Light intensity and dissolved oxygen concentrations are hypothesized to determine the depths where larvae are found during daylight hours. Samples were collected in Yellow Poplar Tree Lake, Millington, Tennessee before and after sunset during a day in March, April, May, July, and September of 2005. Average daytime depth (D_C) and use of daytime refugia (dark or hypoxic waters) significantly increases ($P < 0.05$) with instar stage (I & II < III < IV). IV instars utilized hypoxic waters ($DO_2 < 3.5$ mg/L) more than III instars, while I & II instars did not use this refuge. IV instars remained below maximum light penetration ($1.0 \mu E/m^2/sec$) in May, July, and September, III instars only in July and September, and I & II instars only in September. These behavioral differences among instars are likely due to varying metabolic needs and visual predation vulnerability. Further, IV instar D_C significantly correlates to the critical oxygen threshold for fish ($DO_2 = 3.5$ mg/L) ($R^2 = 0.7826$, $df = 11$, $P < 0.01$) but not to maximum light penetration ($R^2 = 0.183$, $df = 11$, $P > 0.05$), indicating oxygen as the overriding stimulus governing DVM seasonal changes.

EFFECTS OF CADMIUM EXPOSURE ON GLUTATHIONE-S-TRANSFERASE ACTIVITY AND HEAT-SHOCK-PROTEIN-70 LEVELS IN THE GILLS OF THE MANGROVE OYSTER CRASSOSTREA RHIZOPHORAE. *Jennifer Paxson, M. R. F. Marques, D. B. B. Trivella, and G. S. Toledo, Christian Brothers University, Memphis, Tennessee (JP) and University FSC, Florianopolis, Brazil (JP, MRFM, DDBT, GST).* Heat-Shock Protein-70 (HSP-70) and Glutathione-S-Transferase (GST) are among the biomarkers associated with exposure to xenobiotics. These proteins are generally used to help evaluate the toxic effects substances have on both the organism and the aqueous environment it inhabits. In the present work, we report the levels of HSP-70 and the activity of total GST and GST π in the gills of the oyster *Crassostrea rhizophorae* after exposure to different concentrations of Cadmium (Cd). Enzymatic GST activity assays using both 1-chloro-2-nitrobenzene (CDNB) and Ethacrynic acid (ETA) as substrates as well as Western blot analysis with antihuman HSP70Ab were performed to evaluate the response involving these two proteins. Adult oysters were collected and separated into control and Cd-exposed groups. Within the Cd exposed group, the organisms were further separated into three groups in which each was exposed to 100 $\mu g/liter$ of Cd for two days or 200 $\mu g/liter$ of Cd for two or seven days. In general, the level of GST activity was greater in the

exposed organisms compared to the controls, but not statistically significant. The level of HSP-70 was found to be increased in the groups of organisms exposed to Cd for two days in both concentrations (100 µg/liter for 2 days and 200 µg/liter for 2 days). However, in the seven day group, HSP-70 levels were similar to control levels. Even though HSP-70 increased in both of the two day Cd exposed groups, the change was not statistically significant. Future studies will characterize the threshold of these biomarker responses upon heavy metal exposure as well as GST isoforms in this mollusk species. (Supported by MHIRT grant NIH 5T37TW000123-03)

THE ROLE OF THYROID HORMONE IN PHOTOPERIODISM. *Carlesia D. Smith and David A. Freeman, University of Memphis, Memphis, Tennessee.* Siberian hamsters exhibit seasonal cycles of reproduction driven by changes in day length. Day length is encoded endogenously by the duration of nocturnal melatonin secretion from the pineal gland. Short duration melatonin signals stimulate and long duration signals inhibit reproduction. The mechanism by which melatonin signals are decoded at the level of neural target tissues remains uncharacterized. In Siberian hamsters, exposure to short day lengths or injections of melatonin in long days decreases hypothalamic expression of type 2 iodothyronine deiodinase (Dio2) mRNA. Dio2 catalyzes the conversion of the thyroid hormone thyroxine to triiodothyronine (T3). Thus, exposure to short and long day lengths should decrease and increase hypothalamic T3 concentrations, respectively. We tested the hypothesis that injections of exogenous T3 administered to short-day housed hamsters would mimic exposure to long day lengths with respect to gonadal stimulation. Sixteen hamsters gestated and raised in short day lengths that exhibited photoinhibition of the testes were given daily s.c. injections of T3 or saline vehicle for 4 weeks beginning at week 12 of life. Testis size, body mass and pelage scores were obtained weekly for 20 weeks. The results indicate that exogenous T3 induced significant gonadal growth in short day hamsters and delayed spontaneous recrudescence by an interval equal to the number of weeks of exogenous T3 administration. These results suggest that T3 mimics long day exposure in Siberian hamsters and may be involved in decoding the melatonin message.

THE SYNTHESIS OF POTENTIAL CALPAIN INHIBITORS. *Paula Cerrito, Shanta Ali, and Isaac O. Donkor, University of Tennessee Health Science Center, Memphis, Tennessee (PC, SA, IOD) and Christian Brothers University, Memphis, Tennessee (PC).* A series of compounds were synthesized to identify potential calpain inhibitors. Calpain is a calcium dependent cysteine endopeptidase involved in different pathological conditions including stroke, heart attack, Alzheimer's disease, type two diabetes, and some types of cancer. Thus, calpain inhibitors are of therapeutic value. We previously reported 1-(toluene-4-sulfonyl)-pyrrolidine-2-carboxylic acid-(1-formyl-2-phenyl-ethyl)-amide (compound 1) as a potent calpain inhibitor. However, the aldehyde group of compound 1 is readily oxidized in vivo, leading to loss of calpain inhibitory potency. To circumvent this problem and maintain potent calpain inhibition in vivo, we synthesized six new compounds as oxidation-stable derivatives of compound 1. Two of the compounds contained hemiacetal functional groups, two contained ester functional groups, and two contained lactone functional groups. The six new compounds were characterized using thin layer chromatography,

mass spectrometry, proton NMR, and elemental analysis. The calpain inhibitory potency of the compounds will be determined later. The synthesis of oxidation-stable derivatives of compound 1 is important in the development of therapeutic agents to treat diseases that calpain plays a role in the underlying pathophysiology. (Supported by 1 R 15 HL68675-01 and 0255066B)

MECHANISMS OF OSTEROLYSIS. *Andrew Michael, Luciana Pilatti Schwab, and Richard Smith, Christian Brothers University, Memphis, Tennessee (AM), University of Tennessee Health Science Center, Memphis, Tennessee (LPS, RS), and University of Memphis, Memphis, Tennessee (LPS, RS).* Periprosthetic osteolysis is a major cause of joint replacement failure. This degradation of bone adjacent to the prosthesis may cause loosening of the implant and may require additional surgery. Evidence in the literature points to both wear debris particles in the joint space and micro-movement at the bone-implant interface as factors leading to increased activation of osteoclasts and consequently osteolysis. The cells mediating these responses are local macrophages. Our hypothesis is that release of the pro-inflammatory cytokine PGE2 would increase in response to mechanical stimulation even in the absence of particles. In this study, IC-21 macrophages were subjected to mechanical stimulation in combinations of time, frequency, maximum stretch, and various titanium particle concentrations. Prostaglandin E2 released from these macrophages was measured for each set of experimental variables. Collagen coated wells were the best culturing substrate for the conditions tested in this study. Constant application of movement with a frequency of 0.5 Hertz, 10% maximum stretch was found to cause the greatest production of PGE₂ by the macrophages. The addition of a 1:1000 cell:particle ratio produced a similar result. In conclusion, our results supported our hypothesis in showing that the release of the pro-inflammatory cytokine PGE2 would increase in response to mechanical stimulation even in the absence of particles.

THE EFFECT OF TAI CHI ON MEDICATION USE AND MUSCULOSKELETAL DISEASES IN OLDER ADULTS. *Steven L. Moore, Veronica F. Engle, and Lawrence Faulkner, Christian Brothers University, Memphis, Tennessee (SLM) and University of Tennessee Health Science Center, Memphis, Tennessee (SLM, VFE, LF).* With the high prevalence of musculoskeletal diseases in older adults, rising prescription costs, and side effects of arthritis and pain medications, many older adults are turning to Tai Chi for a more convenient, cost effective, and safe treatment. We evaluated the effects of Tai Chi on medication use and musculoskeletal diseases through a subjective review of 20 current (2000–2005) published Tai Chi studies in older adults with musculoskeletal diseases. Inclusion criteria included: a) peer-reviewed, b) indexed, c) publication between 2000 and 2005, (d) data based, e) sample of older adults ≥60 years old, f) Tai Chi intervention, and g) focus on musculoskeletal diseases. A computer literature search was conducted using reputable electronic research databases such as Ovid, PubMed, and CINHALL. Articles that met the inclusion criteria were downloaded and catalogued in a computer file, cited and abstracted in the citation manager Endnote, and their PDFs were saved into a PDF database folder. Each article was reviewed and information from the article was abstracted into a data table with defined categories. The table was analyzed for study methods and outcomes (medication use, musculoskeletal disease signs and symptoms). Twenty studies met the inclusion

criteria. None of the studies addressed medication use as an outcome. Tai Chi was found to be an effective intervention to: a) increase balance, flexibility, and muscle strength, b) reduce pain, c) decrease arthritis symptoms, d) decrease depression and fatigue, and e) improve cardiovascular function. Tai Chi is an effective treatment for older adults with musculoskeletal diseases. Future research should include medication use as an outcome.

INTRASPECIFIC DIFFERENCES IN THE EXPRESSION OF THE AMPA-TYPE GLUTAMATE RECEPTORS IN THE NUCLEUS OF EDINGER-WESTPHAL OF CHICK EMBRYOS AND YOUNG ADULT CHICKS. *Reena S. Patel, Bob J. Dalsania, Raquel S. Pires, Claudio A. Toledo, and Malinda E. C. Fitzgerald, Christian Brothers University, Memphis, Tennessee (RSP, BJD, MECF) and Universidade Cidade de São Paulo, São Paulo, SP, Brazil (RSP, CAT).* Glutamate has been identified as the primary excitatory neurotransmitter within the central nervous system, and it plays an important role in the mediation of synaptic transmission. These actions can be performed through AMPA-type glutamate receptors (GluRs), which are ionotropic and mediate fast neural reactions. Under glutamatergic influence, the Edinger-Westphal nucleus (EW) in birds is responsible for maintaining the control of three ocular adjustments: pupilloconstriction, accommodation, and choroidal blood flow. The aim of this study was to identify the developmental expression of GluRs subunits in EW of chicks (*Gallus gallus*). Immunohistochemical techniques were used to evaluate the expression of the GluRs subunits in the EW of chick embryos from the 10th through the 21st embryonic day (E10–E21) and fifteen and twenty one-day-old (P15 and P21) animals. Antibodies against the subunit GluR1, GluR2/3 (the antibody recognizes a common sequence expressed by GluR2 and GluR3 subunits), and GluR4 were used. The GluR1 and GluR2/3 immunolabel was detected in E10 through E21 consistently. In both P15 and P21 chicks, the subunits showed expression, which was moderate compared to embryonic expression. The GluR4 subunits generally presented strong expression in E10 and E12 chicks compared to that of GluR1 and GluR2/3 expression. The staining for GluR4 changed to less intense on E14 and next on E18. The expression remained moderate for P15 and P21. These results demonstrate a differential expression of the GluRs subunits in the embryonic and post-hatch chickens, suggesting differences between its role as triggering glutamate responses and possibly modeling synaptic connections as well. The differential developmental expression of the GluRs provides information as to the timing of the synaptic connections in the developing visual system. (Supported by NIH MIRT award 5T37TW000123-03)

EFFECT OF GLYCOSYLATION ON CYTOTOXIC T-LYMPHOCYTE EPITOPE PRESENTATION FROM INFLUENZA NEURAMINIDASE. *Sheharyar Minhas and Richard Webby, Christian Brothers University, Memphis Tennessee and St Jude Children's Research Hospital, Memphis, Tennessee.* Studies have shown that a cytotoxic T lymphocyte (CTL) response can be induced by inserting CTL epitopes into the influenza neuraminidase (NA) protein. Prior research has indicated, however, that the presentation of the influenza nucleoprotein (NP₃₆₆₋₃₇₄, ASNENMETM) epitope from the NA protein of the X31 virus induces a weak CTL response compared to a similar approach with the PR8 virus. Through sequence analyses we hypothesize

that glycosylation sites in proximity to the inserted NP epitope might be interfering with the processing and presentation of the X31 epitope, and thus causing a weak immune response. A panel of recombinant viruses with altered glycosylation patterns (X31 NA-NP N69T and X31 NA-NP N79G) was produced to test this hypothesis. Results from intracellular cytokine staining of CD8+ cells from the spleen of infected mice revealed that removal of the glycosylation site at position 79 of the X31 NA resulted in a higher CTL response to the inserted epitope although direct conclusions were difficult due to the unexpected death of control infected mice. In vitro presentation assays were inconclusive and it is still unclear as to the role of glycosylation sites in the induction of CTL responses. (Supported by ALSAC and NIH/NCI 5R25CA23944)

GENETIC, REPRODUCTIVE, AND BEHAVIORAL CHARACTERISTICS OF GIANT PANDAS. *Christie Spencer and Meghan Carr, Christian Brothers University, Memphis, Tennessee and Memphis Zoo, Memphis, Tennessee.* Giant pandas (*Ailuropoda melanoleuca*) are endangered. Although habitat loss is a key issue, poor captive breeding is a continuing threat to the giant panda population. Behavioral abnormalities are often suggested as a cause for the poor reproductive performance. Based on the maturation of the Memphis Zoo pandas and the unexpected timing of the female's estrus cycle events in 2005, our goals were to determine: (1) what genetic and reproductive characteristics in females, and (2) what solitary sexual behavioral characteristics in our male associate with normal reproductive patterns. If we determine which specific conditions and behaviors cue certain reproductive conditions, such as ovulation, it will make future seasons easier to recognize. Recognizing these factors will aid in timing future introductions, thus increasing the female's chance of becoming pregnant. The behavioral data were observed from previously recorded videotapes. Genetic and reproductive output data were collected from *The 2003 International Studbook for Giant Pandas*. We found no apparent relationship between the number of offspring produced by a mother and her daughter. However, most daughters first reproduce within a year of the age when their mothers first gave birth. When applying this to the Memphis Zoo's female giant panda, F507, she did experience an immature estrus during the spring of 2004. Surprisingly, she did not experience a peak estrus during the typical spring season in 2005 but showed a more mature peak estrus in September of that year. If the patterns from the studbook continue, F507 will most likely undergo her first reproduction in 2006 or 2007. Additionally, results showed that changes in the Memphis Zoo's male giant panda's solitary sexual behavior did not seem to be affected by the female's estrus cycle. (Supported by Biodiversity Fellowship from Assisi Foundation Memphis)

CYCLOOXYGENASE METABOLITES AND BRAIN CARBON MONOXIDE PRODUCTION. *Emily DeFur, Michael McNeeley, Oksana Balabanova, and Charles Leffler, Christian Brothers University, Memphis, Tennessee (ED) and University of Tennessee Health Science Center, Memphis, Tennessee (MM, OB, CL).* This study was conducted in vivo to determine if cyclooxygenase (COX) metabolites of arachidonic acid stimulate cerebral carbon monoxide (CO) production that dilates cerebral arterioles in neonatal piglets. Surgically implanted cranial windows in anesthetized newborn pigs were used to measure pial arteriolar diameters and to collect cerebrospinal fluid (CSF)

for prostaglandin (radioimmunoassay of 6-keto-PGF_{1α}) and CO (gas chromatography and mass spectrometry) measurements. Topical arachidonic acid (AA, 2 μM) dilated pial arterioles (51 to 56 μm) and increased both CO (2 fold) and 6-keto-PGF_{1α} (3 fold) concentrations in the CSF. The COX inhibitor, indomethacin, blocked not only the dilation and PGE₂ production, but also the AA induced increase in CO production. Dilation to sodium nitroprusside and constriction to platelet activating factor (PAF), neither of which affected 6-keto-PGF_{1α} or CO concentrations, were not altered by indomethacin. These data suggest that COX metabolite(s) of AA stimulate(s) cerebral CO production that may contribute to cerebrovascular dilation in response to stimuli that activate COX. (Supported by NHLBI R3742851 and NIH RO134059)

GENOTYPING FOR TP53 R337H IN SOUTHERN BRAZIL WHERE IT IS RESPONSIBLE FOR ALMOST ALL CASES OF CHILDHOOD ADRENOCORTICAL CARCINOMA. *Christina L. Brown, Sohaila Boehm Ibrahim Arram, Gislaïne Custódio C. Piovezan, Sérgio Túlio Stingham, Ednéia P. Machado, Guilherme Augusto Parise and Bonald C. Figueiredo, Christian Brothers University, Memphis, Tennessee (CLB) and Federal University of Paraná, Curitiba, Brazil (SBIA, GCCP, STS, EPM, GAP, BCF).* Adrenocortical tumors (ACT) are very rare in children, yet occur in a higher incidence in several states of Southern Brazil. This study was proposed to evaluate the specificity and sensitivity of a genotyping method to screen the germ line TP53 R337H allele in small amounts of blood samples. This study was performed in a state from southern Brazil (Paraná), where this mutation is associated with almost all the ACT cases in children, and where childhood ACT incidence is 12 to 18 times higher than USA and France. A PCR/restriction enzyme based test was used to test blood samples collected in FTA membrane from 20 individuals (19 adults exhibiting the wild-type p53 gene and one child with ACT the TP53 R337H allele previously confirmed by DNA sequencing analysis). Using only 2 punches (circles of 2 mm diameter) of blood-embedded FTA membrane per PCR assay, samples were tested after signing a written consent form. All 20 samples were re-tested four to seven times using the same protocol and revealed the same results; only the child with ACT presented the R337H mutation. Additional experiments were performed replacing chemicals, enzymes, and membranes from 3 other manufacturers. All control samples were negative as expected and the positive mutation also was confirmed using one of the protocols, indicating this protocol is recommended to be used in newborn tests to be initiated in 2005 in Paraná. This test will allow doctors to screen children for the mutation and diagnose ACT early, which is essential for a positive long-term prognosis for these children through medical, hormonal, and imaging exams. (Supported by MHIRT grant NIH 5T37TW000123-03)

ABDOMINAL COMPARTMENT SYNDROME IN A NEWLY DIAGNOSED PATIENT WITH BURKITT LYMPHOMA. *Jennifer M. Hendrick, S. C. Kaste, R. F. Tamburro, F. A. Hoffer, M. Onciu, J. T. Sandlund, R. C. Ribeiro, J. C. Chandler, and S. C. Howard, St. Jude Children's Research Hospital, Memphis, Tennessee (JMH, SCK, RFT, FAH, JTS, RCR, SCH), University of Tennessee School of Health Sciences, Memphis, Tennessee (SCK, MO, JCC) and Christian Brothers University, Memphis Tennessee (JMH).* Burkitt's lymphoma is a rare form of non-Hodgkin's lymphoma that predominately affects young

children. Abdominal compartment syndrome (ACS) is caused by an increase in the intra-abdominal pressure which can lead to intra-abdominal hypertension. Both Burkitt's lymphoma and ACS can decrease renal perfusion to an extent where chemotherapy cannot be optimally delivered. Burkitt's lymphoma and ACS have not been reported together. We present the radiological aspects of ACS associated with Burkitt's lymphoma at diagnosis. We performed a retrospective chart review of one male patient with Burkitt's lymphoma and ACS. The day after the patient was admitted to our institution his clinical condition declined. A therapeutic paracentesis was performed to decrease his intra-abdominal pressure. His clinical condition subsequently improved considerably. Upon radiological recognition of ACS, the possibility of paracentesis to decrease intra-abdominal pressure should be considered to allow optimal delivery of chemotherapy. (Supported NIH grants P30 CA-21765 and P01 CA-20180 and by the American Lebanese Syrian Associated Charities, ALSA)

CHARACTERIZATION OF GENETIC ALTERATIONS IN HIGH GRADE PEDIATRIC GLIOMAS USING FLUORESCENCE IN SITU HYBRIDIZATION. *Melissa Hines, Steven Allen, Erika Proko, Jim Dalton, and Christine Fuller, Christian Brothers University, Memphis, Tennessee (MH), St. Jude Children's Research Hospital, Memphis, Tennessee (MH, SA, EP, JD, CF), Pennsylvania State University, University Park, Pennsylvania (SA) and School of Medicine, University of North Carolina, Chapel Hill, North Carolina (EP).* Little research has been conducted to characterize pediatric gliomas, but there has been an immense amount of research conducted to distinguish the genetic alterations of adult gliomas. In order to characterize high grade pediatric gliomas to a fuller extent, fluorescent in situ hybridization (FISH) using dual hybridizations along with paired control probes on a corresponding chromosome was utilized on 19 collected paraffin embedded pediatric high grade glioma tumor samples from St. Jude Children's Research Hospital. The genes in the tumors that were targeted by FISH included *EGFR*, *PTEN*, *RBI*, *p16/p14ARF*, and *MDM4*. Although *EGFR* is usually amplified in adult GBM, only in 1 out of the 18 pediatric GBM samples had *EGFR* amplification. Other results also suggest there is a definite distinction between the molecular alteration seen in adult high grade gliomas and pediatric high grade tumors. There was also evidence that pediatric de novo and secondary gliomas arise by different molecular pathways; however, the data also showed that the *p16* deletion was equally important in both the primary and secondary high grade glioma formation. Through these results, it is possible that pediatric gliomas can be better understood, and better treatment can be offered by stratification of high grade gliomas, and through the development of novel and targeted therapy. (Supported by NCI Grant 5 R25 CA23944)

SENSITIZATION OF CHRONIC MYELOGENOUS LEUKEMIA CELLS TO GLEEVEC THROUGH REDUCED EXPRESSION OF BCR-ABL KINASE BY LOW-DOSE AD 198 AND AD 288. *Jeremy T. Hunt, Luydmila A. Savranskaya, and Leonard Lothstein, Christian Brothers University, Memphis, Tennessee (JTT) and University of Tennessee Center for Health Sciences, Memphis, Tennessee (LAS, LL).* Chronic myelogenous leukemia (CML) is a type of cancer of the blood and bone marrow that results from the overproduction of immature white blood cells. A kinase called the Bcr-Abl tyrosine kinase is a major factor in

causing the uncontrollable cell growth characteristic of CML by turning on multiple growth signaling pathways. The principal treatment of CML is imatinib myselyate (Gleevec), which binds to and inhibits Bcr-Abl activity. Chronic myelogenous leukemia cells can become resistant to Gleevec through several mechanisms. In an effort to more effectively treat Gleevec-resistant CML cells, N-benzyladriamycin-14-valerate (AD 198) was developed. AD-198 is a PKC activator that rapidly triggers apoptosis in CML cells and is unaffected by mechanisms that confer resistance to Gleevec. The purpose of this study was to determine whether AD 198, in combination with Gleevec, improved cytotoxic efficacy over that seen with the individual agents. Low dose AD 198 was used to determine whether non-apoptotic mechanisms of AD 198 were involved in altering the cell response to Gleevec. We found that sub-cytotoxic doses of AD 198 in combination with Gleevec rapidly decreased the cellular levels of Bcr-Abl and made the cells more sensitive to Gleevec. A similar effect was observed with N-benzyladriamycin (AD 288), the major cellular metabolite of AD 198 whose activity does not involve PKC; thus, the sensitization of CML cells to Gleevec by AD 198 and AD 288 probably involves a PKC-independent mechanism of Bcr-Abl inhibition.

METHYLATION OF DEVELOPMENTAL GENES IN CELL LINES OF DIFFERENT SUBTYPES OF HUMAN RHABDOMYOSARCOMA. *Ashley C. Miller, Rachel N. Stamp, Charlotte A. Peterson, David M. Parham, Raushan T. Kurmasheva, Peter J. Houghton and Craig A. Cooney, Christian Brothers University, Memphis, Tennessee (ACM) and University of Arkansas for Medical Sciences, Little Rock, Arkansas (RNS, CAC, DMP, RTK, PJH, CAC).* In embryogenesis, the PAX gene family expresses a series of highly conserved DNA binding proteins. Some PAX genes and other genes, such as MyoD and Myf, are involved in muscle development. Methylation of the dinucleotide sequence 5'-CG-3' within specific genes affects gene expression without mutating the DNA. Some childhood cancers may arise from developmental dysregulation involving DNA methylation. We hypothesized that methylation of these genes differs between cell lines derived from different subtypes of the muscle-like childhood cancer, rhabdomyosarcoma (RMS). DNA was treated with bisulfite to convert unmethylated cytosines to uracils; and then PCR amplified with primers specific for bisulfite modified DNA. Genes were amplified from two cell lines, one derived from colon cancer and one RMS cell line. We then used gel electrophoresis to correlate product size with that expected for the tested genes. The DNA was sequenced to determine sites of methylation. Several primer sets and procedural modifications were designed and tested to optimize these assays. The optimum temperatures and DNA concentrations were determined for several primer sets using DNA from the colon cancer cell line, WiDr. Following additional refinements of the PCR and DNA purification assays, we intend to begin PCR analysis and DNA sequencing of the RMS cell lines, which will be followed by PCR analysis, DNA sequencing, and analysis of RMS tumor samples. (Supported by Children's University Medical Group Award to DMP, Dept. Pathology Chairman's Discretionary Fund to DMP and CAC, Arkansas Tobacco Settlement Fund to CAP, NIH AG20941 to CAP, and UAMS Biochemistry and Molecular Biology Summer Undergraduate Research Fellowship to ACM)

THE EFFECT OF INTERLEUKIN-1 KNOCKOUT ON THE THICKNESS OF MOUSE OCULAR CHOROID AND SCLERA.

Katrina Thompson, Sharon Frase, and Malinda E. C. Fitzgerald, Christian Brothers University, Memphis, Tennessee (KT, MĒCF) and University of Memphis Integrative Microscopy Center, Memphis, Tennessee (SF). Interleukin-1 (IL-1) is a pro-inflammatory cytokine that plays a role in inflammation, host defense and the neuroimmuno-endocrine network. This interleukin can regulate its own expression by sending cytokines to an area where foreign substances are present. Cytokines help stimulate immune cell proliferation and differentiation. This interleukin activates T-cells, leukocytes, endothelial cells, smooth muscle cells and fibroblasts. IL-1 helps stimulate acute-phase protein synthesis of collagen and adhesion factors on endothelial cells, and leukocytes for diapedesis. This also helps induce coagulation, and helps promote inflammatory characteristics such as vasodilatation. All of these functions could be compromised in a IL-1 knock-out. The choroid is the primary vascular bed of the eye and choroidal thickness and scleral composition both change in myopia and aging. In aging and myopia the choroid thins and in myopia scleral glycosaminoglycan synthesis increases. The aim of this study was to determine if removal of IL-1 through a genetic knock-out would affect choroid and sclera thickness. The IL-1 knock-out and wild type mice were obtained from Dr. K. Hasty at the University of Tennessee. The animals were anesthetized, the eyes were removed, emersion fixed in sodium cacodylate buffered glutaraldehyde, rinsed, dehydrated in an ascending series of alcohols, and embedded in plastic resin. The embedded tissue was sectioned (1 μ m) in a sagittal plane on an ultra microtome and images systematically obtained from sequential regions of the eye section using a digital camera and compound microscope. The thickness was measured from the outer edge of the sclera to the inner edge of Bruch's Membrane beneath the choriocapillaris. Five measurements were made on each of the fourteen regions of each eye. A mean choroid and sclera thickness was determined for each eye and as well as a comparison of each region for the fourteen areas sampled (*t*-test; *P* < 0.05). The wild type choroid and sclera were statistically thinner than those of the knockout mice. The IL-1 knockout led to an increase in choroid/sclera thickness, suggesting increases in either choroidal blood flow or scleral proteoglycans. Further work will be needed to determine if this observed increase in thickness was specific to the choroid or sclera.

RAPID COLLECTION OF GONADOTROPIN-RELEASING HORMONE NEURONS. *Pier-Anne Lachance, DeLacy LeBlanc, Hyunah Roberson, and Gilbert R. Pitts, Austin Peay State University, Clarksville, Tennessee.* Gonadotropin-releasing hormone (GnRH) neurons form the final common pathway for neuroendocrine control of reproduction. Biological rhythms play a key role in GnRH secretion as there is an ultradian pattern of GnRH secretion. In addition, circadian clock genes are found in GnRH neurons, which might be responsible for GnRH secretion regulation. Another regulatory mechanism for GnRH neuron secretion may be synchronization through gap junctions or paracrine substances. Our long term goal is to examine ultradian and circadian patterns of gene expression in GnRH neurons. In the short run, our goal was to develop a protocol that would allow repeated and rapid collection of GnRH neurons from a single culture dish. Immortalized GnRH neurons, GT1-7, were used for these experiments so that large numbers of GnRH neurons could be cultured. GT1-7 neurons were cultured on glass microscope coverslips placed on plastic tissue culture dishes. The coverslips

were removed at 15 min intervals for up to one h. The number of neurons on each coverslip was immediately counted after collection. We determined that the coverslips could be attached to the culture dishes with aquarium silicon. Furthermore, the coverslips had to be collected while the dish was left in the incubator to prevent neuronal death. Our results indicate that the numbers of

live cells on each coverslip did not change during the sampling period ($P > 0.05$). The confluence of the GT1-7 neurons on the coverslips remained constant throughout the sampling period with an average of 656 ± 63 cells/mm² and was similar to the confluence on the culture dish ($P > 0.05$). In conclusion, this technique is appropriate for collecting GT1-7 neurons.