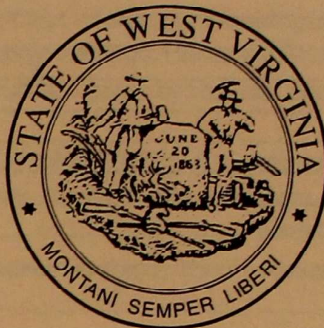


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Abstracts of the
Seventy-Third
Annual Session



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West Virginia Academy of Science
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BIOLOGY

ERIKA M. HARRIS, DR. LESLIE A. QUINN, Dept. of Biology, Wheeling Jesuit University, Wheeling, WV 26003 and DR. WILLIAM D. MCCUMBEE, Dept. of Physiology, Marshall University School of Medicine, Huntington, WV 25755. *In vitro* regulation of vascular smooth muscle cell glucose uptake by vascular endothelial cells.

The purpose of this experiment was to ascertain whether or not cultured vascular endothelial cells (VEC) secrete a paracrine factor in response to cyclic strain which increases glucose uptake by cultured vascular smooth muscle cells (VSMC). Confluent VEC, cultured in collagen-coated flexible-bottomed Flex I culture plates, were subjected to short-term (1 hr.) and long-term (48 hrs.) regulated cyclic strain using a Flexercell Strain Unit. VSMC were incubated in media from stretched and unstretched (control) VEC, and glucose uptake was measured using a 2-deoxyglucose transport assay. VSMC exposed to media from VEC subjected to short-term strain took up significantly more glucose ($p < 0.05$) than those exposed to media from unstretched VEC did. Additionally, VSMC exposed to media from VEC subjected to long-term strain took up significantly more glucose ($p < 0.001$) than those exposed to media from unstretched VEC did. Further studies will be required to confirm that VEC secrete a paracrine factor in response to cyclic strain that increases glucose uptake by VSMC.

TERENCE MESSINGER and DOUGLAS B. CHAMBERS, U.S. Geological Survey, 11 Dunbar St., Charleston, WV 25301. Selected trace metals and organic compounds in stream-bed sediments of the Kanawha River Basin.

Surficial stream-bed sediments were collected from 15 sites in the Kanawha River basin of West Virginia, Virginia, and North Carolina in August-October 1996. Samples were analyzed for a broad

suite of inorganic and organic substances, as part of the U.S. Geological Survey's National Water-Quality Assessment program (NAWQA). Sites were selected to represent important geologic settings and land uses in the basin, which drains parts of the Blue Ridge, Valley and Ridge, and Appalachian Plateaus physiographic provinces. Concentrations of 47 major and trace elements and 100 organic compounds were determined in each sample. Interim regulatory guidance has been issued by Environment Canada for 8 of these trace elements, 6 polycyclic aromatic hydrocarbons (PAHs), 8 pesticides, and total polychlorinated biphenyls (PCBs). This guidance includes 2 sets of criteria, a threshold effects level, a concentration below which toxic effects are rarely expected to occur to aquatic life, and a probable effects level, a concentration above which toxic effects are predicted; an intermediate range exists for all the substances on the list. All 8 of the trace elements for which criteria were available were found in concentrations exceeding the threshold effects level. Nickel, zinc, and lead were detected in concentrations exceeding the probable effects level. Nickel concentrations exceeded the probable effects level in 12 of the 15 samples and exceeded the threshold effects level in the other 3 samples. Zinc concentrations exceeded the probable effects level in 1 sample and exceeded the threshold effects level in 13 other samples. Lead concentrations exceeded the probable effects level in 1 sample and exceeded the threshold effects level in 10 other samples. The other 5 trace metals exceeded the threshold effects level as follows: arsenic in 11 samples, cadmium in 6 samples, chromium in 2 samples, copper in 6 samples, and mercury in 1 sample. Four of the 6 PAHs for which criteria were available were found in concentrations exceeding the probable effects level, and the other 2 were found in concentrations exceeding the threshold effects level. Phenanthrene exceeded the probable effects level in 3 samples, and was measured in concentrations exceeding the threshold effects level in 6 other samples. Benz(a)anthracene exceeded the probable effects level in 2 samples, and was measured in concentrations exceeding the

threshold effects level in 7 other samples. Both pyrene and chrysene exceeded the probable effects level in 1 sample, and exceeded the threshold effects level in 8 other samples. Benzo(a)pyrene was measured in concentrations exceeding the threshold effects level in 9 samples, and fluoranthene exceeded the threshold effects level in 8 samples. The laboratory method reporting level (50 µg/kg for all PAHs) was greater than the threshold effects levels for phenanthrene, benz(a)anthracene, and benzo(a)pyrene, and these 3 compounds were estimated to occur in some samples at concentrations in excess of the threshold effects level. Relative abundance of the 6 different PAHs was similar among sites. The highest concentrations of all 6 PAHs were found in the sample from Peters Creek near Lockwood, W.Va., the second highest concentrations of all PAHs were found in the sample from Bluestone River at Spanishburg, W.Va., and the third highest concentrations of all PAHs were found in the sample from Kanawha River near Winfield, W.Va. Concentrations of PAHs in excess of the threshold effects level were measured in samples from 8 of the 10 sites draining the Appalachian Plateaus physiographic province and from 1 of the 5 sites draining only the Blue Ridge or Valley and Ridge physiographic provinces. As expected in a region with low agricultural intensity, the approximately 30 pesticide analytes were detected at few sites and in low concentrations. Of the 8 pesticides for which criteria were available, p,p'-DDD, dieldrin, endrin, heptachlor epoxide, and lindane were not measured in concentrations above the laboratory method reporting level in any sample. Chlordane and total DDT were detected in some samples, but in concentrations less than the threshold effects level. Sediment from one sample contained p,p'-DDE in a concentration greater than the threshold effects level. Sediment from another sample contained total PCBs in a concentration greater than the threshold effects level.

MARK D. KNOTT, Biology Department,
Shepherd College, Shepherdstown, WV
25443. Regulation of the climacteric in Bosc

pear (*Pyrus communis* L.).

The onset of the climacteric in fruit such as pear, peach and tomato is characterized by an increase in ethylene production, respiration and fruit softening as well as the activation of ripening-associated genes. Several genes that are associated with abiotic stress seem to be expressed at specific times in fruit development and ripening. We are primarily interested in the events that initiate the climacteric and the role of abiotic stress-related genes in ripening. To resolve the molecular events of ripening in Bosc pear, we prematurely induced ripening by subjecting mature, preclimacteric fruit to various polygalacturonase (PG) activities which changed internal turgor, simulating an osmotic stress. This 'stress' signal seemed to accelerate and compact both physiological and molecular aspects of ripening. Treated fruit entered the climacteric 4-5 days before the controls and exhibited a 150% increase in CO₂ exchange, a 300% increase in C₂H₄ exchange, and accumulated about twice as much mRNA homologous to our PG and ACC oxidase DNA probes. Accordingly, the treated fruit softened faster. We also determined that ACC oxidase (ethylene formation) preceded PG (cell wall softening) mRNA accumulation just as ethylene biosynthesis preceded softening. However, since the expression of PG and ACC oxidase genes occurred after the climacteric, it could be concluded that these two genes play no role in the onset of ripening. This experiment represents only the beginning of our investigation into the regulation of fruit ripening. In the future, we will probe for stress-induced/ripening-related proteins such as heat shock and desiccation. These upcoming measures will allow us to determine if a stress response induces the climacteric and the sequence of molecular events in normal ripening.

AARON SECHLER, Biology Department,
Shepherd College, Shepherdstown, WV
25443 and NORM SCHAAD, USDA-ARS,
Fort Detrick, MD 21702. Development of an
Ultra-Sensitive Technique for the Detection
of *Clavibacter michiganensis* subsp.

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sepedonicus Bacterium from Potatoes.

Clavibacter michiganensis subsp. *sepedonicus* (Cms), the causal agent of bacterial ring rot in potatoes, is a Gram positive fastidious bacterium which typically appears as a white mucoid colony on general growth media. Bacterial ring rot is a major problem for seed potatoes. Symptoms include orange coloration of the vascular ring inside the tuber and the production of an orange exudate from the vascular ring when the tuber is squeezed. Late symptoms include necrotic rot of the vascular ring. The United States, Canada, and the European Economic Community have all adopted zero tolerance standards for Cms in seed potatoes, which has required the development of Cms detection techniques. The current serological methods of detection are enzyme-linked immunosorbent assay (ELISA) and indirect fluorescent antibody staining (IFAS) which are not sensitive enough to reach a goal of no Cms in seed potato stock. Also, antigenic variants of Cms are common in samples and can not be detected by either method. The presence of Cms morphologically similar bacteria or cross-reactive bacteria can result in false positives. A more sensitive means of detection involves application of hybridization probes and PCR primers, concurrent with the use of a nested PCR assay which targeted a unique rDNA sequence present in all subspecies of *Clavibacter michiganensis*. From a series of three primer sets, Cms50, Cms72 and Cms85 specific only for Cms, the Cms50 primer was converted for the TaqMan system used in this study. BIO-PCR together with the TaqMan system utilizes two specific PCR primers and a probe to hybridize with the amplified DNA fragment. BIO-PCR specifically enhances growth of Cms prior to use of the TaqMan system. The probe contains both reporter and quencher fluorescent dyes. During PCR, after the probe has hybridized to the target DNA, the 5' nuclease activity of Taq polymerase cleaves the probe and frees the fluorescent reporter dye into solution, which is directly proportional to the amount of amplified DNA. The sensitivity of the TaqMan system has several advantages over ELISA

or IFAS. The period of time necessary for detection of Cms from samples while not shorter, is easier and less labor intensive and the Taqman system does not require DNA extraction nor electrophoresis on Southern blotting for confirmation of detection. Furthermore, no bacterium other than Cms is amplified by the system. Bio-PCR detection of Cms utilizing the TaqMan system may be good enough to replace ELISA and IFAS as the standard means of Cms detection in seed potato stocks, thus achieving the goal of a Cms free stock of seed potatoes.

JERRY T. CREWS, Northeastern Forest Experiment Station, U.S. Forest Service, USDA, Parsons, WV 26287 and LINTON WRIGHT, Monongahela National Forest, U.S. Forest Service, USDA, Elkins, WV 26241. Soil temperature and soil moisture regimes in the central Appalachians.

Soil temperatures and electrical resistances were measured for 3 years at depths of 50 cm at seven sites in three areas at six elevations and four aspects on the Fernow Experimental Forest and at Bearden Knob near Davis, WV. Soil electrical resistance was measured to determine relative soil moisture levels. The mean seasonal soil temperature (MSST) was determined using an average of six readings (three readings taken in mid-December, January, and February to determine winter soil temperature and moisture levels and three readings taken in mid-June, July, and August to determine mean summer soil temperature and moisture levels). The mean seasonal air temperature was collected at one site in each area. A prediction equation was derived from the seasonal soil temperature readings at 50 cm depth ($Y = 15.06 - 0.007X_1 + 0.2295X_2$ where elevation = X_1 in meters and aspect = X_2 in numerical values assigned from true north and $Y =$ mean annual soil temperature). Using the prediction equation the mesic-frigid soil temperature boundary was found to be 1022 meters above mean sea level in the study area. Frigid soils are soils with mean annual temperatures of >0 degrees C and < 8 degrees C

with >5 degree C differences between mean summer and mean winter temperatures at depths 50 cm below the surface. Consideration of aspect led to a mesic-frigid soil temperature boundary 960 meters above mean sea level on a Northwest aspect. The data indicate that forest canopy is also a factor in determining the mesic-frigid soil temperature boundary and soil moisture levels moderate changes in soil temperature. Determination of the extent of frigid soils has implications for successful forestry management in the central Appalachians.

CRAIG W. STIHLER, ANDREW JONES, and JACK L. WALLACE. West Virginia Division of Natural Resources, Elkins, WV 26241. Use of Elkhorn Cave, Grant County, West Virginia, by Virginia big-eared bats (*Corynorhinus townsendii virginianus*).

During the summer, Elkhorn Cave, Grant County, West Virginia, harbors a "bachelor colony" of *Corynorhinus townsendii virginianus*; this species does not appear to hibernate in this cave. Numbers of these bats exiting the cave were monitored on 17 evenings between 12 Jun 1996 and 23 Jun 1997 using night vision equipment. Ultra-sound detectors were used to aid in species identification. Bats were captured in mist nets set at the cave entrance on six nights between 6 Sep 1995 and 30 Jun 1997. Although a few bats were present in the cave in early April, most bats did not arrive at the cave until mid-June (N=66 on 14 Jun; N=92 on 23 Jun). Mist netting in early June resulted in the capture of 27 male and two non-reproductive female *C. t. virginianus*. The number of Virginia big-eared bats at the cave increased unexpectedly in late August (N=159). Both male (N=21) and female (N=17) bats were captured at the cave entrance in mid-September. When released, several males did not fly away, but landed on holding cages containing female bats. Most of the bats left the cave by late October. Winter 1996-97 bat surveys at caves known to serve as hibernacula for *C. t. virginianus* resulted in the band returns for 18 *C. t. virginianus* banded at Elkhorn Cave in 1996.

These bats were observed hibernating in four caves located up to 32 km from Elkhorn Cave. Elkhorn Cave appears to be an important breeding site for this endangered bat.

CRAIG W. STIHLER, WV Div. of Natural Resources, Elkins, WV 26241, EDWIN D. MICHAEL, Div. of Forestry, West Virginia University, Morgantown, WV 26506, and HARRY PAWELCZYK, US Forest Service, Monongahela National Forest, Elkins, WV 26241. Social groupings of northern flying squirrels (*Glaucomys sabrinus fuscus*) in West Virginia.

Glaucomys sabrinus fuscus, a subspecies of the northern flying squirrel, was added to the federal list of endangered species in 1985. At that time, this subspecies was known from 10 specimens collected in Randolph and Pocahontas counties, West Virginia and Highland County, Virginia. Surveys (nest box monitoring and live-trapping) conducted in West Virginia between 1985 and 1996 yielded 769 *G. s. fuscus* captures at 81 sites in six counties. Nest boxes were monitored March-December with most inspections occurring in April-May and October-November. Northern flying squirrels found in nest boxes were aged, sexed, weighed, and ear-tagged prior to release. A total of 558 adult northern flying squirrels, representing 381 social groupings, were observed. The number of individuals present in nest boxes ranged from one to seven squirrels with the following frequencies for each social grouping: 1 male, 27.8% of the groups; 1 female, 28.8%; 2 males, 8.5%; 2 females, 5.6%; 3 males, 1.3%; 3 females, 1.3%; 1 male and 1 female, 9.5%; 1 male and ≥ 2 females, 4.6%; 1 females and ≥ 2 males, 7.8%; all other male/female combinations, 4.8%. The sex ratio of adult squirrels was (male:female) 1:1.16. Forty-nine litters were observed. Mean litter size was 2.5 young (range = 1-5).

DONNA MITCHELL West Virginia Division of Natural Resources. Ward Road, Elkins, WV 26241. Hypogeous fungal records from

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West Virginia.

Twenty-nine taxa of hypogeous fungi have been recorded from WV. Twenty-four of these were collected by the author between 1990 and 1997 from 6 counties. Four species were recorded at the North American Mycological foray held at Canaan Valley State Park, Tucker Co. in 1985, and one species (*Rhizopogon couchii*) was collected by Orson Miller in Pocahontas Co. Included in the author's collection is *Elaphomyces sp. nov.* from Barbour and Tucker Counties, *Elaphomyces sp. nov.* from a shale barren in Pendleton County, and *Hymenogaster sp. nov.* from Pocahontas Co. Prior to a single collection in Pendleton County, *Choiromyces venosus* had been known from only one other North American location (Oregon). *Hysterangium album* is known from one site in Pocahontas Co. and the type locality in New York. All of the author's collections are housed in the Oregon State University herbarium.

JAMIE BREWER, Dept of Biology, Fairmont State College, Fairmont, WV, 26554, MARC FRANKENBERRY and JAMES SHEIL, Dept of Microbiology and Immunology, West Virginia University, Morgantown, WV, 26506. Important features of class I major histocompatibility complex (MHC) molecules for alloreactive T cell recognition.

Cytotoxic T lymphocytes (CTL) have been implicated in both acute and chronic allograft rejection, thereby defining their central role in transplantation immunity. A strong alloreactive CTL response can be elicited by stimulation with three closely-related murine MHC class I molecules. These H-2K^b class I molecules differ by as little as one or two amino acid residues. The location of these discrete changes on the distal surface of closely related MHC class I molecules allows novel interactions with both the antigenic peptide and the T cell receptor of responding alloreactive CTLs. The interaction of the T cell receptor and MHC class I-peptide complex accounts for their ability to induce strong

alloreactive CTLs. In this study we utilized the coordinates of previously defined, x-ray crystallized H-2K^b peptide complexes deposited within the Brookhaven Protein Data Bank. New models containing the discrete amino acid substitutions in the H-2K^b molecule were constructed using a Silicon Graphics UNIX - based computer and software designed by Molecular Simulations, Inc. Following construction of the computer models, these H-2K^b mutant class I molecules were analyzed to show that a difference as small as a single amino acid can result in significant bond alterations. These alterations affect the three-dimensional conformation of the MHC-peptide complex, with new MHC intrachain bonds formed as well as new bonds between the MHC molecule and the antigenic peptide. This analysis indicates that intramolecular alterations within the H-2K^b molecule are able to elicit a strong alloreactive CTL response. These conclusions are supported by results of concurrent studies in our laboratory involving standard 4-hour ⁵¹Cr release assays which employ the above stated H-2K^b mutant strains. This research was supported by a career enhancement award issued by the West Virginia Women and Minorities in the Sciences and the National Science Foundation.

STUART A. WELSH, West Virginia Cooperative Fish and Wildlife Research Unit, West Virginia University, Morgantown, WV, 26506. SUE A. PERRY, Everglades National Park, 40001 State Road 9336, Homestead, FL 33034-6733. JAMES H. HOWARD, Department of Biology, Frostburg State University, Frostburg, MD 21532. Range fragmentation and genetic variation in *Etheostoma tippecanoe* and *E. denoncourti*

The Tippecanoe darter (*Etheostoma tippecanoe*) has an extremely fragmented range with isolated populations widely distributed within the Cumberland and Ohio river drainages. Its range previously included the Tennessee River drainage; however, these populations were recently

described as a new species (golden darter, *Etheostoma denoncourti*), based on morphological data. To determine patterns of genetic variation within and between species, 22 loci in 12 populations of *E. tippecanoe*, and four populations of *E. denoncourti*, were examined with horizontal starch gel electrophoresis. Low allozyme variation occurred across the range of both species. In *E. tippecanoe*, the mean number of alleles per locus (1.02, range 1.0 - 1.2), mean percentage of polymorphic loci (4.52, range 0.0 - 9.1), and mean heterozygosity (0.015, range 0.000 - 0.033), were slightly lower than those in *E. denoncourti* (1.05, range 1.0 - 1.1; 5.68, range 0.0 - 9.1; 0.019, range 0.000 - 0.036). The higher proportion of heterozygotes found in a previous study involving 38 loci and two populations of *E. tippecanoe* suggests that our study underestimated variation. Despite our findings of low allozyme variation, frequency differences at PGM and PGDH separated *E. tippecanoe* and *E. denoncourti*.

BOTANY

RONALD H. FORTNEY, Salem-Teikyo University, Salem, WV 26426, WILLIAM GRAFTON, West Virginia University, Morgantown, WV 26505, and JAMES S. RENTCH, Marshall Univ., South Charleston, WV 25303. An ecological baseline for the Appalachian oak fern (*Gymnocarpium appalachianum* Pryer) in West Virginia.

The Appalachian oak fern (*Gymnocarpium appalachianum* Pryer) is an extremely rare central Appalachian endemic, found mostly on cool, rocky, forested slopes in Pennsylvania, Ohio, Maryland, Virginia, North Carolina, and West Virginia. It was once considered a subspecies of the more common oak fern (*G. dryopteris*); however, recent taxonomic studies have concluded that it is a separate diploid species, while *G. dryopteris* is an allotetraploid hybrid between *G. appalachianum* and *G. disjunctum*, a western North American species. *G. appalachianum* is morphologically distinguishable

from *G. dryopteris* by its pinnae, pinnules, and the pinnulets of mature fertile fronds. In an effort to further clarify the systematics and ecological requirements of this species, a study was initiated in 1996 with the following objectives: 1) to relocate historic populations in West Virginia; 2) to collect and analyze quantitative data on associated plant communities, soils, and topography at all extant sites; 3) to evaluate population viability, including potential threats to extant populations, and 4) to synthesize the general habitat requirements, and limiting factors, of the species. All 12 known historic locations for *G. appalachianum* were visited, with populations located at nine of the 12 sites. At each extant site, the vegetation was quantitatively sampled in 20 m x 25 m quadrats. Importance values were calculated for all vegetation strata. The physical site characteristics measured included slope aspect and steepness, soil pH, concentrations of major and minor nutrients, and percent organic matter. In general, populations were found to be healthy and stable. Eight of the nine sites were forested, with well developed canopy layers, dominated either by hardwoods (e.g., *Quercus rubra*, *Betula lenta*, *B. allegheniensis*, and *Tilia americana*), or a mixture of hardwoods and conifers, that included various mixtures of *Tsuga canadensis*, *Abies balsamea*, *Pinus strobus*, and *P. resinosa*). Common understory dominants were *Acer pensylvanicum* and *T. canadensis*. With the exception of the herbaceous strata at the two Canaan Valley sites, species richness in all strata was low. Recurring site and environmental conditions include the following: 1) on steep, rocky, north facing slopes (often at the base of moist talus slopes, or in high elevation wetlands on slightly elevated areas, e.g., around stumps, tree trunks and logs); 2) under mixed hardwood and conifer dominated canopies; and 3) in well-drained, acidic soils, with both low nitrate concentrations and cation exchange capacities. Interestingly, many of the specimens keyed out to what we believe is *X morseanum*, the hybrid backcross between *G. appalachianum* and *G. dryopteris*. Additional taxonomic studies should be conducted to determine if *G. appalachianum*, *G.*

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dryopteris, and *X. morseanum* occur at all or some of the West Virginia oak fern sites.

JAMES S. RENTCH, School of Engineering and Information Technology, Marshall Univ. Graduate College, South Charleston, WV 25202, HAROLD S. ADAMS, Div. of Arts and Science, Dabney S. Lancaster Community College, Clifton Forge, VA, 24422, ROBERT B. COXE, Robert J. Goldstein & Associates, Raleigh, NC, 27616, and STEVEN L. STEVENSON, Dept. of Biology, Fairmont State College, Fairmont, WV 26554. The vegetation of a Carolina hemlock (*Tsuga caroliniana*) community.

A Carolina hemlock (*Tsuga caroliniana*, Engelm.) community in the Blue Ridge Mountains near Roanoke in southwestern Virginia was sampled in July, 1997. This site was first examined in 1935, and is now protected by the Virginia Nature Conservancy. Standard sampling methods were used to obtain quantitative data on the composition and structure of the tree, small tree, sapling, shrub, seedling, and herbaceous strata. Increment cores were extracted from larger trees (≥ 5 cm dbh) to assess age-diameter relationships, and we made a visual assessment of crown vigor to evaluate stand health. Carolina hemlock is a southern Appalachian endemic, known from northeastern Georgia to southwestern Virginia. It is typically found on dry, exposed, and nutrient poor slopes. Although not of widespread occurrence, it may occur in nearly pure stands at some locations. In comparison to the more mesophytic Canada hemlock (*T. canadensis*), Carolina hemlock is a smaller, slower growing tree. A comparison of the age-diameter regression value ($y=32.3+2.12x$) calculated for Carolina hemlock at this site with values reported in the literature for other tree species indicated that the former displays a slower growth rate than Canada hemlock, red oak (*Quercus rubra*), yellow birch (*Betula lutea*), and black birch (*B. lenta*). The importance values (total possible=100) of Carolina hemlock at this site were 49.2, 89.7, and 38.5 for the tree, small tree, and

sapling strata, respectively. Species richness was low in the tree (9 species), small tree (5), sapling (6), shrub (5), and herb strata (5). Cover values in the shrub and herb strata were also low, and both strata were dominated by low-growing, creeping or climbing plants such as *Parthenocissus quinquefolia*, *Dioscorea villosa*, and *Michella repens*. Although no Carolina hemlock seedlings were present in the sample subplots, this community appeared to be a stable, uneven aged forest, with a peak distribution in the 50 year old (small tree) age class. Three trees older than 150 years were recorded. No standing dead Carolina hemlocks were observed, and 71.8% of the stems had less than 50% damage to tree crowns.

SUSAN MOYLE STUDLAR, Dept. of Biology, West Virginia University, Morgantown, WV 26506 and STEVEN L. STEPHENSON, Dept. of Biology, Fairmont State University, Fairmont, WV 26554. Preparation of a documented checklist of bryophytes for the West Virginia Natural Heritage Program Flora and Fungi Database System.

We are preparing a checklist of West Virginia mosses and liverworts that is vouchered from the West Virginia University Herbarium (Studlar) and the literature (Stephenson). To date, Studlar has annotated and curated 1410 moss species in the WVU Herbarium (about 10 specimens per species), and selected about two voucher specimens per species. She has entered label data (substrate, habitat, locality, reproductive condition, collector, date, and determiner) from these 240 specimens into the Specimen Information database (Natural Heritage Program Database System). Also she has entered into the Checklist Database taxonomic and ecological information: scientific name, common name (coined or selected from published lists), synonyms, classification (family, order, division), habitat, substrate and comments (taxonomic status and rarity). Two rare moss species have been found in the WVU Herbarium so far (one collection each). *Macrocoma sullivantii* (Sullivant's Bark Moss), rare west of the Blue Ridge Mountains was collected

(and misidentified by Fred W. Gray in 1930. *Buxbaumia minakatae* (Round Bug-on-a-Stick) is rare in Eastern North America (Crum and Anderson, 1981). The Natural Heritage Program Checklist of West Virginia Bryophytes, easily updated and readily available (in electronic or printed form), should be useful to biologists, resource managers and the general public in and beyond West Virginia. (Sponsored by the West Virginia Department of Natural Resources Nongame Natural Heritage Program and West Virginia University).

ECOLOGY

SANDRA RAIMONDO and THOMAS K. PAULEY, Marshall University, Huntington, WV, 25755. Foraging habits and feeding niche overlap in five species of plethodontid salamanders.

Gut contents of 175 salamanders representing five species (*Desmognathus fuscus*, *D. monticola*, *D. ochrophaeus*, *Plethodon cinereus*, *P. g. glutinosus*) which occur in the Ridge and Valley Province in West Virginia were analyzed to determine overlaps in feeding niches and foraging habits. Gut contents were removed from specimens and placed in taxonomic categories. Intraspecific comparisons between gut contents of the five species showed no significant dietary overlap between specimens collected at different sites for all species except *P. cinereus*. Intraspecific comparisons based on prey size, however, did show a significant dietary overlap among specimens from different sites. These foraging patterns indicate salamanders search for prey within a particular size range rather than specific prey taxa. There was no significant difference in mean prey size between species and classification of gut content into size categories showed a broad niche overlap in feeding ecology of the species. Food habits of the five species studied are described in detail. The aquatic species *D. fuscus* and *D. monticola* were found to have diets which correlated significantly in both prey taxa and size. The semi-aquatic species, *D. ochrophaeus*,

was found to possess diets overlapping *D. fuscus* and *P. cinereus* in both prey taxa and size. Prey items of *P. cinereus* and *P. g. glutinosus* were found to be similar in size classes.

BETH ANNE PAULEY and THOMAS K. PAULEY, Dept. of Biological Sciences, Marshall University, Huntington, WV 25755. Microdistribution of *Plethodon nettingi* in association with emergent rocks.

Plethodon nettingi is listed as a threatened species by the U.S. Fish and Wildlife Service. Its total range is within 5 counties in eastern West Virginia. There are fewer than 70 disjunct populations known and most populations are at elevations above 3,000 ft. and are associated with emergent rocks or narrow ravines lined with *Rhododendron*. This study examines environmental factors associated with emergent rocks that might regulate the distribution of *P. nettingi*. Two sites with emergent rocks where *P. nettingi* was known to occur were used as study sites and two sites with emergent rocks where *P. nettingi* was known not to occur but within the known range of *P. nettingi* were used as controls. Each site was examined to determine the distance *P. nettingi* extends beyond the rocks. Biological data such as snout-to-vent length, mass, and gender of each species of salamander observed, and environmental factors including air temperature, soil temperature, relative humidity, soil moisture, soil pH, litter mass, and litter moisture were collected along 4 transects in each cardinal direction from the center of the rocks. Significant regulating environmental factors included relative humidity, soil temperature, litter mass, and litter moisture.

ALISON M. ROGERS and THOMAS K. PAULEY, Dept. of Biological Sciences, Marshall University, Huntington, WV 25755. Ecology and natural history of *Rana clamitans melanota* larvae in West Virginia.

The green frog, *Rana clamitans melanota*, is common throughout West Virginia. Literature

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suggests the larvae of this species may not develop in one season. This study examines *R. c. melanota* larval development and transformation. Ten sites were selected representing four habitats including roadside ditches, stream oxbows, and small and large permanent pools in eastern West Virginia. These sites ranged in elevation from 2560 feet to 4000 feet. Aquatic funnel traps and standard dipnet methods were used to collect larvae from each site. Pitfall traps with drift fences were placed around one site to capture newly transformed froglets. Biological data included total length, mass, and life stage of larvae, and snout to urostyle length, right tibia length, and mass of froglets. Environmental data collected at each site included water pH and water temperature. Data collected from January 1997 to December 1997 showed that *R. c. melanota* spend one winter as larvae, and suggest that some may spend two winters. Newman-Keuls multiple comparison test show significant differences at the 0.05 significance level for environmental data from each habitat type and elevation.

ERIC WILHELM and DONALD C. TARTER,
Department of Biological Sciences,
Marshall University, Huntington, WV 25755.
A taxonomic study of the class Insecta in the
Green Bottom Wildlife Management Area
with notes on the life history of *Belostoma*
lutarium (Stal), and *Hesperocorixa lucida*
(Abbott), Cabell County, West Virginia.

Green Bottom Wildlife Management Area (GBWMA), Cabell County, West Virginia, is a wetlands system adjacent to the Ohio River varying in age from 4 to 60 years. This system is annually flooded by the Ohio River, yet insect communities remain unique to each vegetation type or microhabitat. Nine vegetative communities from two wetlands were sampled and compared using chi-square analysis to determine if the insect communities were unique. A total of 34,975 specimens, representing 23 families, were collected over ten sample intervals. Chi-square analysis on seven families common to all sites indicated that each vegetative community yielded unique

proportions of insects. Chi-statistics ranged from $X^2 0.05, 6 = 86.666$ ($P = 0.0000$) for sites 3 and 5 to $X^2 0.05, 6 = 1398.092$ ($P=0.0000$) for sites 1 and 5 (CV: $X^2 0.05, 6 = 12.592$). Low water levels exposed site 6 during July and August preventing sampling; therefore, all data from this site were excluded from analysis. The preliminary survey documented 9 orders, 37 families, 69 genera, and 47 species of the class Insecta. Eleven species of Hemiptera were identified to species, and with the exception of *Notonecta irrorata* all species are West Virginia state records. In this study, the life histories of *Belostoma lutarium* and *Hesperocorixa lucida* were examined to determine oviposition sites, behavior, and number and size class of instars. Both taxa have five instars as determined by size class analysis. *Belostoma lutarium* oviposited a mean of 129.3 eggs on the hemelytra of the male during the months of May, June, and July. *Hesperocorixa lucida* oviposited a mean of 47.6 eggs singly on submergent aquatic vegetation, in early spring (March, April, May).

GAIL L. PERRINE and DONALD C. TARTER,
Department of Biological Sciences, Marshall
University, Huntington, WV 25755.
Ecological life history of *Caenis amica*
Hagen (Ephemeroptera: Caenidae) in a
mitigated wetland, Green Bottom Wildlife
Area, West Virginia.

The ecological life history of the mayfly *Caenis amica* Hagen was examined in a newly mitigated wetland in the Green Bottom Wildlife Management Area (GBWMA), Cabell County, West Virginia from November 1996 to November 1997. The GBWMA was created to mitigate impacts to wetlands, wildlife, and associated recreation acquired by the implementation of the Gallipolis Locks and Dam Replacement Project. The Green Bottom Wildlife Management Area is located 16 miles north of Huntington, West Virginia. Most of the property lies between State Route 2 and the Ohio River in Cabell and Mason Counties. About 67 acres located on the eastern end is south of State Route 2. *Caenis amica* larvae were collected using a

modified Gerking sampler (19" x 19" x 31") (triplicate) and a long handled D-shaped dredge. The following ecological components will be discussed and compared to other species of *Caenis*: foregut analysis, growth, voltinism, sex ratio, emergence period, fecundity, and pH.

JENNIFER A. ADERMAN and DONALD C. TARTER, Department of Biological Sciences, Marshall University, Huntington, WV 25755. Life history, ecology, and low pH tolerance of the freshwater prawn, *Palaemonetes kadiakensis* Rathbun, in a mitigated wetland of the Green Bottom Wildlife Management Area, West Virginia.

An ecological life history study was conducted on the freshwater prawn *Palaemonetes kadiakensis* Rathbun at the mitigated wetland of the Green Bottom Wildlife Management Area, West Virginia, from November 1996 to November 1997.

Palaemonetes kadiakensis is a North American freshwater shrimp also known as the prawn. It prefers slowly moving rivers and streams, or lakes and ponds with dense vegetation. North American freshwater shrimps are probably the most poorly known of our freshwater decapods due to the fact that they have limited commercial value. However, prawns are of great indirect value because they are an important link in the food chain supporting commercial and game fishes. To date, no previous studies on this prawn have been conducted in West Virginia. Green Bottom Wildlife Management Area is overseen by the U.S. Army Corps of Engineers, Department of Natural Resources, and the U.S. Forest and Wildlife Services. It consists of forest, agricultural land, and four wetland types: seasonally flooded basins, inland open freshwater, shrub swamp, and wooded swamp. Prawns were collected using a modified Gerking sampler (19" x 19" x 31") and a standard D-shaped dredge. Three replicate samples were taken on each collecting date. Several ecological parameters, including population density, reproduction, growth, food habits, and low pH tolerance will be discussed and compared to other investigations on *P. kadiakensis*.

JENNIFER WYKLE and DONALD TARTER, Department of Biological Sciences, Marshall University, Huntington, West Virginia 25755. Range extensions and low pH tolerances of the amphipods *Crangonyx rivularis* Bousfield and *C. richmondensis richmondensis* Ellis from the Meadow River wetland complex, Greenbrier County, West Virginia.

The amphipods *Crangonyx rivularis* and *C. richmondensis richmondensis* are recorded for the first time from West Virginia. The record for *C. rivularis* represents a significant southern extension of the known range which was previously limited to Ontario, Canada. The record for *C. richmondensis richmondensis* extends the western range of the subspecies which was previously known from Nova Scotia, Maine, Massachusetts, New Hampshire, South Carolina and Georgia. Acidification can have serious impacts on amphipod populations. Low pH can alter acid-base balance, respiration, and Na and Ca regulation. Using a standard D-shaped dredge, one-hundred amphipods of both species were collected from the Meadow River wetland complex. Amphipods were returned to the laboratory and allowed to acclimate in a controlled environmental chamber for 24 hours. Ten amphipods were placed in each of five duplicate bowls. They were exposed to the following pH values: 1.5, 3.0, 4.5, 6.0, and 7.0 (control). After the 96-hour experiment, the median tolerance limits (T_{lm}) were calculated using the straight-line graphical method and linear regression analysis. Results will be compared with other laboratory and field studies concerning pH tolerance values on amphipods.

CHEMISTRY

MELODY L. BODKINS and SUNDAR NAGA, Department of Chemistry, WVSC, Institute, WV 25112. COGENCH (Conceptualization Of GENeral CHEmistry) Project.

Visualization is the key to studying the abstract

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concepts of undergraduate general chemistry. One needs specific tools to aid in the visualization process. Our research team is developing such visual tools, through the use of the Internet and the computer programming language, JAVA. This enables us to create a fully animated, user interactive guide to basic chemistry concepts. Our COGENCH project, in its initial phase, is concerned with the kinetic theory of matter, according to which: all matter is made up of tiny, invisible particles; these particles are in constant motion all the time, like vibrational motion in solids or translational motion in gases; and the average speed (vigorosity) of the motion of these particles increases as the temperature of that material increases. Students may link to our website and utilize the information presented there in an animated fashion. We hope the students' learning and retaining of material will be greatly enhanced by using the results of our research. To insure that all students may benefit from our efforts, the applets produced by our research team are made available on the World Wide Web under the WVSC chemistry department.

GARY SCHULTZ and SUNDAR NAGA,
Department of Chemistry, WVSC, Institute,
WV 25112. COPHYCH (Conceptualization
of PHYSical Chemistry) Project.

Visualization is the key to studying the abstract concepts of undergraduate physical chemistry. One needs specific tools to aid in the visualization process. Our research team is developing such visual tools, through the use of the internet and the computer programming language, JAVA. This enables us to create a fully animated, user interactive guide to the fundamental concepts of physical chemistry. Our COPHYCH project is at present concerned with the concepts related to Boltzmann distribution. Students may link to our website and utilize the information presented there in an animated fashion. We hope the students' learning and retaining of material will be greatly enhanced by using the results of our research. To insure that all students may benefit from our

efforts, the applets produced by our research team are made available on the World Wide Web under the WVSC chemistry department.

AIMEE R. GUMP and MARK R. FLOOD,
Biology Dept., Fairmont State College,
Fairmont, WV 26554. Determining the
effect of benzo[a]pyrene on frog embryonic
cell growth.

Recently, frogs in the United States have been detected with numerous developmental problems (e.g., extra legs). The suspected cause of these developmental disruptions are pollutants in the environment. A family of detoxication enzymes called the uridine diphosphate glucuronosyltransferases (UGTs) are present in frogs, but their role in detoxication of embryos has not been well defined. Benzo[a]pyrene is an environmental pollutant which is known to induce the production of UGTs. The objective of the proposed project is to determine whether exposure to benzo[a]pyrene causes embryonic frog cell growth to be adversely effected. Embryonic cells from the ICR 134 cell line (ATCC#CCL128) are cultured at 25°C in medium containing 50% Leibovitz's L-15, 40% distilled water and 10% fetal bovine serum. The treatment groups will be exposed to 10^{-3} , 10^{-4} , 10^{-5} , 10^{-6} , or 10^{-7} M concentrations of benzo[a]pyrene in the medium described above. All control and treatment groups will be run in triplicate. Cells will be grown for 2 weeks with cell concentrations being determined daily. Statistical analysis of differences in cell concentration will allow the assessment of whether benzo[a]pyrene is having an adverse effect on frog embryonic cell growth. If benzo[a]pyrene inhibits cell growth, then further investigations will be necessary to elucidate the actual mechanism of these changes. If no effect on cell growth is detected with benzo[a]pyrene exposure, then the UGT system is likely protecting the embryonic cells from these concentrations of this particular environmental pollutant. This is the first step in determining the role of UGTs in the development of embryonic cells from aquatic organisms. This

work is supported by the Beta Beta Beta Research Scholarship Foundation Fund.

ZOOLOGY

DONALD TARTER, JENNIFER WYKLE, and JASON MORGAN, Department of Biological Sciences, Marshall University, Huntington, West Virginia 25755. New record and range extension for *Fabria inornata* (Banks) (Trichoptera: Phryganeidae) from West Virginia.

The phryganeid caddisfly *Fabria inornata* (Banks) is reported for the first time for West Virginia. The documentation of this species represents a southeastern extension of the known range which was previously reported from Illinois, Iowa, Indiana, Minnesota, Michigan, Wisconsin in the United States, and the Canadian provinces of Ontario, Quebec, Northwest Territories, Manitoba, and Alberta. Larvae were collected from the Meadow River wetland complex (Greenbrier County) in dense beds of submerged *Ceratophyllum* using a standard D-frame dip net. Following the addition of *Fabria inornata* to the state checklist, 193 species of caddisflies, representing 16 families and 63 genera, are known from West Virginia.

BARNETT, KIRK G., MICHAEL L. LITTLE, and DONALD C. TARTER, Department of Biological Sciences, Marshall University, Huntington, WV 25755. Preliminary observations on the isozyme patterns and morphometrics of *Gambusia* sp. in the Green Bottom Wildlife Management Area, West Virginia.

Prior to 1988, two subspecies of the mosquitofish *Gambusia affinis* (*G. a. affinis* and *G. a. holbrooki*) were recognized in the warm southern waters along the Gulf and Atlantic coasts of the United States. Based upon electrophoretic and morphometric analysis in 1988, *G. holbrooki* and *G. affinis* were determined to be distinct taxa. Populations west of

Mobile Bay, Alabama were established to be *G. affinis* and those east of Mobile Bay to be *G. holbrooki*. Only two populations of mosquitofish have been found in West Virginia: the Meadow River wetlands, Greenbrier County and the Green Bottom Wildlife Management Area, Cabell and Mason counties. Isometric focusing of glycerol-3-phosphate dehydrogenase isozyme patterns from heart, brain, liver, and muscle tissues, as well as morphometric characteristics are being used to determine the correct designation of the Green Bottom mosquitofish population.

TARA L. ROSE, and DONALD C. TARTER, Department of Biological Sciences, Marshall University, Huntington, WV 25755, and ERICH EMERY, Ohio River Valley Water Sanitation Commission, Cincinnati, OH 45228. Preliminary observations on the distribution of the sucker family (Pisces: Catostomidae) in the Ohio River.

The Ohio River flows 981 mi (1,578 km) southwest from Pittsburgh, Pennsylvania, to empty into the Mississippi River at Cairo, Illinois, crossing four ecoregions and draining 204,000 sq mi (528,000 sq km) in six states. Ichthyofauna in the main channel consists of 102 native species and nine introduced species. Existing Ohio River Valley Water Sanitation Commission data sets indicate distinct longitudinal spatial distribution patterns (both riverwide and within navigational pools) for 15 species in the family Catostomidae in the main channel of the river. Temporal trends in catostomid populations in the Ohio River in response to increasing water quality have also been observed. Defining the trends in sucker distribution is a prerequisite to understanding the origins of these distribution patterns. Distribution trends observed in catostomid population data from 1957 to the present will be explored, and preliminary observations on the probable explanation for these patterns will be discussed. Recommendations for future studies concerning the origin of these distribution trends will also be made.

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WOOTEN, MATTHEW and DONALD TARTER, Dept. of Biological Sciences, Marshall University, Huntington, WV 25755, and ERICH EMERY, Ohio River Valley Water Sanitation Commission, 5735 Kellogg Ave., Cincinnati, OH 45228. A longitudinal comparison of macroinvertebrate assemblages in the Ohio River.

Due to the constraints of working with such a large river system, data reflecting the macroinvertebrate assemblage is limited. Because of the usefulness of macroinvertebrates as pollution indicators, more work needs to be done. This study will compare the macroinvertebrate assemblages of two pools on the Ohio River, Hannibal pool (RM 84.2 - 126.4) representing the upper river, and Smithland pool (RM 846.0 - 918.5) on the lower river. Hester-Dendy multilayer artificial substrate samplers were set the length of each pool and allowed to colonize for six weeks. All specimens collected were identified to the lowest taxonomic level. Community similarities of the macroinvertebrates from the two pools will be determined with an Ecostat computer program. This study is a preliminary step in the development of a river-wide macroinvertebrate index.

ENGINEERING and GEOLOGY

JAMES M. STILES and DONALD D. GRAY, Department of Civil and Environmental Engr, West Virginia University, P.O. Box 6103, Morgantown, WV 26506-6103. Practical numerical modeling of the flow of atmospheric pressure fluidized bed combustor coal ash grout in abandoned mines.

Backfilling abandoned room and pillar coal mines with grout consisting of Atmospheric-pressure Fluidized Bed Combustor (AFBC) coal ash, bentonite and water promises to reduce the environmental problems associated with ash disposal, acid mine drainage, and mine subsidence. In order to accurately estimate the cost of a

proposed grout injection, the open channel flow of the injected grout, a Bingham material, in the mine must be simulated. This presentation will describe Groutnet, a computer program that simulates flow of the injected grout by solving the vertically averaged, two dimensional governing equations for the slow, laminar, gradually varied, open channel flow of a Bingham material. The approximate friction equation used in Groutnet was adapted from the analytical solution for the fully developed laminar flow of a Bingham material in a circular pipe. To illustrate the performance of Groutnet, a 10 hour injection into a hypothetical room and pillar mine with a level floor was simulated. The model grid consisted of 23 rows and 23 columns of 5 m by 5 m model cells with alternating rooms and pillars. Grout was injected in the central intersection at the rate of 0.01699 m³/s and formed a symmetrical mound around the injection model cell. At the end of the simulation, 2,225 m² of the mine floor was covered, and the depth at the injection point was 0.60 m. This simulation required about 51 minutes on a 90 MHz Pentium personal computer. The computer program Groutnet is a practical tool for simulating AFBC ash grout injection into abandoned room and pillar coal mines. Simulations performed by Groutnet have demonstrated a 2,000-fold improvement in performance over three dimensional simulations. Future work will include developing a more realistic friction equation and verifying Groutnet results against bench and field scale injection tests. This work was supported by U. S. Department of Energy Cooperative Agreement No. DE-FC21-94MC2944. This paper does not necessarily represent the views of the DOE.

IAN P. FARRAR, DEWEY D. SANDERSON, JAMES BRUMFIELD and GALINA FET, Department of Geology, Marshall University, Huntington, West Virginia 25755. A Geographical Information System for Yellow Creek, Davis, West Virginia.

The purpose of this study was to establish very high resolution digital maps of wetland vegetation

communities from ground surveying. If the ground truth data can be properly geo-referenced, then it can be used with high resolution digital aerial imagery to map wetland vegetation over large areas. Yellow Creek, near Davis, West Virginia was chosen as a location for one of the control sites. The study included a detailed theodolite survey of the topography, plane table with ultra sonic ranging for vegetation mapping and GPS for UTM geo-referencing at the center of the site. The site was circular with a 32 meter radius. Dominant vegetation types resulted in the mapping of 41 polygonal areas. Collected field data was then converted into digital format for GIS layering. The field vegetation map was digitized using TOSCA, a module of the software package IDRISI. Theodolite data was converted into Cartesian coordinates using a software program called EDM developed by the authors. SURFER mapping software was used to grid the data on a one decimeter (10cm) grid pattern. The data sets were also converted into IDRISI format, a grid-based geographic and image processing system as separate layers. Preliminary results show the vegetation to be very sensitive to subtle changes in elevation of just a few decimeters. Mosses were found in the low water saturated areas giving way to various shrubs in the moist slighter higher elevations. On average, only about 50 centimeters of soil were found overlying the Pennsylvanian sandstone bedrock. Preliminary study of the Yellow Creek site has shown this wetlands to be quite diverse in its plant communities. To adequately study these wetlands over large areas it will be necessary to use very high level ground truth control sites and low level, high resolution geo-referenced imagery.

NATALIE R. RIPSHER and DEWEY D. SANDERSON, Department of Geology, Marshall University, Huntington, West Virginia 25755. A computer based, image analysis of Pennsylvanian age, tidal rhythmites.

This project introduces an alternative approach to the study of sedimentary tidal rhythmites

associated with Pennsylvanian strata involving digital image analysis. The method replaces the measuring of tidal rhythmites in the field or taking core, with instead, the photography of outcrops and digital scanning of the negatives. Differences in the clay and sand content of the lamina make for subtle variations in tonality that can be sensed readily by scanners. The individual lamina within the photographic negatives are converted into pixel traverses by PHOTOSHOP. Lamina thicknesses are determined from variations in pixel reflectance using a software program developed by the authors called PIXLAM. Fourier analysis of lamina thickness revealed periodicities consistent with tidal cycles. Daily tides, spring and neap cycles, apogee and perigee deposits, anomalistic and synodic monthly deposits and semiannual cycles of sedimentation were identified from five outcrops within the Ohio, Kentucky and West Virginia Tri-State area. This method proved successful and provides a useful data collection technique that is less time consuming, accurate and less judgmental than traditional methods.

Nikki Fleshman and Dewey D. Sanderson, Department of Geology, Marshall University, Huntington, WV 25755. The Ancestral and Modern Mud River Valley.

The Mud River has its headwaters in Lincoln County, West Virginia and courses its way to a confluence with the Guyandotte River at Bourboursville, Cabell County, West Virginia. Between the villages of Hamlin and Milton, there is a very prominent, almost anomalous, oxbow with a relatively level, knife-like ridge extending out into the interior of the bend. Upon examining other upstream and downstream bends of the Mud River, several other benches or terraces, but less prominent, were found at approximately the same elevation of approximately 680 feet above sea level. The Mud River enters the ancestral Teays Valley at Milton and thence flows westward in the Teays Valley to the Guyandotte. Remnants of the former Teays Valley are known throughout the corridor between Huntington and Charleston, West

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Virginia. The Teays bedrock valley floor is approximately 680 feet in elevation. This correspondence in elevation suggests that the bench-like features in the Mud River valley formed when the Mud River was a tributary of the Teays River. After the Teays River was abandoned, the Mud River had to establish a new channel in its lower reaches to become a tributary of the Guyandotte River.

FREDERICK GRADY, 1201 South Scott Street, Apt. 123, Arlington, VA 22204 and E. RAY GARTON, Curator, West Virginia Geological Survey, PO Box 879, Morgantown, WV 26507. The Pleistocene Badger *Taxidea taxus* from 5 West Virginia Caves.

Fossil remains of the Badger *Taxidea taxus* a carnivore of the Mustelidae or weasel family is a prairie species that reached its eastern historical limit in Ohio. Remains have been found in 5 West Virginia caves and have also been found as fossils as far east as Maryland and Pennsylvania. One West Virginia site, Hamilton Cave, Pendleton County dates to the middle Pleistocene some 800,000 years before present. The other 4 sites New Trout Cave, Pendleton County, Worm Hole Cave, Pendleton County, Organ Cave, Greenbrier County and Patton Cave, Monroe County are all late Pleistocene and probably less than 100,000 years before present but more than 12,000 years old. All three of the Pendleton County sites also contain the thirteen lined ground squirrel *Spermophilus tridecemlineatus*, another prairie species and a common prey species of the badger. The presence of badger in West Virginia supports the concept of an extension of grasslands eastward during the Pleistocene. These new data indicate that prairie species in West Virginia often co-existed with temperate, northern, and occasionally southern species.

POSTERS

LANDOLT, JOHN C., Department of

Biology, Shepherd College, Shepherdstown, WV, 25443, LINDA GEISER, USDA Forest Service, Siuslaw National Forest, Corvallis, OR 97339 and STEVEN L. STEPHENSON, Department of Biology, Fairmont State College, Fairmont, WV 26554. Dictyostelid cellular slime molds from forest epiphyte communities.

The soil-like material that accumulates beneath epiphytic bryophytes and vascular plants occurring on the trunks and branches of forest trees was investigated for the presence of dictyostelid cellular slime molds. Forest types from which samples were collected included temperate moist coniferous forests of central western Oregon, tropical montane and cloud forests of the Luquillo Experimental Forest in northeastern Puerto Rico, and tropical cloud forests and seasonal dry forests in the Guanacaste Conservation Area in northwestern Costa Rica. Where data for ground sites were also available for comparison, species richness and measurable population densities of dictyostelids were generally lower in the epiphytic communities than on the ground. Epiphytes in forests at the very highest elevations sampled tended to be characterized by the lowest species richness and densities of dictyostelids. (Supported in part by grants from the National Geographic Society, the National Science Foundation, and the West Virginia NASA Space Grant Consortium.)

STEVEN L. STEPHENSON, Dept. of Biology, Fairmont State College, Fairmont, WV 26554, GARY A. LAURSEN, Dept. of Biology and Wildlife, University of Alaska, Fairbanks, AK 99775, and RODNEY D. SEPPELT, Australian Antarctic Division, Kingston, Tasmania 7050, Australia. Fungi of subantarctic Macquarie Island.

Most of what is known about the assemblages of fungi (including slime molds) associated with particular types of terrestrial ecosystems and the fundamental roles that they play in these ecosystems has been derived from studies carried

out in temperate regions of the world. Relatively little is known about fungal biodiversity and ecology in cold-dominated, high-latitude ecosystems. From late January to early May of 1995, we carried out an intensive survey of the fungi of subantarctic Macquarie Island. As a result of our work, the mycoflora of Macquarie island is now known to consist of at least 140 species of basidiomycetes, 55 ascomycetes, and 25 myxomycetes. These totals exceed those known for the entire south polar region prior to the present study. Long distance dispersal by wind and birds undoubtedly accounts for the presence of most of the taxa represented on this remote island. The closest potential source of spore inoculum is New Zealand (including the Auckland Islands and Campbell Island). The vascular flora of Macquarie Island shows a strong biogeographic relationship with New Zealand. We suspect the same will prove true for the fungi, the majority of which are saprobic decomposers of vascular plants and bryophytes. (Supported by grants from the National Science Foundation and the Australian Antarctic Division.)

STEVEN L. STEPHENSON, ADAM W. ROLLINS, and MARIBETH OVERKING, Dept. of Biology, Fairmont State College, Fairmont, WV 26554. Myxomycetes associated with the litter microhabitat in tropical forests of Costa Rica.

Most of what is known about the assemblages of myxomycetes (plasmodial slime molds) associated with particular types of terrestrial ecosystems has been derived from studies carried out in temperate regions of the world. The species associated with tropical forest ecosystems have received very little study. The purpose of the present study is to obtain data on the distribution and ecology of the myxomycetes associated with the litter microhabitat in tropical forests of Costa Rica. Samples of aerial (dead but still attached plant parts) and forest floor litter were collected at three study sites in southeastern Costa Rica. These samples were returned to the laboratory and used

to prepare a series of moist chamber cultures for each study site. Based on results obtained thus far, myxomycetes appear to be relatively common in the litter microhabitat of tropical forests. Samples of aerial litter have yielded 18 species, whereas 14 species have been recorded from samples of forest floor litter. The majority of species appearing in moist chamber cultures on both types of litter are members of the Physarales and Trichiales, with members of the Stemonitales also present but much less common. Among the most consistently abundant species are *Didymium iridis*, *D. squamulosum*, *Lamproderma scintillans*, *Perichaena chrysosperma*, and *Physarum compressum*. (Supported in part by a grant from the National Science Foundation).

DORA GONZALES, JEREMY TRAYLOR, MARCIA HARRISON, Dept. of Biological Sciences, Marshall Univ., Huntington, WV 25755. Growth and gravitropic curvature in *Arabidopsis* mutants which produce excess ethylene.

Arabidopsis thaliana, the common wall cress, is a genetic model for the plant kingdom. Certain mutants of *A. thaliana* exhibit excess production of ethylene, a hormone which inhibits cell growth. Excess ethylene causes short, thick and crooked stems and roots. The purpose of this experiment was to compare the growth rate and gravitropic curvature (bending in response to a change in orientation to gravity) in wild type and ethylene producing mutants. Wild type and mutant (*ctrl-1* and *eto1-1*) seeds were grown in agar in Petri dishes. Dishes were sealed and placed at 4°C for an imbibition period of three days. After imbibition, seedlings were grown under Sho& Gro Brightsticks™ lamps. Growth measurements were taken for stem and root periodically after germination. *Arabidopsis* wild type seedlings grew rapidly during the first week of growth. Seven days after the imbibition period, roots averaged 13.0 mm in length and stems averaged 3.9 mm. The root growth in the *eto1-1* mutant was reduced to 59% of the control. However, stem growth in these

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mutants appears to be slightly greater than the wild type. The *eto1-1* mutant stems had a normal negative gravitropic response (bending away from gravity) and roots a positive gravitropic curvature (towards gravity). The *ctr1-1* mutant exhibited greatly reduced growth in the stem (48% of control) and root (6% of control) as well as little or no response to gravity. These results indicate that increased ethylene produced in both mutant was linked to decreased growth particularly in the roots. Root and stem growth was severely inhibited in the *ctr1-1* mutants. Future research will focus on the comparison of the kinetics of gravitropic curvature and growth rate in both mutants.

JASON FIKES, WARREN REUSCHEL, MARCIA HARRISON, Dept. of Biological Sciences, Marshall Univ., Huntington, WV 25755. Effects of signal transduction regulators on ethylene biosynthesis in pea epicotyls.

Ethylene, a plant hormone functioning in the inhibition of cell and root elongation and the development of axillary buds, is synthesized in response to external stimuli, such as wounding, and is transduced through an unknown signal transduction pathway. In our experiment, we investigated modulators of Ca^{++} /calmodulin signal transduction on ethylene biosynthesis in etiolated stems of *Pisum sativum* L. c.v. Alaska. Subapical stem sections of 7-day old pea seedlings were enclosed in 2-mL vials and treated with CaCl_2 , $\text{CaCl}_2 + \text{A23187}$ (a calcium ionophore which increases cytoplasmic calcium), LaCl_3 (a Ca^{++} channel blocker), and H_2O as a control treatment. Changes in ethylene production rates from the vial headspace were assessed by gas chromatography. For all treatments, wound ethylene peaked at 25-45 min. after incision; the mean peak rate for wound ethylene in control stems was $3.65 \text{ pL g}^{-1} \text{ min}^{-1}$. Stems treated with CaCl_2 demonstrated a doubling of ethylene production rates, while $\text{CaCl}_2 + \text{A23187}$ resulted in the greatest increase in ethylene production rates at nearly three times the control level. Treatment with LaCl_3 resulted in a slight

decrease in ethylene production. These results suggest that cytoplasmic Ca^{++} is linked to the ethylene biosynthetic pathway, possibly in Ca^{++} /calmodulin associations with protein kinases leading to activation of ethylene biosynthesis.

ANNE HUBBARD, BENJAMIN LOWMAN, MARCIA HARRISON, Dept. of Biological Sciences, Marshall Univ., Huntington, WV 25755. Growth and gravitropic curvature in an *Arabidopsis* mutant which under express ethylene.

Arabidopsis thaliana, a member of the mustard family, is the model species used in genetic research and experimental plant biology due to its small size, short life cycle, and small genome. Many mutant forms are available including those that vary in production of the growth inhibitor, ethylene. In this study comparisons were made of both stem and root growth and gravitropism (root or shoot curvature as a result in the change in orientation to gravity) of an *A. thaliana* mutant (*hls1-1*) that exhibits reduced ethylene production and a wild type *A. thaliana*. Seedlings were grown on agar in Petri dishes under GE Sho & Gro Brightsticks™ for 19 days. Growth data was photographed periodically at close range throughout the growth duration. Gravitropism was assessed by measuring root and stem curvature after horizontal placement of seedlings. After 19 days, root growth decreased by 17% for the mutant compared to the wild type and stem growth decreased by 21% for the mutant compared to the wild type. A normal gravitropic (downward curvature for roots and upward curvature for stems) response was observed for both mutant and wild type seedlings. Ethylene measurements showed a 40% decrease in ethylene production from the mutant seedlings compared to the wild type. These results suggest that reduced ethylene production does not increase growth rate or alter gravitropic curvature in the *hls1-1* mutant as would be expected if ethylene inhibited growth levels produced by the seedlings. Further studies will compare the growth kinetics to gravitropic

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curvature under altered ethylene levels in these mutant seedlings.

JACOB OTT and BRUCE EDINGER,
Bioscience Dept., Salem-Teikyo University,
Salem, WV, 26426. Use of
macroinvertebrate fauna to determine
water quality in eastern WV streams;
methods and preliminary findings.

In this poster we outline the biology and use of the taxa and proportions of benthic macroinvertebrates as a measure of water quality, and report preliminary information on the benthic macroinvertebrate community of five eastern West Virginia streams. Using a 500 micron Nytex rectangular dipnet and a traveling 10m kick sample, macroinvertebrates were collected during fall months, preserved, and identified. One hundred randomly chosen invertebrates, using the Hilsenhoff Biotic Index technique, were keyed to genus using Merritt and Cummins. Tolerance values from the updated USEPA Rapid Bioassessment Protocols for each genus were obtained. For each sample, several metrics were calculated: the proportions of the sample accounted for by each order, ratio of Ephemeroptera, Plecoptera, and Trichoptera to Chironomidae abundance, genus diversity and family diversity. The modified Hilsenhoff Biotic Index, which weights tolerance values of each taxon by the abundance of the taxon, will be reported, but has not been calculated to date. By several metrics, the streams showed good water quality. The proportion of the sample comprised of the generally intolerant orders of Ephemeroptera (mayflies), Plecoptera (stoneflies), and Trichoptera (caddisflies) (EPT) ranged from 0.70 to 0.84. The ratio of EPT and Chironomidae abundance varied from 3.3 to 10.1. Genus diversity ranged from 10 to 14 while family diversity ranged from 7 to 13. The comparative value of each of these metrics will be discussed.

SNYDER, EDWARD M., Dept. of Physical
Science, Shepherd College,

Shepherdstown, WV 25443; SEARS,
RACHEL E., Dept. of Environmental
Studies, Shepherd College, Shepherdstown,
WV 25443. Taxonomy of fenestrate Bryozoa
of the Gerster Formation, Northeastern
Nevada.

A diverse and abundant assemblage of fenestrate Bryozoa characterize the Guadalupian (Kazanian) Gerster Formation of northeastern Nevada. Comprehensive analysis of Gerster fenestrate Bryozoa was undertaken employing techniques which distinguish zoarial from zooecial characters both quantitatively and descriptively, and through three-dimensional reconstruction of the zooecial chamber shape and size. Further, unusual preservation in which zooecial chambers were selectively preserved allowed extremely accurate reconstruction of chamber shape and dimension. Zooecial characters exhibit a lower intraspecies variation than zoarial characters, and are heavily relied upon in taxonomic analysis. A total of ten fenestrate species were identified in the Gerster Formation; of these *Rectifenestella microretiformis* Morozova, 1970; *Wjatkella permiana* Morozova, 1970; *Wjatkella wjakensis* (Netschajew, 1893); and *Polypora soyanensis* Morozova, 1970 are all described from Permian age materials in Russia, with most more specifically from the Russian platform. *Rectifenestella microretiformis* Morozova, 1970 also occurs in Primor'áe Province. All the above species are confined to the Lower Kazanian, except *Wjatkella wjakensis* (Netschajew, 1893) and *Polypora kasanensis* Morozova, 1970 which occur throughout the Kazanian. Four new species, one each from the following genera, are recognized and have proven useful in biostratigraphic zonation; *Rectifenestella n. sp. A*, *Wjatkella n. sp. A*, *Reteporidae n. sp. A*, and *Penniretepora n. sp. A*. Images scanned from photograph and directly input into computer were analyzed for comparison qualitatively and quantitatively for taxonomic characters. Adobe Photoshop, Interactive Data Language (IDL) and Adobe Premiere are employed in capture and presentation of the specimen and gathered data. A computer slide show as well as

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poster display will both be employed in this mixed media presentation.

DOUGLAS B. CHAMBERS, U. S. Geological Survey, Charleston, WV, and TERENCE MESSINGER, U. S. Geological Survey, Charleston, WV. Selected contaminants in fish tissues in the Kanawha River Basin

Fish tissues were collected from 15 sites in the Kanawha River Basin of West Virginia, Virginia and North Carolina in August-October 1996. Sites were selected to represent important environmental settings (such as mining land use in the Appalachian Plateau physiographic province) within the basin as part of the U.S. Geological Survey's National Water-Quality Assessment Program. At each site, 10-20 rock bass (*Ambloplites rupestris*) or 7-10 common carp (*Cyprinus carpio*) were collected by electrofishing and sacrificed. All fish were weighed and measured. Both carp and rock bass were collected at one site, New River at Glen Lyn, Va. Whole-fish composite samples were analyzed for 28 organic compounds and liver composite samples were analyzed for 22 trace elements. Of the 28 organic compounds, only 10 were detected in tissue samples from any of the 15 sites. Total polychlorinated biphenyls (PCB's) were the most widespread organic analyte detected in the basin. PCB's were found in tissues from 9 of the 15 sites, and present in the highest concentrations, as high as 1,300 µg/kg in common carp and 990 µg/kg in rock bass. Of the nine organochlorine pesticides detected, each was found at less than half of the sites. No pesticide concentration exceeded National Academy of Sciences/National Academy of Engineering recommended maximum tissue concentrations for whole fish, or U. S. Food and Drug Administration action level concentrations for edible tissues. Nineteen of 22 trace element analytes were detected in samples from at least one of the sites. U. S. Environmental Protection Agency (USEPA) priority pollutants mercury and cadmium were both detected from a sample at the Cranberry River near Richwood, W.Va. site in concentrations significantly higher

than the mean concentration of all samples. Selenium (another USEPA priority pollutant) was detected in concentrations significantly higher in samples from Clear Fork near Whitesville, W. Va., and Bluestone River at Spanishburg, W. Va. than the mean concentration of all samples.

KIRK CAMMARATA, WATARU TAMURA, Bioscience Dept, Salem-Teikyo University, Salem, WV, 26426 and GREGORY SCHMIDT, Botany Dept, University of Georgia, 30602. Sequential reconstitution of LHCII and analyses of pigment binding.

LHCII is the major chlorophyll binding complex in higher plants. Each polypeptide binds 6-8 Chl *a*, 6-7 Chl *b*, and 2-4 xanthophyll molecules. A 3-D model has been proposed (Kuhlbrandt et al., 1994 *Nature* 367:614) based on electron crystallographic data. However, the pigment binding sites have not been identified unequivocally. To help confirm the identity of various pigment binding sites, we are partially reconstituting (Camarata and Schmidt, 1992 *Biochemistry* 31:2779) a bacterially-expressed LHCIIb gene (AB96) with various combinations of pigments. Partially reconstituted complexes can be generated with Chl *a* and xanthophylls or Chl *b* and xanthophylls, but not in the absence of xanthophylls. Partially reconstituted complexes with Chl *b* and xanthophylls sediment in sucrose gradients commensurate with a fully reconstituted (folded) protein. Partially reconstituted LHCII isolated from sucrose gradients was added to supplementary pigments solubilized in ethanol. Non-denaturing SDS-PAGE revealed a mobility shift to larger size and the incorporation of newly added pigment to a "normal" level (Chl *a/b* = 1.14) for Chl *b*/xanthophyll complexes supplemented with Chl *a*. Similar changes were not observed if Chl *a*/xanthophyll complexes were supplemented with Chl *b*. These *in vitro* results are consistent with a sequential assembly/folding model where Chl *b* binding initiates the process. The data do not, however, rule out the other scenario where Chl *a* binds first [consistent with results from Chl *b*-deficient mutants, F.G. Plumley (unpublished)].

Moreover, each of the 3 xanthophylls can independently support a partial reconstitution with Chl *b*, although lutein is the most efficient. Supported in part by a WVEPSCoR Instrumentation Grant to K.C.

MELISSA COBB, DAVID SPEARS, ESTHER GUZMÁN, BECKY COOPER and KIRK CAMMARATA, Bioscience Dept, Salem-Teikyo University, Salem, WV, 26426. Antisense polyphenoloxidase gene construction for downregulation of PPO activity in apple.

Polyphenoloxidase (PPO) is a stress-associated enzyme responsible for the browning reactions of damaged plant tissue. The nuclear encoded enzyme localizes to the thylakoid lumen, but its o-diphenol substrate is found in the vacuole. Chloroplast-related functions have long been speculated for this enzyme. More recent evidence suggests that it may be a metallo-protease. We will use an antisense expression strategy to downregulate PPO activity. Transformed plant tissue with reduced PPO activity: 1) may be useful for tissue culture systems where browning is detrimental (ie. *juncus*); 2) may be of agronomic value if not detrimental to plant viability; and 3) may facilitate elucidation of undiscovered functions for PPO. An approx. 800 bp apple 5' PPO gene fragment, corresponding to DNA sequence encoding the regions between the transit peptide and the copper B binding site, was amplified from Not I digested genomic DNA. This fragment was cloned into the Sma I site of pBluescript and the orientation confirmed by restriction analysis and partial DNA sequencing. The insert was then excised by Bam HI/Hind III digestion and subcloned into Hind III/Bgl II digested pGA643 binary expression vector in an inverted orientation relative to the 35S promoter. We have also established apple callus tissue cultures. These are being multiplied for experiments to determine sensitivity to antibiotic selection. Supported in part by a Spectroscopy Society of Pittsburgh Instrumentation Grant to K.C.

DAVID BERRYHILL and KIRK CAMMARATA, Bioscience Dept, Salem-Teikyo University, Salem, WV, 26426, Culturing wetland plants for gene expression studies related to phytoremediation.

We are interested in using wetland plants for phytoremediation. As part of a strategy to genetically enhance phytoremediation capability, we are looking for genes specifying plant adaptations to stressed environmental conditions which are related to the pollutants to be remediated. Differential Display-Reverse Transcriptase Polymerase Chain Reaction (DD-RT PCR) will be used to identify genes differentially expressed in plants cultured under stress conditions (vs. those cultured in non-stress conditions). We are currently establishing controlled culture systems which will allow us to differentially introduce pollutants to the study plants. *Lemna minor* is being cultured on 1/3 strength Hutners medium with continuous illumination. Experimental cultures are supplemented with concentrations of heavy metal salts (ferrous sulfate, ferric sulfate, zinc sulfate, manganese sulfate) ranging from 0.0001% to 1 % to determine the concentration ranges over which a growth response occurs. Preliminary results suggest that concentrations resulting in an approximate 50 % decrease in plant growth rates are: 0.01 % ferric sulfate, 0.001 % ferrous sulfate, <0.017 % manganese sulfate, and >0.01% zinc sulfate. *Typha* seedlings are being cultured in strength Murashige and Skoog medium (pH 7) supplemented with 2% sucrose and vitamins. For studies of root oxidizing capacity, these plants are transferred to a plug flow aquaculture system designed and constructed by Dr. Wayne Curtis at Penn State University. The continuous flow nutrient solution in this case is 1/20 strength MS medium without sucrose or vitamins.

THOMAS K. PAULEY, ALISON M. ROGERS, BETH A. PAULEY, AND JOHN CAMPBELL, Department of Biological

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Sciences, Marshall University, Huntington, WV 25755 and W. MARK FORD, Timberlands Division, Westvaco Corporation, Rupert, WV 25984. A Study of the Amphibians and Reptiles in the Westvaco Wildlife and Ecosystem Research Forest.

Surveys for amphibians and reptiles were conducted in the Westvaco Wildlife Research and Ecosystem Forest in 1996 and 1997 to determine species present and develop baseline data for future monitoring studies. General surveys in terrestrial habitats produced 8 species of salamanders, 2 species of anurans, and 8 species of snakes. Surveys in aquatic habitats resulted in 8 species of salamanders, 6 species of anurans, 1 species of turtle, and 5 species of snakes. Monitoring studies were conducted in mixed deciduous forests, first- and second-order streams, and various lentic habitats to determine population sizes and aspects of the natural histories of amphibians and reptiles. Studies in mixed deciduous forests involved the use of cover boards along 100 meter transects. Dominant species studied included *Plethodon cinereus* and *Desmognathus ochrophaeus*. Population estimates of both species were determined by mark-recapture studies using the Schnabel Method. Between 1996 and 1997 trees on 3.5 sites were harvested, 1.5 site by a deferment cut and 2 sites by a sawtimber cut, and there were sharp declines in population sizes of both species in the deferment sites. Four streams were monitored during both years of the study and dominant species of salamanders in order of abundance were *Gyrinophilus porphyriticus*, *Eurycea bislineata*, *D. monticola*, *D. ochrophaeus*, and *D. fuscus*. Lentic habitats including permanent pools, temporary pools, and roadside ditches were monitored during both years of the study. Major species studied included *Rana clamitans melanota*, *R. sylvatica*, *Ambystoma maculatum*, and *Notophthalmus v. viridescens*. It was determined that *R. c. melanota* larvae overwinter before transforming the summer after egg deposition. As expected, *R. sylvatica* and

A. maculatum were the first to arrive at the breeding ponds in the spring, and many *N. v. viridescens* adults were found to leave the ponds after the breeding season.

THOMAS K. PAULEY, SANDRA RAIMONDO, BRIAN LINDLEY, MINDY HAMILTON, and JEFFREY HUMPHRIES, Department of Biological Sciences, Marshall University, Huntington WV 25755, Potential impacts on forest salamanders from multiple applications of insecticides.

A long-term study analyzing potential impacts of gypsy moth pesticides on forest salamanders began in 1995. Nine plots were used in the study, three of which were Gypchek, 3 BT and 3 Control. Marking the third year of the study and the first post-treatment year, 1997 data were used to compare biodiversity of terrestrial and aquatic salamanders, environmental parameters (air temperature, relative humidity, soil moisture, soil pH, water temperature, water pH), dietary composition, reproductive potential and stored energy between treatment plots. Terrestrial salamanders were surveyed using cover boards and point survey and night survey methods. Aquatic salamanders were surveyed by juvenile refugia bags, stream survey and night survey methods. A total of 928 salamanders was collected in 1997. The month in which the most salamanders were collected was June and the method by which most were collected was stream surveys. Salamander data collected in 1997 were also compared to those collected the previous years. In 1997, 46 salamanders were found on BT plots, 71 and 53 were found on Gypchek and Control plots, respectively. In 1996, 53 salamanders were collected on BT plots, 57 on Gypchek and 121 on Control. In 1995, 22 salamanders were found on BT, 44 on Gypchek and 62 on control. The only environmental parameter showing significant differences between plots was soil pH (ANOVA, $p=0.05$). This is one part of a three part study (insects, birds and salamanders) funded by the US Department of Agriculture.

BRIAN LINDLEY and THOMAS PAULEY,
Department of Biological Sciences,
Marshall University, Huntington, WV 25755.
Impacts of environmental conditions on the
abundance of salamanders on the
Allegheny Mountains of Virginia and West
Virginia.

Many studies have shown that soil moisture is a determining factor on the distribution of forest salamanders. In this study, we compared surface abundance of terrestrial salamanders in nine sites in the Monongahela National Forest (MNF) in West Virginia with nine sites in the George Washington Forest (GWNF) in Virginia. Sites in the GWNF have a west-to-east and east-to-west pattern of frontal air flow resulting in a rainshadow effect. This area of Virginia is considered to be the most arid in the state. In the MNF, westerly winds predominate allowing cold northern and warm southern air masses to meet resulting in mesic conditions. Precipitation exceeds 53 inches annually in this part of West Virginia. The 18 study sites were surveyed once per month from May to October in 1995 and 1996 in a study supported by the USDA-Forest Service to determine potential effects of insecticide applications used to control gypsy moth invasions on nontarget forest species. Environmental results showed that the GWNF sites had higher air and soil temperatures, lower relative humidity, and higher light intensity than the MNF sites. No differences were found for litter moisture, soil moisture, and soil pH between the two states. In the GWNF, 19 salamanders (4 species) were observed compared to 283 salamanders (7 species) in the MNF.

JEFFREY HUMPHRIES, Marshall
University, Huntington, WV 25755, SCOTT
G. BLACKBURN, WV Natural Heritage
Program, P.O. Box 67, Elkins, WV 26241 and
THOMAS K. PAULEY, Marshall University,
Huntington, WV 25755. Conservation and
current status of the eastern hellbender in
West Virginia.

The eastern hellbender (*Cryptobranchus alleganiensis alleganiensis*) has been found in 22 counties in West Virginia. The majority of these records are over 30 years old and only recently have efforts been made to determine the current distribution of the hellbender, especially in comparison to historical localities. Throughout the eastern U.S. biologists have noticed declines in hellbender populations, attributed to the loss or degradation of aquatic habitats caused by human activities. These include: siltation from logging, mining, road construction and urban development, as well as river impoundments, thermal and chemical pollution, acid mine drainage and eutrophication. The hellbender is an Endangered Species in Ohio, Illinois, Indiana and Maryland, and is believed to be extirpated from Illinois, Iowa and Kansas. Though thought to be "secure", very little is known about the current status of the hellbender in West Virginia. We will use both mark-recapture and radio-tracking methods to study hellbenders at specific sites in West Virginia. This study's aim is to provide natural history, population demography, and distribution data to the West Virginia Natural Heritage Program so that future conservation efforts may be implemented.

BETH PAULEY, BEN LOWMAN, JEFF
GINGER, AND DONALD TARTER,
Department of Biological Sciences,
Marshall University, Huntington, WV
25755. Low pH tolerance of aquatic insects
from high-elevation acidic bogs in West
Virginia.

In the Appalachian region, acidification from acid mine drainage and acid precipitation creates major problems for aquatic insects. Low pH can alter respiration, acid-base balance, and sodium and calcium regulation. Aquatic insects have varying levels of acid tolerance. The purpose of this investigation was to determine the acid tolerances of a mayfly (*Eurylophella bicolor*) and an alderfly (*Sialis iola*) under laboratory conditions. Both species were collected from the Otter Creek National Wilderness Area, Randolph County, West

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Virginia. Using a standard D-shaped dredge, larvae of both species were returned to the laboratory and allowed to acclimate in a controlled environmental chamber for 24 hours. Ten larvae of both species were placed in each of five duplicate bowls. They were exposed to the following pH values: 8.0 (control), 6.5, 4.5, and 3.0. After a preliminary test, an additional pH value (1.5) was added to the alderfly experiment. After 96-hours, the median tolerance limits (TLm) were calculated using the straight-line graphical method and linear regression analysis. These limits were determined using a 50 percent mortality rate. The low pH median tolerance limits were 4.35 and 1.70, mayfly and alderfly, respectively. To test these pH values, an additional experiment was conducted on acclimated larvae for both species. Results will be compared with other laboratory and field studies involving pH tolerance values of benthic populations.

RONALD H. FORTNEY and DAVID STURM,
Dept. of Bioscience, Salem-Teikyo
University, Salem, WV 26426. A chronology
of post logging plant succession between
1945 and 1997 in Canaan Valley, WV.

Canaan Valley, a high elevation valley in the Allegheny Mountain Section of the Appalachian Physiographic Province, contains one of the largest inland freshwater wetland ecosystems of bogs, marshes, wet meadows, and swamps in the eastern United States. Because of its physiographic setting and past influences from glacial activities, it is an exceptional example of a refugium for biota of a prior climatic period. Like most high elevation mountainous areas in the Appalachians, its original forest, which was predominantly *Picea rubens*, was essentially clear cut and burned early in the 1900's. Following the logging era, most of the well-drained areas on the valley floor was converted to agricultural use, while most wetlands and steep mountain sides were permitted to succeed naturally. Canaan's vegetation was intensely studied in the 1970's and 1980's by several biologists. However, no attempt was made to track

the chronological succession of vegetation types through the 1900's. This study uses aerial black and white and color photography, plus ground truthing activities to produce a series of GIS-based vegetation maps for the valley. The three map series (1945, 1975 and 1997) is based on 1945 and 1957 US Dept. of Agriculture produced black and white photography and 1989 US Dept. of Interior produced color aerial photography. Physiognomic cover classes and dominant species were used to classify plant community types and to construct plant successional models. Overall, successional trends appear to follow rather predictable patterns for upland and lowland habitats. Between 1945 and 1997, there was a marked increase in the area covered by northern hardwood species and *Spiraea alba* and *Hypericum densiflorum* shrub thickets, but only a limited increase of *P. rubens* dominated forests. Beaver activity along waterways became an important factor in determining successional trends (and retrogression), causing shrub swamps to be converted to wet meadows and marshes. *Populus grandidentata* groves on uplands and *P. tremuloides* groves in lowlands, which were once important community types, decreased in area between 1945 and 1997, showing limited recruitment success since 1975. Bogs and old fields displayed the least amount of successional change. However, there is little evidence to suggest that *P. rubens* will replace hardwood species in the upland habitats of Canaan Valley.

LESLEY BAKER, ROB HOSTUTTNER,
WHITNEY REED, SETH TOMBLYN,
SHELLY VANCE, and DONALD TRISEL,
Fairmont State College, Fairmont, WV. The
science of cloning: history, techniques,
ethics, and the future.

Although asexual reproduction of plants, fungi, and some invertebrates is a natural part of these organisms' life cycles, the successful cloning of vertebrate animals is the cause of major debate throughout the international community. This procedure involves the transfer of a somatic cell

nucleus into an egg cell which has had its nucleus removed. Medical and ethical concerns are at the center of this controversy. The techniques may be beneficial in situations of infertility or endangered species, but there are obvious concerns about interfering with the natural evolution of species and with our desire to "play God". While legislators are rushing to limit future research in cloning, the scientists are eager to improve techniques and continue the advancement of this science.

DONALD E. TRISEL AND DAVID L. GORCHOV, Fairmont State College, Fairmont, WV and Miami University, Oxford, OH. Maple trees versus shrubs that are weeds.

Lonicera maackii, native to northeastern Asia, was introduced to North America as an ornamental plant. Non-cultivated shrubs now occur in numerous parts of the eastern USA. Previous research has shown a reduced herb layer under dense stands of this shrub, thus *L. maackii* may affect natural areas via competition and/or allelopathy. A field experiment was conducted to test the effects of established *L. maackii* on the survival and growth of transplanted *Acer saccharum* seedlings. The experiment examined competition for light (by removing *L. maackii* shoots) and competition for below-ground resources (by trenching around the transplanted seedlings). *Acer* seedling survival was significantly reduced in the presence of *L. maackii* shoots and roots. With increased competition for light and increased densities of *L. maackii*, surviving *Acer* seedlings grew larger leaves and taller stems. The increased mortality of tree seedlings indicates that *L. maackii* may negatively impact the natural regeneration and cause a loss of biodiversity in natural areas invaded by this shrub.

TARA MARKER, Dept. of Biological Sciences, Wheeling Jesuit University, Wheeling, WV, 26003 and BETTY THACKER, Dept. of Biological Sciences, Wheeling Jesuit University, Wheeling, WV,

26003. The distribution of neural cell adhesion molecules on differentiated and undifferentiated NG108-15 cells.

The objective of this research was to ascertain whether or not there is a difference in the distribution of neural cell adhesion molecules (N-CAMs) on the surfaces of differentiated and undifferentiated NG108-15 cells. NG108-15 cells were grown on coverslips in a medium that inhibits the differentiation of NG108-15 cells (G+) or a supplemented medium that induces differentiation of the cells (B27 or N2). The Avidin Biotin Complex (ABC) method and Evan's blue counter-staining techniques were utilized for N-CAM detection. Standard protocols for cell fixation, the ABC method, and counter-staining techniques were tested and then modified for the detection of N-CAMs on the surfaces of NG108-15 cells. The data supports that there is no observable differences in the distribution of N-CAMs on the surfaces of differentiated and undifferentiated NG108-15 cells.

MEGAN MASLOSKI, Dept of Biological Sciences, Wheeling Jesuit University, Wheeling, WV, 26003 and BETTY THACKER, Dept of Biological Sciences, Wheeling, WV 26003. Development of standard protocol for the differentiation of ES-D3 embryonic stem cells into neurons.

The objective of this research was to develop a standard protocol for the maintenance and differentiation of ES-D3 embryonic stem cells into neurons. The cells were grown in a medium comprised of Dulbecco's Modified Eagle Medium containing high glucose, L-glutamine, and pyridoxine hydrochloride. Fetal Bovine Serum was added to this medium; it was then supplemented with antimyotic. The stem cells were grown on an STO fibroblast feeder layer that was treated with Mitomycin C in order to stop its growth and differentiation once the stem cells were introduced into the culture flask. Several experiments were run to ascertain the optimum concentration of Mitomycin C in the growth medium. Stem cells

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were treated with Retinoic Acid, which is known to produce neuron-like projections. Studies were done to ascertain the optimum concentration of Retinoic Acid as well. Preliminary results show that the fibroblasts do not respond well to freezing and thawing cycles, so these should be limited to an absolute minimum. It is suspected that the Retinoic Acid produces neurons because it stimulates Hawk's genes, which are regulator genes for cell differentiation. Research was performed in the cell lab at Wheeling Jesuit University under the direction of Dr. Betty Thacker.

ALLISON BINKOWSKI, Dept of Biology,
Wheeling Jesuit University, Wheeling, WV,
26003. Distribution of Neuronal Markers of
NG108-15 Cells Grown in Different Media.

Fixed NG108-15 cells that had grown in G+ media and N2 supplemented media were treated with fluorescent chemicals to ascertain whether they differed in their placement of the neuronal markers, MAP2 and *tau*. The Avidin-Biotin Complex (ABC) Method was employed as well as use of the fluorescent microscope to make these observations. Cells grown in G+ media emitted more fluorescence when treated with MAP2 than cells grown in the N2 supplement treated with *tau*. Also, the neurites of the G+ cells, if any, emitted fluorescence where the highly differentiated N2 cells did not. Therefore, the neurites of NG108-15 cells do not exhibit neuronal character, but instead must be a characteristic of the glial nature of the cells. All research was completed at Wheeling Jesuit University under the direction of Dr. Betty Thacker.

CLINT J. SPRINGER, WALTER J. CYBULSKI III, and WILLIAM T. PETERJOHN, Dept. of Biology, West Virginia University, Morgantown, WV, 26506. Effects of Ambient and Enhanced UV-B Radiation on the Growth and Biomass of *Quercus rubra*.

Due to the ongoing depletion of stratospheric ozone a rise in ultraviolet-B (UV-B, 280-320 nm)

radiation is occurring. A number of studies have been performed on crop species to find the consequences of increases in solar UV-B, yet not many studies have been initiated on native species. In this study *Quercus rubra* was used to determine the effects of UV-B on stem mass, leaf mass, root mass, leaf number, and root/shoot ratios. The *Q. rubra* was grown in the West Virginia University Greenhouse (39° N, 70° W) under six lamp banks. Half of the lamp banks supplied ambient levels of UV-B (5.98 kJ m⁻²), while the other half supplied UV-B (kJ m⁻²) representing a 25% ozone depletion in Morgantown, WV. Although no significance was found, a decrease in stem, leaf, and root mass was present in the replicates exposed to elevated UV-B. The decreases in biomass may be attributed to the fixation into UV-B protective compounds such as flavonoids.

KERRIE LOMAN, BRANDY DORSEY,
RONALD H. FORTNEY, Dept. Of
Biosciences, Salem-Teikyo University, WV
26426. A review of the ecology and threats of mile-a-minute plant (*Polygonum perfoliatum* L.) In North America.

Polygonum perfoliatum, known as the Mile-A-Minute plant (MAM), is native to temperate regions of southeast Asia. Although it was introduced in North America in the 1800's, it was not until the 1980's and 1990's that it became an invasive, exotic wood spreading out in med-Atlantic states from a site in eastern PA. The MAM is now present or becoming a problem in eight states (PA, MD, NJ, WV, DE, VA, CT and OH) and the District of Columbia. In southeast Asia it is not known to be an invasive plant. The purpose of this poster is to examine the status of the MAM plant in North America and to evaluate the ecological and agricultural threats associated with the species. Information was compiled from several journal articles, fliers, technical reports, and internet sources. Local experts were also contacted for their unpublished information on MAM. The MAM is a self-pollinating, vine-like plant that utilizes small hooks to climb. It typically occurs in moist

disturbed habitats--roadsides, forest clearings, and old fields. Although an annual, it can grow extraordinarily fast in one season, overgrowing vegetation. It is this aggressive growth that outcompetes other plants and makes this species a threat to species diversity and agricultural related interests, including Christmas tree nurseries, orchards, timber production areas, and ornamental shrubs. The MAM plant requires a minimum cold period of eight weeks at maximum temperature between 5° C and 10° C to germinate. This means that there are 18 more possible states that the MAM plant could successfully germinate in. Two major uncontrollable sources of distribution of the MAM are water and birds. Achenes that survive partial digestion are viable and the seeds are buoyant for up to seven days in water. Fortunately, there are possible sources of control. The plant has an extremely fragile root system which allows for mechanical removal. There is also a parasitic competitor, *Cucusta gronovii*, which suppresses the growth of the MAM. Several herbicides can also be applied to hinder ontogenesis, but such efforts may not be successful in controlling the MAM. Current research areas are biological controls and ecological impacts.

CATALINA TEBA and E.C. KELLER, JR.
(West Virginia University, Morgantown, WV 26506-6057) Comparitive Studies of Human Mortality: Differences between Northern and Southern West Virginia.

Many factors play roles in determining how long an individual may live, such as their surrounding environment, genetic makeup, diet, stress, and countless others. In this study we have examined the question concerning the role that geographical location plays in the distribution of mortalities. By examining the death certificate records of West Virginian residents from 1959 until 1981 we compared the cause of deaths in northern areas to those in southern parts of West Virginia. We predicted that since there are generally broad differences in occupations in the north compared to those in the south that perhaps this could be

influencing the different mortality trends. The results of our research established there was a significant differences in the causes of death in the north compared to those in the south. Residents in the north had a higher incidence of more "old age" mortalities; such as cerebral vascular disease, genital/urinary cancers, heart disease, malignant neoplasms of digestive organs and peritoneum, hernia, major cardiovascular diseases, malignant neoplasms, and other diseases of arteries, arterioles, capillaries. In contrast we found residents of the south had a significantly higher incidence of one mortality, pneumoconiosis due to silica and silicates.

BRIAN J. SCHMIDT and E.C. KELLER, JR.
(West Virginia University, Morgantown, WV 26506-6057) Cardiovascular Mortality Trends in West Virginia.

This study of resident human mortality focuses on general cardiovascular mortality trends in West Virginia. These data were compiled by the West Virginia Department of Health from cause of death entries on death certificates. The broad category of cardiovascular disease was divided among five major sub-groups in order to ascertain the relationship among the respective mortality trends (1982-1994). The groups are cerebrovascular disease, arteriolosclerosis, other arterial disease, heart disease, and hypertension. Preliminary results indicate that cerebrovascular mortality is the most frequently occurring mortality in recent years (1985-1994). However, the cerebrovascular mortality shows a downward trend, resulting in the narrowing of the gap between it and other arterial disease mortality. Mortality due to other arterial diseases indicated an initial increase (1983-1985) followed by a moderate downward trend. The third type of cardiovascular mortality, heart disease, showed a modest upward trend (1983-1985) and, thereafter, a period of stability. Mortality due to arteriolosclerosis, after a modest increase, showed a moderate decrease. Mortality due to hypertension remained at low levels and was relatively stable throughout the period.

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JEFFREY PUMPHREY, ROBERT KRISTOVICH, and ALBERT MAGRO, Division of Science, Mathematics and Health Careers, Fairmont State College, 1201 Locust Avenue, Fairmont, WV 26554-2470. Estrogen receptor dependent assays of xenoestrogenic compounds.

Xenoestrogenic compounds are environmental pollutants known to have hormone-like effects in fish, wildlife and humans. Among the compounds known to function as estrogenic agonists are pesticides, non-steroidal stilbenes and phenyl analogs. The activity of these estrogen mimics results from direct binding to the steroid site of the nuclear estrogen receptor with IC_{50} values of 1-10 μ M. An objective of this study was to develop an in culture cell growth assay utilizing estrogen dependent human breast MCF-7 cells. It was found that stimulation of MCF-7 cells by xenoestrogenic compounds significantly increased the growth of the MCF-7 cells as compared to non-stimulated controls. An additional objective was to develop an enzyme-linked assay which tests the binding of xenoestrogenic compounds to purified estrogen receptor. The estrogen receptor was biotinylated and bound to avidin treated microtiter plates. Estrogen was covalently bound to alkaline phosphatase by the mixed anhydride method and used as a competitive ligand in the enzyme-lined assay. It was determined that non-steroidal estrogen mimics competed effectively against estrogen-alkaline phosphatase for the steroid binding site of the purified estrogen receptor. It was concluded that both the MCF-7 cell growth assay and the estrogen receptor enzyme assay are effective assays for the screening of xenoestrogenic compounds.

AMY SARVER and E. C. KELLER, West Virginia University, Department of Biology, Morgantown, WV. Cancer Mortality Trends in West Virginia, by Sex, 1959 to 1994.

Increasing mortality rates due to malignant neoplasms have long been a concern for human

populations. This study concerns the investigation of mortality trends due to malignant neoplasms in West Virginia, by sex, for the years 1959 to 1994. West Virginia death records were obtained from the WV Department of Health for the years in mention and assessed. Using the Manual of the International Statistical Classification of Disease, Injuries, and Cause of Death (WHO 1957, 1967, 1979), deaths due to malignant neoplasms were counted. Malignant neoplasms of the respiratory system, digestive organs and peritoneum, and reproductive organs and urinary system were also counted. Any malignant neoplasm that did not fall into these categories was calculated as other. These results were plotted and analyzed for increasing or decreasing trends. Overall trends for malignant neoplasms have increased in West Virginia from 1959 to 1994, with respiratory neoplasms showing the greatest increase, especially for females. Malignant neoplasms of digestive organs and peritoneum showed a slight increase, as neoplasms of the reproductive organs and urinary system remained relatively constant. These findings generally correspond to national findings concerning malignant neoplasm, although more recent studies (1990 to 1996) on the national level have shown more decreasing trends in overall cancer. Further studies should be conducted to determine trends in West Virginia of breast, prostate, and other major types of cancer.

