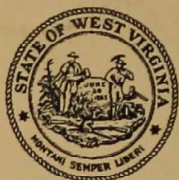


Volume 68, Number 1

# Proceedings of the West Virginia Academy of Science 1996



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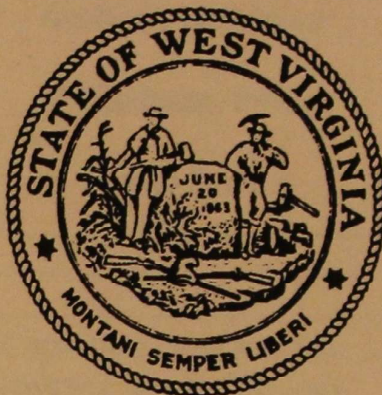
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**"Risks and Regulations: Prospects for Food Safety and  
Health Protection in the 21st Century"**

Moderator: Dr. Karl D. Fezer, Dept. of Biology, Concord College

Speakers/Panelists from the United States Food and Drug Administration

Dr. Ronald J. Lorentzen, Strategic Manager for Risk Assessment and Risk  
Communication

"Risk Assessment and Risk Management: The Art and Science"

Dr. Joseph M. Madden, Strategic Manager for Microbiology

"Microbiological Hazards in Food and Water: Contemporary and Ancient  
Enemies"

Dr. Albert E. Pohland, Strategic Manager for Research

"Natural Toxins and Environmental Contaminants: Risks and Realities"

Dr. Nega Beru, Biotechnology Policy Branch

"Biotechnology in the 21st Century: Attitudes and Allergies"



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1996 Annual  
Meeting



## BIOCHEMISTRY - CHEMISTRY

KRISTA EVANS, Shepherd College, Shepherdstown, WV 25443 and  
PAMELA HAWLEY-NELSON, Life Technologies, Inc., 8717 Grovemont  
Circle, Gaithersburg, MD 20884-9980. A rapid mammalian cell transfection  
reporter using jellyfish mutant Green Fluorescent Protein  
in pGreenLantern<sup>TM</sup>-1.

Green fluorescent protein (GFP) from *Aequorea victoria* jellyfish emits green fluorescence when excited with blue light. No additional substrates are required for fluorescence which allows this protein to be detected noninvasively in live cells. Expression vectors utilizing wild-type GFP as a reporter gene have exhibited lower expression levels in mammalian cells when compared to  $\beta$ -galactosidase, a standard reporter protein, which utilizes enzymatic amplification. A new mammalian cell transfection reporter vector was constructed, pGreenLantern<sup>TM</sup>-1, which employs "humanized" codons and a Ser<sup>65</sup> to Thr point mutation in its cGFP under control of a CMV enhancer/promoter showing increased fluorescence and gene expression over wild-type GFP in transfected cells detected by microscopy using fluorescein-isothiocyanate filter conditions. In LipofectAMINE<sup>TM</sup> transfected CHO-K1 cells, pGreenLantern-1 exhibited 70% positive fluorescent cells in 24 hours as compared with a wild-type vector, pRAY1, which exhibited 22% positive fluorescent cells at 48 hours. Wild-type GFP requires 48 hours in mammalian cells to develop peak fluorescence. Due to the increased fluorescence intensity, it is possible to quantitate the expression of this mutant GFP using a fluorescence plate reader. Transfected CHO-K1 cells were trypsinized and counted as well as stained *in situ* with Trypan Blue. No reduction in cell viability could be associated with high levels of GFP expression compared to high level expression of  $\beta$ -galactosidase, another common reporter protein. Therefore, pGreenLantern-1 gives rapid detection of positive transfected mammalian cells with increased sensitivity over wild-type GFP vectors with no adverse effects on living cells.

MARK R. FLOOD, Biology Dept., Fairmont State College, Fairmont WV 26554 and GAROLD S. YOST, Dept of Pharmacology and Toxicology, University of Utah, Salt Lake City, Utah 84112 Cloning of an UDP-glucuronosyltransferase from an ethanol-induced liver.

Phase II enzymes, including the UDP-glucuronosyltransferase (UGT) enzymes, can be induced by various drug treatments. Previously, an ethanol-induced rabbit UGT enzyme has been identified and characterized (Hutabarat and Yost, Arch. Biochem. Biophys. 273:16, 1989). We report here the isolation and cloning of a full length cDNA clone for an UGT expressed during the ethanol-induced state. The ethanol-induced state was accomplished by the treatment of a male New Zealand White rabbit with 10% ethanol:90% water solution for 14 days prior to



tissue collection. Total RNA was isolated from liver samples via standard guanidium thiocyanate extraction procedures. mRNA was isolated from a Poly (A) Quik™ (Stratagene) oligo dT column. Northern blot hybridizations with a 51 bp oligonucleotide, corresponding to a UGT conserved region, indicated that there were higher levels of UGT mRNA expression with ethanol-induced animals than control animals. A rabbit liver cDNA library was constructed from the ethanol-induced liver mRNA using a ZAP-cDNA synthesis kit (Stratagene). The library was screened using the oligonucleotide. After tertiary screening, positive clones were packaged into a bluescript vector and placed into XL1-Blue cells using an *in vivo* excision protocol. XL1-Blue cells containing the vector and insert were then subjected to a plasmid DNA preparation. The double-stranded plasmid cDNA has been completely sequenced and this 2.3 kb insert belongs to the UGT1 family. The open reading frame is 1590 nucleotides long, which corresponds with an expected protein of 530 amino acids long. When compared to the rat cDNA for UGT 1\*06, there is near complete identity. However, the predicted protein sequences are only 82 % similar. Northern blot hybridizations indicated that this UGT clone corresponds to a gene that is constitutively expressed in control rabbits and ethanol-induced animals. Work is continuing in this area to determine the function of the expressed protein from this cDNA clone and also to obtain a full-length cDNA clone of an ethanol-induced UGT. The UGT clones we are isolating may correspond to a constitutively expressed UGT that is induced by consumption of ethanol. The UGT enzymes are responsible for metabolism of various therapeutic drugs in humans, and changes in metabolism could be a concern to individuals who consume ethanol while on some drug treatment regimes. This work was supported by Grant AA06555 from the USPHS, National Institutes of Health.

DIANNE K. ANESTIS, Dept. Of Biology, Marshall University, and Dept. Of Pharmacology, Marshall Univ. School of Medicine, Huntington, WV 25755.  
Small mammals as bioindicators of environmental toxicity: a review.

Empirical data suggest that animals may be more sensitive indicators of environmental toxicity than humans. Mammals, especially small mammals, living in close proximity to humans in polluted environments may serve as important bioindicators of hazards to human health. Ecotoxicologists observe and measure the reactions of animals to polluted environments to extrapolate potentially harmful effects to humans. The large quantity and diversity of synthetic pesticides in use since the Industrial Revolution have caused serious environmental contamination and harm to wildlife, sometimes via bioaccumulation. Although some earlier, more environmentally abusive patterns of toxicant uses have diminished due to increasing environmental awareness, small mammals are still at the forefront of exposure to compounds and their active metabolites. Our ecosystem is limited in its ability to absorb and detoxify large quantities of man-made waste products, many of which have proved harmful. The intelligent utilization of our limited resources requires a fundamental knowledge of human



impact on our environment. In addition, the study of populations of small mammals helps us understand the importance of maintaining biodiversity to avoid concomitant, potentially detrimental, shifts in the food web. In view of recent proposals to weaken some environmental regulations and the Endangered Species Act, monitoring is essential to assess biodiversity and a species supportive role in the ecosystem. The health of animal populations is an important measure of environmental quality. Small mammals, as well as other wildlife species, are valuable bioindicators that can help humans assess and responsibly modify our impacts on the environment.

TANYA C. WARWICK and MICHAEL J. PANIGOT, Department of Chemistry, West Virginia State College, Institute, WV 25112-1000. Effects of ester protecting groups on the ortho-tolyl alkylation of acylated glycosyl halides by benzylmagnesium chloride.

Attempts to directly prepare benzyl-C-glucoside by direct benzyl Grignard displacement of bromide from acetobromoglucose yielded a 3:1 mixture of the unexpected ortho-tolyl alkylation product to the desired benzyl alkylation product. This was avoided by using a glucosyl bromide protected as the tetrabenzyl ether. This starting material has significant drawbacks in terms of cost and additional chemical manipulations to arrive at the final desired product. The rearrangement is presumed to be due to the participating group at the C-2 position of the pyranose ring as well as the hydroxymethyl group at the C-6 position. Glucose pentabenzoate has been prepared by standard methodology as have similar compounds with substituted benzoate esters. Additionally they have been converted to the substituted and unsubstituted perbenzoylated glycosyl bromides. These compounds will be reacted under conditions analogous to that previously used for acetobromoglucose and the benzyl to ortho-tolyl ratio will be determined by  $^1\text{H}$  NMR. Benzyl to ortho-tolyl alkylation comparisons between these compounds and acetobromoglucose will be investigated both in terms of the steric and electronic demands of the benzoate ester at C-2.

## BIOLOGY

SARAH MEADS, Shepherd College, Shepherdstown, WV, 25443 and ROCCO CIPRIANO, National Fish Health Research Laboratory, Kearneysville, WV, 25430. Characterization of *Aeromonas salmonicida* isolated from small mouth bass (*Micropterus dolomieu*) in Chaumont Bay from eastern Lake Ontario.

A characterization study was done to confirm an *Aeromonas salmonicida* outbreak in small mouth bass during July 1995 in the Chaumont Bay of eastern Lake Ontario. This bacterial isolate was compared with that of a known isolate of *A. salmonicida* obtained from Atlantic salmon. The two were compared using Coomassie Brilliant



Blue (CBB) agar and antimicrobial sensitivity testing. It was confirmed that the bacteria found in the sample obtained from the small mouth bass in Lake Ontario was *A. salmonicida*. It was found that the two strains were similar biochemically and by antibiotic testing. Differences were observed in the lack of the A-layer, CBB colony color, and a 50kd protein in the small mouth bass strain. The absence of the A-layer, which has been found to be associated with virulence, would also explain the longevity of the brook trout inoculated with the small mouth bass isolate.

HAJENGA, JEFFREY J., and MARY ETTA HIGHT, Dept. of Biological Sciences, Marshall University, Huntington, WV 25755. New records of shrews, *Sorex hoyi*, *S. dispar*, and *Cryptotis parva* in West Virginia.

Eight species of shrews (Family Soricidae) are members of the small mammal fauna in West Virginia, some of which are rare or of uncertain status. Seven species were captured in a thesis study to assess the distribution, ecology, and natural history of shrews in Mercer and Summers counties by pitfall trapping. New county records were obtained for three species not previously known from southern West Virginia. Three new county records were established: Pygmy Shrew (*Sorex hoyi*), and Long-tailed Shrew (*S. dispar*) from Mercer Co. and Least Shrew (*Cryptotis parva*) from Summers Co. This represents the first documented capture of Least Shrew within WV since 1969. *Sorex hoyi*, first reported from Tucker Co. (Michael, Proc. W. Va. Acad. Sci. 58:37), now appears to be wide-spread in the state, but it is not abundant anyplace. In 34,213 trapnights 653 shrews were captured, yielding records of only five Pygmy Shrews, two Long-tailed Shrews, and two Least Shrews. This study shows that these species are limited in distribution by both microhabitat requirements and interspecific competition for resources.

JOHN BURTON, WES GLADWELL, and MICHAEL LITTLE, Department of Biological Sciences, Marshall University, Huntington, WV 25755. The development of genetic markers that can be used to analyze the effects of anthropogenic stress on genetic diversity of fish populations.

Recently, investigators have documented a relationship between environmental disturbance and loss of genetic diversity. These studies have used starch gel electrophoresis to assay allelic numbers and genotype frequencies for fish populations and have associated genetic decline with a reduction in environmental quality. Although starch gel electrophoresis is an efficient method that can be used to assay genetic diversity of a large number of loci, the allelic identifications obtained from one study are not easily transferred to another. This is a serious problem in environmental assessment that may involve several studies conducted over a long period of time. In this study, we are using isoelectric focusing to isolate alleles of mottled sculpin, *Cottus bairdi*, and banded sculpin, *C. carolinae*, from five West Virginia streams that have a history of anthropogenic stress. The isoelectric points of alleles of LDH-A, LDH-B, LDH-C, MDH-A, MDH-B, and



PGM from these populations have been labeled relative to protein standards and recorded as PI values.

MICHAEL LITTLE, DOUGLAS CHAMBERS and ALAN TENNANT,  
Department of Biological Sciences, Marshall University, Huntington, WV  
25755. Fish species diversity in the tributaries of the Cheat River.

A number of investigators have described a relationship between habitat diversity and fish species diversity and have reported a correlation between the distribution of adult and juvenile fishes and such factors as stream depth, current, and substrate. In this study, we examined the diversity of fish species and their relative abundance in the major tributaries of the Cheat River, Monongehala River system. The Cheat River drains the higher elevations of northern West Virginia and is fed by tributaries with rocky channels, steep gradients, and high velocities. Thirty two sections of ten Cheat River tributaries were sampled by electrofishing and all fish greater than 40 mm identified on site. Fish were collected from stream sections that were transitional riffle/pools with predominantly cobble bottoms and midstream depths of .2 to .5 m at the time of sampling. The dominant species in streams surveyed were blacknose dace, *Rhinichthys atratulus*, mottled sculpin, *Cottus bairdi*, stoneroller, *Camptostoma anomalum*, and longnose dace, *Rhinichthys cataractae*. Fish species diversity differed significantly between Laurel and Glady Forks and all other tributaries. Blacknose dace were 34% of Cheat River fish sampled but constituted only 3% of fish from Glady and Laurel Fork.

JASON BARNETT, JAGAN V. VALLURI, and H. WAYNE ELMORE,  
Department of Biological Sciences, Marshall University, Huntington, WV  
25755. Protein synthesis and ethylene production in sandalwood callus cultures exposed to drought and heat shock.

Plant cell cultures exposed to environmental stress conditions synthesize a unique group of stress proteins. These proteins seem to play a role in protecting the plant cell. A knowledge of the molecular responses such as gene expression during cellular adaptation would be helpful and might lead to the identification of the altered putative genes. Sandalwood callus was maintained on Murashige and Skoog medium supplemented with 1 mg/L 2,4-D and 1 mg/L benzyladenine. A group of rapidly growing sandal calli were exposed to elevated temperatures ranging from 32C to 45C. Another group of calli were subjected to low water potentials ranging from -0.4 MPa to -2.5 MPa. Protein synthesis induced by heat shock and drought stress were assessed by SDS-polyacrylamide gel electrophoresis. After 48 h of exposure to stress, a 30% reduction in callus volume was observed in both groups of calli. In vivo labeling of stressed tissue with <sup>35</sup>S-methionine revealed a diverse array of low molecular weight proteins on SDS-PAGE gels at 36, 40 and 45C after 6 to 8 hours of heat shock. Although most appeared to be synthesized only during heat shock, some were detectable at low



levels in control tissue. New protein synthesis was also detected in tissue subjected to low water potentials. At low water potentials and elevated temperatures ethylene production was greatly inhibited.

## BOTANY

RONALD H. FORTNEY, Dept of Natural Science and Math, Salem-Teikyo University, Salem, WV 26426. A revision of the pteridophytes of West Virginia.

The *Flora of West Virginia* by P. D. Strausbaugh and E. L. Core was first published in four parts between 1952 and 1964. The second and last revisions were published between 1970 and 1977, making the second edition of Part I twenty-six years old and Part IV nineteen years old. In 1995, a group of botanists formed a committee whose objectives were to write the third edition of the *Flora* and to publish an atlas on the vascular plant taxa of West Virginia. The first section being revised is Part I, which includes the ferns and fern allies (pteridophytes), grasses, sedges, lilies, and orchids; of these groups, the ferns are the first group to be completed by the committee. In the third edition of the *Flora*, it is doubtful that any other major group will be taxonomically changed to the extent of the pteridophytes. The user of the third edition will find numerous nomenclatural changes and reassignment of genera to new families for both the fern allies and true ferns. The revised section on ferns and fern allies generally follows the treatment for pteridophytes in the *Flora of North America Volume 2 Pteridophytes and Gymnosperms*.

In the second edition of Part I, 75 vouchered pteridophyte taxa were recognized at the species level. In the revised treatment, 79 species are recognized as occurring in the state and supported by vouchered records. Based on citations in the *Flora of North America*, an additional six species are noted as possibly occurring in the state. For most of these species, West Virginia is clearly within their normal range, but locations within the state have not been documented with vouchered specimens.

Among the notable family changes are those within the Polypodiaceae. Once the largest family of true ferns in West Virginia, with 20 separate genera, it now includes two genera. The new families described for the Polypodiaceae are Pteridiaceae, Dennstaedtiaceae, Thelypteridaceae, Blechnaceae, Aspleniaceae, and Dryopteridaceae. There are, also, changes at the genus and specific epithet levels within these groups, e. g., *Athyrium angustum* and *A. asplenioides* are now recognized as *A. filix-femina* var. *angustum* and *A. filix-femina* var. *asplenioides*.

Another family with major changes is the Lycopodiaceae. Four genera are now recognized within this family--*Huperzia*, *Lycopodium*, *Diphasiatrum*, and *Lycopodiella*.



The revised treatment of pteridophytes will be published in 1996. This publication is intended to be a final draft of the new treatment of pteridophytes in the *Flora*. Botanists are encouraged to use this draft to test new keys and to search for new and old localities for rare and interesting pteridophytes.

JAMES RENTCH, West Virginia Graduate College, S. Charleston, WV 25202; RONALD H. FORTNEY, Dept. of Natural Science and Math, Salem-Teikyo University, Salem, WV 26326; STEVEN L. STEPHENSON, Dept. Of Biology, Fairmont State College, Fairmont, WV 26554; AND HAROLD S. ADAMS, Div. of Arts and Science, Dabney S. Lancaster Community College, Clifton Forge, VA 24422. Vegetation study of the Bluestone River Gorge in southern West Virginia.

The vegetation of 13 sites within the proclamation boundaries of the Bluestone National Scenic River and within the Bluestone Gorge area of Pipestem State Park and Bluestone Wildlife Management Area were examined in a study conducted during the summers of 1994 and 1995. Three transects were run from rim to riverbank. Upper, mid, and lower slope plots were established, permanently marked, and sampled using standard community sampling techniques. In addition, one floodplain plot along the Bluestone River was sampled.

North, northeast, and east facing slopes were dominated by tulip poplar (*Liriodendron tulipifera*) in the higher plots, and basswood (*Tilia americana*) and buckeye (*Aesculus flava*) in the lower plots. Relatively few oaks occurred in the canopy, small tree, and sapling strata. Soils were well-buffered and nutrient-rich (Ca, Mg, Mn), circumneutral (pH 6.5-6.6), and low in iron and aluminum. South and southwest slopes were oak dominated; chestnut oak (*Quercus prinus*) and white oak (*Q. alba*) at the higher slope positions, and red oak (*Q. rubra*) and white oak (*Q. alba*) were the leading dominants at lower slope positions. Lower slopes somewhat resembled north slopes, with basswood, poplar, and hemlock (*Tsuga canadensis*) present. Soils at lower slope positions were more acidic (pH 4.4-5.5), higher in iron and aluminum, and lower in organic matter and exchangeable cations. While maples were present in the canopy in eight plots, the understory was dominated by sugar maple (*Acer saccharum*) in the small tree, sapling, and seedling strata at all slope positions and aspects. Dogwood (*Cornus florida*) also formed a prominent component in the small tree strata.

The principal gradients determining community composition appeared to be pH, which correlated negatively with aspect, iron and aluminum ( $r < 0.805$ ,  $p = 0.05$ ) and positively with calcium, boron, magnesium, and manganese ( $r < 0.803$ ), and aspect. Aspect correlated negatively with soil cations ( $r < 0.782$ ,  $p = 0.025$ ), and positively with seedling density ( $r = 0.830$ ,  $p = 0.005$ ).



DONALD E. TRISEL, Department of Biology, Fairmont State College, Fairmont, WV 26554 and DAVID L. GORCHOV, Department of Botany, Miami University, Oxford, Ohio 45056. Competitive effects of *Lonicera maackii* on native tree seedlings.

*Lonicera maackii* (Rupr.) Maxim., native to eastern Asia, was introduced to North America ca. 1920 as an ornamental plant. Non-cultivated shrubs of *L. maackii* now occur in Ontario and at least 23 states of the eastern US, growing at densities of up to 6800 shrubs/ha in secondary forests. Because there is a reduced herb layer under dense stands of this shrub, *L. maackii* may be disrupting the natural succession of forests and old fields through allelopathy and/or competition. Field experiments were conducted to examine the effect of *L. maackii* shoot pruning and root trenching on the growth and survival of native tree seedlings. Pruning the shoot of *L. maackii* significantly increased the survival of *Acer saccharum* (Chi-square,  $p=.02$ ) and *Fraxinus americana* ( $p=.03$ ). Trenching the roots of *L. maackii* did not significantly affect survival of any of the species. A combined trenching and pruning treatment further increased survival over shoot pruning alone in *A. saccharum* ( $p=.001$ ). Competition for light appears to be the most important competitive effect of *L. maackii* on tree seedling survival. Root competition becomes important to seedling survival when light is not limiting.

ERIC W. EWING and DAN K. EVANS, Herbarium, Dept. of Biological Sciences, Marshall University, Huntington, WV 25755 and PAUL J. HARMON, West Virginia Natural Heritage Program, Division of Natural Resources, Elkins, WV 26241. A morphometric study of the *Scutellaria ovata* Hill (Lamiaceae) complex in West Virginia with emphasis on shale barren taxa.

Elements of the *Scutellaria ovata* complex in West Virginia are reported to include *S. ovata* var. *pseudoarguta*, *S. ovata* var. *rugosa*, *S. ovata* var. *virginiana* and *S. ovata* ssp. *ovata*. All but *S. ovata* ssp. *ovata* occur the shale barren habitat of eastern West Virginia and western Virginia. *S. ovata* in var. *pseudoarguta* has been proposed as a threatened element in North America. This study was undertaken to determine the morphological characters most useful in separating taxa in the complex. Nineteen characters were assessed in 104 herbarium specimens and the data subjected to principal component and canonical discriminant analyses and to Duncan's model of analysis of variance. Specimens were grouped according to collection site in order to determine intra- and inter-population variation. For further analysis plants were grouped taxonomically according to variety or subspecies. Variation between sites was minimal. Plant height, leaf length and width, petiole length, internode length, stem width and leaf cordateness separate the subcomplex *S. ovata* var. *pseudoarguta* - *S. ovata* var. *rugosa* from *S. ovata* var. *virginiana* and *S. ovata* ssp. *ovata*. The latter taxa are separated by leaf width, floral bract length, raceme length and raceme internode length. Finally, *S. ovata* var. *pseudoarguta* and *S. ovata* var. *rugosa* are separated only by leaf pubescence.



AMY MORRIS and DAN K. EVANS, Herbarium, Dept. of Biological Sciences, Marshall University, Huntington, WV 25755. Floristics, plant community structure, and bank profiles for five sites along the Middle Ohio River.

River banks are diverse ecological areas under constant disturbances by both natural and man-made causes. Bank erosion and stability may be related to vegetative cover and species composition. Floristics, plant community studies, and bank profiles were studied at five sites in the Greenup-Gallipolis navigation pools of the middle Ohio River. Community types and structure were determined by plot studies of three strata including herbs, shrubs and trees along transects from the water's edge to top of bank. Riverbank profiles were established by surveying microelevations along the same transects. Voucher specimens were collected for all species along study transect and in adjacent areas. Results are compared to a similar study completed in 1978.

REBECCA A. HALL-RUTHERFORD and THOMAS E. WEAKS, Department of Biological Sciences, Marshall University, Huntington, WV 25755. Periphyton of the tributaries of the New River Gorge National River.

Periphyton community structure was studied in ten tributaries of the New River Gorge National River. This area is a large tourist attraction for West Virginia for sporting, recreation, white water rafting, etc. Five of these streams receive moderate to high levels of industrial and domestic organic pollutants. Industrial waste are primarily generated by white-water rafting operations. The other five streams do not receive industrial waste and are generally free of domestic pollutants. The streams were sampled four times during the year. The pH, chlorophyll, temperature, dissolved oxygen, turbidity, conductivity and alkalinity was measured. Periphyton was identified and the cell density was established for each stream. Periphyton community indices and coefficients was calculated for water quality indicators in order to identify possible pollution sources.

JOSEPH E. BOGGS and THOMAS E. WEAKS, Department of Biological Sciences, Marshall University, Huntington, WV 25755. A study of the periphyton and phytoplankton communities of the Big Sandy River.

Phytoplankton and periphyton communities were studied along the entire length of the Big Sandy River. Sampling stations (10) were located 5km apart. Sampling was conducted seasonally using an 80 micron phytoplankton net and Catherwood diatometers. The algae (live and preserved) were identified, enumerated and analyzed to infer community structure, composition and the factors which effect them. Seston values were determined and used in the evaluation of biomass. An analysis of photosynthetic pigments was performed and was used as a measure of productivity. The dominant groups were blue-green algae (cyanobacteria), green



algae and diatoms. Periphyton seston values were characterized by a noticeable increase at the uppermost stations. Phytoplankton seston values remained fairly constant along the entire length of the river.

## ECOLOGY

HAROLD S. ADAMS, Division of Arts and Sciences, Dabney S. Lancaster Community College, Clifton, Clifton Forge, VA 24422 and STEVEN L. STEPHENSON, Dept. Of Biology, Fairmont State College, Fairmont, WV 26554. West Virginia Forests with red spruce component.

Quantitative data on the composition and structure of the tree stratum (stems  $\geq 10$  cm DBH) were obtained from a 20 by 50 m (0.1 ha) plot in each of thirty-seven forest stands in West Virginia with red spruce as a minor or major component of the canopy. More stands (16) were located on ridgetops than at any other topographic position, with another twelve on lower slopes. Over 60 percent of the stands were located on southeast or northwest exposures. Elevations of the stands ranged from 914 m to 1463 m, with the average being 1147 m. Basal area ( $\text{m}^2/\text{ha}$ ) of all trees ranged from 17.4 to 52.4 (average = 35.2), whereas tree density ( $\text{N}/\text{ha}$ ) ranged from 370 to 2120 (average = 813). The importance value (IV, maximum = 100) of red spruce ranged from 1.2 to 100 (average = 41.4). Other consistently important components of the canopy in the stands we sampled were yellow birch (average IV = 14.8, present in thirty-one stands), hemlock (average IV = 11.7, present in twenty-two stands and often the single most important species present), and balsam fir (average IV = 10.0, but present in only eight stands). DECORANA ordination of the thirty-seven stands resulted in an X-axis extending from hardwood domination and/or admixture to stands with balsam fir, whereas Y-axis variation extended from strong dominance ( $\text{IV} \geq 95$ ) by red spruce to stands with hemlock as a major component. The Y-axis also correlated strongly with elevation, with high red spruce dominance towards higher elevations and high hemlock dominance towards lower elevations. (This research was supported in part by funds provided by the USDA Forest Service.)

NATHAN D. MISSEL, Dept. Of Biology, Fairmont State College, Fairmont, WV 26554, HAROLD S. ADAMS, Division of Arts and Sciences, Dabney S. Lancaster Community College, Clifton Forge, VA 24422, and STEVEN L. STEPHENSON, Dept. of Biology, Fairmont State College, Fairmont, WV 26554. Patterns of seed fall across spruce/hardwood ecotones in the mid-Appalachians.

We are currently investigating the ecotonal relationships of red spruce and surrounding hardwoods in the mountains of Virginia and West Virginia. In 1993, seed traps (each with a total collection area of  $0.25 \text{ m}^2$ ) were established at four localities to assess seed fall characteristics. Traps were placed at three positions



along permanent transects (each consisting of a series of contiguous 100 m<sup>2</sup> plots) that extend across the spruce/hardwood ecotone at each locality. Three traps were placed outside (but within 5 m) of each end and at the center of each transect, for a total of nine seed traps at each locality being studied. Collections were made of seed fall during the period of late June to late November or early December in each of the past three years. Seeds in the traps were sorted and tallied according to species. A total seed fall of 2,599,900 spruce and hardwood seeds per hectare was calculated from the 1993 data, whereas a total almost 32 times that number (82,284,800) was calculated for 1994. The vast majority of these seeds were those of hardwood species (88.2% and 93.7%, respectively). The only red spruce germinal seedlings tallied in the ecotonal segment in 1994 (417 per hectare, representing only 2.3% of the red spruce seeds tallied in the preceding year in that segment). Hardwood germinal seedlings were found in all segments of the transect during both 1994 and 1995; their numbers represented <0.1% (hardwood segment in 1995) to 6.6% (hardwood segment in 1994) of the total hardwood seeds tallied in each of the previous years. (This research was supported in part by funds provided by the USDA Forest Service.)

JENNIFER L. ROBERTS, Salem-Teikyo University, Salem, WV 26426; ROBERT B. COXE, National Biological Service, West Virginia Cooperative Fish and Wildlife Unit, Morgantown, WV 26506; RONALD H. FORTNEY, Salem-Teikyo University, Salem, WV 26426; MICHELLE JENKINS, Fairmont State College, Fairmont, WV 26554; DAVID M. LAWRENCE, Lamont-Doherty Earth Observatory of Columbia University, Palisades, NY 10964; SUE A. PERRY, National Biological Service, West Virginia Cooperative Fish and Wildlife Unit, Morgantown, WV 26506; REBECCA E. SANDERS, Fairmont State College, Fairmont, WV 26554; AARON P. SMITH, Fairmont State College, Fairmont, WV 26554; STEVEN L. STEPHENSON, Fairmont State College, Fairmont, WV 26554; and NEIL THORNE, National Biological Service, West Virginia Cooperative Fish and Wildlife Unit, Morgantown, WV 26506. Forest vegetation of West Virginia.

During the 1995 field season, quantitative data on the composition and structure of the tree stratum (stem  $\geq$  10 DBH) were obtained from 197 twenty by fifty meter (0.1 ha) plots located throughout the state of West Virginia. Most of the study area is within the Appalachian Plateau physiographic province, but a portion falls within the Ridge and Valley province. The data were subjected to detrended correspondence analysis (DCA) and two-way indicator species analysis (TWINSpan) in an effort to identify classifiable units and also to determine species distribution patterns and composition gradients across the landscape. TWINSpan delineated 21 forest types, which were named on the basis of the leading dominants. Oaks (*Quercus* spp.) occurred as the leading dominants in five forest types, tulip poplar (*Liriodendron tulipifera*) in four types, and sugar maple (*Acer saccharum*) in three types. Although forests over much of West Virginia



have been referred to as oak-hickory, hickory (*Carya* spp.) occurred as a leading dominant in only three types and was never the most important species present. Results from DCA ordination indicate that the distribution of forest types is most closely related to the environmental complex-gradients associated with differences in site moisture conditions and elevation.

BRADLEY M. YURISH and FRANK S. GILLIAM, Department of Biological Sciences, Marshall University, Huntington, WV 25755-2510. Dynamics of soil and forest floor nitrogen in a nitrogen-saturated central Appalachian hardwood forest.

The potential for excess nitrogen (N) deposition to disrupt biogeochemical cycles, reduce water quality, and potentially induce forest decline has been recognized. This study examined N dynamics in mineral soil over three years (and in O2 horizons over one year) in three watersheds of the Fernow Experimental Forest, WV: WS7 (25 yr-old, untreated); WS4 (mature, untreated); WS3 (25 yr-old, treated). WS3 has received aerial applications of  $(\text{NH}_4)_2\text{SO}_4$  since 1989 (~54 kg N/ha/yr). Samples of mineral soil and organic (O2) horizons were incubated *in situ* using buried polyethylene bags. Although there were few significant differences among watersheds for monthly rates of net nitrification and mineralization for soil or O2 horizon, seasonal patterns of these rates exhibited consistently increasing trends over the 3-yr study, regardless of treatment. Available N pools ( $\text{NH}_4^+$  and  $\text{NO}_3^-$ ) for soil were generally significantly higher on WS3 than on untreated watersheds throughout the growing season for all 3 yr. Lack of differences in rates of mineralization and nitrification among watersheds support conclusions of earlier studies that the untreated watersheds had become N saturated prior to the initiation of this study, apparently the result of high ambient inputs of N in this region. Data also show that further increases of N deposition to a N-saturated system can increase substantially  $\text{NH}_4^+$  and  $\text{NO}_3^-$  pools in the mineral soil. Finally, close correlation of seasonal  $\text{NO}_3^-$  pools with seasonal changes in concentrations of stream  $\text{NO}_3^-$  and  $\text{Ca}^{++}$  suggest that later stages of N saturation in central Appalachian hardwood forests may result in base cation depletion from the soil.

CACKA, JENNIFER L., Davis and Elkins College, Elkins, WV 26241 and TED R. ANGRADI, USDA Forest Service, Parsons, WV 26287. Ecology of a cave-resurgence-spring run ecosystem at the Fernow Experimental Forest, Tucker County, West Virginia.

We examined the macroinvertebrate fauna of a connected cave stream (Big Spring Cave), spring resurgence, and spring run ecosystem at the Fernow Experiment Forest. Samples of the macroinvertebrate communities in each habitat were collected in June and July 1995. Organic matter, water quality and substrate characteristics were also determined. Organisms were identified to family or genus. We found significant differences in community structure among the three



habitats. Macroinvertebrate abundance and taxonomic richness was lowest in the cave stream. Of six taxa collected in the cave, the isopod *Caecidotea* was most abundant. The resurgence was dominated by the amphipod *Gammarus minus* and non insect taxa, although aquatic insects were present. The spring run was the richest of the three communities. *Gammarus minus* was the dominant organism in the run, however the abundance and body size of *G. minus* decreased with distance downstream from the resurgence. The number of insect taxa in the spring run increased with distance from the resurgence. We attribute the downstream increase in diversity in the spring run to increasing habitat variability in terms of temperature, water chemistry, and flow. Our study shows that adjacent, linked woodland lotic habitats may support distinct benthic communities.

ANDREA HENRY and DONALD TARTER, Department of Biological Sciences, Marshall University, Huntington, WV 25755 . Low pH tolerance, under acute static bioassay conditions, of the amphipod *Crangonyx gracilis* Smith from Green Bottom Swamp, Cabell County, West Virginia.

Acidification can have major impacts on amphipod populations. Certain amphipods (e.g. *Gammarus pulex*) are limited to waters with pH>5.7, while other amphipods (e.g. *Gammarus* spp.) can tolerate stream pH values ranging from 2.2-3.2. The amphipod *Crangonyx richmondensis laurentianus* is found in small acid lakes (pH=6.0) in the St. Lawrence drainage basin. One-hundred amphipods were collected from Green Bottom Swamp, a naturally occurring wetland of 58 ha, using a standard D-shaped dredge. The water temperature was 4 C. 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 limit (TLm) was calculated using both the straight-line graphical interpolation method and linear regression analysis. Results will be compared with other laboratory and field studies concerning pH tolerance values on amphipods.

ROBERT W. HOOD and DONALD C. TARTER, Department of Biological Sciences, Marshall University, Huntington, WV 25755 and TED R.

ANGRADI, Northeastern Forest Experiment Station, Parsons, WV 26287. Longitudinal, vertical, and seasonal variation of the macroinvertebrate fauna within the interstitial substrate of the elk lick run drainage, on the Fernow Experimental Forest, Parsons, West Virginia.

Multi-level basket samplers were placed in the substrate on first through fourth order streams on the Fernow Experimental Forest, Parsons, West Virginia. Samplers were colonized by macroinvertebrates over three month intervals, collected and are in the process of being analyzed. Seasonal, longitudinal, and vertical distribution are being analyzed to better understand the structure and



function of the macroinvertebrate community within the interstitial substrate and important influencing factors. Surber samples were also taken for comparison between surface and artificial basket samples. Weekly routines measuring temperature, dissolved oxygen, depth and surface velocities were taken to characterize conditions at each sampler. Monthly water samples were taken at each site along with seasonal inter-gravel flow measurements using a calcium sulfate dissolution techniques. Preliminary data show most macroinvertebrates decreasing in number with depth, and the family Chironomidae increasing in relative abundance with depth.

## ZOOLOGY

STUART A. WELSH and SUE A. PERRY, West Virginia Cooperative Fish and Wildlife Research Unit, National Biological Service, West Virginia University, Morgantown, WV 26506-6125. Habitat partitioning in a diverse assemblage of darters in the Elk River drainage, West Virginia.

We used direct observation (snorkeling) to collect data on habitat use for a total of 10 species (*Etheostoma blennioides*, *Etheostoma caeruleum*, *Etheostoma camurum*, *Etheostoma pellucida*, *Etheostoma tippecanoe*, *Etheostoma variatum*, *Etheostoma zonale*, *Percina caprodes*, *Percina macrocephala*, and *Percina sciera*) from five sites in the Elk River drainage system, West Virginia. These data were collected during periods of low flow from July through September, 1995. We used canonical discriminant analysis to quantify microhabitat use and examine differences in microhabitat use among species based on 10 habitat variables. *Percina caprodes* and *Percina macrocephala* were typically suspended above the substrate and used deeper habitats than *Etheostoma* species. *Etheostoma* species differed in habitat use in water velocity, and in their position relative to the substrate (i.e., under, between, and on top of substrate). Overlap in habitat use among darters likely increases in streams during summertime low flows when available habitat decreases. However, our results indicate that differences in microhabitat use occur among these closely-related species during low flow conditions.

D. A. CINCOTTA and T. E. OLDHAM, Division of Natural Resources, Wildlife Resources Section, Elkins, WV 26241; and, S. A. WELSH, WV University Cooperative Fish and Wildlife Research Unit, Morgantown, WV 26506. The status of several introductions of fishes in the New River drainage of West Virginia.

The native ichthyofauna of the upper Kanawha River system (usually referred to as the New River drainage) is described in the fishery literature as fairly unique, but depauperate. This description is substantiated by the presence of seven endemic fishes in the upper drainage and the fact that the lower drainage harbors approximately twice the number of native fishes than above. These faunal



differences have been largely interpreted by researchers as the result of the dispersal barrier created by several falls (primarily Kanawha Falls at Gauley Bridge, WV), and the rigorous gradient and environment that exists in the lower Gauley and New river gorges. Due to numerous introductions by man, the numbers of species in the upper and lower Kanawha drainages are nearly equal today. A recent comprehensive source on West Virginia fishes usually depicts these drainage additions as sparse and does not discuss their abundance. This paper updates the status and distribution of several New River introductions in West Virginia; it will emphasize data for *Cyprinella galactura*, *Luxilus albeolus*, *Nocomis leptocephalus*, *Notropis telescopus*, *Etheostoma caeruleum*, and *Percina roanoka* in the Greenbrier River, and *Phoxinus oreas*, *Gambusia affinis*, and *Ameiurus melas* in the Gauley River.

MATT MCREYNOLDS, DONALD TARTER and MIKE LITTLE,  
Department of Biological Sciences, Marshall University, Huntington, West  
Virginia 2755. Preliminary observations on isozyme patterns and meristics in  
mosquitofish (Pisces: Poeciliidae) from West Virginia and Virginia.

Prior to 1988, two subspecies of the mosquitofish *Gambusia affinis* (*G. a. affinis* and *G. a. holbrooki*) were recognized along the Gulf and Atlantic Coasts of the United States. Based on electrophoretic analysis, subsequent investigators proposed the original taxonomic designation recognizing *G. holbrooki* and *G. affinis* as separate species. Populations in drainages west of Mobile Bay should be considered *G. affinis*, and those east of this divide being *G. holbrooki*. The only population of the mosquitofish in West Virginia is found in the Meadow River wetlands (1392 ha) located at the western end of Greenbrier County. Using heart, skeletal muscle, and brain tissues, two isozyme patterns (malate dehydrogenase and lactic acid dehydrogenase) were used to determine the correct specific designation of the population in the Meadow River wetlands. These results were compared to a population of *G. holbrooki* from an Atlantic coast drainage in Virginia. Additionally, counts (e.g. dorsal and fin rays, etc.) were used to separate the two species.

KEVIN D. BRITTINGHAM and DONALD C. TARTER, Department of  
Biological Sciences, Marshall University, Huntington, WV 25755 and TED R.  
ANGRADI, Northeastern Forest Experiment Station, Parsons, WV 26287. A  
comparison of the benthos in weir ponds draining watersheds of the Fernow  
Experimental Forest.

A comparison of the benthos in weir ponds draining watersheds of the Fernow Experimental Forest, Parsons, West Virginia, was conducted in October and November of 1971 and 1994. The collection in 1971 was done by Steve Harris for his thesis work at West Virginia University. Organisms collected with an Ekman dredge were keyed to genus and densities calculated for a m<sup>2</sup>. Taxa density and taxa



richness were compared among ponds and between studies (1971 vs. 1994). Data showed that the collection in 1971 had fewer numbers but greater taxa richness when compared to the collection in 1994. It also showed that there was no correlation between the 1971 and 1994 collections when used in a Spearman's rank correlation test. For the 1994 data, there was a correlation between the fauna of the control weir 4 to weir 1 and 3, but no correlation with weir 6. Overall there are some significant differences in the weir pond populations when comparing 1971 to 1994 collection dates.

DONALD C. TARTER and ERICA F. MIDKIFF, Dept. of Biological Sciences, Marshall University, Huntington, WV 25755. Fall spawning and preliminary observations on the growth rates of larval and juvenile grass pickerel, *Esox americanus vermiculatus*, in the Green Bottom Wildlife Management Area, West Virginia.

The grass pickerel, *Esox americanus vermiculatus*, is listed as Undetermined on the Vertebrate Species List of Concern in West Virginia. Lentic, vegetated area required for spawning are being reduced by residential, agricultural, and industrial developments. Green Bottom Swamp, a naturally occurring wetland of 58 ha, and a nearby mitigated wetland of 29 ha provide spawning habitat for the grass pickerel. Based on gonosomatic indices, histology, and young-of-the-year, spawning normally vegetation associated with the buttonbush community. The threshold temperature for spawning was approximately 9 C. On 13 April 1994, 1 post sac larva (PYSL) (22.0 mm) was collected in beds of *Potamogeton crispus* and *Ceratophyllum demersum* from the old swamp. Between 18 April and 21 June 1994, 55 juveniles (25.7-98.8 mm;  $\bar{x}=55.1$ ) were collected from the same nursery area. No fall spawn was observed in the old swamp. Between 02-09 December 1994, 7 PYSL (14.0-20.6 mm,  $\bar{x}=18.8$ ) and 8 juveniles (25.6-30.3 mm,  $\bar{x}=28.3$ ) were collected from beds of *Polygonum* sp. in the mitigated wetland. The water temperature ranged from 8-13 C. Lengths of fall spawned grass pickerel (25.6-27.0 mm) approximated the lengths of spring spawned grass pickerel (22.0-25.7 mm) providing evidence of the fall spawn in the mitigated area. The unusually warm weather in November may have triggered the spawn. Comparisons of lengths of larval and juvenile grass pickerel will be made with fall spawned grass pickerel from Fleming Creek, Michigan. Additionally, growth rates (mm/day) comparisons will be made between larval grass pickerel in Oklahoma and West Virginia.

DONALD C. TARTER and ERICA F. MIDKIFF, Dept. of Biological Sciences, Marshall University, Huntington, WV 25755 and DIANE R. NELSON, Dept. of Biological Sciences, East Tennessee State University, Johnson City, TN 37601. New distributional records, including two state records, of tardigrades (Phylum: Tardigrada) from mosses in the Monongahela National Forest, West Virginia.



The Monongahela National Forest stretches over 830,000 acres of the Allegheny Mountain Range in West Virginia. The Forest is mountainous; elevations range from 900-4860 feet. Mosses were collected from trees and rocks in the following areas: (1) Cranberry Glades Botanical Area (3400 ), a land covered with a bog, bog forest, shrubs and mosses, and (2) Dolly Sods Wilderness Area (4000 ), a tundralike region that forms the eastern section of the Allegheny Plateau. Two state records were established; *Echiniscus virginicus* Riffin and *Macrobiotus spallanzanii* Maucci were identified from the moss *Ulota crispa* collected from the back of a birch tree in the Cranberry Glades Botanical Area. New distributional records from this area include *Pseudechiniscus suillus* Ehrenberg and *Macrobiotus hufelandi* Schultze. Twelve species of tardigrades have been identified from this area. Additionally, *Minibiotus intermedius* (Plate) has been recorded for the first time from the Dolly Sods Wilderness Area. Eight species of tardigrades have been reported from this area. Altogether, 26 species of tardigrades have now been identified from the Monongahela National Forest. Elevational and slope trends will be made of the tardigrade fauna from the Monongahela National Forest.

DONALD TARTER and ERIC WILHELM, Department of Biological Sciences, Marshall University, Huntington, WV 25755 and JEFFREY BAILEY and MICHAEL NOWLIN, West Virginia Bureau of the Environment, St. Albans, WV 25177. New record and range extension for *Anisocentropus pyraloides* (Trichoptera: Calamoceratidae) from West Virginia.

The caddisfly *Anisocentropus pyraloides* is recorded for the first time from West Virginia. This record represents a northwest extension of the known range which was previously limited to the coastal plain and lower Piedmont at the northern end of its range. Larvae were collected from the Meadow River (Greenbrier County) among snags (e.g. logs) using a standard D-frame dip net. The water was approximately 1 m deep, and the elevation is 732 m above sea level. The number of caddisfly species now known from West Virginia is 191.

## GEOLOGY

E. RAY GARTON, Curator, Geology & Natural History Museum, WV, Geological Survey, PO Box 897, Morgantown, WV 26507; and FRED GRADY, Dept. of Paleobiology, MRC 121 NHB, Smithsonian Institution, Washington, D.C. In search of the West Virginia cave from which President Thomas Jefferson described the bones of a Pleistocene Age, extinct ground sloth *Megalonyx*.

In 1796 President Thomas Jefferson was sent some bones from a cave in Greenbrier County, Virginia, now West Virginia. Jefferson described these bones, a femur fragment, ulna, radius, and some foot bones, as a new genus of mammal,



*Megalonyx*. Jefferson reported the bones were found in a cave by saltpetre workers and gave the cave owner's name as Frederick Cromer, an apparent misspelling of Frederick Gromer. Correspondence between Jefferson and John Stuart who sent him the bones indicated the cave was about five miles from Stuart's home and contained saltpetre vats. While Organ Cave has been previously cited at the location of this discovery it can be eliminated as it was never owned by Gromer. The discovery of two letters written by Tristram Patton, the next owner of the cave, indicates the cave was in Monroe County near Second Creek. Monroe County was separated from Greenbrier County shortly after the discovery of the bones. Patton described the cave and indicated more bones were there. This information and other material accumulated over several years leads to the unmistakable conclusion that the cave in which the *Megalonyx* was discovered is actually Haynes Cave in Monroe County. The evidence became even stronger when two fragments of a *Megalonyx* scapula were discovered in Haynes Cave in 1993.

E. RAY GARTON, Curator, Geology & Natural History Museum, WV, Geological Survey, PO Box 897, Morgantown, WV 26507; and FRED GRADY, Dept. of Paleobiology, MRC 121 NHB, Smithsonian Institution, Washington, D.C. A second record of the extinct armadillo, *Dasypus bellus*, from West Virginia.

The discovery of some 50 osteoderms and other bone fragments of the extinct armadillo, *Dasypus bellus* from Alaina P Cave in Berkeley County represents the second record of this genus and species from West Virginia. The new discovery is about 200 kilometers (120 miles) northeast of the only previously reported specimen which was a single band osteoderm from Organ Cave, Greenbrier County. The specimen from Alaina P Cave represents a northeastern range extension for the species. Both movable band and buckler osteoderms are represented in the Alaina P collection along with some as yet unidentified skeletal fragments. Some of the movable band osteoderms are quite large in comparison with single specimen from Organ Cave. So far no other species have been found in Alaina P Cave. The age of *Dasypus bellus* cannot be determined beyond the probability of being middle to late Pleistocene, ca. 500,000 to 12,000 years before present.

EBERHARD WERNER, P. O. Box 795, Morgantown, WV 26507-0795.  
Fracture system on the Chestnut Ridge anticline, West Virginia.

To determine the distribution of fractures in the rocks of the Chestnut Ridge anticline, orientations of natural fractures in 12 outcrops of several stratigraphic formations along West Virginia Route 7 were measured, and lineaments were mapped from side-looking airborne radar (SLAR) mosaics. The data from outcrops contained concentration maxima in five directions: (means) N81°W, N52°W, N22°W, N16°E, and N67°E. Not all of these orientations are present at



each outcrop, nor within each of the five formations present in outcrop. Lineaments for the same area have primary orientations of N50°-70°W, N10°-20°W, and N35°-40°E, although each of the joint maxima is represented by one or more lineaments. These results do not fully agree with an earlier study by Hough in this general area (West Virginia Geological Survey Report of Investigations 19, 1960), who found the dominant maxima in both joints and lineaments to be approximately north-south and east-west. These orientations appear in the present study in only the outcrops of the Allegheny Group, stratigraphically the uppermost formations in the study area. Of special interest in the results of this study are the following two items: 1) although the systematic (first-formed) joints, as defined on the basis of continuity, surface characteristics, and regularity of the fractures, in most of the outcrops appear to be the cross-fold set, in one outcrop the systematic set parallels the fold trend; 2) in another outcrop, the systematic set is disrupted in the vicinity of a covered section, which may be indicative of faulting, although there is no SLAR lineament passing through this area.

DAVID W. VALENTINO, Dept. of Phy. Sci., Concord College, Athens, WV 24712. Ductile gravitational collapse structure: an example from the Piedmont of Pennsylvania.

Andersonian theory generalizes that the maximum principle compressive stress (<sup>1</sup>) to be equal to lithostatic stress, while the intermediate (<sup>2</sup>) and minimum (<sup>3</sup>) compressive stresses are subparallel to the surface of the earth during the development of extensional structures. Structural escape under such a stress field occurs at a low angle and aparallel to <sup>3</sup>. Geologic structures that develop under extensional stress conditions are directly related to divergent tectonism on the scale of the lithosphere, or gravitational collapse on a considerably smaller scale. An example of extensional geologic structure was analyzed from the region of southeastern Pennsylvania and it is concluded that these structures are the result intracrustal gravitational collapse immediately after Late Paleozoic crustal thickening related to the Alleghanian orogeny.

Exposures to conjugate kink-bands and cleavage, and conjugate box-folds were used to determine the orientation of the paleo- bulk shortening and bulk elongation directions relative to present geographic coordinates. Ductile conjugate structures develop with the bulk shortening direction as the obtuse bisector between the structures, and the bulk elongation direction as the acute bisector. With minimal ductile modification of the box-fold and cleavage angles the bulk shortening and elongation directions approximate the orientation <sup>1</sup> and <sup>3</sup>. The cleavages occur locally and regionally adjacent to a crustal arch (the Tucquan antiform) that had up to 7 km of structural relief prior to erosion. Identical metamorphic mineral recrystallization assemblages for the conjugate cleavages and microstructures related to crustal arching, suggest that all these structures developed during the same thermal event. The geographic position of the extensional structures,



adjacent to a substantial crustal bulge, with extensional structural escape direction directory away from the over thickened region leads to the conclusion that extension was related to gravitational collapse of the Tucquan arch. Although gravity driven crustal deformation has been illustrated for discrete structures in numerous mountain chains world wide, these structures generally occur at shallow crustal levels where the rocks deform brittly with low confining pressures. This example of possible gravitational collapse structures from southeastern Pennsylvania may be significant because the recrystallization assemblage indicates that the conjugate cleavages developed under greenschist facies condition (probably greater than 400°C). In conclusion, gravity driven deformation may be operative as a mechanism for deep crustal deformation as well as in the shallow regions of the crust.

DAVID W. VALENTINO, Dept. of Phy. Sci., Concord College, Athens, WV 24712. An analysis of garnet chemical zoning and inclusion trails using calibrated two-dimensional false color x-ray maps.

Metamorphic porphyroblasts, particularly garnet, have proven to be particularly useful in structural and metamorphic studies of orogenic belts. Many geothermobarometric techniques are dependent on chemical fractionations between garnet and other matrix minerals, and similarly important, microstructural analysis of garnets with spiral-shaped inclusion trails are used to constrain the kinematic and strain history of deformed regions. However, very few studies have attempted to correlate the internal chemical variability of garnet or other porphyroblasts with the development and orientation of different types of inclusion trails, and none has utilized full two-dimensional quantitative data.

There are two cardinal competing models in the geologic literature for development of metamorphic crystals containing geometrically complex inclusion trails: the "Rosenfeld model" for contemporaneous growth and deformation ("rolling") and the "Bell model" for post-deformation porphyroblast growth that encloses preformed microfolds. This study includes implementation of a technique for calibration of two-dimensional false-color x-ray maps for a suite of lower greenschist facies garnet with relatively simple inclusion patterns from the central Appalachian Piedmont to address these competing hypotheses for synkinematic garnet growth. After calibration, the quantitative maps were used to develop a two-dimensional kinematic and growth history for each garnet crystal by correlating discrete chemical zones with structural micro-fabrics. Systematic unmasking of the compositional range that includes the garnet porphyroblast revealed minute anomalies in the chemical zoning that correlate with inclusion trail disparities. For example, a compositional jump of about 2% occurs in FeO in an otherwise smooth zoning pattern. This jump correlates directly with the location in the garnet where inclusion trails are bent. The chemical gap was also observed viewing the composition of MnO. This compositional gap at the precise location where the



inclusion trails are curved suggests that there was a hiatus in growth of the garnet, or resorption during the warping of the matrix foliation. Similarly, near the edge of the same garnet a compositional jump occurs at the inflection point in the inclusion trail geometry again suggesting a growth hiatus or resorption accompanying folding of the external matrix foliation. These preliminary findings support the "Bell model" for episodic growth and deformation. In addition to documenting the detailed geometry of chemical zoning and inclusion trails for these garnet porphyroblasts, this analysis included identification of the mineralogy and chemistry of these inclusions and the two-dimensional geometry and chemical variation of pressure fringes or strain shadows external to the porphyroblast using the same data sets.

DEBBIE HOPKINS, Dept. Phys. Sci., Concord College, Athens, WV 24712.  
The New York-Alabama Magnetic Lineament: a continental arc?

The source of the New York-Alabama Lineament (NYAML) has been revealed on reprocessed industry reflection data in eastern Tennessee and probably accounts for the source of the lineament along its length. Two-dimensional models of the gravity and magnetic data indicate that the wedge-shaped block northwest of the steep magnetic gradient is characterized by higher density and magnetic susceptibility than the block to the southeast. The characteristics are consistent with continental arc intrusive rocks comprising the wedge.

Evidence of deformation in the crust southeast of the wedge consists of strong, consistent west-dipping reflection that do not penetrate the wedge. Mylonites are the most likely source of the reflectivity and were probably produced during Grenville orogenesis. The absence of deformation within the wedge indicates that the wedge was relatively unaffected by convergence. I propose that the wedge formed as a continental arc on the Grenville margin in response to subduction of North American oceanic crust. Following collision, the arc rose as a block along boundary faults while low angle west-dipping shear zones formed in eastern Tennessee and Ohio on both sides of the NYAML. Furthermore, the upward displacement of the arc emplaced the higher density, higher susceptibility root adjacent to the slightly lower density shallower crustal rocks on both sides.

The length and linearity of the NYAML can be explained by this model using strictly convergent tectonics, but might be more likely if transpression and possible anatexis played a role. Rapid uplift of the arc rocks might have resulted in depressurization and remelting along contacts with the adjacent terranes. Such anatexis melts could have facilitated transpression at a time when the vectors for plate motion favored lateral slip.



## POSTERS

HOPE M. SACCO, DR. BEN M. STOUT III, DAVE M. SAVILLE, AND BRIAN M. SIKARSKIE. Department of Biology, Wheeling Jesuit College, Wheeling WV 26003. Stream geomorphology and riparian forest composition in the Wheeling Creek Watershed.

The purpose of this study is to examine relationships between riparian forest composition and stream geomorphology. The ultimate objective is to determine whether the type of vegetation composing the riparian corridor effects water quality and to determine how wide of a vegetation corridor is needed to protect the watershed. Within belt transects along 83 Wheeling Creek Watershed sites the riparian condition on each side of the stream was characterized by measuring bank and flood plain width, bank and flood plain slope, percent forest cover, diameter at breast height (DBH) of identified trees, and by qualitatively describing herbaceous vegetation and land use. Other geomorphic data were obtained from previous studies using a GIS. 46 tree species were identified. Tree species richness was highest in fourth order streams. Dominant canopy species demonstrated a geomorphological gradient from small to large order streams. Sugar maples, *Acer saccharum* Marsh were present throughout the watershed but appear to dominate riparian communities along first and second order communities. Maximum sugar maple DBH occurred at sites ranging in elevation from 260-380m, about 70km from the point of confluence of Wheeling Creek with Ohio River. Slippery elm, *Ulmus rubra* dominated riparian communities along third and fourth order streams. Most slippery elms occurred 9-36km from the mouth and between 215-354m in elevation, with maximum elm DBH occurring where catchment area was 788.4m<sup>3</sup>. Boxelder, *Acer negundo*, was dominant along fourth through sixth order streams, 2.6-48km from the mouth and about 208-300m in elevation. Characterization of vegetation at relatively undisturbed sites provides a predictive model of the expected riparian community along Wheeling Creek streams. The goal of this study is to reconstruct disturbance history, which is critical to the understanding of site-specific water quality and watershed-ecosystem processes.

AMY PIKO, WALTER J. CYBULSKI III, BEN M. STOUT III. Department of Biology, Wheeling Jesuit College, Wheeling, WV, 26003. The relationship between stream order and leaf litter decomposition in the Wheeling Creek watershed.

Following the principles set forth by the River Continuum Concept, leaf shredding macroinvertebrates should dominate small, low order streams. This was tested in the Wheeling Creek watershed using leaf decay coefficients (-K) as an indicator of leaf processing rates by shredders. Decay rates should gradually decrease as stream orders increase. Macroinvertebrate and basin geomorphological data were obtained from on-going studies of Wheeling Creek watershed. Decay coefficients



were found by using coarse-mesh bags filled with 10g of sugar maple (*Acer saccharum*) leaves. The bags were placed at 15 sites representing first through sixth order streams in three separate sub-basins in the Wheeling Creek watershed. Samples were then removed from each site at approximately 45 day intervals for 6 months. Decay rates ranged from 0.00443 to 0.00814 with slowest processing occurring in a first order stream and fastest in a fourth order stream. Decay coefficients also varied between sub-basins. For instance, -K of Big Wheeling Creek increased with increasing stream order; whereas, -K decreased with increasing stream order in Little Wheeling Creek. Shredder density declined from headwaters to mouth, but decay rate showed no correlation with shredder number. In Wheeling Creek watershed, decay coefficients appear to be independent of stream order.

BRIDGET AMMONS, DAVID SAVILLE, AND BEN STOUT III.  
Department of Biology, Wheeling Jesuit College, Wheeling, WV, 26003. Re-establishment of *Justicia americana* in the Wheeling Creek ecosystem.

*Justicia americana*, or the waterwillow is a dominant emergent plant which contributes to the primary production, nutrient uptake, and habitat diversification in Appalachian river and stream basins. A detailed investigation of the Wheeling Creek watershed has revealed the absence of *Justicia americana* from the Middle and Little Creek tributaries that have been subjected to 150 years of human settlement. Conditions necessary for waterwillow re-establishment were tested in July 1995 by the introduction of 600 plants in the Wheeling Creek tributaries at 15 sites. At each site there were 5 plots each containing 8 plants. Plants were monitored regularly thereafter. Of the fifteen variables measured, rhizome growth and survivorship appeared to best indicate transplant success. Rhizome growth is the cumulative length of new rhizomes per plot while survivorship is the number of surviving plants. A total of 68% of plots exhibited rhizome growth. The average number of rhizomes was 4.1 per plot, and the average length of the rhizomes was 26.51 cm per plot. An average of 6.4 stems per plot survived, indicating 80 % survivorship for the growing season. Water depth, forest canopy cover, and distance between individual plants had a significant influence on transplant success. The optimum water depth for transplant success was 10-20cm. Rhizome growth was found to be dependant on forest canopy cover and distance between individual plants, but survivorship did not show as strong of a dependance. Transplant success was found to be independent of velocity, width of stream, and the distance of the plot from the bank.

ANGELA D. EDMUNDS and STEVEN L. STEPHENSON, Dept. of Biology, Fairmont State College, Fairmont, WV 26554. Myxomycetes associated with the litter microhabitat in tropical forests of Costa Rica.



Myxomycetes (plasmodial slime molds) are eukaryotic, phagotrophic bacterivores usually present and often abundant in terrestrial ecosystems. Most of what is known about the assemblages of myxomycetes 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 major purpose of the present study is to obtain data on the distribution and ecology of the myxomycetes associated with the little microhabitat in tropical forests of Costa Rica. During November of 1995, 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. The majority of the species appearing in moist chamber cultures 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. quamulosum*, and *Physarum compressum*.

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; JOHN C. LANDOLT, Dept. of Biology, Shepherd College, Shepherdstown, WV 25443; and RODNEY D. SEPPELT, Australian Antarctic Division, Channel Highway, Kingston, Tasmania 7050, Australia. Dictyostelid cellular slime molds on subantarctic Macquarie Island.

Macquarie Island (54°30' S, 158°57' E) is an oceanic island 34 km long and 2.5 to 5.5 km wide located 1000 km southeast of Tasmania. The island, composed of oceanic crustal rocks lifted above sea level during the middle to late Pleistocene, lies close to but north of the Antarctic Convergence. The nearest land masses are the New Zealand shelf island groups of the Auckland Islands and Campbell Island, lying 640 and 700 km to the northeast, respectively. Macquarie Island is characterized by an equable oceanic climate that is cool, moist, and windy with low temperatures (annual mean of 4.8°C, range 3.3°C), a mean annual precipitation of 895 mm, high humidity (89% average), and a mean wind speed of 9.3 sec<sup>-1</sup>. While wind, cloud cover, precipitation and relative humidity vary little throughout the year, there is a marked annual cycle in daylength from about 7 hours in mid-winter to 17 hours in mid-summer. During the period of early February to early April of 1995, samples of all of the major types of plant communities found on the island. These included tussock grassland, short grassland, herbfield, fernbrake, mire, and feldmark communities. There are apparently no previous reports of dictyostelids from the south polar region, but *Dictyostelium mucoroides* var. *Stoloniferum* was recovered from soil/litter and litter samples collected in tussock grassland, herbfield, and fernbrake communities. Densities were almost invariably low (<50



clones/gram), but one study site yielded 135 clones/gram. The most diverse plant communities on Macquarie are mixed herbfield communities that occur on raised coastal terraces, and the majority of positive samples were collected from these communities. No clones were recovered from samples collected at higher elevations (generally >200 meters) on the main plateau of the island. In general, both frequency of occurrence and density were higher for samples of moist, decomposing litter found around (and sometimes still attached to) the bases of such plants as *Pleurophyllum hookeri* and *Stilbocarpa polaris* than for samples collected from the soil/litter interface zone. In addition to samples specifically collected for isolation of dictyostelids, single clones were recorded from each of two moist chamber cultures prepared with samples of rabbit dung in the first instance and *Pleurophyllum* leaf litter in the second instance. (This research was funded in part by a grant from the National Science Foundation. We acknowledge logistical support provided by the Australian Antarctic Division. Permission to work on Macquarie Island was granted by the Tasmanian National Parks and Wildlife Service.)

GREGORY FARRIS, MICHELLE JENKINS, CLAYTON STEED and ALBERT MAGRO, Division of Science, Mathematics and Health Careers, Fairmont State College, Fairmont, WV 26554. Analysis of high titers of antibodies by indirect enzyme-linked immunosorbent assay.

The aim of this study is to develop and maximize conditions for an indirect enzyme-linked immunosorbent assay (ELISA) capable of analyzing very high titers of high affinity antibodies. Mice were immunized with bovine serum albumin (BSA) in saline diluted 1:1 with Freund's complete adjuvant. The mice were injected subcutaneously with 10 $\mu$ g of BSA in a volume of 0.01 mL and boosted on days 15 and 30 following the primary injection. Serum samples were taken seven days following the last injection of BSA. Indirect ELISA techniques were utilized to analyze the serum samples from the hyperimmunized mice. Two ELISA techniques were compared. Both of the ELISA techniques utilized alkaline phosphatase enzyme acting upon p-nitrophenyl phosphate as the substrate. The optical density of the resultant p-nitrophenol was determined by the use of microtiter plates that were read at 420 nm in a MR 4000 Dynatech microplate reader. In the determination of the titers of the mouse anti-BSA antibodies, one ELISA technique used goat anti-mouse IgG(Fc specific) antibodies conjugated to alkaline phosphatase. The other ELISA technique used goat anti-mouse IgG(Fc specific) antibodies conjugated to biotin, followed by an avidin-alkaline phosphatase conjugate. Various parameters of the two ELISA techniques were compared, with the primary objective being to maximize significant differences between dilution data points at very high titers of the mouse anti-BSA antibodies.

REBECCA SANDERS, Department of Biology, Fairmont State College, Fairmont, WV 26554; AARON SMITH, Department of Biology, Fairmont



State College, Fairmont, WV 26554; and TIMOTHY WARNER, Department of Geology and Geography, West Virginia University, Morgantown, WV 26506-6300. Remote sensing of phenological characteristics of Mixed Mesophytic Forests of West Virginia.

The aim of this project is to map, using remote sensing, hardwood forest associations in the Mixed Mesophytic Forests of west-central West Virginia. This is challenging due to the similarity of the spectral reflectance of different hardwood species during most of the year. However, data that incorporates phenological variations such as spring bud-break and fall coloration could overcome this limitation. We acquired spring, summer and fall Landsat Thematic Mapper satellite images of the study area, and co-registered them to a common map projection. Field verification of the satellite imagery was obtained from the WV Global Analysis Project (GAP) data. This consisted of 0.1 hectare vegetation surveys, which were geo-referenced using Global Positioning Satellite (GPS) technology. The analysis of the satellite imagery involved forest classification of each image separately, as well as the combined multi-temporal data set.

Preliminary results indicate that phenological characteristics improve classification accuracies greatly. In particular, the incorporation of fall phenology seems to be more important than that of spring. Use of phenological characteristics requires, however, that imagery be collected during short intervals of time, which may vary from year-to-year. For example, our results might have been improved if slightly later dates of satellite imagery were available. Further improvements in accuracy should also be possible by incorporating digital elevation data to stratify the imagery based on topographic site. These improvements should make it possible to use remotely sensed imagery for the reliable regional-scale classification of deciduous forests. Support is gratefully acknowledged from the Department of Energy (Grant #DE-FG21-95MC32159) and the State of West Virginia for funds distributed through the National Research Center for Coal and Energy. We also thank the WV GAP project for the field data.

TERRY L. HAMRICK and PROTIP K. GHOSH, Department of Geology, Marshall University, Huntington, WV., 25755. A geochemical study of the impact of acid mine drainage on Sandlick Creek, Bruno, Logan County, West Virginia.

Striking evidences of the effects of acid mine waters on a drainage system are seen in Sandlick Creek, Bruno, Logan Co., West Virginia. Two tributaries converge at the headwaters of Sandlick Creek, one of which, the South Fork, is strongly impacted by acidic seepage ( $\text{pH} < 3.0$ ) from abandoned coal-mining activities on the ridge forming its southern valley walls. Neutralization of the pH by mixing with waters of the North Fork ( $\text{pH} \sim 7.0$ ) produces extensive precipitation of white gelatinous Al which coats the bed of Sandlick Creek for over 0.8 km. Turbulence of the water caused by bed roughness produces pockets of foam as air mixes with



the precipitating Al. Detailed geochemical analyses of the waters and precipitates have been done using the ICP-AES and Ion Chromatograph, and the dissolved species and precipitates have been characterized by X-ray Diffraction and NMR techniques.

A groundwater seep located approximately 0.4 km above the confluence acts as the point source for the low pH of the South Fork. Immediately below the seep dissolved Al and Fe in the South Fork increase by factors of 500X and 30X, respectively. The combination of a rise in pH and exposure to oxygenated waters leads to the production of a rusty brown precipitate of Fe over a distance of 300 m, and correlates with a change in the dissolved Fe values from 1.3 to 0.23 mg/L. The Al content, however, remains constant at 25 mg/L for over 900 m until it drops dramatically to 7 mg/L at the confluence.  $\text{SO}_4$  content, as expected, is high at 630 mg/L at the seep and falls to 250 mg/L at the confluence. Si and Mn mimic the behavior of Fe, although on a much lesser scale (12/5 and 8/1 mg/L, respectively).

As expected, the brown precipitate is dominantly Fe, whereas the white precipitate is dominantly Al.

The influence of pH and dissolved constituents on the biological diversity is shown by some preliminary studies by Chris White of the biology department at Marshall. He finds that the South Fork shows the presence of only 4 taxa, whereas the North Fork shows 21 taxa. This study has been supported by a Marshall Summer Research grant to PKG. The ICP-AES used in this study was obtained through a NSF-ILI grant to PKG.

ESTHER GUZMAN, MONTRELL SEAY, JAMES NUTTER, KIRK CAMMARATA, and SUZANNE ROGERS, Molecular Biology/Biotechnology Program, Salem-Teikyo University, Salem, WV 26426-0500.  
Polyphenol oxidases in apple and *arabidopsis*.

Plant polyphenol oxidase (PPO) is being studied in preparation for genetic manipulation. PPO is responsible for the "Browning" reaction of damaged plant tissue. Disruption of cellular compartmentalization permits interaction of the vacuolar o-dihydroxy phenolic substrates with the plastid-localized enzyme, resulting in the formation of quinones and polymerized secondary products. The polymerization localizes microbial infection and suggests a "Defense role" for PPO, but other functions related to PPO's chloroplast thylakoid location have long been debated as well. Our laboratory is studying PPO in *arabidopsis* and *malus* (apple). The former is a model system facilitating genetic manipulation and the latter is important agronomically.

PPO activity is found in both types of plants. Substrate specificity studies show that 4-methyl catechol is preferred over dihydroxyphenylalanine, dopamine, and catechol. However, substrate auto-oxidation makes determinations of the apparent



$K_m$ 's unreliable. Genomic hybridization analyses are being used to study the PPO gene families in each plant species. A 1.2 kb EcoRI fragment of the potato PPO-P1 gene is being used as the hybridization probe. However, weak hybridization signals at low stringency (5 X SSC, 0.1 % SDS, 25 C) indicate a low degree of sequence similarity between the potato, apple, and *arabidopsis* genes. A more suitable hybridization probe is being sought. The potato PPO gene is also being engineered for bacterial expression. Activity reconstitution and site-directed mutagenesis will then be used to study the observed latency and activation of PPO.

TODD DIEHL and DAVID VALENTINO, Dept. of Phys. Sci., Concord College, Athens, WV 24712. Punctuated aggradational cycles (PAC's) in the Upper Mississippian Avis Limestone of the Hinton Group, West Virginia.

The Avis member is a marine carbonate unit (1-15 m thick) that resides within the fluvial and deltaic sequences of the Upper Mississippian Hinton Group in the Appalachian Basin of southern West Virginia. Below the Avis, the Hinton Group consists of fining upward sequences of conglomerate to red mudstones that are interpreted to be fluvial meander-belt deposits. Above the Avis, the Hinton consists of a coarsening upward sequence of gray-black shales to interlayered thin-bedded sandstone and shale that grades upward into thick-bedded sandstone capped off by massive red mudstone bearing plant fossils. This sequence is interpreted to represent river-dominated deltaic sedimentation. Near and within the Bluestone River gorge the Avis member consists of a 15 m thick sequence of interlayered fossiliferous lime mudstone, grainstone and dolostone that define meter-scale lithofacies cycles that resemble punctuated aggradational cycles.

Overall the Avis limestone represents a marine transgression in the eastern Appalachian basin, however, in detail there are at least three lithofacies sequences in the Avis that are interpreted as smaller scale transgressive-regressive cycles. The repeated lithofacies sequence (<5 m thick) are characterized by a series of fossil-rich limemudstone that grades upward into grainstone, then capped off by dolostone with no trace of fossils. The lower most cycle is dominated by spiriferida-bearing limemudstone that progressively grades upward to fossil-free limemudstone and dolomitic shale. The second cycle contains larger forms of spiriferida in possible life position in a limemudstone, that grades upward into a fossiliferous grainstone. The stratigraphically highest cycle in the Avis (>5 m thick) contains abundant fossils in the lowermost beds that grade upward into skeletal grainstone containing gastropods, bryozoa, and crinoidal debris. The skeletal grainstone is capped off by dolomitic shale. The dolomitic shale is in an abrupt contact with gray clastic shale that occurs at the base of sequence of red shale with an upward increasing abundance of sandstone beds (the upper deltaic section). From southwest to northeast the carbonate sequences in the Avis thins to three discrete limemudstone beds (<1 m thick) in a section dominated by red shale with rare sandstone beds. The abrupt transition from carbonate- to clastic-dominated



rocks occurs over 2500 m in the area of the Bluestone State Park. This lithofacies transition is interpreted as the lateral transition from marine- to terrestrial-dominated environments going from offshore to onshore along the basin margin.









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