Volume 65, Numbers 2, 3 and 4

Proceedings of the West Virginia Academy of Science 1993

Papers of the Sixty-Eighth Annual Session

Proceedings of the West Virginia Academy of Science 1993

Vol. 65–No. 2,3 and 4

PAPERS OF
THE SIXTY-EIGHTH ANNUAL SESSION

Davis and Elkins College
Elkins, West Virginia
April 3, 1993

Printed by
Marshall University Printing Services
June 1997

Cover Design
Meredith Pearce
West Virginia University
Office of Publications
Morgantown

West Virginia Academy of Science, Inc.

The West Virginia Academy of Science was founded at Morgantown, November 28, 1924. The Academy was incorporated under the Code of West Virginia on May 9, 1959 as "a nonstock corporation, which is nor organized for profit but for the advancement of learning and scientific knowledge."

MINUTES OF THE ANNUAL MEETING

Minutes of the sixty-eighth annual meeting of the West Virginia Academy of Science held on the campus of Davis and Elkins College in Elkins, West Virginia on April 3, 1993.

8:50 AM The Academy was welcomed by Dr. Abbott Brayton, Vice President and Dean of the Faculty of Davis and Elkins College.

10:00 AM Executive Meeting

1. Meeting was called to order by President Weaks.
2. Those present were Andy Cook, Treasurer; Ralph Booth, D & E host; Craig Stamm, NIOSH ; Al Magro, 1994 Host at Fairmont State College; and Phil Cottrill, Recording Secretary.
3. A discussion concerning $12.00 per page charge for the Acid-Rain Symposium participants was considered. It was decided that the $12.00 per page charge should apply to everyone.
4. Ralph Booth is working on a revision of the Academy By-Laws. Suggestions for revision should be sent to Ralph Booth.
5. The rate charged libraries for the Academy Proceedings was discussed.
6. President Weaks discussed the printing costs of the publications and compared the costs with the Tennessee and Kentucky Academies. The costs are high. The Academy Proceedings are now being published at the Marshall University Printing Service.
7. Andy Cook reported that membership in the Academy is growing.
8. Send recommendations concerning dues to President Weaks before the October 1993 meeting.
9. The October planning meeting will be at Fairmont State College on October 24, 1993. President Weaks will call the executive board members if a different date is needed.
10. Andy cook moved that the 1994 meeting of the WV Academy be held on April 16, 1994. The motion was seconded by Ralph Booth. A discussion followed. The motion was defeated.
11. Andy Cook moved that the 1994 meeting be held on 4-16-94 (first choice) or 4-23-94 (second choice) or 4-30-94 (third choice). The motion was seconded by Phil Cottrill. Motion passed.
12. Al Magro (1994 Fairmont host) asked if a symposium with the theme " Main Challenges to the Environment" could be held in conjunction with the Fairmont meeting? There was no dissent.
13. Treasurer's report: Andy Cook said there was over $500.00 unaccounted for in favor of the Academy in the treasurer's books.

11:30 AM General Business Meeting, President Weaks presiding.

1. Jim Van Gundy (Davis & Elkins Campus Coordinator) was thanked for the day's meeting arrangements.

2. Andy Cook's financial report revealed the $500 error in favor of the Academy.
3. The Proceedings by being printed at Marshall University saved the Academy approximately $3,000 over the previous printer.
4. It was announced by Andy Cook that the Academy owned a Certificate of deposit dated October 7, 1992 in the amount of $7712.34. The CD is still in the name of Roy Clarkson the previous treasurer. It was discussed as to how the Academy could get the CD transferred to the next treasurer.
5. It was moved by Don Tarter, seconded, and passed that the Academy transfer the CD ($7,712.34) from Roy Clarkson to the new treasurer.
6. Andy Cook, the outgoing treasurer, was awarded a plaque for his service to the Academy.
7. Tom Pauley nominated Ian Jenness of Davis & Elkins College to the office of treasurer. The nomination was seconded and Ian was voted into the treasurer's position.
8. The 1994 meeting will be at Fairmont State College. Al Magro will be the host. The 1995 meeting will be at Marshall University. Ralph Taylor will be the host. The 1996 meeting will be at Concord College. Carl Fezer will be the host.
9. Craig Stamm reported that the 1991 Proceedings and 1992 Proceedings are being prepared.
10. Ed Keller asked to be relieved of the editor's position. The Academy needs volunteers.

12:06 PM Luncheon meeting

1. The outstanding science teacher awards were given to Dr. Sharon Erickson Harmon, of Morefield High School in Hardy County and Mr. Carol J. O'Connell of Harts High School in Lincoln County.
2. A plaque was given to Jim van Gundy and Davis & Elkins College for coordinating this meeting.

4:00 PM Awards Presentation

1. The Dupont Best Student Paper ($200.00) went to Nocole Turrill of Marshall University. The topic was "Variations of Herb Layer - Soil Relationships in Young vs. Mature stands of Northeastern West Virginia Forests.
2. Davis and Elkins runner-up paper ($100.00) went to Deepika Walpita of West Virginia University. The topic was Enzymatic Repair of Oxidative DNA Damage in Actively Growing and Quiescent Human Neuroblastoma Cells."
3. The Academy Best Poster Award went to Daniel W. Chaffin of Marshall University. The topic was " Tissue Localization Methods in Plants: New Applications.

The meeting adjourned at 4:15 PM

Table of Contents

Papers

Abstracts of the 1993 Annual Meeding

Relationships of Burrow Size, Soil Temperature, and Body Size and Temperature in the Salamanders *Plethodon wehrlei* and *Plethodon cinereus*

Thomas K. Pauley and E.C. Keller, Jr.

Department of Biological Sciences, Marshall Unviersity, Huntington, West Virginia 25755, and Department of Biology, West Virginia Unviersity, Morgantown 26506

ABSTRACT: Habitat analysis of adult sympatric salamander species, *Plethodon wehrlei* and *Plethodon cinereus*, revealed a significant negative correlation between cloacal temperatures and burrow size for *P. cinereus*, but not for *P. wehrlei*. Burrow size showed a significantly positive relationship with snout/vent length for the combined data set. Adult *P. wehrlei* utilized larger burrows than adult *P. cinereus*. Differentiation of burrow utilization appears to be critical in microhabitat partitioning.

INTRODUCTION

This study examines the association among soil temperature and burrow size and certain morphological attributes of two adult species of *Plethodon* in the eastern United States. The study was designed to determine possible influences of selected environmental factors on the microdistribution of two sympatric salamander species of unequal adult sizes. *Plethodon cinereus* is a small woodland salamander with a broad distribution that extends from southern Canada to southern North Carolina; *P. wehrlei* is a larger species that has a more limited distribution extending from southern New York to northern North Carolina. For the two species, relationships were examined among burrow sizes, soil temperature, head width, snout-vent lengths, and cloacal temperatures.

The general hypotheses examined in this study were that burrow size and/or environmental temperature can affect densities of adult salamanders and determine, in part, patterns of co-existence.

Studies on microdistribution of salamanders have addressed competition and territoriality (Fraser 1967b; Hairston 1980; Jaeger 1971a, 1980a,; Jaeger, et al. 1982; Kaplan 1977; Wells 1980; Wrobel, et al. 1980), chemical cues used in communication (Jaeger 1981; Jaeger and Gergits 1979), soil moisture (Taub 1961; Jaeger 1971b; 1980b; Pauley 1977, 1978a), and soil depth (Jaeger 1970). Bogert (1952) performed a comparative study on substrate temperatures of several species of salamanders and found no significant differences between the mean substrate temperatures of two or more species inhabiting the same area. Heatwole (1962) found that *Plethodon cinereus* was excluded from certain forest types when temperatures exceeded an upper tolerance limit . Pauley (1978b) determined that soil temperature played an important role in habitat partitioning between *P. cinereus* and *P. wehrlei*.

Jaeger (1974) demonstrated that burrows are important resources in interspecific competition between similarly sized salamanders (*P. cinereus* and *P. shenandoah*) because burrows provide shelter from desiccation and predators. Fraser (1976b) studied interactions between large and small plethodontids and found that the larger *P. punctatus* was excluded from areas where soil and rocks were tightly packed (resulting in smaller burrows). In contrast, the smaller *P. hoffmani* utilized these areas. Fraser suggested that, when in sympatry with *P. punctatus, P. hoffnmani* should be able to find nest sites without interference from *P. punctatus*. Maiorana (1978) also found that larger sized species of salamanders were more prevalent in areas with larger crevices. She observed that *Aneides aeneus* and other larger salamanders were found under larger cover objects. Brandon and Huheey (1971) and Southland (1986) determined that adult *Desmognathus monticola* and *D. quadramaculatus* use streambank burrows as refuges from which to forage for food. Because adults use burrows, Southerland suggested that juveniles are forced to use various cover objects, thereby partitioning streambank habitats between age classes.

Food has been suggested as a major resource in habitat partitioning (Jaeger 1972, Fraser 1976a). Fraser (1976b) and Maiorana (1978) agreed that, although food plays an important role in regulating the microdistribution of salamanders, it is not the only factor involved in habitat partitioning. Hairston (1987) stated that detailed analysis of burrow use, as a limiting resource for habitat partitioning, has not been carefully considered.

METHODS AND MATERIALS

The study site is on a hillside with mixed deciduous trees located in Doddridge County, West Virginia, 3.2 kilometers west of the Harrison/Doddridge County line. The site is triangular in shape with north and northwest aspects, and there are 5 microreliefs consisting of 3 benches and 2 slopes. Elevation ranges from 274-366 m. *P. cinereus* is the dominant species on the north-facing slope and *P. wehrlei* is the dominant species on the northwest-facing slope.

In a random sampling of adult *P. wehrlei* and *P. cinereus* over a 3-year period from March through October 1974-1976, cloacal temperatures were recorded using a telethermometer with a 1.9 mm probe. Each salamander was held on the soil with a pair of forceps covered with rubber tubing to help prevent the investigator's body temperature being transferred to the salamander. On-site soil temperature was immediately recorded where each salamander was collected. A vernier caliper was used to measure snout-vent length, head width, and diameter of the burrow at the soil surface occupied by each salamander.

Burrow openings (holes) were measured at each collection site to the nearest 0.01 mm. The head width of each salamander captured near burrow openings was also measured at the widest point (posterior end of the jaws). Comparisions were made between burrow sizes and the head widths of salamanders found nearest to each burrow opening.

Statistics used to characterize the field data were the mean, standard deviation, maximum value, minimum value, and coefficient of variation. Two-tailed t-tests were completed between species. One variable (the ratio between snout-vent length to head width[1]) was examined to determine if there were a non-linear relationship between one or more of the physical variables examined and the non-linear transformation of the body size variable. Linear correlation coefficients (r) were computed to evaluate the relationships among the 6 variable (listed in Table 1) in a pair-wise fashion. Co-variance analysis was completed on head widths as adjusted by respective body lengths to examine the difference between head widths of the 2 species independent of differences in body lengths. Laboratory data were interpreted by examining the percentage of occurrence.

RESULTS

During the day, *P. cinereus* was found beneath flat stones and rotting logs, while *P. wehrlei* was found beneath flat stones. The soil in the area where *P. cinereus* was found had few rocks and, therefore, few crevices that could be used as burrows. The substrate in the area where *P. wehrlei* occurred had numerous sandstone rocks that resulted in many crevices and burrows.

As shown in Table 1, only mean head widths and snout-vent lengths were found to be significantly different between the 2 species (t-test, $P<0.01$). The co-variance analysis revealed a significant difference between the head widths of the 2 species ($P<0.01$) even after adjustment for body lengths.

Three pairs of variances were shown to be significantly different using the variance ratio test: viz., head widths, snout-vent lengths, and cloacal temperatures (each with $P <0.01$).

Results from correlations analyses of the field data (Table 2) show that there was a very strong positive linear relationship between soil temperatures and cloacal temperatures ($r=0.91$ and $r=0.97$; $P <0.01$ in both cases). This was true for both species separately and for the data of both species combined. Likewise, there were very strong linear relationships between head width and snout-vent lengths ($r=0.71$, $r=0.77$, and $r=0.92$; respectively for*P. cinereus, P. wehrlei*) for each species and for the combined data of both species (Table 2). NOTE: We used the combined data in an attempt to obtain information on generalized relationships regardless of species.

Two other significant negative correlations occurred in the *P. cinereus* data, viz., the size of the burrow vs. soil temperature and the size of burrow vs. cloacal temperature ($r=0.45$ and $r=-0.33$, respectively; each $P <0.05$, Table 2). A fifth significant correlation was observed for the data of both species combined, between burrow size and the snout-vent length/head width ratio (r=.0.26; P value <0.05, Table 2). The modest correlation found in the joint species data, and not in the data of each species, is probably due to the increased number of observations and is similar to some of the results shown by Fraser's (1976b) combined data. The correlation is undoubtedly an indication of the general relationship between the size of a salamander and the size of an associated burrow.

DISCUSSION

Because *P. wehrlei* is larger than *P. cinereus*, the significant inter-species differences in head width and snout-vent length were expected. When in sympatry, *P. cinereus* and *P. wehrlei* partition habitats with *P. cinereus* inhabiting cooler areas (Pauley 1978b). However, in this study cloacal and soil temperature measurements for each species in its respective habitats were not significantly different.

The difference between the variances of the 2 species for head width and snout-vent length are undoubtedly due to size differences, since coefficients of variation for these two characteristics are essentially equal in the two species. The significant difference in cloacal temperature variances is probably due not only to general body size differences, but is perhaps due to innate temperature variation in *P. wehrlei* (cloacal temperature C/V value of the *P. wehrlei* sample is considerably greater than that of *P. cinereus*, thus indicating that something other than size was causing these differences).

Previous investigators have determined that soil temperature measurements are excellent surrogate variables for salamander body temperature measurements, and this study supports these findings. For example, Bogert (1952) showed that the cloacal temperature of *P. glutinosus* was about 3.0 ^{0}C higher than that of the corresponding substrate. In the present study, it was observed that average cloacal temperatures were 1.4 ^{0}C for *P. wehrlei* and 1.7 ^{0}C for *P. cinereus* higher than their associated soil temperatures. There is no obvious reason for differences among these temperatures, except perhaps species size or some other unmeasured variable. In the field, soil temperatures of narrow burrows of *P. cinereus* were significantly lower than the wider burrows of *P. wehrlei.* Previously, Pauley (1977) noted that soil in *P. wehrlei* burrows was less moist than soil in *P. cinereus* burrows. Since moist soil tends to dissipate more heat than drier soil (Spurr and Barnes 1964), burrows in moist soil will remain cooler than burrows in drier soil.

The ratio of average burrow width to average head width is somewhat higher for *P. cinereus* (ratio = 2.32) than for *P. wehrlei* (ratio = 1.69); hence *P. wehrlei* appears to fill a greater portion of its burrow than does *P. cinereus*. However, a causal relationship of this species-specific association is not evident.

The positive correlation between length/width ratio and burrow size supports the hypothesis that burrow size is an effective factor in habitat partitioning. Selection of burrow size by these salamanders appears to be a function of body size. Apparently, shorter and smaller individuals select more narrow burrows while relatively longer and larger individuals select wider burrows. This burrow selection might also indicate that the depth of burrows is also an important factor in the selection of burrows by a salamander. *P. wehrlei* is wider and longer than *P. cinereus* and this might account, in part, for the significant negative correlations between burrow size and soil and cloacal temperatures in *P. cinereus*.

We conclude that the size of burrows is an effective habitat partitioning agent for these two sympatric species of unequal size.

LITERATURE CITED

Bogert, C. M. 1952. Relative abundance, habitats, and normal thermal levels of some Virginian salamanders. Ecology, 33:16-30.

Brandon, R. A. and J. E. Huheey. 1971. Movements and interactions of two species of *Desmognathus* (Amphibia: Plethodontidae). Amer. Midl. Natur., 86:86-92.

Frasir, D. F. 1976a. Empirical evaluation of the hypothesis of food competition in salamanders of the genus *Plethodon*. Ecology, 57 (3): 459-471.

Fraser, D. F. 1976b. Coexistence of salamanders in the genus *Plethodon*: A variation of the Santa Rosalia Theme. Ecology, 57(2): 238-251.

Hairston, N. G. 1980. Evolution under interspecific competition: Field experiments on terrestrial salamanders. Evolution, 34 (3): 409-420.

Hariston, N. G. 1987. Community ecology and salamander guilds. Cambridge University Press, New York. 230pp.

Heatwole, H. 1962. Evironmental factors influencing local distributions and activity of the salamander, *Plethodon cinereus*. Ecology, 43:360-472.

Jaeger, R. G. 1970. Potential extinction through competition between two species of terrestrial salamanders. Evolution, 24:632-642.

Jaeger, R. G. 1971a. Competitive exclusion as a factor influencing the distributions of two species of terrestrial salamanders. Ecology, 52 (4): 632-637.

Jaeger, R. G. 1971b. Moisture as a factor influencing the distribution of two species of terrestrial salamanders. Oecologia, 6:191-207.

Jaeger, R. G. 1972. Food as a limited resource in competition between two species of terrestrial salamanders. Ecology, 53 (3): 535-546.

Jaeger, R. G. 1980a. Density-dependent and density-independent causes of extinction of a salamander population. Evolution, 34 (4): 617-621.

Jaeger, R. G. 1980b. Microhabitats of terrestrial forest salamanders. Copeia, 2:265-268.

Jaeger, R. G. 1981. Dear enemy recognition and the costs of aggression between salamanders. Am. Nat., 117(6): 926-974.

Jaeger, R. G. and W. F. Gergits. 1979. Intra-and interspecific communication in salamanders through chemical signals on the substrate. Anim. Behav., 27:150-156.

Jaeger, R. G., D. Kalvarsky, and N. Shimizu. 1982. Terrestrial behavior of the red-backed salamander: Expulsion of intruders. Anim. Behav., 30:490-496.

Kaplan, D.L. 1977. Exploitative competition in salamanders: Test of a hypothesis. Copeia 1977 (2):234-238.

Kirk, R. E. 1982. Experimental Design: Procedures for the Behavioral Sciences. First Edition. Brooks and Cole Publ. Monterey, Ca.

Maiorana, V. C. 1978. Difference in diet as an epiphenomenon: Space regulates salamanders. Can J. Zool., 56 (5):1017-1025.

Pauley, T. K. 1977. The microdistribution of two sympatric species of salamanders, *Plethodon wehrlei* and *Plethodon cinereus*. Ph.D. diss., West Virginia University. 109 pp.

Pauley, T. K. 1978a. Moisture as a factor regulating habitat partitioning between two sympatric *Plethodon* species. J. Herpetol., 12 (4): 491-493.

Pauley, T. K. 1978b. Temperature and isolation as factors regulating the partitioning of habitats of two sympatric *Plethodon* species. Proc. W. Virginia Acad. Sci., 50 (4): 77-84.

Southerland, M. T. 1986. Behavior interactions among four species of the salamander genus *Desmognathus*. Ecology, 67:175-181.

Spurr, S. H. and B. V. Barnes. 1964. Forest Ecology. Second Edition. The Ronald Press Company, New York. 571 pp.

Taub, F. B. 1961. The distribution of the red-backed salamander, *Plethodon c. cinereus*, within the soil. Ecology 42 (3): 271-275.

Wells, K. D. 1980. spatial associations among individuals in a population of slimy salamanders (*Plethodon glutinosus*). Herpetologica, 36(3): 271-275.

[1]Although there are some statistical difficulties in the use of the ratio variable, Kirk (1982) explains that it is appropriate to use them as normal variates if they do not approach zero.

Table 1. Sum of Squares. Means, number of observations, standard deviations, maximum and minimum values, and coefficients of variation for the size of burrows, snout-vent length, head width, cloacal temperature, soil temperature, and the ratio of snout-vent length to head width[1], for populations of P. wehrlei and P. cinereus.

	Variable	x	N	s	Max.	Min.	C/V%
P. wehrlei	Burrow size (mm)	13.31	38	5.43	33.0	6.5	40.8
	Head width (mm)	7.86	38	0.91	9.6	6.1	11.6
	Snout-vent (mm)	54.85	38	6.44	65.0	40.7	11.7
	Cloacal temp. (°C)	12.10	38	4.44	20.6	4.4	36.7
	Soil temp. (°C)	10.69	38	3.81	17.8	4.1	35.7
	Length/width	6.98	38	----	----	---	---
P. cinereus	Burrow size (mm)	11.73	52	5.43	29.6	4.3	46.3
	Head width (mm)	5.06	61	0.56	6.4	4.1	11.1
	Snout-vent (mm)	40.34	61	4.54	49.3	30.9	11.3
	Cloacal temp. (°C)	11.87	61	2.93	16.7	5.8	24.7
	Soil temp (°C)	10.15	61	2.93	16.7	5.8	24.7
	Length/width	8.70	61	----	----	---	---

[1] These ratios were calculated for each salamander.
x= population mean, N= number of individuals examined, s= standard deviation, max.= maximum value observed, min.= minimum value observed, C/V%= coefficient of variation (s/x̄).

Table 2. Linear coefficients (r) among soil temperatures, cloacal temperatures, burrow sizes, SVL (Snout-vent length), head width, and svl/head width in P. wehrlei, P. cinereus, and both species combined. N is between 38 and 61 for each species and 115 overall. See Table 1 for exact N values.

Variables	Cloacal temp.	Burrow size	SVL	Head width	SVL/Head width
Soil temp.					
P.c.[1]	0.91**	-0.45**	-0.06	-0.04	-0.07
P.w.[2]	0.97**	0.21	-0.11	-0.12	-0.01
c&w[3]	0.94***	-0.10	0.01	0.03	0.04
Cloacal temp.					
P.c.	1.00	-0.33**	-0.17	-0.13	0.02
P.w.	1.00	0.21	-0.15	-0.18	-0.04
c&w	1.00	-0.04	-0.07	-0.05	0.02
Burrow size					
P.c.	---	1.00	-0.04	0.13	0.12
P.w.	---	1.00	-0.12	-0.04	0.18
c&w	---	1.00	0.07	0.14	0.26*
SVL					
P.c.	---	---	1.00	0.71**	---
P.w.	---	---	1.00	0.77**	---
c&w	---	---	1.00	0.92**	---

[1] P.c. = P. cinereus data only [2] P.w. = P. wehrlei data only [3] c&w = both species
* 0.05 >p >0.01 ** p< 0.01 *** p< 0.001

A Predictive Model for Cultural Resource Site Location on the Monongahela National Forest.

W. Hunter Lesser.
Monongahela National Forest

ABSTRACT: A model for predicting prehistoric and historic site locations on the Monogahela National Forest was developed and implemented in 1992. The model is based on preliminary correlations first proposed in the Forest Cultural Resource Overview in 1978 and refined by field inventory data over the past decade. The predictive model may be used as a planning tool to be quantified, tested and refined to enable land resource managers to focus cultural resource inventory efforts in areas most likely to contain significant sites.

INTRODUCTION

The following predictive model for cultural resources is based on preliminary correlations first proposed in the Monongahela National Forest Cultural Resource Overview in 1978 and refined by field inventory data over the past decade. Cultural resource inventories since 1981 have greatly expanded our understanding of prehistoric and historic site distribution patterns on the Monongahela National Forest. Intensive inventory of contiguous areas of 5,000 or more acres provided much of the data used in the preparation of this model.

The purpose of the predictive model is to provide a planning tool which may be quantified, tested and refined to enable land resource managers to focus cultural resource inventory efforts in areas most likely to contain sites potentially eligible for the National Register of Historic Places. A working predictive model is particularly desirable due to the increasing demands of inventory, evaluation, enhancement and interpretation of cultural resources as required by Federal Law.

The Monongahela National Forest appears to be particularly suited for application of such a predictive model due to the severe topographic and physiographic constraints limiting human settlement in the area. The model should be considered as dynamic in nature and will be subjected to change and refinement.

In the original Monongahela National Forest Cultural Resource Overview, Davis (1978) noted that evidence of past human activity was not scattered randomly over the land surface, but was patterned to reflect both the cultural and environmental limitations operating upon past human groups. He further stated that extent of human use of the National forest and that topography was the dominant environmental aspect controlling human spactial distribution. Davis delineated six broad topographic/environmental zones which are relevant to human exploitation of the Monongahela. They are: (1) primary stream valleys, (2) secondary stream valleys, (3) valley and upland slopes, (4) valley and upland slopes, (4) upland benches, (5) ridge and mountain tops, and (6) unique environmental zones (Davis 1978: 152-158).

The environmental zones delineated by Davis have been retained in the predictive model with some modifications. They are referred to as cultural resource sensitivity zones. His categories of "primary stream valleys" and "secondary stream valleys" have been changed to "stream valleys with floodplains and terraces" and stream valleys without developed floodplains and terraces." The zones are ranked according to the expected probability of cultural resources occurring within them. These rankings are HIGH (resources generally occur within the zone), MEDIUM (resources may occur within the zone but are not frequent) and LOW (resources generally do not occur within the zone. Cultural resource sensitivity rankings are inclusive for both prehistoric and historic sites unless otherwise indicated.

Descriptions of the cultural resource sensitivity zones in the Monongahela National Forest predictive model follow.

1. Stream valleys with floodplains and terraces: this zone contains valleys with well developed floodplains and terraces. It has a cultural resource sensitivity ranking of HIGH. Primary stream valleys with well-developed Pleistocene terraces will have the highest sensitivity. There are few such formations within the current boundaries of the Monongahela National Forest. Terraces near the confluence of secondary streams also will have HIGH sensitivity. Secondary stream tributary confluences with associated level ground may be considered to have MEDIUM sensitivity for historic resources such as small logging era sites.

2. Stream valleys without floodplains and terraces: this zone contains valleys without well-developed floodplains and terraces. It has a cultural resource sensitivity ranking of LOW. This zone contains valleys with V-shaped stream channels with flow debris and other evidence of geologically frequent flushing of sediments.

3. Valley and upland slopes: this zone, as noted by Davis (1978), contains the majority of land within the Monongahela National Forest. It has a cultural resource sensitivity ranking of LOW. Valley and upland slopes are the lowest sensitivity areas in the Forest. Land within this zone is moderately to steeply sloped and is expected to show minimal evidence of habitation. Important exceptions to the above are discussed under cultural resource sensitivity zone #7 (unique environmental zones).

4. Upland benches: this zone may be generally rated as MEDIUM sensitivity. Those benches associated with good water sources and a lack of surface rocky debris may be considered to have MEDIUM sensitivity. Benches located in areas with otherwise minimal level ground will have higher sensitivity as will those adjacent to chert outcrops. Larger benches will generally have higher sensitivity than small ones. Small benches less than 25 meters square and those covered by boulder fields should be considered to have LOW sensitivity.

5. Ridge and mountain tops: are generally rated as LOW to MEDIUM sensitivity. In the western or more heavily dissected portion of the Allegheny Plateau, very

broad, extensive ridge lines may be considered MEDIUM to HIGH sensitivity. These may have been used as travel routes by prehistoric groups due to the V-shaped, rock and vegetation choked character of the major stream drainages in the area. Narrow ridge tops (less than 50 meters in width) and discontinuous ridge formations should be considered LOW sensitivity. Broad ridge tops (greater than 50 meters in width) containing extensive surface rock or boulder fields or exhibiting minimal soil development and evidence of heavy soil erosion are LOW sensitivity.

5. Ridge and mountain tops: these sites are generally rated as LOW to MEDIUM sensitivity. In the western or more heavily dissected portion of the Allegheny Plateau, very broad, extensive ridge lines may be considered MEDIUM to HIGH sensitivity. These may have been used a travel routes by prehistoric groups due to the V-shaped, rock and vegetation choked character of the major stream drainages in the area. Narrow ridge tops (less than 50 meters in width) and discontinuous ridge formations should be considered LOW sensitivity. Broad ridge tops (greater than 50 meters in width) containing extensive surface rock or boulder fields or exhibiting minimal soil development and evidence of heavy soil erosion are LOW sensitivity.

6. Gaps and saddles: these lower elevation areas of ridge lines have been given their own category due to HIGH sensitivity and their hypothesized use as a predictive indicator for site density in a particular area. Large gaps separating two prominent stream drainages can be considered as highest sensitivity for prehistoric sites and certain historic resources, such as logging camps. Smaller gaps and those dividing less prominent drainages or with steep approaches from below will have lower sensitivity (Lesser and Brashler 1987). A high frequency of gaps in a ridge system will lower the sensitivity for individual gaps in the area. If high sensitivity gaps in a study area do not contain sites, the probability of other prehistoric cultural resource sites in the area appears to be very low. Therefore, topographic gaps may be useful for prediction of site density in a large previously uninventoried study area.

7. Unique environmental zones: included within this catagory are upland wetlands, bogs, prominent spring resources, rock exposures with overhangs (rockshelters), caves and chert-bearing limestone outcrops. These features all have a cultural resource sensitivity ranking of HIGH. Wetlands and bogs have been shown to have HIGH sensitivity for prehistoric sites (particularly Paleo-Indian and Archaic Periods) in a systematic inventory of the western Maryland coal region by Wall (1981). Little work has been conducted in such settings on the Monongahela National Forest but larger wetlands with associated level ground should be considered as HIGH sensitivity for prehistoric sites at present. Prominent springs are usually associated with small benches or gaps and should always be considered as HIGH sensitivity for cultural resources.

Valley and upland slopes containing rock outcrops with overhangs (rockshelters) are considered to be HIGH sensitivity areas, particularly those with southern exposures. Cave entrances and passages should be considered likewise.

Chert-bearing limestone outcrops on slopes should also be considered as HIGH sensitivity (Lesser 1988, Robertson et al. 1990).

Application of the Predictive Model

The predictive model is applied by designating all landforms within a particular project area under one of the seven topographic/environmental zones. The zones are then ranked according to the expected probability of cultural resources as indicated in the predictive model as HIGH, MEDIUM or LOW. This information is compiled and provided to a field crew before the cultural resource inventory begins. The actual inventory then follows standard survey procedures recommended by the State Historic Preservation Office.

Testing the Predictive Model

The predictive model can be tested over time to evaluate its overall effectiveness and to reveal areas of refinement. Testing the model may be accomplished in numerous ways. Possibly the most effective and certainly the most cost effective method would be to monitor areas after completion of earth-disturbing projects to determine the effectiveness of the model in site prediction. The Monongahela National Forest Plan requires that cultural resource project and site-specific monitoring be conducted yearly. Therefore, this type of work is already mandated. A more scientifically rigorous method of testing the model would be to use a traditional complete coverage approach to resurvey areas already inventoried with the model. This would provide an excellent baseline comparison on effectiveness of the model, but is less desirable in that it would require a duplication of effort at significant expense. Another approach would use the complete coverage method to examine previously uninventoried areas. Data generated from this strategy could be used in a comparative fashion to suggest areas of refinement in the model. Rigorous sample survey strategies could also be used in the same manner.

CONCLUSIONS

Utilizing a predictive model for cultural resource inventory of large land masses has already proven to be an extremely cost-effective strategy for compliance with the National Historic Preservation Act on the Monongahela National Forest. Further refinement of the model through testing should enable land resource managers to more effectively predict and avoid impacts to significant archaeological sites on the Monongahela National Forest.

LITERATURE CITED

Davis, R. P. S., Jr. 1978. Final Report: A Cultural Resource Overview of the Monongahela National Forest, West Virginia. West Virginia Geological and Economic Survey, Morgantown, West Virginia.

Lesser, W.H., and J. G. Brashler. 1987. Can We Go Beyond Site Distribution? Cultural Models and Lithic Scatters From the West Virginia Uplands. Pages

189-202 in Upland Archaeology of the East: A Third Symposium. Cultural Resources Report No. 87-1. USDA Forest Service, Southern Region.

Lesser, W. H. 1988. Preliminary Investigations at Files Run Quarry (46RD114): Lithic Procurement and Reduction in the Tygart Valley Uplands. West Virginia Archeologist 40:24-39.

Robertson, J.A., G. W. Lantz, J. Schuldenrein and T. L. Benedict. 1990. Cultural Resources Evaluation of Selected Prehistoric and Historic Archaeological Sites, Monongahela National Forest, Randolph and Pocahontas Counties, West Virginia. Report on File, USDA Forest Service, Monongahela National Forest, Elkins, West Virginia.

Wall, R. D. 1981. An Archaeological Study of the Western Maryland Coal Region: The Prehistoric Resources. Maryland Geological Survey, Baltimore.

Growth of Oak and Maple on Soil Amended with Fluidized Bed Waste[1]

Bahm, D. and R. F. Keefer[2]
Div. Plant and Soil Sciences, West Virginia University, Morgantown, WV 26506

ABSTRACT: Two fluidized bed combustion wastes (FBW) were compared with ground agricultural limestone as lime sources for establishment of red maple (*Acer rubrum* L.) and red oak (*Quercus rubra* L.) seedlings on an acidic soil (pH 5.1) in a pot experiment. After 21 weeks, soil chemical and plant properties were evaluated. Target pH levels were 6 and 7, but levels reached were about 5.2 and 6.2 for the two rates of lime sources used. This was probably due to the poor efficiencies of these sources to neutralize acidity. Addition of FBW increased soil pH and exchangeable bases and decreased acidity, exchangeable Al, and extractable Fe and Mn. However, added salts in FBW also increased soil electrical conductivity by about 1 mmho over the control that could retard root growth and nutrient uptake. Leaf tissue Ca increased above the control for all treatments. Maple seedlings survived better than oak. All seedlings seemed to grow best in the unamended acidic soil even though the final pH was 4.7; however, there was very little change in plant height and no consistent change in nutrient levels for the FBW treatments. Therefore, these preliminary studies show that FBW has potential to be used as a source of lime for the production of maples and oaks, but further studies are necessary.

INTRODUCTION

The large amounts of coal used for electrical power generation in Appalachia yield annually over 80 million tons of coal combustion wastes, e.g., fly ash, bottom ash, and fluidized bed combustion waste (FBW) (Rehage and Holcombe, 1990). Power generating companies are urgently seeking ways to use or dispose of large amounts of these wastes. Applying them to land as a liming material may be an alternative to disposing of these wastes in a landfill or monofill. Fluidized bed waste has been used as a soil amendment for production of corn (Holmes et al. 1979), forage crops (Cochran et al. 1991 and Stout et al. 1979) and apples (Korcak, 1980a), but not for hardwood tree species, such as maple or oak. Mature forests in West Virginia and much of Appalachia consist of maple and oak. Perhaps FBW could be used as a liming source to improve growth conditions of native vegetation. Therefore, the objective of this study was to evaluate the effects of two fluidized bed combustion wastes as liming sources for establishing maple and oak tree seedlings on an acidic mine soil in West Virginia.

---

[1]Published with the approval of the Director of the West Virginia Agricultural and Forestry Experiment Station as Scientific Paper NHo. 2487.
[2]Graduate Student and Professor, repectively.

MATERIALS AND METHODS

Experimental Design and Treatment

Soil and Amendment-- Topsoil, that would be applied to a surface mined site (table 1), was collected from a stockpile at the Patriot mine site in Osage, West Virginia. The soil was air-dried and sieved to <2mm. Along with agricultural limestone, two fluidized bed wastes-- one from a stockpile near Albright, West Virginia, the other from Morgantown Energy Associated (MEA) power generation plant (Table 2) were selected. The two FBW had similar pH, electrical conductivity, neutralization potential, and texture. Micronutrient concentrations were comparable, except MEA FBW had approximately twice the diethylenetriaminepentaacetic acid (DTPA)-extractable Fe as Albright FBW.

These treatments were compared with ground agricultural limestone added to reach the same pH levels. Each rate of soil amendment was mixed with five kg of the soil and placed in pots lined with plastic bags. Each soil treatment was equilibrated for three cycles of wetting and drying and remixed to ensure uniformity before planting.

Experimental Design-- The study consisted on seven treatments using a control with nothing added and three liming sources (Albright FBW, MEA FBW, and ground agricultural limestone) each at two rates (Table 3). Pots were arranged in a randomized block design with nine blocks per species with each block being a replicate containing all treatments for one tree species. Blocks were periodically re-arranged to minimize variability of solar radiation and other environmental factors.

Tree Selection and Planting -- First year seedlings of two native hardwood species (red maple and red oak) were obtained from Pike's Peak Nursery in Penn Run, PA. The Choice of species was based on their natural distribution in the area, growth rate, and rooting characteristics. Seedlings were planted after soil equilibration and pots were placed outside a greenhouse and watered daily.

Harvesting -- After 21 weeks, plant heights were measured and all leaves were collected and leaf area was determined with a LI-3100 Area Meter (Anon. 1987). The leaves of both species were dried in a forced air oven at 65°C for plant tissue analyses. Each pot was moved into the greenhouse, tops and roots were removed, the soil was air-dried, ground to <2mm, and a 200 g soil subsample was stored in a plastic bag.

Soil and FBW Analyses -- Soil was analyzed before the experiment (Table 1) and after harvest. Aluminum, P, and DTPA-extractable elements were analyzed by an inductively coupled plasma (ICP-AES) unit, Perkin Elmer Model 400. Exchangeable bases were extracted by NH_4OAc (Soil Survey Staff, 1984). Calcium, Mg, Na, and K were analyzed by atomic absorption on a Perkin Elmer Spectrophotometer Model 5000. Sample pH was measured in a 1:1 soil:water ratio (McLean 1982) using a Fisher Model 805MP pH Meter. Electrical conductivity

(Rhoades 1982) was measured using a VWR Scientific Model 1052 EC meter. Exchangeable acidity was extracted with KCl and titrated with 0.1 *N* NaOH (Thomas 1982). Particle size distribution was determined by the Versol method of Sobek et al. (1978); P by two methods: (a) Mehlich 1 (double acid) and (b) that outlined by Chang and Jackson (1957) and modified by Singh (1965); and organic C by acid dichromate digestion (Soil Survey Staff, 1984). Lime requirement was evaluated by the modified $Ca(OH)_2$ double buffer method of McLean (1982) and neutralization potential of soil was analyzed by the method of Smith et al. (1974) modified using 0.5 *N* HCl, microwave digestion, and auto titration.

Prior to the experiment, the fluidized bed wastes were chemically analyzed in a manner similar to the soil tests for pH, EC, and DTPA-extractable elements. Neutralization potential for FBW was determined using a CEM MDS-200 microwave digestion unit using a modified Smith et al. (1974) method.

Plant Analysis -- A 0.5 g sample of dried plant material was digested overnight, using 10 mL of concentrated HNO_3 that was accelerated using pressure and temperature by CEM microwave methods (Anon. 1991). Plant nutrients were analyzed with the ICP-AES unit. Potassium was analyzed using emission spectrophotometry.

Statistical Analysis -- Data were analyzed statistically by the analysis of variance (ANOVA) using the 0.05 level of significance.

RESULTS AND DISCUSSION

All maple seedlings survived for the control (no amendment) and for the lower liming rate for all amendments, but only 89% did for the higher liming rate. All oak seedlings on the control plants survived. Oak survival at the lower liming rate was 90% for Albright FBW, 67% for MEA FBW, and 90% for agricultural lime. Oak survival for the higher liming rate was less than the lower, i.e., 67% for both FBWs and 55% for agricultural lime.

Plant height of oak trees seemed to be higher than that of maples (Table 3); however, there were no significant differences among the treatments for the oaks. On the other hand, maple trees showed differences in plant height among the treatments. The control showed highest growth, but this was not significantly different for the low rate for MEA FBW, low rate for lime, and high rate for Albright FBW. The low rate of Albright FBW, high rate of MEA FBW, and high rate of lime all showed significantly lower growth.

Leaf area of maple trees was larger than that of oaks (Table 3), with a maximum value of 538 cm^2 for maples and 430 cm^2 for oaks. The low rate of Albright FBW and lime were the only treatments that showed significantly smaller leaf area than the control for the maple trees. Plant data (Table 4) indicate that these two liming sources gave higher values for Ca than the control. The high level of extractable Fe (24 mg/kg-- Table 2) in the MEA FBW was shown to be bound with P at harvest (170 mg/kg-- Table 5), whereas even though the Albright FBW had lesser amounts of DTPA-extractable Fe initially (14 mg/kg-- Table 2), it apparently remained available for plant uptake as there was less Fe-bound P in the Albright FBW treatment at harvest (154 mg/kg-- Table 5). The high Mn concentrations in the control plants for both the maple (Table 4) and oak (Table 6) resulted from low pH. It is possible that the FBW materials reacted with soil Mn rendering it less available for plant uptake, as all liming sources reduced leaf Mn concentrations compared to the unamended control.

For the oak trees, significantly smaller leaf area values than the control were evident for Albright FBW low rate and MEA FBW high rate. Plant data for these treatments (Table 6) appeared to have higher levels of Mg, P, and lower levels of K than the control. It is possible that imbalance of major nutrients in the nutrition of the oak trees caused the growth to be less than desired.

Soil pH levels of all treatments were higher than the controls. This agrees with an apple experiment by Korcak (1985). Normally, one would expect these pH levels to be more beneficial to plant growth. However, the apple trees in the Korcak study grew less well than the control in some cases. Korcak (1980b) showed a reduction in growth of apple seedlings, but could not explain it based on soil properties or plant tissue analyses. Fluidized bed waste has been shown to increase alkalinity and salinity (electrical conductivity) and may reduce plant growth (Terman et al. 1978; Holmes et al. 1979; and Korcak 1980a). Salinity was increased from 0.5 mmhos in the control to about 1.3 mmhos in the FBW treatments. In the present pot experiment for five months, neither the alkalinity nor the salinity appeared to be high enough to cause poor plant growth.

The desirable growth of the red maple tree seedlings undoubtedly related to an abundance of small feeder roots. This species can be considered acceptable for reforestation on soils amended with FBW. Most red oak seedlings also survived. They did not have as extensive root systems as that of the maples, but the oaks were larger initially with a definite taproot.

SUMMARY AND CONCLUSIONS

Compared to an unamended control soil, mixing of fluidized bed waste (FBW) at two rates with topsoil significantly increased pH (from 4.7 in the unamended control to about 5.1 and above in the FBW treatments), soluble salts measured by electrical conductivity, and soil Ca, but decreased soil acidity, exchangeable Al, extractable Fe and Mn, and Mn concentration in maple and oak leaves grown thereon. Magnesium concentrations in tree leaves were considerably higher for the amended soils, especially for MEA FBW, probably as a result of the high Mg concentration in this material. Phosphorus concentrations in leaves were also increased over the control for the FBW treatments. All liming sources reduced leaf Mn concentrations compared to the unamended control.

Red maple trees grew satisfactorily probably due to their well developed feeder root system. They can be considered acceptable for reforestation on soils limed with FBW. The lack of feeder roots in the oaks and/or an imbalance of relatively high levels of P and Mg and low levels of K may have been responsible for limiting their growth during the five month growth period.

The data show that FBW has potential for use as a liming source for red maple and red oak seedling establishment. However, further studies are needed to determine the most effective rate of FBW as a soil amendment. Use of this material may be limited by its high salt concentration.

LITERATURE CITED

Anonymous. 1987. LI-3100 Area Meter, Instruction Manual. Pub. No. 7903-20. LI-COR, INC.

Anonymous. 1991. Microwave sample preparation system. *In* Application Manual, CEM corp., Matthews, NC.

Bauder, J. W., and G. W. Gee. 1982. Particle-size analysis, pp. 383-412. *In* A. L. Page, R. H. Miller, and D. R. Keeney (eds.), Methods of Soil Analysis-- Part 1. Physical and Mineralogical Methods, Am. Soc. Agron., Madison, WI.

Chang, S. C., and M. L. Jackson. 1957. Fractionation of soil phosphorus. Soil Sci. 84:133-144.

Cochran, F. W., H. D. Perry, J. P. Fontenot, and V. G. Allen. 1991. Nutrient utililzation by sheep fed forage grown on soil treated with fluidized bed combustion residue. J. An. Sci. 69:318-327.

Holmes, M. A., D. C. Martens, and J. N. Jones, Jr. 1979. Growth and sulfur sorption of corn on soil amended with fluidized-bed waste, pp. 973-979. In M. K. Wali (ed.), Ecology and Coal Resource Development, Vol. 2, Pergamon Press, New York, NY.

Korcak, R. F. 1980a. Fluidized bed material as a lime substitute and calcium source for apple seedlings. J. Environ. Qual. 9:147-151.

Korcak, R. F. 1980b. Effects of applied sewage sludge compost and fluidized bed material on apple seedling growth. Commun. Soil Sci. Pl. Anal. 11:571-585.

Korcak, R. F. 1985. Effect of coal combustion wastes used as lime substitutes in nutrition of apples in three soils. Plant Soil 85:437-441.

Lindsay, W. L. and W. A. Norvell. 1978. Development of a DTPA soil test for Zn, Fe, Mn, and Cu. Soil Sci. Soc. Am. J. 42:421-428.

McLean, E. O. 1982. Soil pH and lime requirement, pp. 199-224. *In* A. L. Page, R. H. Milller, and D. R. Keeney (eds.), Methods of Soil Analysis, Part 2. Chemical and Microbiological Properties, Am. Soc. Agron., Madison, WI.

Rehage, J. A., and L. J. Holcombe. 1990. Environmental performance assessment of coal ash use sites: Little Canada structural ash fill. Electric Power Research Institute, Palo Alto, CA Rept. EN-6532.

Rhoades, J. D. 1982. Soluble salts, pp. 1671-1680. *In* A. L. Page, R. H. Miller, and D. R. Keeney (eds.), Methods of Soil Analysis-- Part 2. Chemical and Microbiological Properties, Am. Soc. Agron., Madison, WI.

Singh, R. N. 1965. The fate and distribution of phosphorus applied to Davidson clay loam. Ph. D. dissert., Virginia Polytechnic Institute and State Univ., Blacksburg, VA.

Smith, R. M., W. E. Grube, Jr., T. Arkle, Jr., and A. Sobek. 1974. Mine spoil potential for soil and water quality. U. S. Environ. Protection Agency, Cincinnati, OH, EPA-670/2-74-070.

Sobek, A. A., W. A. Schuller, J. R. Freeman, and R. M. Smith. 1978. Field and laboratory methods applicable to overburden and minesoils. U. S. Environ. Protection Agency, Cincinnati, OH EPA-600/2-74-054.

Soil Survey Staff. 1984. Procedures for collecting soil samples and methods of analysis for soil survey. USDA Soil Conservation Service, Washington, DC Soil Survey Investigations Rept. No. 1.

Stout, W. L., R. C. Sidle, J. L. Hern, and O. L. Bennett. 1979. Effects of fluidized bed combustion waste on the Ca, Mg, S, and Zn levels in red clover, tall fescue, oats, and buckwheat. Agron. J. 71:662-665.

Terman, G. L., V. J. Kilmer, C. M. Hunt, and W. Buchanan. 1978. Fluidized bed boiler waste as a source of nutrients and lime. J. Environ. Qual. 7:1471-150.

Thomas, G. W. 1982. Enchangeable cations, pp. 159-166. *In* A. L. Page, R. H. Miller, and D. R. Keeney (eds.), Methods of Soil Analysis-- Part 2. Chemical and Microbiological Properties, Am. Soc. Agron., Madison, WI.

Wolf, A. and D. Beegle. 1991. Recommended soil tests for macronutrients-- phosphorus, potassium, calcium, and magnesium, pp. 25-34. *In* Recommended soil testing procedures for the Northeastern United States, Agric. Expt. Sta. Univ. Del., Newark, DE 19717-1303, Bul. No. 493.

Table 1. Some selected chemical and physical properties of the original soil

Soil Property	Value	Method Reference
pH (1:1 soil:water)	5.12	McLean, 1982
	(mmhos)	
Electrical Conductivity	0.07	Rhoades, 1982
	($cmol_c$/kg)	
Ca (NH_4OAc extraction)	4.64	Soil Survey Staff, 1984
Mg (NH_4OAc extraction)	1.77	Soil Survey Staff, 1984
K (NH_4OAc extraction)	0.11	Soil Survey Staff, 1984
Na (NH_4OAc extraction)	0.08	Soil Survey Staff, 1984
Exchangeable Al (KCl extraction)	2.43	Thomas, 1982
Exchangeable H^+ (KCl extraction)	2.63	Thomas, 1982
Cation Exchange Capacity	12.76	Rhoades, 1982
	(mg/kg)	
Phosphorus (Double Acid)	43.4	Wolf and Beegle, 1991
Zn (DTPA extraction)	0.91	Lindsay and Norvell, 1978
Cu (DTPA extraction)	1.52	Lindsay and Norvell, 1978
Mn (DTPA extraction)	34.8	Lindsay and Norvell, 1978
Fe (DTPA extraction)	31.5	Lindsay and Norvell, 1978
	(%)	
Organic Carbon (dichromate digestion)	0.43	Soil Survey Staff, 1984

% sand	% silt	% clay	Texture	Method Reference
25.1	45.7	29.2	Clay loam	Bauder and Gee, 1986

Table 2. Some chemical analyses of fluidized bed wastes (FBW)

	FBW Used		
	Albright	MEA	Method Reference
pH (1:1 soil:water)	12.6	12.4	McLean, 1982
	-----(mmhos)-----		
Electrical Conductivity	5.74	5.66	Rhoades, 1982
	-----(mg/kg)-----		
Zn (DTPA extraction)	0.07	0.13	Lindsay and Norvell, 1978
Cu (DTPA extraction)	0.64	0.61	Lindsay and Norvell, 1978
Mn (DTPA extraction)	BDL*	BDL	Lindsay and Norvell, 1978
Fe (DTPA extraction)	14.14	23.64	Lindsay and Norvell, 1978
	$CaCO_3$ Equivalent (%)		
Neutralization Potential	17.72	16.03	Smith et al., 1974

*BDL = below detection limit (1.4 μg/kg)

Table 3. Plant height and leaf area of maple and oak seedlings grown on soil treated with three lime sources at two levels

Liming		Plant height		Leaf area	
Source	Rate*	Maple	Oak**	Maple	Oak
		------(cm)------		-------(cm)------	
Control	0	65.0 a	68	538 a	430 a
Albright FBW***	1	52.0 b	58	385 b	134 b
MEA FBW	1	55.0 ab	66	484 ab	310 ab
Limestone	1	55.0 ab	57	337 b	299 ab
Albright FBW	2	56.2 ab	57	419 ab	237 ab
MEA FBW	2	54.8 b	67	507 a	114 b
Limestone	2	54.0 b	63	396 ab	314 ab

Data with the same letters within a column are not statistically different at the 0.05 level.

* Rate 1 = Albright FBW 6.22 g/kg; MEA FBW 6.36 g/kg; lime 1.40 g/kg.
Rate 2 = Albright FBW 11.74 g/kg; MEA FBW 12.0 g/kg; lime 2.62 g/kg.
** Data not statistically different at the 0.05 level.
*** FBW = fluidized bed combustion waste

Table 4. Selected plant nutrients in leaves of maple seedlings grown on soil treated with three lime sources at two levels

Liming							
Source	Rate*	P	Ca	Mg	Fe	Mn	Zn
		--------------(g/kg)------------			-------------(mg/kg)-----------		
Control	0	1.13 a	14.8 d	5.2 c	538 bc	4206 a	18.1 a
Albright FBW**	1	0.98 abc	19.8 c	6.1 c	696 ab	1899 b	7.9 a
MEA FBW	1	0.92 bc	20.7 bc	8.8 b	485 bc	1446 bc	12.5 a
Limestone	1	1.05 abc	21.8 b	5.9 c	862 a	1936 b	29.2 a
Albright FBW	2	0.93 bc	9.7 e	5.6 c	423 c	314 cd	0.5 b
MEA FBW	2	1.10 ab	27.1 a	11.0 a	442 c	179 d	7.7 ab
Limestone	2	0.87 c	20.9 bc	4.2 c	608 bc	816 cd	34.2 a

Data with the same letters within a column are not statistically different at the 0.05 level.

* Rate 1 = Albright FBW 6.22 g/kg; MEA FBW 6.36 g/kg; limestone 1.4 g/kg.
Rate 2 = Albright FBW 11.74 g/kg; MEA FBW 12.0 g/kg; limestone 2.6 g/kg.

** FBW = fluidized bed combustion waste.

Table 5. Soil phosphorus fractionation of selected treatments after harvest

Liming Source	Rate	Al-bound	Fe-bound	Ca-bound	Total P
		-------------------(mg/kg)-------------------			
Control	0	11	174	16	216
Albright FBW	2	20	154	30	219
MEA FBW	2	25	170	25	234
Limestone	2	17	158	19	210

No water soluble P was detected (detection limit 0.;076 mg/kg)

Table 6. Selected plant nutrients in leaves of oak seedlings grown on soil treated with three lime sources at two levels

Liming Source	Rate*	P	Ca	Mg	Fe**	Mn	K
		---------------(g/kg)---------------			------------(mg/kg)---------		
Control	0	1.13 b	15.3 b	2.2 c	352	6186 a	10.2 a
Albright FBW***	1	1.36 ab	17.0 a	3.5 ab	526	3833 ab	9.2 b
MEA FBW	1	1.33 ab	16.1 ab	4.6 a	717	3183 b	11.2 a
Limestone	1	1.09 bc	19.9 a	3.6 ab	530	3374 b	10.9 a
Albright FBW	2	1.49 a	12.9 b	2.8 bc	394	2501 b	12.0 a
MEA FBW	2	1.38 ab	11.8 b	4.8 a	495	2122 b	9.9 ab
Limestone	2	0.89 cd	17.6 a	3.2 bc	514	2033 b	10.7 a

Data with the same letters within a column are not statistically different at the 0.05 level.

* Rate 1 = Albright FBW 6.22 g/kg; MEA FBW 6.36 g/kg; limestone 1.4 g/kg.
Rate 2 = Albright FBW 11.74 g/kg; MEA FBW 12.0 g/kg; limestone 2.6 g/kg.

**Data not statistically different at the 0.05 level.
*** FBW = fluidized bed combustion waste.

Table 7. Selected properties of soil treated with three lime sources at two levels (mean of both seedlings) after harvest

Liming Source	Rate*	pH	EC**	H^+	Al	K	DTPA-extractable Fe	DTPA-extractable Mn
			(mmhos)	------(cmol/kg)-------			-------(ppm)------	
Control	0	4.71	0.51	2.68	0.24	0.27	54	46
Albright FBW***	1	5.21	0.92	0.90	0	0.30	37	29
MEA FBW	1	5.24	0.86	0.84	0	0.30	41	31
Limestone	1	5.28	0.58	0.88	0	0.32	43	34
Albright FBW	2	6.06	1.50	0.48	0	0.27	24	20
MEA FBW	2	6.16	1.07	0.48	0	0.28	26	20
Limestone	2	6.56	0.66	0.46	0	0.26	24	24
LSD (0.05)		0.05	0.10	0.07	0.01	0.02	2	2

Data with the same letters within a column are not statistically different at the 0.05 level.

* Rate 1 = Albright FBW 6.22 g/kg; MEA FBW 6.36 g/kg; limestone 1.4 g/kg.
Rate 2 = Albright FBW 11.74 g/kg; MEA FBW 12.0 g/kg; limestone 2.6 g/kg.

** EC = electrical conductivity
*** FBW = fluidized bed combustion waste.

The Occurrence and Specific Associations of Spina Bifida with Environmental/Socio/Economic Attributes in the Counties of West Virginia

E. G. Jessen and E. C. Keller Jr.

ABSTRACT: Spina Bifida is a congenital disease that belongs to a class of diseases known as neural tube defects. The causes of spina bifida are unknown, but generic, gender, and environmental factors are all thought to play significant roles.

Correlation analysis were computed among the counties on the incidence of spina bifida and 287 environmental/socio/economic variables. In order to examine the incidence of spina bifida in relation to the environment, the relative incidence of spina bifida in West Virginia was examined by county. This paper is concerned with two questions: 1) what, if any, geographical, social, or environmental factors are related to the incidence of spina bifida and 2) are there "hot spots" of the occurrence of spina bifida in West Virginia?

The highest areas of occurrence of spina bifida were generally located in the northeastern (not including the eastern panhandle) and north-central part of West Virginia. This study also showed a higher incidence of spina bifida in counties that are less affluent, less populated, and have a higher incidence of Caucasians an the population. Also, counties that are less economically productive have a higher incidence of spina bifida. Environmentally, the acidity and iron content of the natural waters of the state are strongly associated with the occurrence of spina bifida. Finally, geologically, the older geological formations (in the eastern non-panhandle portion of the state) are positively associated with the occurrence of spina bifida.

INTRODUCTION

Spina bifida is generally considered to be a congenital disease that belongs to a class of diseases known as neural tube defects (NTD). Developmentally, spina bifida occurs in one of three ways: 1) spina bifida occult, (the least severe and least common), 2) meningocele; and 3) meningomyelocele. In occulta, the spinal cord remains in the vertebral canal, with "no protrusion of the underlying soft tissue." In meningocele, "swelling occurs over the site of the spinal defect consisting of meninges nerve roots", but the spinal cord remains in the canal. Meningomyelocele is the most severe form and also involves a swelling containing nerve root and meninges, but "the spinal cord has left the vertebral canal" (Coffey, 1970).

A singular cause of spinal bifida is unknown, but genetic, gender, and environmental factors appear to play significant roles. In regard to environmental influences, when a higher incidence group migrates to other areas of the world, they tend to have a reduced incidence with succeeding generations in the new locations (Leck, 1984). Spina bifida is usually more common in whites than in African Americans or Orientals, which is evidence that there is a generic aspect to the etiology of spina bifida (Leck, 1984). Further evidence for an environmental aspect indicates that the incidence is greater for African Americans who live in the southeastern U.S. (0.31/1000 births) as compared to the incidence of spina bifida in other areas of the U.S. (less than 0.1/1000 births). However, this incidence is still lower than the incidence for whites in the southeastern U.S. (0.8/1000 births). There is more evidence that spina bifida is influenced by the environment, Caucasians who live in the western U.S. have a lower incidence of spina bifida (0.23/1000 births) than do Caucasians who live in the southeastern U.S. (0.8/1000 births) (Greenberg, et al., 1982). Spina bifida is more prevalent in women than in men. In a compilation of studies in the British Isles, North America, Hungary, Mexico City, Bombay, Japan, and Israel, the average percentage of men having spina bifida was 42.2 (Elwood and Elwood, 1980), with a high of 50% in Mexico City (Stevenson et al., 1966) and a low of 33% in Rochester, Minnesota (Haynes et al., 1974).

The incidence of spina bifida has periodically peaked at specific places and times. Factors that have been studied in regard to the incidence of the anomaly are: drug use, illness, socioeconomic factors, diet, and drinking water. Kelsey et al., (1978) found that women who smoke during pregnancy have a higher risk of congenital defects (NTD) in their children (a 1.8 "relative risk" for those smoking more than 20 cigarettes a day). In this same study, the differences remained when controlling for social class, parity (number of previous children), and maternal age at the time of the child's birth.

The incidence of occurrence of spina bifida varies throughout the world ranging from a high of 4.5/1000 births in Belfast, Ireland, to lows of 0.12/1000 births in Japan, Taiwan, and Singapore (Elwood and Elwood, 1980). A compilation of studies done in various countries, on the prevalence of spina bifida, is presented in Table 1.

A number of studies have been shown a relationship between the incidence of spina bifida and lower socioeconomic groups (for instance, unskilled and semiskilled workers) (Naggan and MacMahon, 1967). Richards et al. (1972) controlled for other factors (geographical region, maternal age, and ethnic group) and found the incidence among socioeconomic groups to be similar.

In a study in Great Britain (Fedrick, 1976), a "higher risk" of children with spina bifida was observed when the parents worked as truck drivers, members of the military, transport workers, decorators, painters, or worked in the paper or print trades. Furthermore, rubber workers were found to have increased risks to have children with NTD (Aylett, Robert, and Lloyd, 1974).

Numerous studies have been conducted concerning illness and nutrition during pregnancy and the incidence of NTD's. Using case-control studies, Lawrence, et al. (1968) found a positive relationship between pregnant women having influenza and thereafter having an increased number of children with NTD's and consumption of cereals, bread, ice cream, cooked meats, and canned meats. Fedrick (1974) studied tea drinking and its relationship to anencephalus, (another class of NTD'S), and found there was a greater risk for anencephalus in the children of parents who drank more than two cups of tea per day. Recently, studies have determined that preconceptual vitamins may actually prevent NTD. As previously mentioned, vitamin deficiencies are thought to contribute to NTD (Knox, 1972).

The relationship between water quality and the incidence of NTD'S has also been examined. Wilson, Watson, and Richards (1973) performed a case-control study in Glasgow, Scotland, matching social class, parity, age, and the section of the city in which the case or control individuals lived. No difference was found between the mineral content of tap water in the homes of cases and controls. Morton, Elwood, and Abernathy (1976) studied the mineral content of the waters in South Wales and found that increased levels of aluminum were correlated with increased incidence of NTD's. Fedrick (1976) found that the incidence of anencephaly was inversely associated with hardness, pH, and calcium levels of water. Lowe et al. (1971) found that the frequency of anencephaly, in general, and of spina bifida specifically, were negatively correlated with water hardness. Elwood (1997) examined the effects of water composition on anencephaly and found and inverse association between the concentration of magnesium in drinking water and the incidence of anencephaly.

In order to examine the incidence of spina bifida in a smaller, but environmentally diverse region, its relative incidence was determined for the counties of West Virginia. West Virginia's major environmental resource extraction industry is coal mining. Thirty-four of the fifty-five counties of the state have active mining.

This paper is concerned with two major questions viz., 1) what if any, geographical, social, or environmental factors appear to be related to the incidence of spina bifida in the counties of West Virginia and 2) are there "hot spots" of the higher incidence of spina bifida in West Virginia?

METHODS

The incidence of spina bifida for each county (Toler, 1998 and personal communication) was gathered in 1987 and 1988 by extensive survey and visitation throughout the 55 counties of West Virginia. These data were used in computing the incidence of spina bifida per 1000 births by using the 1987 population estimates for each county, as estimated by the U.S. Bureau of the Census and obtained from the West Virginia Department of Health.

Individual mortality records were obtained from the West Virginia Department of Health for the years 1959-1984, excluding 1966. From these data, the average human mortality rates were calculated on the forty major types of mortality, as given in The Manual of the International Statistical Classification of Diseases, Injuries, and Cause of Death for all 55 West Virginia counties (WHO, 1957, 1967, 1977). The death records of only West Virginia residents were used. Since, there was only a small percentage of non-white resident death records in the data base, they were included in the study. Although, we recognize the etiology of some types of mortality maybe somewhat dissimilar, we have chosen to utilize the data according to the conventions used by the West Virginia Department of Health and the manuals quoted above.

The source of the other variables used in these correlation analyses is given in the appendix. Both linear and curvi-linear correlation analyses were computed among a variety of social, environmental, and economic variables for each county vs. the incidence of spina bifida within each county vs. the incidence of spina bifida within each county. Certain mathematical scaling transformations were utilized for the spina bifida variable, in an attempt to detect significant non-linear associations. For the geographical variables, the data were calculated as the percentage of the area of a county, covered by the specific surface geographical characters.

Certain combined variables, such as *durable goods produced and the average annual wage*, in the counties, were determined via a factor analyses program of the Statistical Analysis System (SAS) of the West Virginia Network for Education Computing (WVNET). These factors were utilized because they accounted for a moderate to high degree of variation in the set of independent variables analyzed.

RESULTS

Geographical Distribution of Spina Bifida among the Counties of West Virginia

In West Virginia, the highest incidence levels of spina bifida are generally clustered in the 17 counties which are in the north central section of the state (Figures 1 and 2). Generally, the northern panhandle counties (Hancock, Brooke, Ohio, and Marshall Counties) and the eastern panhandle counties (Jefferson, Berkeley, Morgan, Hardy, Hampshire, Mineral, and Grant), along with the counties along the Ohio River, and most of the southern counties have considerably lower incidence levels of spina bifida than the "hot spots" with the exception of Brooke, Monroe, and Morgan counties (Figure 1, 2, and 3).

Pocohontas, Pendleton, and Preston, counties along the Virginia border, (west and southwest of the eastern panhandle counties) have the highest incidences of spina bifida in the state with 0.40/1000 births, 0.38/1000 births, and 0.36/1000 births, respectively (Figures 2 and 3). Pleasants and Tyler counties have the lowest incidence level in the southern part of the state with 0.31/1000 births. The eleven counties that border the Ohio River have incidence levels of less than 0.10/1000 births with the exception of Brooke county which has an incidence of 0.13/1000 births (Figure 2 and 3). The eastern panhandle has a fairly low incidence of less than 0.11/1000 births except for Morgan county which has a spina bifida incidence of 0.19/1000 births. Lewis and Webster counties are the two exceptions to the north central "high incidence" cluster, since they have incidences of 0.05/1000 births and 0.08/1000 births, respectively (Figure 2, 3, and 11).

Associations of Spina Bifida and other Health Characteristics

The rate of individuals having visual impairments is significantly and positively associated with the square root transformation of the rate of occurrence of individuals with spina bifida (Figure 4). Also, the occurrence of persons having impairments of internal organ systems is negatively correlated to the rate of the occurrence of individuals with spina bifida. Similarly, the occurrence of persons with limb impairments and the occurrence of individuals who are profoundly mentally retarded. show a negative correlation with spina bifida. These statistical analysis indicate that all of the above relationships are of a non-linear nature (Figure 4).

The relationship among the rate of occurrence of spina bifida and various socioeconomic factors (the combined variables used were previously constructed by the use of factor analysis and all combined variables are italized below) and are presented in Figure 5. Most of the factors have negative correlations with the rate of occurrence of spina bifida.

The lowest negative correlations observed with the rate of occurrence of spina bifida is the rate of adults with disabilities receiving social security. The combined variables, *total industry non-durable/durable goods production and the rate of the* *production of durable goods/average wage*, are also negatively associated with the rate of occurrence of spina bifida (Figure 5). The log rate of the combined variable, *per capita income/average annual wages*, also shows a negative associations with the rate of spina bifida. Another combined variable, *average liquor sales/ population density*, is also negatively correlated with the incidence of spina bifida, as is the combined variable, *production rate of wholesale/retail/construction* activities. The most significant negative correlation occurs with the rate of occurrence of spina bifida and the combined variable, *rate of total assets/total wages/retail sales* (Figure 5).

There were three positive correlations in Figure 5:1) the log rate of the combined variable, *the amount of surface and underground coal production*, showed the lowest significant positive correlation, 2) the rate of aged adults on social security with the rate of spina bifida, and 3) the rate of government aid for exceptional children had the most significant positive correlation.

In Figure 6, in regards to environmental attributes, the rate of the occurrence of spina bifida was negatively associated with the degree of alkalinity of natural (non-drinking) waters and the number of active particulate emitters. The combined variables, *acidity/iron/pH* and the *zinc/dissolved oxygen levels* in natural waters (squared transformations) are positively and significantly associated with spina bifida.

The association of selected genetic and related variables with the rate of spina bifida is shown in Figure 7.. The proportion of native born individuals of native parentage and African-Americans is negatively correlated to the rate of the incidence of spina bifida (log transformation). Conversely, the proportion of Caucasians in the population, also a log transformation, is positively associated with the rate of occurrence of spina bifida. The gene frequency for tongue rolling (squared transformation) and the gene frequency for hair type are also positively associated with the rate of occurrence of spina bifida (Figure 7).

Five of the West Virginia counties with the highest rates of spina bifida, lie along the eastern portion of the state, excluding the eastern panhandle, and the eastern edge of the Allegheny plateau (Pendleton, Preston, Monroe, Barbour, and Pocohontas). These counties contain a range of geological formations from the Ordovician to the Pennsylvanian eras. When the proportion of the geological formations in each county were correlated with the rate of the occurrence of spina bifida, both positive and negative relationships were found (Figures 8, 9, and 10). The Greenbrier Formation and the Chemung Group are positively associated with the rate of the occurrence of spina bifida. This occurs in four of the give "high rate" counties. The McKenzie Formation and igneous intrusives are also positively correlated with the rate of spina bifida (Figures 8 and 9). Igneous instrusives are found only in Pendleton county and are of a small areal extent.

Negative correlations were found between the occurrence of the New River Formation (Pennsylvanian), Pocahontas Formation, Bluefield Formation and the rate of occurrence of spina bifida. In the five "high rate" counties, the New River and Pocahontas Formations are only present in one county, Pocahontas. The Bluefield Formation occurs to a minor degree in three of the counties that have "high rates" of the occurrence of spina bifida (Figures 8 and 10).

DISCUSSION

West Virginia has two general "hot spots" of the occurrence of spina bifida. One "hot spot" has a higher level of occurrence (greater than 0.16/1000 births) and is in the northeastern portion of the state (includes Preston, Taylor, Tucker, Pendleton, Pocohontas, Upshur, Randolph, and Barbour counties). The other "moderate hot spot" is centered in the north central part of the state and includes Doddridge, Ritchie, Gilmer, Calhoun, Roane, Harrison, Braxton, Wirt, and Clay counties (Figure 11).

The incidence of spina bifida in the counties of West Virginia corresponds with those found in Asian countries viz., Singapore, Taiwan, and Japan and with the Jewish population of Quebec. The overall West Virginia rate is clearly on the lower end of the worldwide incidence range.

The examination of the characteristics of the 55 counties provides some indication as to the possible causes of the differential rates of the incidence for spina bifida in West Virginia.

The modest positive correlation of the three health impairments viz., limb impaired, rate of profoundly mentally retarded, and rate of impairment due to various internal systems, do no appear to be etiologically linked to the occurrence of spina bifida (Figure 4). However, there is a positive correlation with the rate of persons with vision impairments.

The correlations of the socioeconomic characteristics and the occurrence of spina bifida indicate two patterns. The eight negatively associated characteristics: viz., total industry, per capita income, population density, retail sales, rate of adults with disabilities on social security, production rate of durable goods, average annual wage, and the rate of total county assets, indicate a lower incidence of spina bifida in hose areas of the state that are generally more affluent, have greater population densities, and are economically more productive. The three positively associated characteristics: viz., rate of government aid for exceptional children, rate of aged adults on social security, and surface and underground coal mining are all associated with high rates of spina bifida. Thus, areas that are generally more affluent, have greater population density, and are economically more productive, generally have lower rates of spina bifida.

Most counties in the coal fields of West Virginia have a low rate of spina bifida.(Figures 1 and 3). Pendleton, Doddridge, and Monroe have little or no coal mining. Pocahontas county also has very little coal mining. Preston and Barbour counties have coal mining, but the coal is in more recent geological formations than the above counties (McCollock, Aston, and Smith, 1986). It is important to note that Preston, Taylor, Upshur, Braxton, and Clay counties are partly located on the Allegheny Formation (Pennsylvanian), which is a known acid producer (personal communication, Jane McColloch).

The geographic formations, which were negatively associated with the incidence of spina bifida, lie generally to the west of the group of formations that are positively correlated with the incidence of spina bifida. This is located along the wastern border fo the state, excluding the eastern panhandle. The geographis formations having a positive correlation with spina bifida lie mainly in the more geologically recent coal fields that have little or no coal mining. Greenbrier county does have coal mining but it is very limited. It is necessary to emphasize that the

Greenbrier Formation appears to be a major factor in defining the "hot spots" of West Virginia. In Greenbrier county, a large portion of which is underlain by the Greenbrier Formation, the incidence of spina bifida is low (0.03/1000 births). Thus if the Greenbrier Formation would be significantly associated with the incidence of spina bifida, one would expect the incidence of Greenbrier county to be high, which it is not. Recall this paper only deals with th esurface geological formations. There is need to examine not only what is occurring within the formations below the Greenbrier, Chemung, and the McKenzie Formations (such as the Tonoloway and the Tuscarora), but also what is the connection among the lower formations, and the incidence of spina bifida.

In regards to the general environment profiles and the incidence of spina bifida, there is a higher incidence of spina bifida in non-industrialized regions where there is higher acidity and iron in the natural waters of these areas. This is in agreement with the relationship between the occurrence of spina bifida and the contents of drinking water which was previously mentioned. It is interesting to speculate about the characteristics of the ground water in the "hot spots" of the counties of West Virginia. This question is currently being examined by the authors.

The genetic associations shown in this paper support the earlier findings that the affliction is more prevalent in whites than in African-Americans (Greenburg et al., 1982). The relationship of the rate of native born individuals of native parentage might be indicative of a small amount of inbreeding. On-the-other-hand, two single gene traits (hair type and ability to roll one's tongue) are shown to be somewhat associated with the incidence of spina bifida. There does not appear to be any rational reason for these latter associations.

CONCLUSIONS

The occurrence of spina bifida in the counties of West Virginia is generally in the low-end of the range of the incidence statistics throughout the world. The highest incidence of spina bifida is generally located in the northeastern and north central parts of West Virginia. There is a higher incidence of spina bifida in counties having higher proportions of Caucasian population, the individuals therein are also less affluent, and the counties are less populated, less economically productive, receive little government aid, and have little or no coal mining. Two of the counties have modest coal mining, but do not appear to have a significant positive relationship to the occurrence of spina bifida. The older geologic formations in the eastern part of the state, excluding eastern panhandle, are positively associated with the incidence of spina bifida, while just to the west of these formations, the incidence of spina bifida is lower and the spina bifida rates negatively associated with these other formations (Figure 9 and 10). Also, the greater the acidity and iron content of the natural waters of the counties, the greater is the association with the increased incidence of spina bifida.

Further research is needed to examine the geological formations, surface and ground water characteristics of West Virginia in relation to the incidence of spina bifida.

Acknowledgments: We wish to express our appreciation to Cathy Riffon, Jane and G. McColloch in the preparation of the paper.

LITERATURE

Aylett, M.J., C.J. Roberts, and S. Lloyd. 1974. Neural Tube Defects in a Country Town. Br. J. Soc. Med. 28: 177-179.

Callahan, James. 1923. History of West Virginia, Old and New; Chicago, The American History Society, Inc.

Coffey, V. 1970. Spina Bifida in Ireland. Journal of the Irish Medical Assn. 63:343-348.

Elwood, J. M. and J. H. Elwood. 1980. *Epidemiology of Anencephalus and Spina Bifida*. Oxford University Press.

Elwood, J. M. Anencephalus and Drinking Water Composition. Am. J. Epidemiology. (1977). 105:460-467.

Fedrick, J. 1974. Anencephalus and Maternal Tea Drinking: Evidence For Possible Association. Proc. Roy. Soc. Med. 67: 6-10.

Fedrick, J. 1976. Anencephalus in Scotland 1961-1972. Br. J. Prev. Soc. Med. 30: 132-137.

Greenberg, F., J. M. Levy, and G. P. Oakley, Jr. 1983. Estimates of birth Prevalence Rates of Spina Bifida in the United States From Computer Generated Maps. Am. J. Obstet. Gynecol. 145(5): 570-573.

Haynes, S. G., J. B. Gibson, and L. T. Kurland. 1974. Epidemiology of Neural-Tube Defects and Down's Syndrome in Rochester, Minnesota, 1935-1971. Neurology. 24: 691-700.

Kelsey, J. L., T. Dwyer, T. R. Holford and M. B. Bracken. 1978. Maternal Smoking and Congenital Malformations: an Epidemiological Study. J. Epidem. Community Hlth. 32: 102-107.

Knox, E. G. 1972. Anencephalus and Dietary Intakes. Br. J. Prev. Soc. Med. 26: 219-223

Laurence, K. M., C. O. Carter, and P. A. David. 1968. Major Central Nervous System Malformations in South Wales. II. Pregnancy Factors, Seasonal Variation, and Social Class Effects. Br. J. Pre. Soc. med. 22: 212-222.

Leck, I. 1963. Incidence of Malformations Following Influenza Epidemics. Br. J. prev. soc. Med. 17: 70-80.

Leck, I. 1984. The Geographical Distribution of Neural Tube Defects and oral Clefts. Brit. Med. Bull. 40: 390-395.

Lowe, C. R., C. J. Roberts, and S. Lloyd. 1971. Malformations of Central Nervous system and Softness of Local Water Supplies. British Medical Journal (1971) 2:357-361.

Manual of the International Statistical Classification of Diseases, Injuries, and Cuases of Death. 1957, 1967, 1977. (Three publications). World Health Organization. Geneva.

Morton, M. S., P. C. Elwood and M. Abernethy. 1976. Trace Elements in Water and Congenital Malformations of the Central Nervous system in South Wales. Br. J. prev. soc. Med. 30: 36-39.

Naggan, L. and MacMahon. 1967. Ethnic Differences in the Prevalence of Anencephaly and Spina Bifida in Boston, Massachusetts. new Engl. J. Med. 227: 1119-1123.

Richards, I. D. G., C. J. Roberts, and S. Lloyd. 1972. Area Differences in Prevalence of Neural Tube Malformations in South Wales, Br. J. Pre. Soc. Med. 26: 89-93.

Stevenson, A. C., H. A. Johnson, M. I. P. Stewart, and D. R. Golding. 1966. Congenital Malformations--A Report of a study of Series of Consecutive Births in 24 Centres. Bull. WHO 34(1): 1-125.

Toler, D. 1988. Sister chromatid exchange in patients with spina bifida: Effects of age and position of black lesions. unpub. diss. West Virginia University.

West Virginia Department of Health Computer Tapes. 1959-84 (excluding 1966). Charleston, WV.

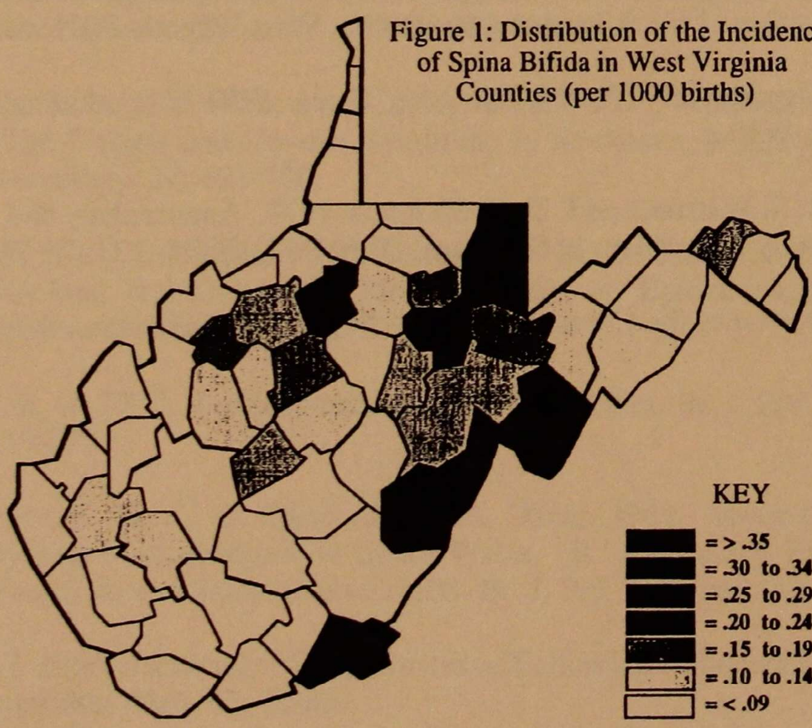
Wilson, T. S., R. S. Watson, and I. D. G. Richards. 1973. Anencephaly and Water Suply--a Study of Problem in Glasgow. Hlth. Bull. (Edinb.) 31: 76-78.

United States Bureau of the Census: 1987 WV Population Estimates.

TABLE ONE
Summary of the Ranked Incidence of Spina Bifida throughout the World

Incidence	Location	Reference
4.5/1000 births	Belfast, Ireland	Elwood and Elwood, 1980
3.27/1000 births	Ireland	Elwood and Elwood, 1980
2.91/1000 births	Great Britain	Elwood and Elwoos, 1980
2.461/1000 births	New Brunswick, Canada	Elwood and Rogers, 1975
1.65/1000 births	Alexandria, Egypt	Stevenson et al., 1966
1.63/1000 births	Budapest, Hungary	Czeisel & Revesz, 1970
1.2/1000 births	United States	Elwood and Elwood, 1980
1.08/1000 births	Bombay, India	Stevenson, et at., 1966
1.02/1000 births	Caucasian pop. Hawaii	Morton, Chung & Mi, 1967
0.94/1000 births	Mexico City, Mexico	Stevenson et al, 1966
0.84/1000 births	British Columbia	Trimble & Baird, 1978
0.80/1000 births	Japanese pop Hawaii	Morton, Chung & Mi, 1967
0.55/1000 births	Scandinavia	Elwood and Elwood, 1980
0.38/1000 births	Finland	Elwood and Elwood, 1980
0.33/1000 births	Jewish pop. Quebec	Horowitz & McDonald, 1969
0.12/1000 births	Japan, Taiwan, Singapore	Elwood and Elwood, 1980

Figure 1: Distribution of the Incidence of Spina Bifida in West Virginia Counties (per 1000 births)



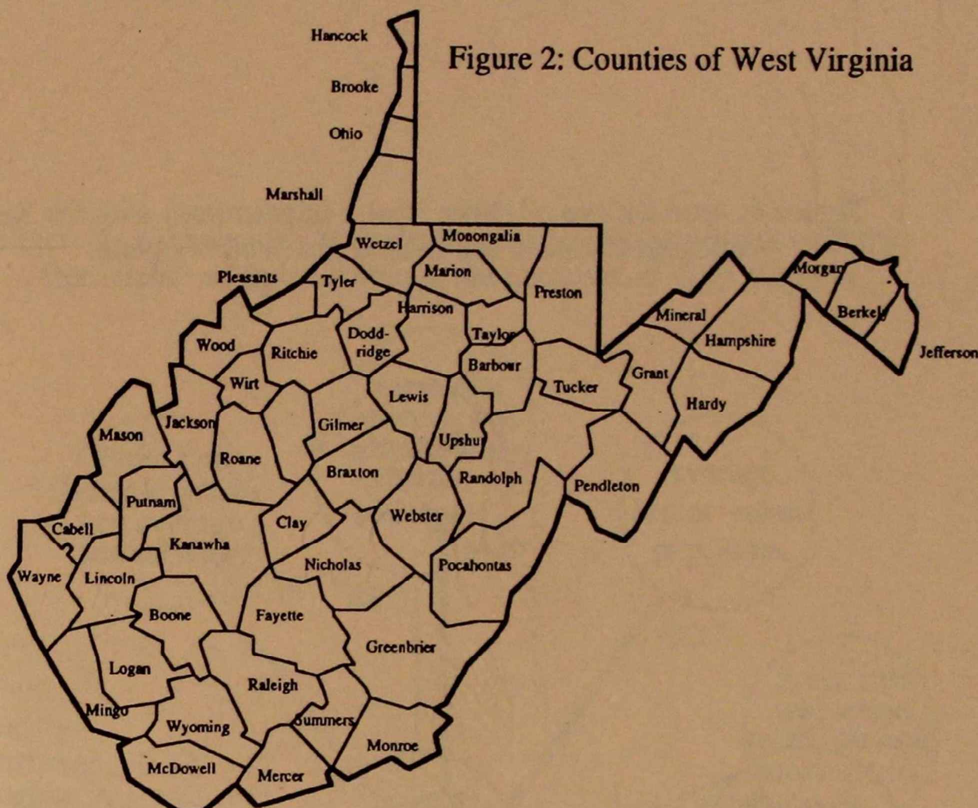


Figure 2: Counties of West Virginia

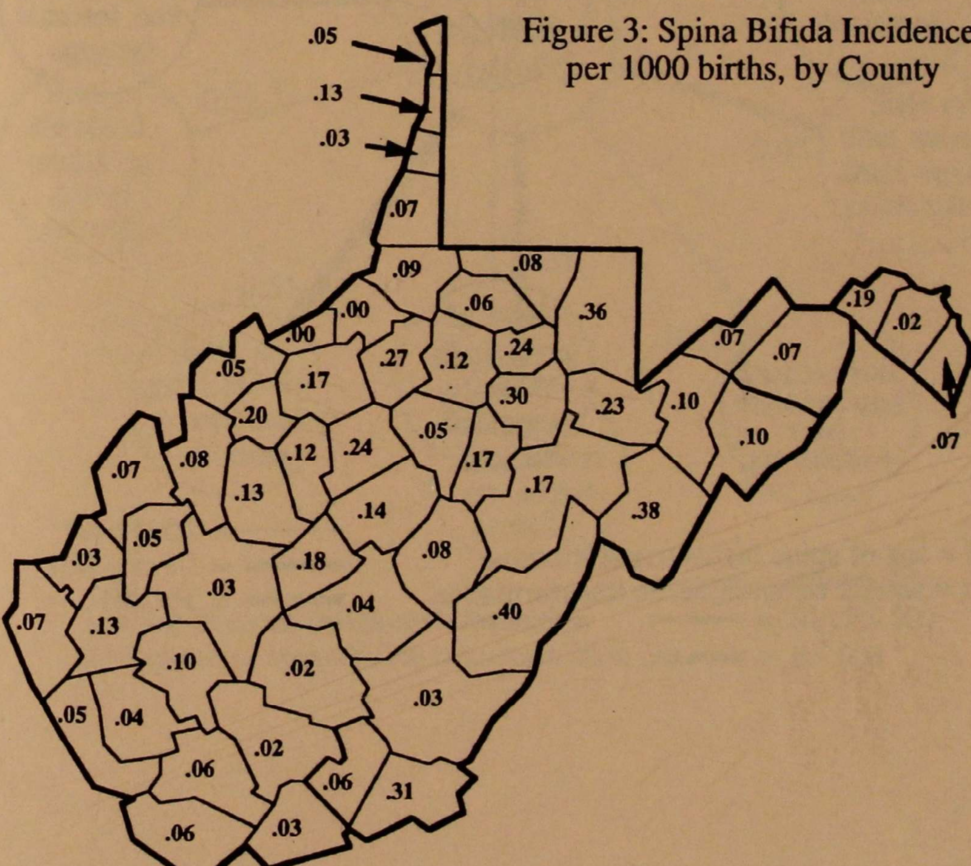


Figure 3: Spina Bifida Incidence per 1000 births, by County

Figure 4: Associations of Other Health Impairments with the Rate of Spina Bifida in the Counties of West Virginia.

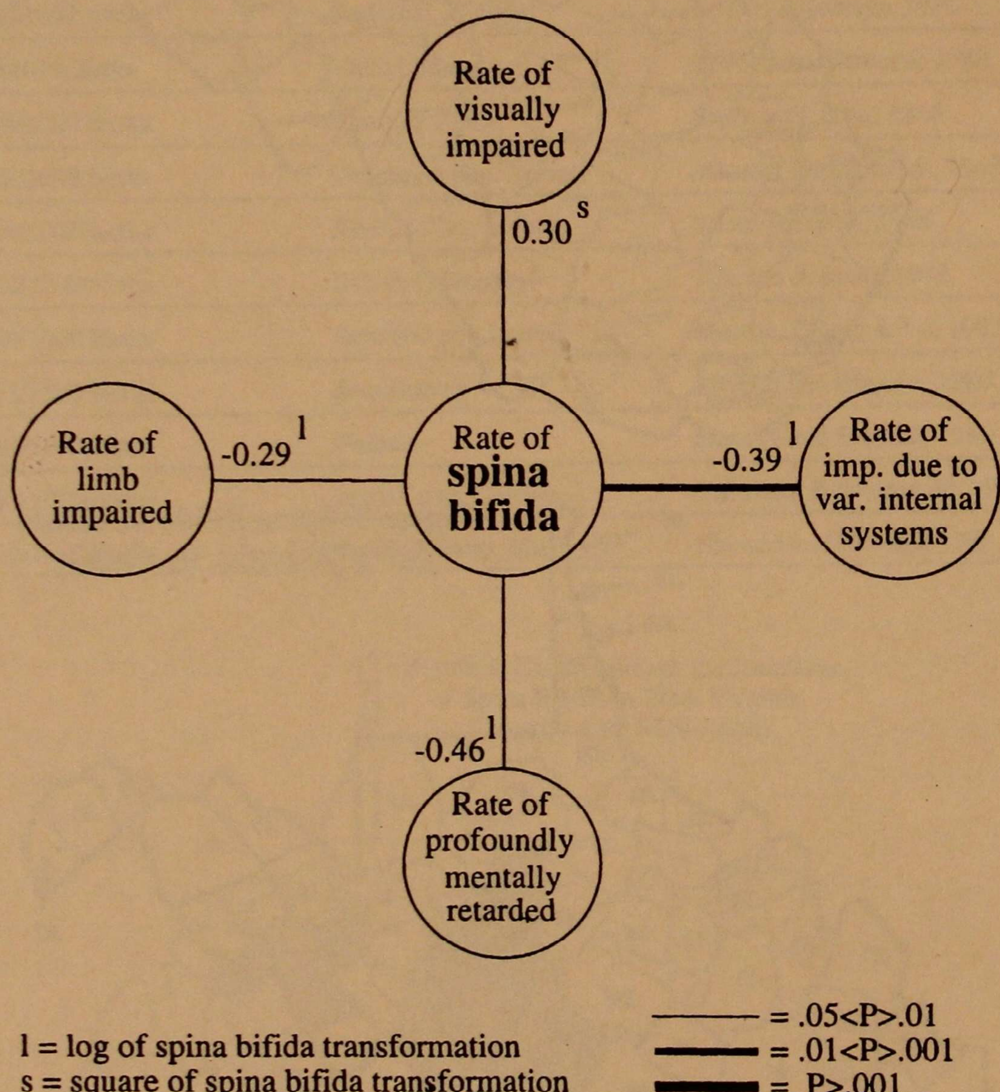
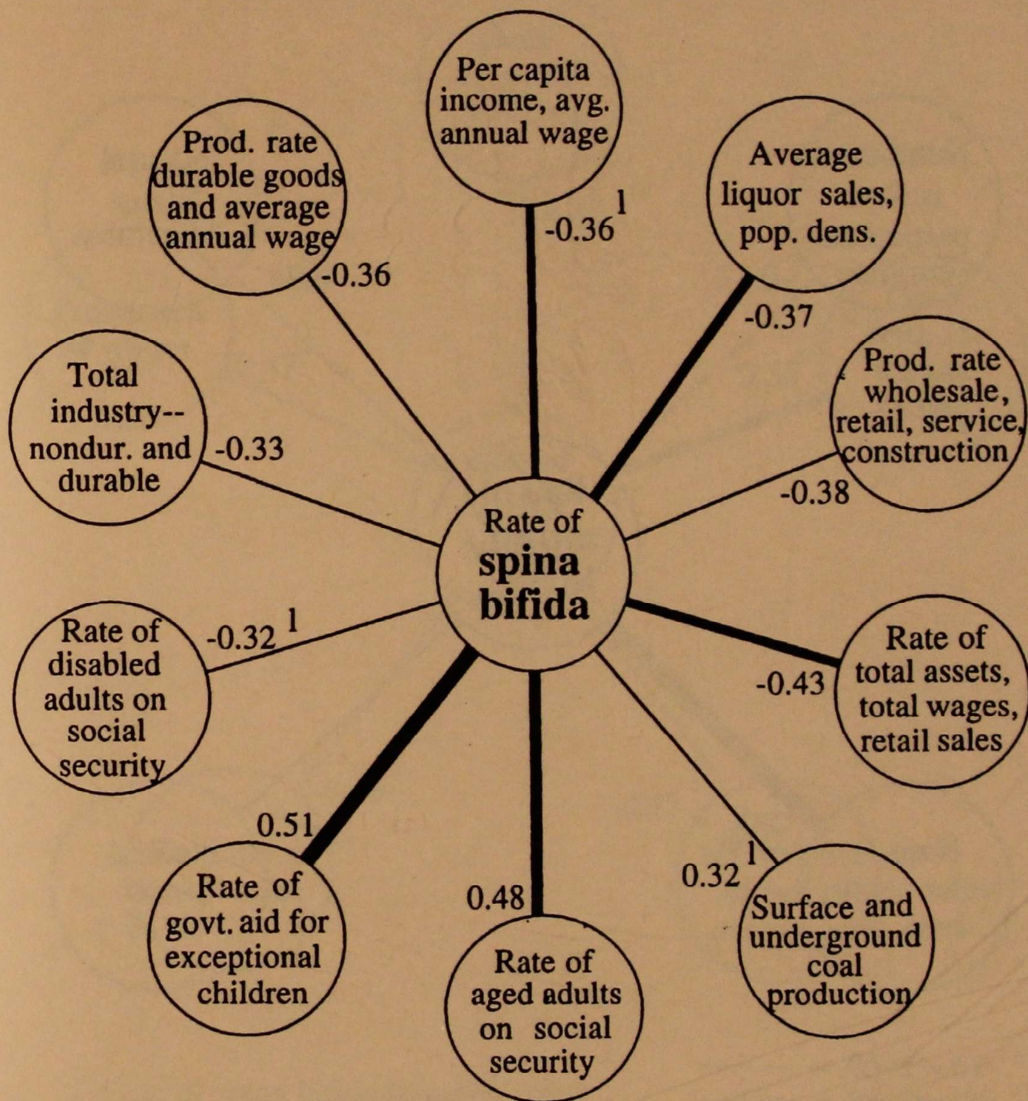


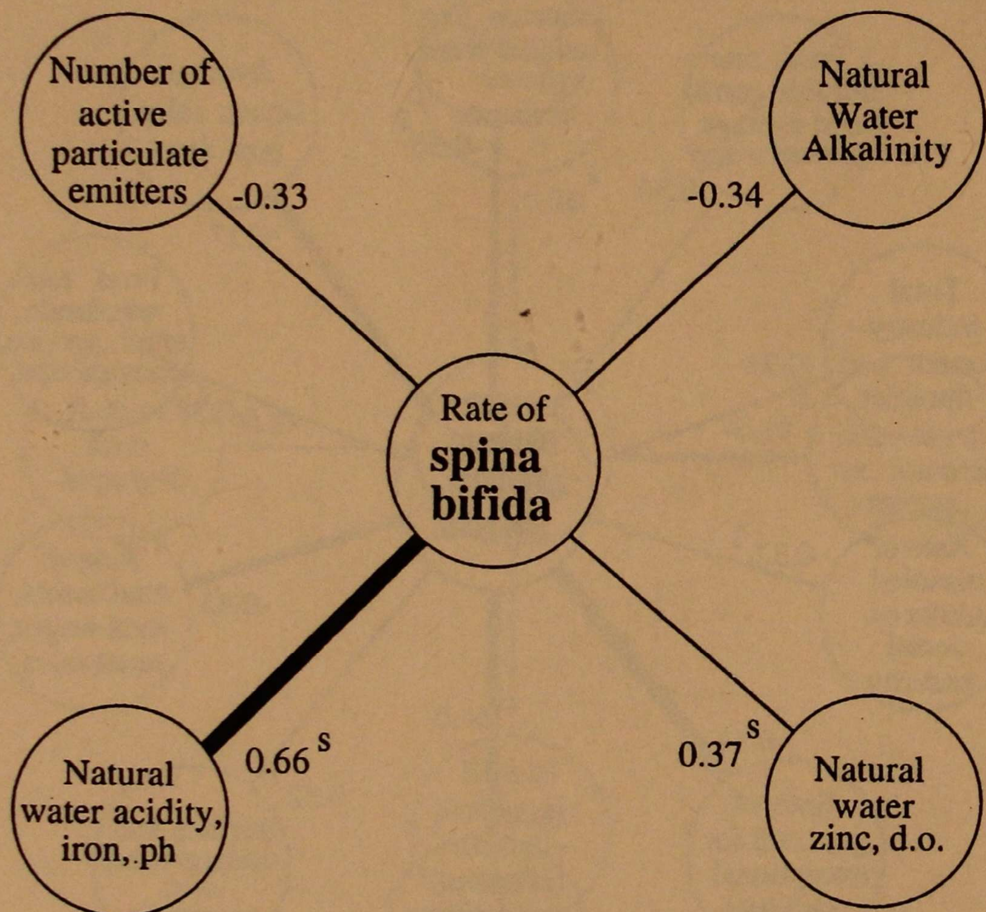
Figure 5: Associations of Socio-Economic Characteristics with the Occurrence of Spina Bifida in the Counties of West Virginia



1 = log of spina bifida transformation
s = square of spina bifida transformation

= .05<P>.01
= .01<P>.001
= P>.001

Figure 6: Associations of Environmental and Personal Aspects of Individuals with the Occurrence of Spina Bifida in the Counties of West Virginia



l = log of spina bifida transformation
s = square of spina bifida transformation

(thin line)	= .05<P>.01
(medium line)	= .01<P>.001
(thick line)	= P>.001

Figure 7: Associations of Genetic and Related Attributes with the Rate of Occurrence of Spina Bifida in the Counties of West Virginia

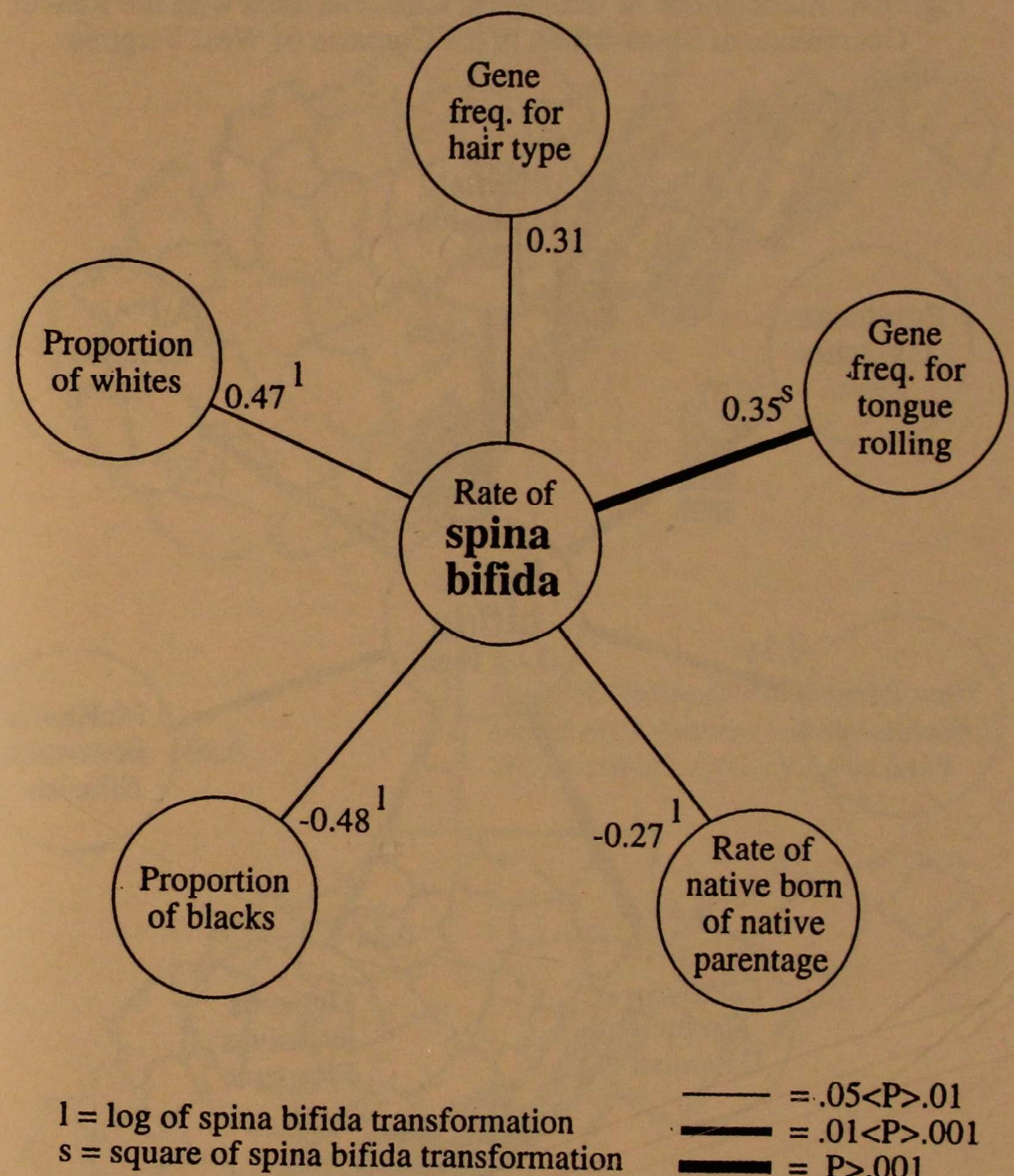


Figure 8: Associations of Geological Characteristics with the Rate of Occurrence of Spina Bifida in the Counties of West Virginia

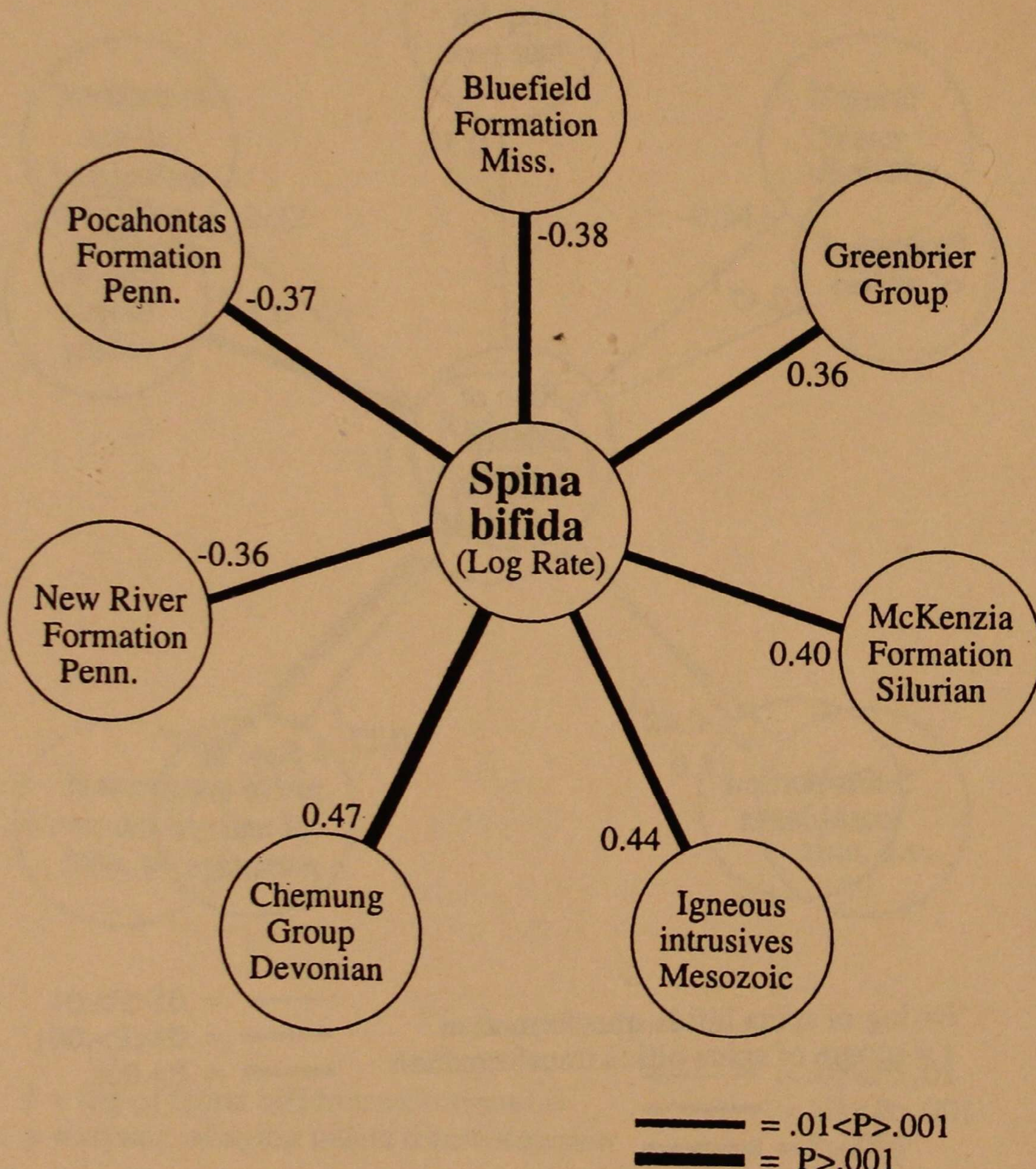


Figure 9: Geological Formations Positively Associated with the Occurance of Spina Bifida in West Virginia

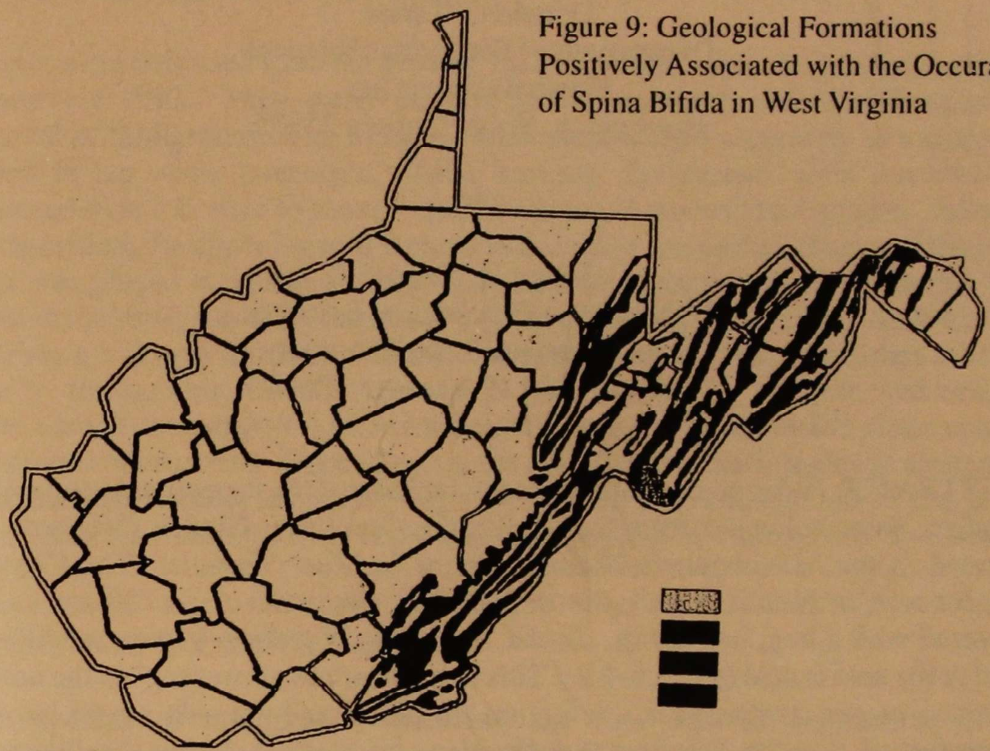
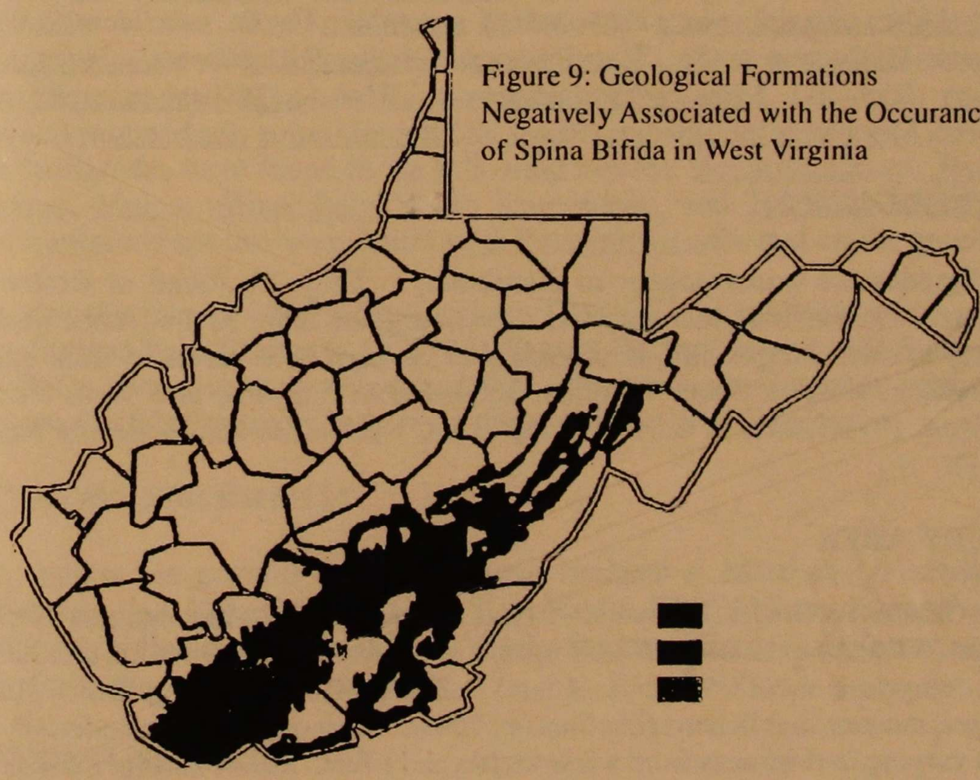


Figure 9: Geological Formations Negatively Associated with the Occurance of Spina Bifida in West Virginia



First records of tardigrades (Phylum: Tardigrada) from mosses in the Cranberry Glades Botanical Area in the Monongahela National Forest, Pocahontas County, West Virginia

Donald C. Tarter
Department of Biological Sciences
Marshall University
Huntington, West Virginia 25755

and

Diane R. Nelson
Department of Biological Sciences
East Tennessee State University
Johnson City, Tennessee 37614

ABSTRACT: Nine species of tardigrades, including two state records and one new species, were collected from mosses in the Cranberry Glades Botanical Area located in the Monongahela National Forest in West Virginia. The Cranberry Glades area, located at an elevation of 3400 feet, comprises about 750 acres of land covered with a bog, bog forest, shrubs, and areas of sedges, mosses, and lichens. Soil in the area is acid (pH, 3.8-6.2). This bog, known as a "muskeg" in the northern sections of our continent, is a refugium for plants and animals whose ancestors were forced into the Southern Appalachians by glaciers during the Pleistocene period. The following species of tardigrades, including the state records, Macrobiotus pseudofurcatus Pilato and M. spectabilis Thulin, were identified from mosses: Diphascon n. Sp., Hypsibius convergens (Urbanowicz), Isohypsibius sattleri (Richters), Macrobiotus harmsworthi Murray, Milnesium tardigradum Doyere, Minibiotus intermedius (Plate), and Ramazzottius oberhaeuseri (Doyere).

INTRODUCTION

Tardigrades are cosmopolitan in distribution. They are found in freshwater, marine, and terrestrial microhabitats. The tardigrade fauna of the United States is poorly known. Regionally, tardigrade species have been recorded from eastern Tennessee, western North Carolina, southeastern Virginia, and West Virginia (Nelson, 1975; Tarter et al., 1989; Tarter and Nelson., 1990; Tarter and Nelson, 1991).

STUDY AREA

The Cranberry Glades Botanical Area is located in the Monongahela National Forest, Pocahontas County, West Virginia. It is located at an elevation of 3400 feet and comprises about 750 acres of land covered with a bog, bog forest, shrubs, sedges, mosses, and lichens (Darlington, 1943). Most of the area is underlain with peat varying in thickness from a few inches to 11 feet. Acidic soil (pH, 3.8-6.2) is characteristic of the area. The bog is a refugium for plants and animals whose ancestors were forced into the Southern Appalachians by glaciers during the Pleistocene Period.

MATERIALS AND METHODS

Mosses were collected from the ground, rocks, and trees in the bog forest during the summer of 1992. They were returned to the laboratory for identification and removal of tardigrades. The moss samples were soaked separately in a stoppered funnel in tap water overnight. After soaking, the mosses were removed and squeezed over a beaker to remove the remaining water and tardigrades. After the debris settled, the upper layer of water was decanted and a small aliquot of the lower layer was placed in a petri dish and searched for tardigrades. If the sample were positive for tardigrades, the sample was fixed by adding hot ethanol. An Irwin loop (200 um x 500 um) was used to transfer the tardigrades from petri dishes to slides. Finally, tardigrades were mounted in Hoyer's mounting medium and oriented under a small coverslip (#1) for identification under an Olympus BH phase contrast compound microscope. Depending on the abundance of tardigrades in a sample, a representative number (usually 5-10) was removed from each sample. Tardigrades were identified mainly with keys and descriptions in Ramazzotti and Maucci (1983).

RESULTS AND DISCUSSION

Nine species of tardigrades, including the new species *Diphascon* n. sp., representing seven genera (Diphascon, Hypsibius, Isohypsibius, Macrobiotus, Milnesium, Minibiotus, Ramazzottius) were identified from mosses in the Cranberry Glades Botanical Area (Table 1). Two species, Macrobiotus pseudofurcatus andM. Spectabilis, are state records. The most common species were Hypnum convergens and Ramazzottius oberhaeuseri.

The tardigrades were found in the following mosses: Brachythecin sp., Hypnum pratense, Mnium affine, Polytrichum jumperinum, and Thiudium recognitum. Five tardigrade species were found in T. Recognitum collected on the ground.

Based on this investigation at the Cranberry Glades Botanical Area and the records from Spruce Knob (Tarter et al., 1989), Seneca Rocks (Tarter and Nelson, 1991) and Dolly Sods (Tarter and Nelson, 1992), 24 species of tardigrades, including a new species of Diphascon, have been reported from West Virginia.

ACKNOWLEDGEMENTS

The authors are grateful to the following students at Marshall University for assistance: Amy Kokesh, Steve Love, Kim Martin, Cheryl Nease, Nancy Powers, and Brent Williams. We thank Tom Weaks for identification of mosses, and Lu Ann South for typing the manuscript.

LITERATURE CITED

1. Nelson, D. R. 1976. Ecological distribution of Tardigrada on Roan Mountain,

Tennessee--North Carolina. Mem. Ist. Ital. Idrobiol. 32 Suppl.:225-276.

2. Darlington, H.C. 1943. Vegetation and substrate of Cranberry Glades, West Virignia. Bot. Gaz. 104(3):371-393.

3. Ramazzotti, G., and W. Maucci. 1983. II Phylum Tardigrada 3rd ed. Mem. Ist. Ital. Idrobiol. 41:1-1012.

4. Tarter, D., K. Ruggles, S. Gillenwater, and D. Nelson. 1989. First records of water bears (Phylum: Tardigrada) from West Virginia. Proc. W. Va. Acad. Sci. 61(2-4):96-99.

5. Tarter, D. C., and D. R. Nelson. 1990. An altitudinal comparison of the tardigrade fauna (Phylum: Tardigrada) from mosses on Spruce Mountain, West Virginia. Proc. W. Va. Acad. Sci. 62 (2-4):134-150.

6. Tarter, D., and D. Nelson. 1991. Tardigrade fauna (Phylum: Tardigrada) in mosses and liverworts from Seneca Rocks in the Monongahela National Forest, Pendleton County, West Virginia. Proc. W. Va. Acad. Sci. 63:(2-4) 109-112.

Table 1. Checklist of tardigrades from Cranberry Glades Botanical Area, Pocahontas County, West Virginia.

Class Eutardigrada
- Order Parachela
  - Family Macrobiotidae
    - Macrobiotus harmsworthi Murray, 1907
    - M. pseudofurcatus Pilato, 1972
    - M. spectabilis Thulin, 1928
    - Minibiotus intermedius (Plate, 1888)
  - Family Hypsibiidae
    - Diphascon n. sp.
    - Hypsibius convergens (Urbanowicz, 1925)
    - Isohypsibius sattleri (Richters, 1964)
    - Ramazzottius oberhaeuseri (Doyere, 1840)
- Order Apochela
  - Family Milnesiidae
    - Milnesium tardigradum Doyere, 1840

The West Virginia Speleological Survey As a Source of Water-Related Information

Eberhard Werner, P.O. Box 795, Morgantown, WV 26507

INTRODUCTION

West Virginia, like most states, contains areas underlain by rocks that are soluble in natural waters. The resulting solution features -sinkholes, caves, sinking streams, and large springs - are characteristic of a type of terrain known as karst. The flow of water above and below ground in such areas is unusual when compared to areas underlain by other rock types. It is difficult to obtain systematic information regarding water flow, especially ground water, in normal terrains; karst terrains with their more complex flow patterns present an even greater information gathering problem to planners and developers.

Early in 1975, while construction was in progress on Corridor H in Randolph County, caves were encountered during excavation of the roadbed. These caves are the upstream portions of conduits which feed springs used by the federal fish hatchery at Bowden (Garton, 1977). As is typical of karst conduits, there is no filtering of sediment once surface water enters them, and the springs serving the hatchery became muddy, which resulted in a massive fish kill. Additional problems developed with the road and bridge construction because of the poor foundation conditions typical of karst areas which are often not detected by conventional engineering investigations. This situation was readily predictable by any karst geologist. Although unknown to the highway department at the time, geologists familiar with the special features of karst terrains, and reasonably familiar with the terrain on which the highway was being built, were available for consultation.

A similar problem occurred at the state fish hatchery at Edray in Pocahontas County during the construction of West Virginia Route 150 (Werner, 1983). Here muddy water from the construction area flowed into sinkholes feeding underground conduits which led to the springs serving as the hatchery water supply. This area had already been extensively studied by karst researchers and water flow patterns were well known at the time. Unfortunately, no consideration was given to these flow patterns in planning the route or, subsequently, in routing the runoff from the highway construction.

Water contamination problems other than muddy water exist in the karst areas. A common technique for disposal of waste is to dump the waste into a nearby sinkhole. In some cases, whole communities dump into one large sinkhole, such as the sinkhole above Huntersville in Pocahontas County. Often the drainage from these sinkholes travels quickly without filtration to a nearby spring or well that might serve as a drinking water source. The complexity of the flow paths prevents an easy determination of connections (for example, see Jones, 1973).

The total outcrop area of soluble rocks (almost all limestones) in West Virginia is a relatively small percentage (about 10%) of the total surface, but the area influenced by karstic water flow is much greater. Karst flow systems dominate ground water flow in much of the eastern third of West Virginia, and conduit flow systems are the rule in the outcrop belt of the Mississippian Greenbrier group of limestones. In other limestone outcrop areas, flow may be through smaller openings such as only slightly enlarged fractures, or through a relatively diffuse, almost Darcyan, system. Often karstic water flow is the dominant form of flow even when the limestones do not outcrop in the area such as in eastern Hampshire and Hardy counties where the Helderberg limestones underlie the outcropping Oriskany sandstone. Presence or absence of potentially soluble carbonate rocks in outcrop does not necessarily define a karst groundwater flow system.

Although water contamination problems are common in karst areas, these are not the only water-related problems. Sinkholes are characteristic of karst terrains, but many are caused or aggravated by concentrated water flow. Routing of stormwater or wastewater into karst sinkholes often precipitates catastrophic collapses at the site of the water entry or above the subterranean channels through which that water flows. Delineating these channels cannot usually be done by conventional hydrologic investigations; it requires special knowledge of karst terrains. The flow of water, both above and below ground, in areas influenced by karst development is often radically different from that of other areas.

Until relatively recently, most of the West Virginia karst areas were farming areas or forest land, and had a relatively low population density. Now, however, more people are moving into these areas, especially in the eastern panhandle, the Greenbrier valley of the southeastern part of the state, and recreational areas such as Canaan Valley. As development occurs in these areas which are underlain by rocks susceptible to solution, more and more problems are expected. Prediction of such problems by conventional engineering or even geological techniques is often difficult. Because karst terrains usually have different hydrogeologic characteristics from other rock terrains, different methods of investigation are called for. Generally, more extensive investigations are required, and more detail is needed.

In this country, until recently, most karst studies have been done by amateur scientists, or by professionals on their own time. The study of geomorphology, and of karst in particular, has been considered as something of a stepchild of both geology and geography, with neither discipline fully accepting it as legitimate. Funding for such studies has not been high or frequent. One result of this situation is that although a great deal of information has been collected dealing with the karst terrains, little of it appears in the "conventional" literature. Those who work in the field are generally familiar with the sources while those not working in karst areas, are usually completely ignorant of its existence. Although this situation has changed in recent years, there are still many geologists and engineers working who date from earlier times. In many cases, knowledge of recharge areas and subterranean water flow paths through the karst could be sufficient to eliminate or at least reduce the frequency or severity of the type of problems mentioned above.

In an effort to preserve and disseminate the information collected by karst researchers in West Virginia, the West Virginia Speleological Survey (WvaSS) was founded in 1971. WvaSS is a study group of the National Speleological Society, and is incorporated as a non-profit organization. The primary activities of the groups are to informally coordinate karst research activities within the state, to serve as an umbrella organization for publications dealing with caves and karst studies, and to provide continuity for the constantly changing group of individuals working in the West Virginia karst, many of whom do not reside within the state.

At this writing (1993), eleven monographs have been produced as a series of Bulletins with several more in progress. Most of these bulletins deal with caves in particular areas, but there have also been monographs on cave-dwelling animals, a bibliography of West Virginia karst literature, and several in progress on archaeology, history, and paleontology.

Aside from the publications distributed, the WVaSS keeps a systematic information file on caves within the state which includes locations and important characteristics. For those planning construction projects in karst terrains in the state, this file may provide information to circumvent major problems such as those that have occurred int he past as noted above. Presently, a file is also being kept on the reports of animals in or near caves; this includes several threatened and endangered species. Other files are in the planning stage. In all cases, the WVaSS will serve to refer thôse in need of information and expertise to individuals who might provide help. The WVaSS maintains contacts with investigators in all aspects of karst studies, including the traditional scientific disciplines of archaeology, history, and management of caves and karst terrains. This allows the WvaSS to provide sources not only of hydrogeologic data of an area, but also of related matters as well. Other organizations maintain more complete lists of contacts within the state for specific disciplines, but the WVaSS seeks to be informed about all aspects of a particular type of terrain-karst.

LITERATURE CITED

1. Garton, E. Ray. 1977. The effects of highway construction on the hydrogeologic environment at Bowden, West Virginia. In Hydrologic problems of karst regions.

2. Jones, William K. 1973. Hydrology of limestone karst in Greenbrier County, West Virginia. West Virginia Geological and Economic Survey.

3. Werner, Eberhard. 1983. Effects of highways on karst springs - an example from Pocahontas County, WV. In Environmental Karst. Percy H. Dougherty, Editor. Cincinnati: GeoSpeleo Publications. P. 3-13.

(The present contact address for the West Virginia Speleological Survey is P. O. Box 200, Barrackville, WV 26559; any address changes will also be filed with the National Speleological Society, Cave Avenue, Huntsville, AL 35810.)