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Changes in Vegetation and Bobwhite Quail and Eastern Cottontail Rabbit Use in a Converted Fescue Field

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ABSTRACT

A pair of approximately 8.1 ha (20 acre) tall fescue (*Festuca arundinacea*) dominated fields at Kleber Wildlife Management Area (KWMA), Owen County, KY, were chosen to determine the effects of converting fescue-dominated vegetation to an orchard grass/legume mixture on bobwhite quail (*Colinus virginianus*) and eastern cottontail rabbit (*Sylvilagus floridanus*) habitat and utilization. One field was treated and 1 remained untreated for comparison. The treated field showed increased plant species diversity (66 spp. on untreated/101 spp. on treated), a higher percentage bare ground the year of treatment (10.4% on untreated/24.4% on treated), and a greater abundance of legumes (6 spp. of legumes on untreated, none having >1.0% total cover/8 spp. of legumes on treated, 6 having >1.0% total vegetative cover). The treated field had 27 plant species providing at least 1.0 per cent cover while the untreated field had 10 species. An index value was calculated to compare the value of the vegetation of the fields to bobwhite quail and eastern cottontails. The treated field was rated at 59 points and the untreated field rated at 14 points (the higher the index value, the better the habitat). Quail and rabbit use of the treated field increased while the untreated field remained unutilized. Costs involved to implement the fescue conversion and mosaic mowing were \$182.15/ha plus approximately 20 manhours/ha labor.

INTRODUCTION

Eastern cottontail rabbit and northern bobwhite quail are 2 of the most important game species in Kentucky. Recent data indicate the eastern cottontail rabbit to be the third most sought-after game species in the state with approximately 160,000 rabbit hunters harvesting 1.5 million cottontails annually (1, 2). Rabbit hunting provides an estimated 1.4 million mandays of recreation to Kentucky sportsmen while contributing about 23.7 million dollars to local economies (1, 2). The northern bobwhite quail is the number 1 game bird in Kentucky and ranks 6th in popularity as a Kentucky game species (1).

Northern bobwhite quail and eastern cottontail rabbit populations have shown long-term declines in Kentucky over the last 3 decades (3). These population trends are gen-

erally attributed to habitat losses due to major changes in land-use practices such as urban sprawl, intensification of commercial agricultural tillage practices, and widespread conversion of open lands, pastureland and hayland to "KY 31" tall fescue (*Festuca arundinacea*) dominated stands. Of the approximately 7.0 million acres of grassland in Kentucky, 5.5 million acres have been planted to "KY 31" tall fescue cover (4).

Wildlife biologists have long recognized the low wildlife habitat value provided by dense sod forming grasses such as "KY 31" tall fescue (5-13). The highly competitive and invasive growth of "KY 31" results in reduced plant species diversity within hayland/pastures and old fields. The dominance of "KY 31" over other pasture and old field species has resulted in an almost uniform coverage of Kentuc-

ky's open lands. A second problem noted with "KY 31" is the thick, rank growth habit characteristic of the species which restricts movements of wildlife species and retards plant succession.

More recent evidence has revealed a third problem associated with KY 31, or more appropriately, with the endophytic fungus (*Acremonium coenophialum*) which lives in "KY 31". Lacefield et al. (14) and Siegel et al. (4) reported nearly 80% of the tall fescue fields in Kentucky are infected with the endophyte, at a rate of 80% or higher. Data indicate a diet of endophyte-infected "KY 31" impacts the reproductive potential of laboratory animals and livestock (i.e., 15-21) through decreased sperm and egg counts, smaller than normal litter sizes, lowered lactation rates, poor weight gains, elevated body temperatures, and abortion or absorption of fetuses. Sadler (8) found cottontail rabbit reproduction and survival were reduced when rabbits were kept in outdoor pens vegetated with tall fescue. Betsill et al. (7) reported cottontails in North Carolina avoided areas containing fescue.

Recognizing that lowered reproductive potential reported in laboratory animals and livestock may apply to wild cottontails and other wildlife species living in fescue-dominated habitat, the Kentucky Department of Fish and Wildlife Resources (KDFWR) recommends conversion of "KY 31" tall fescue fields to other cover types by private landowners interested in improving wildlife habitat on their land. Terrain often dictates no-till conversion methods be used. In conjunction with fescue conversion, KDFWR often recommends mowing practices be implemented to improve interspersed of escape cover.

This study was initiated to determine the effect of converting "KY 31" tall fescue-dominated fields to another grass/legume mixture, coupled with mosaic pattern mowing to improve escape-cover interspersed, on northern bobwhite quail and eastern cottontail rabbit populations. The costs associated with no-till conversion of tall fescue were also documented.

STUDY AREA

The Kleber Wildlife Management Area is located approximately 35.4 km northeast of Frankfort, within the Outer Bluegrass phys-

iographic region, in Owen and Franklin counties, Kentucky. This 929 ha area is characterized by steep rolling hills with narrow flat ridge tops and narrow stream valleys. Elevations range from 198 to 274 m msl. Approximately 60% of the area is wooded, with eastern red cedar (*Juniperus virginianus*) dominated old fields and woodlands and oak-hickory dominated woodlots. The remaining 40% of the area is old-field type open lands dominated by tall fescue. Kleber Wildlife Management Area (KWMA) was chosen as a study site because habitat conditions were very similar to those typically found on privately owned land in many parts of the state.

MATERIALS AND METHODS

Two fescue-dominated fields (1 control, 1 conversion), each about 8.1 ha in size, were chosen for study on KWMA. Baseline data documenting bobwhite quail and cottontail rabbit use and vegetative composition were gathered for both fields for 1-year prior to treatment (1987). A fescue conversion project was implemented on 1 field in 1988. Vegetative composition and quail and rabbit use were monitored on both fields through 1990.

Vegetative Sampling.—Vegetative sampling was conducted annually on both fields during September. These data were collected 1 year prior to treatment (1987) and for 3 years following treatment (1988-1990). Forty 1-m² sample plots were positioned at 30.5 m intervals along line transects. Transects were designed to bisect all slopes and aspects present and were marked with steel fence posts. A compass bearing from 1 post to the other was followed to make the yearly sample points as consistent as possible. Ocular estimates were made of per cent total vegetative cover, per cent bare ground, average vegetation height, and per cent total cover contributed by each plant species.

Individual species were ranked by per cent total cover contributed in each treatment type. Plant species with >4.0% total cover were assigned a subjective numerical rating of good (=2), fair (=1) or poor (=0) as food and cover for quail and rabbits based on literature review (i.e., 5, 11-13, 22-41). An overall numerical index value for each field was derived by summing index values for each plant species providing at least 4.0% total cover.

Quail and Rabbit Sampling.—Flush-drive censuses (42) were conducted in September for 1 season prior to treatment (1987), during the treatment year (1988) and for 2 years (1989–1990) following treatment. Surveys were conducted by spacing personnel approximately 6 m apart and traversing the fields in a manner to cover each area as entirely as possible. Number of quail coveys, total birds per covey and the number of rabbits flushed were determined. Efforts were made to watch the direction of flush and subsequent movement of animals to eliminate recounting.

Fescue Conversion.—One field served as a fescue-dominated control and the other field was converted from a fescue-dominated vegetative cover to a grass/legume mixture. On 5 March 1988, the treatment field was limed in accordance with soil test recommendations. On 28 March 1988, a prescribed fire was used on the entire treatment field to remove heavy litter and duff from the ground. On 14 April 1988, approximately 50% of the area was sprayed with glyphosphate (Roundup) at a rate of 3.9 liter/ha to kill the vegetation. Portions of the field were not sprayed due to steepness of the slopes. A no-till drill was used to plant a mixture of orchard grass (*Dactylis glomerata* at 5.8 kg/ha), ladino clover (*Trifolium repens* at 3.9 kg/ha), red clover (*Trifolium pratense* at 2.4 kg/ha) and Korean lespedeza (*Lepedeza striata* at 3.9 kg/ha) on 26 April 1988. Total costs were recorded and a cost/area rate determined.

Mosaic pattern strip-mowing was conducted during late July 1988 and late July 1990 on the treatment field to control woody plant encroachment and create a mosaic of herbaceous and early succession woody cover that would provide more optimum quail and rabbit habitat (9, 39).

RESULTS AND DISCUSSION

Baseline vegetative sampling, completed in September 1987, showed the 2 fields to be similar in vegetative composition prior to treatment. Both fields were dominated by "KY 31" tall fescue and bluegrass (*Poa pratensis*). The untreated field also had a fairly high component of prairie dropseed (*Sporobolus asper*).

A total of 115 plant species was identified from the vegetative sampling during all years of the study. Sixty six plant species were doc-

umented in the untreated field and 101 in the treated field (Table 1). Forty one (41) species were unique to the treated field and six species were only found in the control field (Table 1).

The fescue conversion to the chosen grass/legume mixture was completed in April 1988 and can be considered only a partial success. The kill on the fescue was not total, and fescue came back as a codominant cover species in the treated field during the study period. Germination and survival of the planted grass/legume mixture was poor due to the severe 1988 drought; orchard grass was essentially lost but clovers and Korean lespedeza survived better. Korean lespedeza survived the drought better, providing 10.6% and 6.7% vegetative cover in the treated field for 2 years following treatment. Legumes responded well to treatment, resulting in 6 species of legumes with at least 1% total cover on the treated area. No legumes documented in the control field provided 1% cover.

Conversion was successful at increasing overall plant diversity and the number of dominant plant species (Tables 1, 2). The treated field had 27 species of plants providing at least 1.0% cover compared to 10 species on the untreated field. Likewise, when looking at plant species providing 4.0% or more cover, the treated field had 11 species while the untreated field had 4 species (Table 2). An index to post-treatment quality of plant cover showed the untreated area to have an index of 4 for quail and 10 for rabbit; for an overall rating of 14. The treated area had an index of 27 for quail and 32 for rabbit; an overall rating of 59 (Table 2).

While the percentage of bare ground remained nearly constant and averaged 11.2% on the untreated site, bare ground increased from 14.4% pretreatment to 24.4% on the treated field the year immediately following treatment. However, during the 2 years post-treatment the per cent bare ground was lower (7.8%) on the treated field than in the untreated field. Ideal quail habitat should have been between 30% and 60% bare ground to provide adequate space for feeding and movement (39).

With the exception of the decrease in bare ground in years 2 and 3 following treatment, all the vegetation changes observed on the

TABLE 1. Plant species identified on fescue converted and control fields at the Kleber WMA, Owen County, Kentucky.

Species	Common name	Untreated	Treated
<i>Acalypha rhomboidea</i>	3-Seeded Mercury	x	x
<i>Achillea millefolium</i>	Yarrow	x	x
<i>Agrimonia pubescens</i>	Agrimony	x	x
<i>Allium</i> sp.	Wild Onion	x	—
<i>Ambrosia artemisiifolia</i>	Ragweed	x	x
<i>Andropogon virginicus</i>	Broomsedge	x	x
<i>Antennaria plantaginifolia</i>	Pussy-toes	—	x
<i>Apocynum cannabinum</i>	Indian Hemp	—	x
<i>Asclepias syriaca</i>	Big Milkweed	—	x
<i>Asclepias tuberosa</i>	Butterfly Weed	x	x
<i>Aster ericoides</i>	Aster	x	x
<i>Aster patens</i>	Aster	—	x
<i>Barbarea</i> sp.	Mustard	—	x
<i>Blephilia ciliata</i>	Blephilia	x	x
<i>Campsis radicans</i>	Trumpet Vine	—	x
<i>Carex</i> sp.	Sedge	x	x
<i>Carya ovata</i>	Shagbark Hickory	—	x
<i>Celtis occidentalis</i>	Hackberry	—	x
<i>Chrysanthemum leucanthemum</i>	Ox-eye Daisy	x	x
<i>Cirsium arvense</i>	Canada Thistle	x	x
<i>Cirsium vulgare</i>	Bull Thistle	—	x
<i>Coreopsis lanceolata</i>	Wingstem	—	x
<i>Cornus obliqua</i>	Silky Dogwood	—	x
<i>Crataegus</i> sp.	Hawthorn	—	x
<i>Croton</i> sp.	Doveweed	x	—
<i>Dactylis glomerata</i>	Orchard Grass	—	x
<i>Danthonia spicata</i>	Poor Man's Grass	x	—
<i>Daucus carota</i>	Queen Anne's Lace	x	x
<i>Desmodium paniculatum</i>	Tick Trefoil	x	x
<i>Dianthus armeria</i>	Deptford Pink	x	—
<i>Digitaria sanguinalis</i>	Crabgrass	—	x
<i>Dipsacus sylvestris</i>	Teasel	—	x
<i>Elymus virginicus</i>	Wild Rye	—	x
<i>Eragrostis capillaris</i>	Love Grass	x	x
<i>Erigeron annuus</i>	Daisy Fleabane	x	x
<i>Euonymus americanus</i>	Strawberry Bush	x	x
<i>Eupatorium serotinum</i>	Boneset	—	x
<i>Euphorbia corollata</i>	Flowering Spurge	x	x
<i>Euphorbia maculata</i>	Euphorbia	x	x
<i>Festuca arundinacea</i>	Tall Fescue	x	x
<i>Fragaria virginiana</i>	Wild Strawberry	x	x
<i>Fraxinus americana</i>	White Ash	—	x
<i>Gleditsia triacanthos</i>	Honey Locust	—	x
<i>Gnaphalium obtusifolium</i>	Gnaphalium	x	x
<i>Helianthus mollis</i>	Sunflower	—	x
<i>Houstonia purpurea</i>	Houstonia	—	x
<i>Hypericum punctatum</i>	St. John's Wart	x	x
<i>Ipomoea pandurata</i>	Morning Glory	x	x
<i>Lespedeza procumbens</i>	Trailing lespedeza	x	x
<i>Lespedeza striata</i>	Korean lespedeza	x	x
<i>Lespedeza virginica</i>	Virginian Lespedeza	x	—
<i>Lonicera japonica</i>	Honeysuckle	x	x
<i>Ludwigia</i> sp.	Rattlebox	—	x
<i>Lysimachia quadrifolia</i>	Whorled Loosestrife	—	x
<i>Maclura pomifera</i>	Osage Orange	—	x
<i>Melilotus alba</i>	White Sweet Clover	x	x
<i>Melilotus officinalis</i>	Yellow Sweet Clover	x	x
<i>Monarda fistulosa</i>	Monarda	x	x
<i>Osmorhiza</i> sp.	Sweet Sicily	—	x
<i>Oxalis stricta</i>	Wood Sorrel	x	x

TABLE 1. Continued.

Species	Common name	Untreated	Treated
<i>Panicum capillare</i>	Panic Grass	x	x
<i>Panicum clandestinum</i>	Deer Tongue	x	x
<i>Panicum microcarpon</i>	Panic Grass	x	x
<i>Parthenocissus quinquefolia</i>	Virginia Creeper	x	x
<i>Paspalum</i> sp.	Paspalum	x	x
<i>Physalis</i> sp.	Ground Cherry	x	x
<i>Plantago lanceolata</i>	Plantain	x	x
<i>Plantago major</i>	Plantain	x	x
<i>Poa pratensis</i>	Bluegrass	x	x
<i>Potentilla simplex</i>	Cinquefoil	x	—
<i>Prunus serotina</i>	Black Cherry	—	x
<i>Quercus prinus</i>	Chestnut Oak	—	x
<i>Rhus copallinum</i>	Winged Sumac	—	x
<i>Rhus glabra</i>	Smooth Sumac	x	x
<i>Robinia psuedoacacia</i>	Black Locust	—	x
<i>Rosa carolina</i>	Carolina Rose	x	x
<i>Rosa multiflora</i>	Multiflora Rose	x	x
<i>Rubus</i> sp.	Blackberry	x	x
<i>Rubus flagellaris</i>	Dewberry	x	x
<i>Rubus pensilvanicus</i>	Raspberry	x	x
<i>Rudbeckia hirta</i>	Black-eyed Susan	x	x
<i>Ruellia caroliniensis</i>	Wild Petunia	x	x
<i>Rumex acetosella</i>	Dock	x	x
<i>Sanicula canadensis</i>	Sanicula	—	x
<i>Setaria glauca</i>	Foxtail	x	x
<i>Smilax bona-nox</i>	Greenbriar	—	x
<i>Solanum carolinense</i>	Horse Nettle	x	x
<i>Solidago altissima</i>	Field Goldenrod	x	x
<i>Solidago nemoralis</i>	Gray Goldenrod	—	x
<i>Spiranthes cernua</i>	Ladies Tresses	x	—
<i>Sporobolus asper</i>	Prairie Dropseed	x	x
<i>Symphoricarpos orbiculata</i>	Coralberry	x	x
<i>Toxicodendron radicans</i>	Poison Ivy	x	x
<i>Tridens flavus</i>	Greasy Grass	x	x
<i>Trifolium pratense</i>	Red Clover	—	x
<i>Trifolium procumbens</i>	Yellow Hop Clover	x	x
<i>Trifolium repens</i>	White Clover	—	x
<i>Ulmus alata</i>	Winged Elm	—	x
Unknown mint	Sticky Purple Stuff	x	x
<i>Verbascum thaspus</i>	Woolly Mullein	—	x
<i>Verbena simplex</i>	Verbena	—	x
<i>Verbesina occidentalis</i>	Crownbeard	—	x
<i>Vernonia altissima</i>	Ironweed	x	x
<i>Viburnum prunifolium</i>	Black Haw	x	x
<i>Viburnum rifidulum</i>	Black Haw	x	x
<i>Viola</i> sp.	Violet	x	x
<i>Vitis vulpina</i>	Frosty Grape	—	x
Total species		66	101

treated field would be considered beneficial to quail and rabbits utilizing the area.

The mosaic mowing pattern implemented on the treated field had a positive impact on vegetation important to quail and rabbit. Escape cover resulting from the establishment of species such as blackberries, raspberries, dew-

berries (*Rubus* spp.), coralberry (*Symphoricarpos orbiculatus*), goldenrod (*Solidago altissima*), sweet clover (*Melilotus officinalis*), and crown beard (*Verbesina occidentalis*) was developed in desired patterns by mowing the treated field. Herbicide treatment released woody species from the fescue domination.

TABLE 2. Plant species with >4.0% cover found in 1 m² plots on fescue converted and control fields at the Kleber WMA, Owen County, Kentucky indexed to indicate value for food or cover for bobwhite quail and cottontail rabbits.

Species	Food value		Cover value		Quail index	Rabbit index	Total index
	Quail	Rabbit	Quail	Rabbit			
Converted field							
<i>Festuca arundinacea</i>	poor	poor	poor	poor	0	0	0
<i>Poa pratensis</i>	poor	good	fair	good	1	4	5
<i>Lespedeza procumbens</i>	good	good	fair	fair	3	3	6
<i>Lespedeza striata</i>	good	good	fair	fair	3	3	6
<i>Symphoricarpos orbiculata</i>	fair	good	good	good	3	4	7
<i>Solidago altissima</i>	poor	poor	fair	good	1	2	3
<i>Melilotus officinalis</i>	good	good	good	good	4	4	8
<i>Tridens flavus</i>	fair	fair	fair	fair	2	2	4
<i>Rubus</i> spp.	fair	fair	good	good	3	3	6
<i>Ambrosia artemisiifolia</i>	good	good	good	good	4	4	8
<i>Panicum capillare</i>	good	good	fair	fair	3	3	6
					27	32	59
Control field							
<i>Festuca arundinacea</i>	poor	poor	poor	poor	0	0	0
<i>Poa pratensis</i>	poor	good	fair	good	1	4	5
<i>Sporobolus asper</i>	poor	good	good	good	2	4	6
<i>Solidago altissima</i>	poor	poor	fair	good	1	2	3
					4	10	14

Mowing controlled woody invasion and provided escape cover approaching the optimum distribution of within 100 m of other cover types (9, 39).

There was a change in utilization of the fields by quail and rabbits following fescue conversion; no use was detected in surveys prior to treatment. The fall following treatment, at least 1 covey of quail had become established on the treated field and 1 rabbit was flushed during the survey. The next fall, a covey of birds was again found on the treated area and 3 rabbits were flushed. During the last survey period no quail were found; however, 5 rabbits were observed. It is assumed these animals represent an increase to the local populations. The numbers should be considered conservative. Each year rabbits were flushed which could not be positively identified as different from a previously flushed rabbit and were not counted. The flush-survey method has been shown to find approximately 50% of a quail population (41). Therefore, there were likely 2 coveys of quail using the treated field for 2 years immediately following treatment.

After the initial year following treatment, habitat quality for quail declined due to the lack of bare ground (39). By the 1990 survey, the vegetation on the treated field may have

become too thick for quail utilization: This suggests a need for vegetative disturbance on a 3-year rotation in order to keep ground level vegetation open enough for quail use.

No quail or rabbit use was found on the untreated field until the last survey. During that survey a pair of quail were flushed from a multiflora rose thicket in a draw crossing the field. It is hypothesized that these quail were simply using the brushy corridor to cross the area and were not residents of the field.

This single replication study cannot make conclusive statements on the value of no-till fescue conversion to rabbit and quail populations. However, the results support the practice of converting fescue to other plant cover types. Native and planted legumes, which are a major food source for quail and rabbits, (9, 12, 13, 22, 23, 26, 27, 30, 31, 36, 39, 43, 44), responded very favorably. Bare ground increased initially, to allow better movement and feeding by quail.

Cost analysis for the fescue conversion and mosaic pattern mowing are shown in Table 3. The costs for this project totaled \$182.15/ha for chemicals, no-till equipment rental, and seed for the area treated. About 20 manhours/ha labor were required to accomplish the task.

The costs for seed and no-till drill rental on this project were more than half the total ex-

TABLE 3. Cost analysis of a no-till fescue conversion project on a field at Kleber WMA, Owen County, Kentucky. Costs based on 1988 prices.

Item	Direct \$ cost	Manhours	Tractor fuel used
Prescribed fire		5 hr/ha	
Herbicide treatment			
Chemical	\$81.90/ha 3.9 liters/ha "Roundup" \$21/liter		
Spraying		7.5 hr/ha	0.4 ha/liter
No-till seeding			
Drill rental	\$7.75/ha	7.5 hr/ha (seeding)	0.4 ha/liter
Seed	\$92.50/ha per mix used on area		
Mowing		7.5 hr/ha/yr	0.4 ha/liter
Total costs	\$182.15/ha	20 hr/ha (implementation) 7.5 hr/ha/yr (mosaic maintenance mowing)	

penses. Due to the extreme drought conditions experienced during the 1988 growing season, very little resulted from planting the orchard grass/legume mixture. However, the grasses, forbs and legumes released by the burning and spraying alone provided excellent cover and food for bobwhite quail and eastern cottontails. This suggests little need for planting a grass/legume mixture on fescue conversion sites where enhancing wildlife habitat is the primary objective.

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Human Sex Ratio and Factors Influencing Family Size in Hunan, China

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ABSTRACT

In 1990, sex ratio and family size data were obtained on 1,002 families of university students from Hunan Province, China. Each student respondent provided data on the parental, present, and projected generations. Secondary sex ratios (males per 100 females) were 114.6, 101.3 and 110.0 and average numbers of children were 4.32, 3.36, and 1.65 for the parental, present, and projected generations, respectively. Disparity between observed and expected combinations of sexes of children among families of the parental and present generations indicated a preference for both sexes and for male children. No significant correlations were found between sexes of children within families. Student respondents expressed strong preferences for family size and for specific combinations and permutations of sexes of children. The most desired family consisted of 2 children including both sexes with the first-born being male. For the projected generation, the resulting sex ratio (110) would not create a serious imbalance of the sexes and the resulting average family size (1.65 children) would be supportive of China's population control effort.

INTRODUCTION

The increasing human population continues to be a global threat and a national concern for many countries. The human population is a function of the number of families and family size. Control efforts are largely directed toward family size. Reduction in family size appears to be the result of deliberate family planning.

Greater understanding of the human sex ratio and of the factors that influence family size would contribute to family planning. In some populations, evidence has been found that the combinations of sexes of existing children influenced the parents' decision to have additional children. In some cultures fewer families had additional children after both sexes were represented. In other cultures, the likelihood of additional children was influenced by the number of sons among existing children. Survey results from diverse cultures have revealed that individuals have definite preferences for family sizes consisting of specific combinations and permutations of sexes of children.

The China population is estimated to be approximately 1.1 billion and to be increasing at the rate of 1.4 percent annually, a rate that

would double the population in 49 years (14). Approximately 27 per cent of the population is under the age of 15 years. As the most populous country in the world, China's population control will have a substantial impact on the world population (22). Although the Chinese Government has given major emphasis to family planning since the early 1970s, the State Council's call for "only one child per couple" was issued in 1980 (21). It was acknowledged that implementation of the one-child-per-couple measure may result in social problems; however, that control would enable China to keep its population within a 1.2 billion limit.

The objective of the present investigation was to extend the basic studies of the human sex ratio and of the influence of combinations of sexes of children on family size to a population which has reached unequaled size and which has been subjected to unparalleled control measures.

POPULATION AND PROCEDURES

In 1990, data were obtained on sex of children and family size for 1,002 university students from Hunan Province, China. The students were enrolled at one of 3 educational institutions—Hunan Medical University, Changsha Medical School, or Hunan Teachers

University. Each student completed a data form providing information on the parental, present, and projected generations.

For the parental generation, information was collected on the number of children by sex for both the maternal and paternal parents. These data were used to calculate average family size and sex ratio, and for comparison of the observed and expected composition of sexes of children within various family sizes. For the present generation, information was obtained on sex of children by order of birth, permitting exploration of associations between sexes of children and the effects of combinations and permutations of sexes of children on family size. Linear correlation was used to test associations between sexes and chi-square for a 2×2 contingency table was used to test the independence of combinations of sexes and family size (17). For the projected generation, each student respondent indicated the desired number of children and the preferred combination and permutation of sexes. Using these data, average desired family size and the resulting sex ratio were calculated.

RESULTS

Binomial distribution.—The number of families of sizes 1 through 4 and their various combinations of sexes for the parental and present generations were determined. Expected frequencies of the various combinations of sexes were calculated using the observed sex ratio for each generation in the binomial expansion. In the parental generation, the only significant divergence between observed and expected combinations of sexes occurred in 2-children families. The combination of 1 male and 1 female was detected more frequently than expected. In the present generation, the only significant difference between observed and expected combinations occurred in three-children families. The combination of 2 males and 1 female appeared more frequently than expected. Disparity between observed and expected distributions of combinations of sexes within various family sizes was not found in other populations (8, 10, 13, 15).

Correlations between Sexes.—Correlation coefficients were calculated between sexes of consecutive and nonconsecutive children within families of the present generation (Table 1).

TABLE 1. Correlation coefficients between sexes of children of different births in the present generation.

Family size and birth order	Number	Correlation
All families		
Birth 1 vs. birth 2	778	-0.022 (ns) ^a
Birth 1 vs. birth 3	585	-0.039 (ns)
Birth 2 vs. birth 3	585	-0.052 (ns)
Families of two children		
Birth 1 vs. birth 2	193	-0.064 (ns)
Families of three children		
Birth 1 vs. birth 2	291	-0.051 (ns)
Birth 1 vs. birth 3	291	-0.111 (ns)
Birth 2 vs. birth 3	291	-0.112 (ns)

^a ns nonsignificant at the 0.05 level of probability.

Those associations were explored among the first three births for all families and for families consisting of only two or three children. All of the coefficients were low in magnitude, negative in sign, and nonsignificant. Sexes of successive children have been found to be positively correlated in some subsets of the population (4, 8, 10, 15), but not in others (5, 13).

Sex Ratios and Average Family Sizes.—Secondary sex ratios (males per 100 females) were 114.6 and 101.3 for the parental and present generations, respectively. If the true ratio is greater than 100, as indicated by the parental ratio, the ratio for the present generation was biased downward by the inclusion of an equal number of female and male respondents. These sex ratios are within the range of those reported for other subsets of the human population. Examples of ratios reported for other populations include: 101 for Black Americans (13), 106 for White Americans (18), 107 for Brazilians (8), 107 for Japanese (10), and 106 for Libyans (2). Sex ratios by order of birth were 91.4, 95.5, 117.5, and 98.6, respectively, for the first 4 births in the present generation (Table 2). The ratio of 117.5 for the third birth was significantly different from the others, but did not differ significantly from the overall ratio of 101.3 for the present generation. No biological explanation is evident for the aberrant ratio for the third birth.

Average numbers of children were 4.32 for the parental and 3.36 for the present generations. These averages were lower than corresponding values reported for Brazilian (8) and Libyan (2) populations. However, they were

TABLE 2. Sex ratios (males per 100 females) by order of birth in the present generation.

Birth order		Family		Combined for birth
		Stopped	Continued	
1	Number	20	778	798
	Sex ratio	122.2	90.7	91.4
2	Number	193	585	778
	Sex ratio	114.4	89.9	95.5
3	Number	291	294	585
	Sex ratio	136.6	101.4	117.5
4	Number	159	135	294
	Sex ratio	144.6	62.6	98.6

approximately equal to average numbers of children (4.56 and 3.32) reported for a United States population (15). The decrease in average number of children (4.32 to 3.36) from the parental to the present generations in the China population is considerably less than the corresponding decrease (5.16 to 2.43) reported for a Japanese population (10).

Combinations of Sexes and Family Size.—Composition of sexes of the first 2 children had no significant effect on family size (Table 3). However, the proportions of families stopping with 3 and 4 children were influenced by the sexes of existing children. For both 3 and 4 children families, the percentages of families that ceased to have additional children were higher when both sexes were present than when only 1 sex was present. Presence of both sexes in the first 2 children has been associated with smaller families in Britain (19) and in the United States (7, 13). That association was not found in another United States study (3), in Brazil (8, 20), in Japan (10), or in Libya (2). The influence of the composition of sexes of children on family size in the present study is similar to that reported for Black and Appalachian populations in the United States (13). In those populations, the composition of sexes of the first 2 children had no influence on family size; however, the presence of both sexes in the first 3 or 4 children reduced the likelihood of additional children.

In addition to the preference for both sexes, there was preference for a prevalence of male children. Higher percentages of families ceased having children when existing children were all males than when all were females and when there were at least 2 male children among the first 3 or 4 children (Table 3). Also,

TABLE 3. Influence of composition of sexes of existing children on family size in the present generation.

Sex composition	Family stopped		Family increased	
	No.	%	No.	%
First child				
Outcomes (ns) ^a				
m	11	2.9	370	97.1
f	9	2.2	408	97.8
Total	20	2.5	778	97.5
First two children				
All combinations and permutations (ns)				
mm	46	26.1	130	73.9
mf	46	23.8	147	76.2
fm	57	27.9	147	72.1
ff	44	21.5	161	78.5
Total	193	24.8	585	75.2
First three children				
All combinations and permutations (*)				
mmm	29	46.0	34	54.0
mmf	40	59.7	27	40.3
mfm	45	55.6	36	44.4
fmf	49	59.8	33	40.2
mff	28	42.4	38	57.6
fmf	31	47.0	35	53.0
ffm	45	50.0	45	50.0
fff	24	34.3	46	65.7
Total	291	49.7	294	50.3
First four children				
All combinations (**)				
Four males	8	50.0	8	50.0
Three males, one female	42	63.6	24	36.4
Two males, two females	66	62.3	40	37.7
One male, three females	37	44.0	47	56.0
Four females	6	27.3	16	72.7
Total	159	54.1	135	45.9

* and **, $P < 0.05$ and 0.01 , respectively, that increases in family size are independent of sexes of existing children; ns, $P > 0.05$ that increases in family size are independent of sexes of existing children

the sex ratio for the last birth within families (Table 2) indicated that families more often stopped having children after the birth of a male than after the birth of a female child. Park (16) reported a male preference for Korean families. The percentages of families producing a fourth child were 75.8, 80.6, 83.9, and 87.0, respectively, when the numbers of males in the first 3 children were 3, 2, 1, and 0. Gray (6) found evidence of the male preference among families of a United States population.

Projected Generation

Data for desired family size, combinations, and permutations of sexes of children are pre-

TABLE 4. Desired family size, combination, and permutation of sexes of children.

No. children	Combination of sexes	Respondents		Permutation of sexes	Respondents	
		No.	%		No.	%
0		68	8.5			
1	1m	123	15.4			
	1f	92	11.5			
	Total	215	26.9			
2	2m	13	1.6			
	1m, 1f	464	58.1	mf	306	38.3
				fm	158	19.8
	2f	2	0.2			
	Total	479	60.0			
3	3m	0	0.0			
	2m, 1f	15	1.9	mmf	8	1.0
				mfm	7	0.9
				fmm	0	0.0
				mff	2	0.2
		0	0.0			
		0	0.0			
	2	0.2				
3f	0	0.0				
Total	19	2.4				
>3		17	2.1			

sented in Table 4. Approximately 8.5 per cent of the respondents wanted no children. In similar studies of other subsets of the human population, the percentages of respondents wanting no children ranged from a low of 0.0 for Nigeria (11) to a high of approximately 8.4 for a United States population (6). In the present study, the most desired family size (60% of respondents) consisted of two children which was also the most preferred family size in the United States (6, 12), in Japan (10), and in Brazil (9). Only 2.4% of the Chinese respondents wanted three children; only 2.1% wanted more than three children. The resulting average family size of 1.65 children was lower than that reported for other subsets of the population—United States 2.60 (6) and 2.46 (12), Brazil 2.76 (9), Japan 2.35 (10), Nigeria 4.88 (11) and in Libya 4.40 (1).

The Chinese respondents expressed strong preference for both sexes of children. For example, 58.1% of the respondents wanted 1 female and 1 male; whereas, 1.8% wanted either 2 females, or 2 males. In addition to the preference for both sexes, there was a preference for male children as evidenced by greater frequencies of 1 male over 1 female in 1-child families, of 2 males over 2 females in 2-child families, and of 2 males and 1 female

TABLE 5. Sex ratios, by order of birth, resulting from desired family size, combination, and permutation of sexes of children.

Desired family size	Number of respondents	Sex ratio by order of birth (males: 100 females)			Overall births
		1	2	3	
1	215	134			134
2	479	199	56		105
3	19	850	89	90	148
>3	17	112	183	112	112
Overall sizes	730	179	59	100	110

over 1 male and 2 females in 3-children families. Approximately 64% of the respondents wanted the first-born child to be male. These preferences for both sexes and for the first child to be male are compatible with results from studies of other populations (1, 6, 11, 9, 12). Since few Chinese respondents wanted 3 or more children, it was not possible to explore the preference for alternation of sexes (mfm, mfmf) that has characterized other subsets of the population.

Sex ratios (males per 100 females) resulting from desired family sizes, combinations, and permutations of sexes of children by desired family size and order of birth are given in Table 5. Preference for the first-born to be male was again evidenced by the ratio of 179 for the first birth overall family sizes. The sex ratio (110) overall family sizes and birth orders was within the range of the observed ratios for the parental (114.6) and present (101.3) generations. The 110 ratio would result in more equal proportions of females and males for the Chinese population than has been reported for other populations. Ratios for desired families in other populations have ranged from a low of 121.3 for Brazil (9) to a high of 167.0 for Nigeria (11).

DISCUSSION

The China population was selected because of its unequaled size and its recent subjection to intensive family planning measures. The population control efforts have been directed toward limiting the number of children per family. Utilization of college and university students did not result in a totally representative sample of the China population; however, the study did include the parental generation of the college students as well. Other

reported studies have, likewise, utilized college students and their families.

The China population had some similarities and some differences when compared with other populations. Similarities with certain other populations included: magnitude of observed sex ratios, lack of significant correlations between sexes of successive children within families, average number of children per family, magnitude of decrease in family size between parental and present generations, influence of both sexes and male children on family size, and preferences for 2-children families consisting of both sexes with the first-born being male.

The China population differed from others in that the observed and expected frequencies of combinations of sexes within families were not always equal. Discrepancies were observed in both the parental and present generations. Those departures were indicative of parental preferences for both sexes and for male children. Although there was evidence of preference for male children in the present generation and of strong preference for the first-born to be male in the projected generation, the resulting sex ratio (110) for the projected generation is closer to equal numbers of males and females than that reported for any other population. In fact, the 110 ratio for the projected generation is lower than the 114.6 observed ratio for the parental generation. Closeness of the sex ratio for the desired families may be a result of China's recent emphasis on the fact that ancestral lines are continued through female as well as male children (21). If the knowledge of human reproduction reaches the level where parents can have their choice of sexes, the resulting imbalance of sexes should be less in the China population than in other populations where the sex ratio for desired families is higher. For example, the resulting ratio for a projected generation of Nigerian families was 167 males per 100 females (11).

In the present study, respondents wanted an average of 1.65 children per family, which is the smallest desired family size of any of the populations that have been studied. However, the 1.65 children per family is far greater than the one child per family encouraged by China's present population policy (22). Approximately 27% of respondents wanted a 1-child

family. Wenruo (21) conceded that the fertility rate for the end of this century is still expected to be approximately 1.5 children per couple.

The China Government initiated vigorous population planning programs in the early 1970s and has greatly intensified those programs since 1980. In the present study, the parental generation was born around 1950; the present generation of college students was born around 1970. Thus, both generations preceded the intensive family planning impact. It would be enlightening to study the sex ratio of the smaller families of the 1980s.

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Decomposition of Nerve Gas and Mustard Gas Analogs Using Nicotine and Ultrasound

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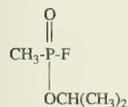
ABSTRACT

There are appreciable amounts of mustard gas (HD) and the nerve gases GB and VX stored in Madison County, Kentucky. This research employed nicotine and ultrasound in a study of the chemical decomposition of chemical-physical analogs of these toxic agents. The insecticide malathion was selected as an analog of VX due to the presence of phosphorus-sulfur bonds in both compounds and similar physical properties. 2-Chloroethyl ethyl sulfide was used as the analog of mustard gas (HD), bis(2-chloroethyl) sulfide. Nicotine alone was found to effect a complete decomposition of these compounds, but the reaction with 2-chloroethyl ethyl sulfide was slow. The application of ultrasound to the nicotine-analog mixtures induced a substantial increase in the rate of analog reaction, thus indicating this method has the potential for practical, "closed-loop" demilitarization of the corresponding warfare agents.

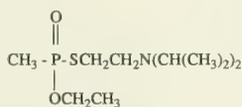
INTRODUCTION

There are 8 locations about the continental United States, and one at Johnston Island, where some 25,000 tons of chemical warfare agents are stored (1). These agents need to be demilitarized for two reasons, (1) to be in compliance with international treaties on destruction of these agents, and (2) because the containers for these agents are gradually corroding and present a long-term hazard to persons living or working in the vicinity of the storage areas.

The second reason is of particular concern to the residents of central Kentucky, and especially those in proximity to the Lexington-Blue Grass Army Depot, where the nerve gases GB (I) and VX (II) are stored as well as the vesicant agent mustard gas or HD (III).



I (GB)



II (VX)



III (HD)

Some of these agents are contained in rockets that also hold both explosive bursters and propellant chemicals that make them doubly dangerous as they age. Safe destruction and/or disposal of these agents is complicated by the fact that the storage and pro-

posed disposal sites are close (2-3 miles) to civilian housing, businesses, and schools. This proximity leaves absolutely no margin for human error or mechanical accident or malfunction in demilitarization, all possibilities in agent destruction by the army-favored incineration process.

The proposed incineration of the chemical agents in Kentucky, and at several other storage sites around the country, has been opposed by citizen's groups for a variety of reasons:

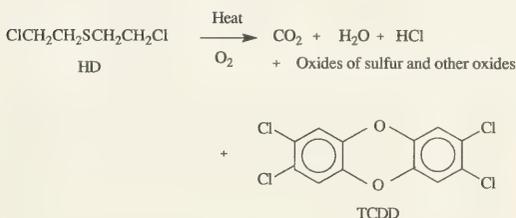
(1) There is concern because the incineration process requires an "open-loop" system. There is a finite possibility that due to error or accident a major emission of agent could escape from the incinerator stack. Residents of this area believe there would be so many unprotected people close to the incinerator that the possibility of adequate warning and safe evacuation is, arguably, impossible. Such a disaster would thus result in the loss of many lives and the magnitude of the tragedy would be incalculable.

(2) Minor amounts of agent might persist in stack effluent even though the health consequences of this continued exposure to trace amounts of chemical warfare agent have never been adequately studied.

(3) There is a logical concern that after the disposal of the chemical warfare agents no effort would be made to dismantle the incinerator. Rather, such an expensive facility would be converted into a general toxic waste incin-

erator and be perpetually used to burn commercial as well as government toxic wastes. It would serve as a magnet for the transportation of toxic wastes from all of the Eastern United States into central Kentucky.

(4) The incineration of mustard gas (HD) has the potential of forming small amounts of the very toxic and persistent 2,3,7,8-tetrachlorodibenzodioxane (TCDD or "dioxin") and other polychlorinated dibenzodioxanes. In animal tests, TCDD is found to be embryotoxic, teratogenic and perhaps carcinogenic (2, 3). In fact, the organizations Physicians for Social Responsibility and the Environmental Defense Fund have jointly released recommendations for a new national policy to reduce dioxin pollution. These recommendations include a requirement that all incinerators operate under conditions that produce virtually no dioxins (4). The general reaction for TCDD production during incineration is shown below:



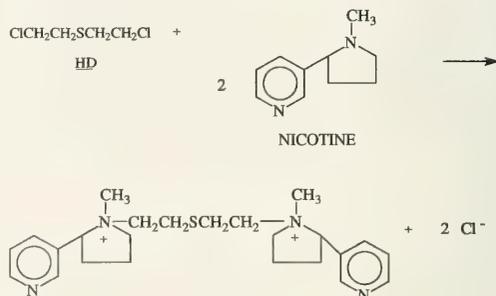
DISCUSSION

The army has stated its intention to use a demilitarization method that will decompose all of the different types of warfare agents by the same process. Their position is that incineration is the only current process that meets that criterion. In part, the argument has some validity. In the past, some GB agent has been satisfactorily demilitarized by using aqueous hydroxide or hypochlorite solutions. However, unlike GB, VX and mustard gas (HD) are not readily or completely soluble in water (5, 6). Therefore, it is difficult to attain complete decomposition of these agents at low temperature-low pressure in aqueous solutions by hydrolysis (7).

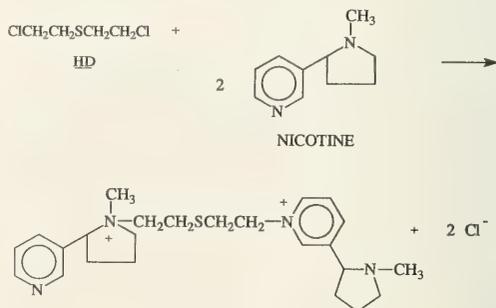
We propose that since all of these agents have a leaving group subject to removal by nucleophilic attack, all should be able to be decomposed by simple mixing with an effective nucleophile in which they are soluble. We fur-

ther suggest that the natural alkaloid nicotine possesses these solubility-nucleophilic properties, thus presenting a novel approach to demilitarization. In addition, we have studied the use of sonochemistry as a method to enhance the decomposition of these agents. Below are listed the expected reaction of each of the agents with nicotine:

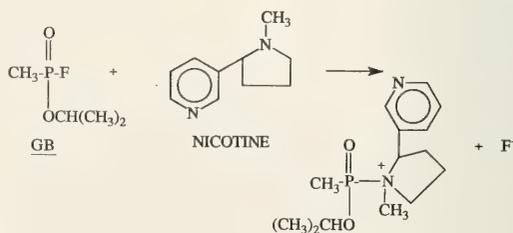
MUSTARD GAS (HD) + NICOTINE



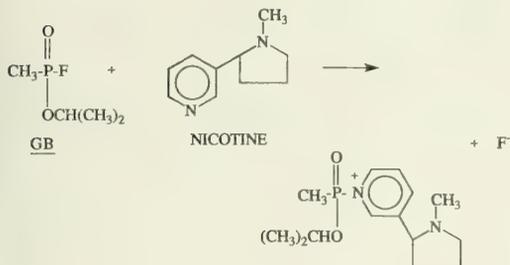
In addition, there are some less likely by-products where the pyridine moiety rather than the N-methylpyrrolidine moiety of nicotine displaces one or two chlorines. The reaction that follows shows a one-chlorine displacement by that process:



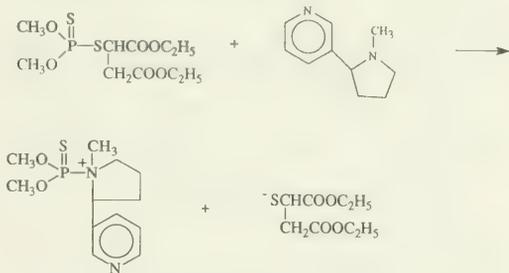
GB + NICOTINE



Plus small amounts of:

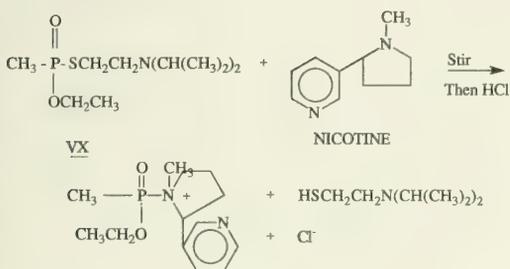


MALATHION + NICOTINE

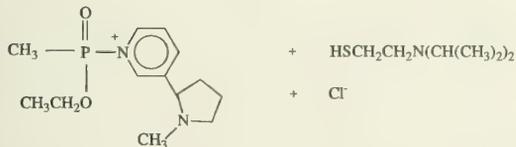


Plus small amounts of this product:

VX + NICOTINE



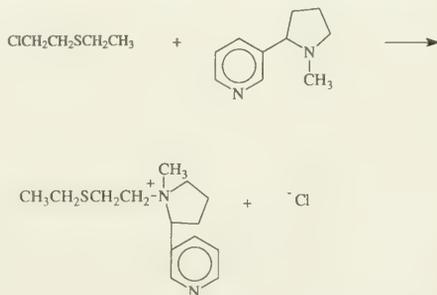
and small amounts of:



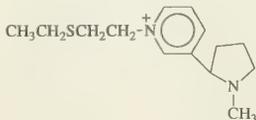
Due to the extreme toxicity and unavailability of the warfare agents, it has not been possible, to date, for us to perform experiments on the agents themselves. Rather, it has been necessary to use commercially available compounds that are reasonable chemical analogs for this research. The insecticide malathion was selected to serve as the analog of VX since it has a similar phosphorus-sulfur bond, similarly sized substituents on the phosphorus atom, and similar solubility properties. Its chemical reactivity is sufficiently close to that of VX to cause it, and especially its oxidized metabolite malafoxon, to possess some anticholinesterase activity (8). The equations for the expected decomposition of malathion by nicotine are given below:

2-Chloroethyl ethyl sulfide was the logical choice to employ as the experimental analog of mustard gas (HD or bis(2-chloroethyl)sulfide). The sensitive 2-chloroethyl sulfide functional group is the same in both compounds, just one in the analog and 2 in mustard gas, and they have obvious similarities of size and solubility. The expected nucleophilic displacement reactions of nicotine and 2-chloroethyl ethyl sulfide are shown below:

2-CHLOROETHYL ETHYL SULFIDE + NICOTINE



Plus small amounts of the isomer below:



MATERIALS, INSTRUMENTATION,
AND METHODS

Reagents:

Benzene—The benzene was freshly distilled prior to each use (Fisher, #B-245, ACS certified, b.p. 80°C).

2-Chloroethyl ethyl sulfide—This compound was used as purchased without further purification (Aldrich Chemical Co., #24,264-0, Assay 98% purity, b.p. 156–157°C).*

Malathion—This compound was used as purchased without further purification (Chem Service, #PS-86, Assay 98% purity, b.p. 156–157°C).*

Nicotine—This compound was used as purchased without further purification (EM Science, #NX0370-1, Assay 98% purity, b.p. 123–125°C).*

Instrumentation:

Gas Chromatograph/Mass Spectrometer—Hewlett Packard 5890 GC and 5970 MS with an automatic sampler 7673A.

Column—J & W Inc., DB-5, 50 m × 0.20 mm ID, 0.33 micron polysiloxane film.

Sonochemical Reactor—Ace Glass Incorporated #9830 Sonochemical Reaction Assembly with #9818 microtip.

Experimental Procedure:

All reactions were carried out without heating, except for any heat increase caused by the sonochemical probe. The reactions without sonochemistry were carried out at room temperature, 20–22°C. The reactions with sonochemistry reached 40–41.5°C. This temperature differential accounts for only a portion of the observed rate increase with sonochemistry. Current theory on sonochemical effects holds that as transient, minute cavitation bubbles at the surface of the probe collapse they generate enormous local pressures and temperatures. Even though the microbubble collapse may generate temperatures well over 1,000°K, the macroscopic temperature change is only some 15–20° (9).

In all sonochemical procedures the probe was programmed to alternately pulse for 2 seconds and rest for two seconds.

In all experiments, 5.0 microliter samples

were removed for analysis, diluted in 6.0 ml of benzene, and the concentration of analog determined by GC/MS. The small sample size caused some slight, but acceptable scatter of data points. Each reaction was monitored until no trace of the analog could be detected.

Separation of components was carried out for malathion at an initial column temperature of 150°C, increased 10°C/min. to a maximum of 280°C. For 2-chloroethyl ethyl sulfide, initial column temperature was 100°C, increased by 10°C/min. to a final temperature of 230°C.

Malathion With Nicotine.—0.330 grams (0.001 mol) of malathion was mixed with 0.324 grams (0.002 mol) of nicotine in a 3.0 ml reaction vial fitted with a water-cooled condenser. The reaction was stirred continuously with a magnetic-spin vane except for sample removal periods.

Malathion With Nicotine Plus Sonochemistry.—3.301 grams (0.01 mol) of malathion was mixed with 3.245 grams (0.02 mol) of nicotine in a cylindrical reaction vessel, open at the top with a sonochemical probe inserted.

2-Chloroethyl Ethyl Sulfide With Nicotine.—0.249 grams (0.002 mol) of 2-chloroethyl ethyl sulfide was mixed with 0.649 grams (0.004 mol) of nicotine in a 3.0 ml reaction vial fitted with a water-cooled condenser. The reaction was stirred continuously with a magnetic-spin vane except for sample removal periods.

2-chloroethyl Ethyl Sulfide With Nicotine Plus Sonochemistry.—1.246 grams (0.01 mol) of 2-chloroethyl ethyl sulfide was mixed with 3.24 grams (0.02 mol) of nicotine in a cylindrical reaction vessel, open at the top with a sonochemical probe inserted.

EXPERIMENTAL RESULTS

Malathion:

The experimental results for the reaction of malathion and nicotine, with and without the sonochemical probe, are summarized in Figure 1. Without sonochemistry the 0.33 gram sample (0.001 mol) of malathion was completely decomposed in 600 minutes, with a reaction half-life of 99 minutes. The decay rate was exponential, with $y = 113.9 \times 10^{-0.004x}$ in the best-fit curve.

Using the sonochemical reactor, 3.30 grams (0.01 mol) of malathion was decomposed in 42

* Gave a single peak upon GC analysis.

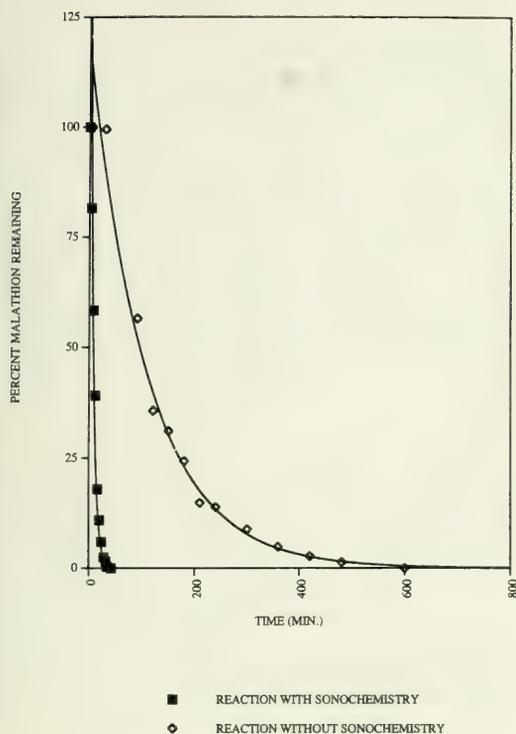


FIG. 1. Malathion + nicotine. ■—reaction with sonochemistry; ◇—reaction without sonochemistry.

minutes, the reaction half-life dropping to only 9.6 minutes. Again, there was an exponential decay rate for the malathion. The best-fit curve had $y = 176.7 \times 10^{-0.068x}$.

Even though the reaction was carried out in an open vessel, there was no measurable loss of reactants by evaporation during the reaction.

2-Chloroethyl ethyl sulfide:

The experimental results for the reaction of 2-chloroethyl ethyl sulfide are displayed on the graph in Figure 2. The decomposition of 0.249 grams (0.002 mol) of this compound by nicotine alone was quite slow, taking 290 hours for completion. The reaction half-life was approximately 150 hours. It is obvious from Figure 2 that the decomposition rate of this compound is not exponential like that of malathion. In fact, the best-fit curve is a second-order polynomial type that has the sign of the change in slope with time opposite to that of the malathion curve. The 2-chloroethyl ethyl sulfide reaction with nicotine begins slowly,

suggesting an induction period. This is probably due to a solvent effect because as the reactants move toward the transition state there must be forming charges. Since both reactants are neutral, either an S_N1 or an S_N2 mechanism requires charge formation that is not effectively stabilized by the neutral mixture. As the reaction proceeds and ions are produced the reaction environment is more amenable to the forming charges in the transition state.

The application of a sonochemical probe to this reaction effected a substantial rate increase. The 1.246 gram (0.10 mol) sample of 2-chloroethyl ethyl sulfide was completely decomposed in 41 hours compared to 290 hours without sonochemistry. The reaction half-life was reduced to 15.5 hours in comparison to the 150 hours required without sonochemistry. There was no loss of reactants from the open reaction vessel by evaporation.

CONCLUSIONS

This work demonstrates that analogs of VX nerve gas and mustard gas (HD) can be completely decomposed chemically using nicotine as both reactant and reaction solvent. Also, the experimental results demonstrate that using a sonochemical probe affords a great increase in reaction rate. Additional rate increases would be readily effected by lengthening the time of the sonochemical pulse and increasing the reaction temperature. Further studies to optimize solvent polarity should provide even greater rates, especially in the case of mustard gas (HD) decomposition. One can conclude that with the above mentioned enhancements in method and with additional research the use of nicotine for the demilitarization of the aforementioned warfare agents could be a viable process.

The process does have several features and advantages that address the major concerns of the residents of central Kentucky and the recommendations of the Kentucky Citizens Advisory Commission, appointed by Governor Brereton Jones, with respect to demilitarization of the chemical agent stockpile.

(1) It would permit a "closed-loop" system so no gases could escape into the atmosphere.

(2) The demilitarization reactions could be carried out at low temperature-low pressure by a batch process. No transfer of reaction products into transport or storage containers

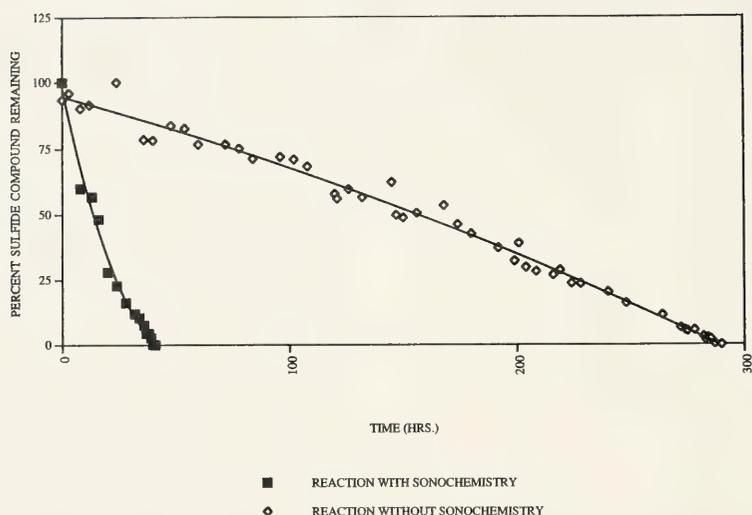


FIG. 2. 2-Chloroethyl ethyl sulfide + nicotine. ■—reaction with sonochemistry; ◇—reaction without sonochemistry.

would need to occur until the products could be assessed for the presence of residual warfare agent, thus providing this process with a level of safety not possible with incineration. We further propose that dedicated railroad tank-cars could be constructed that would serve both as "reaction vessels" and as a means to transport the reaction products to a toxic waste dump. This would minimize the number of times transfer of the chemicals would be required.

(3) The proposed chemical demilitarization reagent nicotine will mix well with GB, VX and HD and thus improve the effectiveness of destroying these agents completely. All of these agents could be demilitarized in the same batch reactors (tank-cars), thus adding an additional economical benefit to this process.

(4) A closed-loop batch reactor of the type that would be used for this process would not be useful for the destruction of most industrial toxic wastes and therefore would not serve to be converted into a general toxic waste disposal unit after the chemical warfare agents are demilitarized.

(5) Chemical demilitarization of the agents would not produce TCDD, "dioxin."

(6) The reaction products are non-volatile salts or high-boiling liquids, substantially reducing any hazard in handling or transportation.

There are other non-chemical benefits that would be afforded by applying this demilitarization method to the Chemical Stockpile Disposal Program:

(1) The Department of Defense and its executive agent for chemical stockpile disposal, the U.S. Army, would benefit. Their support of this project, or even support for a serious study of the method, would indicate a willingness to explore alternatives to the incineration program and thereby improve their credibility with the civilian populations surrounding the eight continental storage sites. This is no small matter since incidents at storage-disposal sites over the past few years have reduced their credibility and recent revelations about secret government tests with radioactive agents on civilians in the 1940s and 1950s will make it even more difficult for these agencies to convince the civilian population that concern for their health has a top priority.

(2) The use of nicotine as the demilitarizing reagent in the destruction of the chemical warfare agents would provide an economic boon to the tobacco farmers not only of Kentucky, but also of several other tobacco-growing states, some with depressed economies, as well as to the businesses and workers associated with the recovery of nicotine from tobacco.

The work reported in this paper suggests that additional research needs to be accom-

plished. Specifically, the warfare agents themselves need to be used and the demilitarization conditions optimized. In the process of optimizing the conditions, an effort should be made to verify the reaction mechanisms and to insure the absence of reversibility. In addition, bioassays of the mixture of reaction products need to be made to determine general toxicity (LD_{50}) values. Reaction mixtures from mustard gas decomposition need to be assayed for residual vesicant activity and those from the nerve gases checked for residual antiacetylcholinesterase activity.

ACKNOWLEDGMENTS

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Oocyte Staging in Paddlefish, *Polyodon spathula*

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ABSTRACT

Oocyte size, germinal vesicle position, and pigment distribution of ova were examined as indicators of progress toward ovulation for hormonally injected paddlefish, *Polyodon spathula*. A boil and cut procedure to identify germinal vesicle position in oocytes was useful for estimating stage of oocyte maturation. Pigment pattern was a functional alternative for identifying the germinal vesicle position, because it could be substituted for the boil and cut procedure. Ova size was of no practical significance in relation to estimating potential spawning success or ovulation time. Latent time to ovulation for females injected with Luteinizing-Hormone-Releasing Hormone analogue was shorter than that of females injected with paddlefish pituitary extract. Information on the rate of germinal vesicle migration relative to water temperature would facilitate prediction of ovulation time following hormonal injection.

INTRODUCTION

Artificial propagation of paddlefish is an important tool for resource management. It may be used to enhance natural populations (1) or to produce stock for aquaculture (2). Natural stocks have been impacted by environmental perturbations and by recent exploitation for the caviar market (3, 4). Some governmental agencies are assessing the status of various natural populations, and are developing plans to revitalize the affected populations.

In most warmwater species, ova viability deteriorates relatively quickly after ovulation. Consequently, the quality of ova produced by induced spawning can be improved by the ability to accurately predict time of ovulation. Convenience to hatchery personnel can also be enhanced. Techniques used to examine intra-ovarian eggs are related to species specific characteristics. During final maturation of oocytes, cytological reorganization is usually associated with visible changes. The usefulness of these changes as predictors of ovulation depends on the ability to relate identifiable stanzas with time-related sequences (5). An important event associated with these changes is migration of the germinal vesicle (GVM) to-

ward the periphery of the oocyte to complete the first meiotic division (6, 7, 8).

Coalescence of oil globules and related redistribution of cellular components results in spontaneous ova clearing in many marine fishes. For example, this has been used as an important tool to predict ovulation in striped bass, *Morone saxatilis* (9). However, cytological redistribution and germinal vesicle (GV) position are not easily viewed in ova of most freshwater species because their oocytes are opaque.

The yolk of some fish oocytes can be cleared with one of several solutions so that the germinal vesicle can be seen (10). However, this method is not satisfactory for oocytes of fish species that have pigmented eggs. Lutes et al. (11) located GV positions in the eggs of white sturgeon, *Acipenser transmontanus* by heat-hardening the oocytes and cutting them along the animal-vegetal axis. The objectives of this study were to test the applicability of the technique used to identify GV position in sturgeon oocytes, in paddlefish oocytes, and to examine other functional alternatives.

MATERIALS AND METHODS

Initially, a seasonal series of oocytes was sampled from wild adult paddlefish in Grand

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Lake, Oklahoma (12). Oocytes and pituitaries were collected from fish being processed for caviar by licensed commercial fishermen. Pituitaries were frozen for later use in spawning, while ova were examined fresh.

Ten oocytes from each mature female were measured along the vertical axis, the pigment distributions were noted, and then the oocytes were boiled for 3–5 minutes to harden them. Similar to the methodology used for sturgeon by Lutes et al. (11), the boiled oocytes were bisected through the animal-vegetal pole axis, and the position of the GV was recorded. Some ova were preserved in formalin (5–10%) for several weeks to see if they would harden sufficiently for cutting. Several clearing solutions were also used with fresh oocytes to test their effectiveness to provide a view of the GV. In a few tests, commercial bleach was used as a follow-on treatment to a clearing solution. The solutions included an alcohol-xylene treatment (13), Stockard's solution (14), and a serra solution (15). Stoeckel and Neves (10) found these solutions to be effective in clearing oocytes of 9 teleosts. The serra solution has been widely used in Europe for clearing oocytes of common carp, *Cyprinus carpio* (16).

Artificial propagation trials were conducted at the Aquaculture Research Center, Kentucky State University. Broodstock in spawning condition were netted and transported to the hatchery facility, where they were hormonally injected following the procedures of Graham et al. (17) and Semmens (18). Fresh-frozen paddlefish pituitaries or super-active analog of Luteinizing Hormone-Releasing Hormone, LH-RHa (Sigma) were used as inducing agents. A priming dose (1/10 of the total) and a resolving dose (9/10) were administered 12–24 hr apart. Intra-ovarian eggs were sampled through a small ventral incision as described by Doroshov et al. (19) at the time of priming and in some instances at the resolving injection. Pigmentation patterns and GV positions of these oocytes were examined.

RESULTS AND DISCUSSION

Oocyte Treatments

Ova were examined from 93 mature (>15 kg) females monthly from November through April (Table 1). Oocyte size spanned nearly the same range in November as in April just

TABLE 1. Paddlefish oocyte size collected from fresh ovarian samples during the prespawning period.

Month	Mean height (mm) (n = 10 ova)	Range	Number of fish
November	2.46	2.30–2.72	20
December	2.49	2.35–2.77	18
January	2.55	2.44–2.89	14
February	2.63	2.48–2.94	20
March	2.70	2.31–2.92	15
Spawning	2.71	2.56–2.79	6

prior to spawning. There was a slight increase in mean size from November through April, but this change would be of no practical value in staging oocytes for induced ovulation. Ova size during the prespawning period was similar to that reported for paddlefish in Louisiana (20).

Heat-hardening of paddlefish oocytes and subsequent bisection resulted in 2 egg halves that were satisfactory for locating the GV. The GV could be clearly seen as a fine-textured, round object within the more granular yolk. None of the formalin-fixed oocytes were hardened enough to be cut without distorting the egg. Further, none of the solutions cleared paddlefish ova so that the GV could be seen because the dark pigment screened any change in yolk opacity. Treatment with bleach somewhat reduced the pigment intensity, but also distorted the oocytes so that they could not be used for staging.

Oocyte Staging

In the period just preceding natural spawning, the GV was in a central position (Fig. 1, right couplet of Stage I) and pigment was unequally distributed (Fig. 1, left couplet of Stage I). Pigment appears to be more concentrated near the oocyte periphery rather than being dispersed in the yolk. Yolk in the animal hemisphere is more uniform in particle size, than that of the vegetal hemisphere. The animal hemisphere also has a much greater concentration of pigment than the vegetal hemisphere. The latter is whitish-grey, while the former is charcoal colored. Transition of pigment distribution at the equatorial circumference is distinct, and often appears as a shadowy ring at the equator. Position of the centrally located GVs usually corresponds to the zone of transition between the vegetal and an-

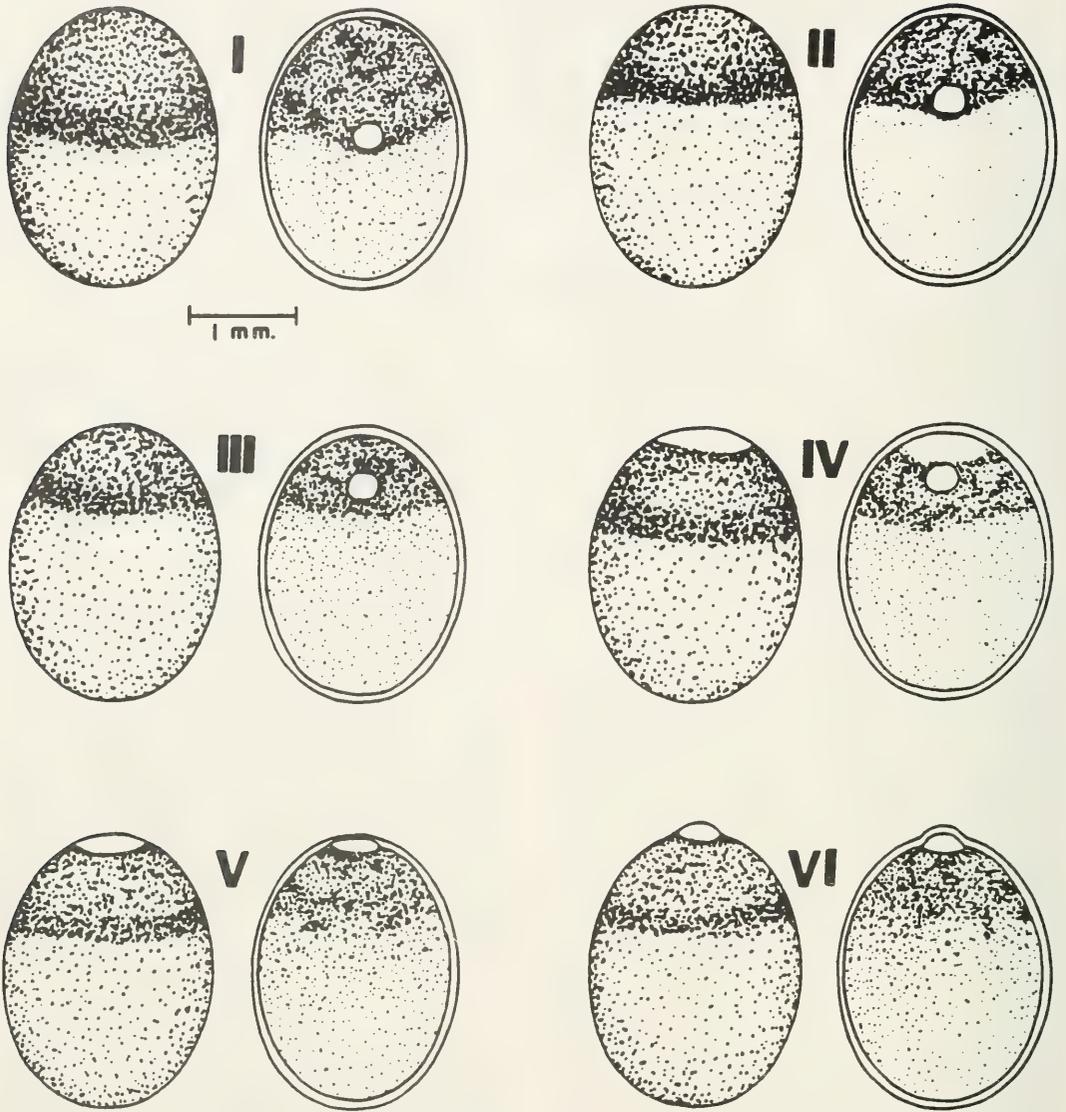


FIG. 1. Paddlefish oocyte stages (I-VI); the left illustration of each pair is representative of the surface pigmentation and the right illustration of each pair is of a bisected oocyte showing yolk distribution and germinal vesicle (GV) position. Stage I typifies an oocyte with a centrally located GV prior to resumption of meiosis-I; Stage II illustrates the slightly displaced GV at the initiation of germinal vesicle migration (GVM) and pigment redistribution after the resumption of events leading to meiosis-I; Stage III shows the GV in a position in the upper region of the oocyte near the animal pole; Stage IV depicts the clearing of the animal pole region in relation to the GV position; Stage V is characterized by further pigment change and the disappearance the GV at germinal vesicle breakdown (GVBD); Stage VI shows the appearance of an ovulated egg.

imal hemisphere pigmentation. This relationship is maintained as the GV migrates toward the animal pole.

The GV was in the central position (Stage I) throughout the fall and early spring. Migration toward the animal pole occurred rapidly,

and just prior to ovulation. As the GV migrates (GVM), the pigment discontinuity also moves poleward (Fig. 1, Stage II). Pigment near the transition is often more concentrated, giving the appearance of a ring. The position of the pigment margin does not continue to move as

TABLE 2. Paddlefish oocyte stage, inducing agent, and temperature related to latent period for ovulation.

Oocyte stage at injections*			Ovulating Agent**	Temp. (C)	Ovulated/injected	Latent period (hr)	
Prime	Interval (hr)	Resolve				Resolve	Total
I	24, 24***	II, III	P	13-14	0/2	both died	
II	24, 24***	III, IV	P	13-14	2/2	18-19	66
III	24	—	P	13-14	1/1	25	49
IV	22	—	P	17-18	1/1	18	40
III	24	—	L	13-14	1/3	15	39
III	12	IV	L	17-18	6/6	24-26	36-38
III-IV	12	V	L	15-16	8/9	24-28	36-40
III-IV	22	—	L	18-19	5/5	19	31
IV	22	—	L	17-18	2/2	13.5	35

* See Figure 1.

** P = fresh-frozen paddlefish pituitaries; L = LH-RHa

*** Second resolving injection.

the GV shifts further to approximately $\frac{1}{2}$ - $\frac{2}{3}$ the distance to the pole (Fig. 1, Stage III) and there is a more uniform dark pigmentation, rather than the ring-like pattern seen earlier. However, Stage-II and Stage-III oocytes are difficult to differentiate without direct reference to the GV position. As GVM continues, a clear area appears at the animal pole (Fig. 1, Stage IV). Consequently, at Stage IV, a relatively broad band of pigment occupies the middle two-thirds of the animal hemisphere and the GV is now located at the polar edge of the pigment band. Appearance of the clear area at the animal pole is indicative of Germinal Vesicle Breakdown (GVB), and subsequently completion of the first meiotic division and ovulation. The clear area of the oocyte contracts slightly prior to ovulation, and the GV is no longer visible in gross cross-section (Fig. 1, Stage V). The ovulated egg (Fig. 1, Stage VI) has a distinct elevated protuberance at the animal pole/micropylar area, and the pigment is slightly more diffuse than that of earlier stages.

Application to Propagation

Direct observation of oocyte GV position, or the changing pattern of pigmentation can be used to stage oocytes of paddlefish. Position of the GV was used as an indication of maturational activity, once GVM had been initiated. However, because the GV is centrally located for most of the prespawning period these characteristics are useful as indicators of approximate time to ovulation only after some displacement has occurred.

Oocytes from females collected early in the spring-time spawning cycle were typically in

Stage I or II. Some females that were collected when spring-time ambient temperatures were approaching the spawning range, and that had oocytes with a centrally located GV (Stage I), were not ovulated even after multiple injections (Table 2). Oocyte maturation (GVM) was stimulated by two resolving injections but these fish died before ovulation occurred. However, when GVM was proceeding (Stage II), responsiveness to induction was more predictable. Twenty four of 28 females with oocytes at Stage III or IV were induced to ovulate within 31 to 49 hr after the priming injection. Two factors, temperature and inducing agent, appeared to influence the timing of ovulation.

Because temperature affects maturational rate of oocytes in fishes, incorporation of a temperature component could improve predictive power of time to ovulation of hormonally injected paddlefish. Only general inferences can be made from the present data. Higher temperatures within the normal spawning range did result in shorter times to ovulation (Table 2). Temperature influence on developmental rate for the Russian sturgeon, *Acipenser guldenstadti*, is described by Ginsberg and Dettlaff (21). Staging under different temperature regimes may provide more insight into establishing a GVM rate and improve the usefulness; however, the inducing agent must also be considered.

In our study, the latent period for females injected with LH-RHa was generally shorter than that of females injected with pituitary extract. Further, the response to LH-RHa stimulation appeared to be less temperature sen-

sitive than that of pituitary induction. Semmens (18) also reported shorter latent periods for paddlefish injected with LH-RHa compared to pituitary stimulation. The reciprocal response would be expected based on the pathways and modes of action of the 2 agents. Rottmann and Shireman (22) reported that latent time to ovulation of Chinese carps was longer for LH-RHa than for pituitary induction.

CONCLUSIONS

Environmental factors influence the progress of gamete maturation. Intraovarian samples can provide a window to the process and indicate the status of the maturational trajectory. Whether a female is responding to an inducing agent, or will need additional stimulus to ovulate are pertinent questions during artificial propagation. The position of the GV, or in the case of paddlefish, the ova pigment pattern, can be used to provide more information during induction, or can be used as tools to examine more detailed relationships. In the latter context, a GVM-rate/temperature relationship would be useful in the artificial propagation of paddlefish. With this information, other factors, such as a more thorough comparison of the efficacy of inducing agents could be examined. Applicability of staging techniques may provide useful tools in facilitating artificial propagation of related fishes threatened with extirpation such as the Chinese Paddlefish, *Psephurus gladius*.

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Egg and Larval Development of the Striped Fantail Darter, *Etheostoma flabellare lineolatum* (Agassiz), and Duskytail Darter, *E. Percnurum* Jenkins, with Comments on the *Etheostoma flabellare* Species Group

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ABSTRACT

The *Etheostoma flabellare* species group comprises 6 recognized forms, including *E. f. lineolatum* and *E. percnurum*. The wide ranging *E. f. lineolatum* and the relict *E. percnurum* both exhibit large spherical yolk sacs, with a mid-ventral vitelline vein plexus; 34-36 total myomeres; well developed jaws at hatching; and head not deflected over the yolk sac. Eggs of *E. f. lineolatum* range between 2.0-2.8 mm in diameter. Hatching in *E. f. lineolatum* occurs between 4.5-5.9 mm TL and first ray formation occurs at 5.0 mm in the pectoral fins. *Etheostoma percnurum* has the largest eggs of the group with diameters between 2.6-3.3 mm. Larvae hatch between 4.5-5.4 mm TL and possess six incipient rays in the pectoral fin. Fin ray formation occurs at later length intervals than *E. f. lineolatum*, except for the spinous dorsal fin. Orbit shape and initiation of squamation for *E. percnurum* is similar to *E. kennicotti*.

The fantail darter, *Etheostoma flabellare*, is the most widespread member of the subgenus *Catonotus*, ranging from the Great Lakes basin, south through the upper and middle Mississippi basin, and east onto the central Atlantic slope (1). This highly variable taxon, consists of 3 to 5 subspecies. However, *E. f. lineolatum*, was not recognized by McGeehan (2) because of the erratic geographical distribution of striped and non-striped forms. *Etheostoma flabellare* inhabits gravelly or rocky riffles (occasionally slow runs or pools) in headwater creeks to moderate-sized rivers, and is potentially sympatric with at least 11 other species of *Catonotus* (1, 3, 4). In contrast, the duskytail darter, *Etheostoma percnurum*, is a relict species restricted to only 4 large streams of the upper Tennessee and middle Cumberland River drainages, where it inhabits rocky pools (4, 5). It is accorded threatened status by the state of Tennessee (6), and endangered in Virginia (7, 8). It oc-

curs sympatrically with only 1 species of *Catonotus*, *E. flabellare*. The fantail and duskytail darters represent distributional extremes within *Catonotus*, but are closely related, comprising, with *E. kennicotti*, the *E. flabellare* species group (3, 9).

There is a growing body of comparative information for distinguishing larvae and juveniles of different species of *Catonotus* (10, 11). Embryonic and larval development of *E. flabellare flabellare*, the subspecies occupying the Great Lakes and upper Ohio River basins (2), has been characterized by Lake (12), Cooper (13), Auer (14), and Paine (15); however, descriptions are lacking for other subspecies. Also, given the high degree of sympatry between *E. flabellare* and other species of *Catonotus*, comparative data are needed for separating their larvae. Layman (5) described larval stages of the duskytail darter but did not report his observations of embryonic development or provide meristic and morphometric characteristics of larvae. Such information may become critical in implementing future recovery plans for this jeopardized species. Larval

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characters may also prove to be useful in resolving phylogenetic relationships within the *E. flabellare* species group.

This paper describes larval development of *Etheostoma flabellare lineolatum*, the striped fantail darter, a widespread taxon found in the lower Ohio River basin and tributaries of the Mississippi River, and embryonic and larval development of *E. percunrum*, the duskytail darter. Meristic, morphometric, and pigmentation features are used to separate these taxa from *E. f. flabellare*, *E. kennicotti*, and other sympatric species of *Catonotus*.

MATERIALS AND METHODS

Laboratory cultured and wild collected specimens were studied for differences in morphology, meristics, pigmentation, and sequential development relative to size. A series of meristic and morphometric features was measured from 128 eggs and 237 larvae and early juvenile striped fantail darters, and 20 eggs and 24 larvae and early juvenile duskytail darters.

A total of 21 morphometric and nine meristic characters was measured for each specimen following methods outlined in Simon (10). Eggs and embryos were preserved in 5% formalin, while larvae were preserved in 10% formalin after removal from nests. The middle of the spawning episode (i.e., fertilization of ova) was used as time zero in estimating ages of embryos. All measurements were made to the nearest 0.1 mm using a dissecting microscope with an ocular micrometer. Measurements in the text are expressed as a proportion of total length (TL) unless otherwise noted. Illustrations were delineated following Sumida et al. (16).

Embryonic and larval descriptions of the duskytail darter were based on adults collected and spawned by Layman (5) from the Tennessee River drainage, Little River, Blount County, Tennessee. Little River contains 1 of 3 extant populations for the species in the upper Tennessee River drainage. Eggs were obtained from nests in Little River and from spawnings of aquarium-held adults. All eggs were incubated in aquaria at 18–27°C. Larval descriptions of striped fantail darter were based on adults collected and reared by Simon (17) from the Mississippi River drainage, Spring Coulee Creek, Vernon County and

Coon Creek, La Crosse County, Wisconsin; Root River, Fillmore County, Minnesota; and upper Iowa River, Houston County, Iowa. Eggs were obtained from spawnings of aquarium-held adults and incubated at 2 temperatures (20° and 23°C) in the laboratory. Larvae of both species were fed live *Artemia* nauplii.

SPECIES ACCOUNTS

Striped fantail darter, *Etheostoma flabellare lineolatum* (Agassiz)

Eggs.—Three size classes of ova were observed in the dissected ovary of *E. flabellare lineolatum*. The smallest ova ranged 1.0–1.5 mm ($n = 13$, $\bar{x} = 1.3$ mm), and were spherical, opaque, and pale yellow. The intermediate sized ova were ovoid, opaque, and pale yellow and ranged 1.5–2.0 mm ($n = 15$, $\bar{x} = 1.6$ mm). Mature eggs collected from Spring Coulee Creek, Wisconsin, the Rock River, Illinois, and the upper Iowa River, Iowa, were all equivalent in diameter and ranged from 2.0–2.8 mm ($n = 100$, $\bar{x} = 2.4$ mm). Mature eggs are spherical, demersal, and adhesive with translucent yellow yolk. Generally, mature eggs have a single oil globule, a narrow perivitelline space, an unsculptured chorion, and are unpigmented. Based on presence of various size classes of ova and aquarium observations, the striped fantail darter is considered a multiple spawner depositing more than a single clutch of eggs.

Eggs are attached to the undersides of slab rocks in the margins and slower portions of riffles and raceways (2, 12, 18, 19, 20).

Embryonic Development.—Embryonic development for the fantail darter has been previously described by Lake (12), Cooper (13), and Paine (15). Eggs incubated at 20°C hatched after 240 ± 18 hr (9.25–10.75 days); at 23°C, hatching occurred in 144 ± 10 hr (5.50–6.50 days). Lake (12) reported eggs of *Etheostoma f. flabellare* to hatch in 30–35 days at 17°–20°C; after 21 days at 21°–22°C; and in 14–16 days at 26°C.

Larvae

Morphology.—The size of initial formation for selected structures is summarized for larval and early juvenile *E. f. lineolatum* with morphometric features shown in Table 1. At 4.5–5.9 mm, newly hatched, well developed pec-

TABLE 1. Morphometry of *Etheostoma flabellare lineolatum* larvae and early juveniles grouped by selected intervals of total length (N = sample size). Characters expressed as percent total length or head length^(a) with a single standard deviation.

Total length	N	Length (% TL)					Depth (% TL)				
		Standard	Prealanal	Snout ^(a)	Eye ^(a)	Head	Head	Shoulder	Anus	Caudal Peduncle	
4.5-6.9	61	89.7 ± 2.2	54.0 ± 2.9	7.9 ± 3.0	47.0 ± 5.5	18.4 ± 2.1	19.6 ± 1.4	32.2 ± 4.9	10.4 ± 1.4	6.5 ± 0.8	
7.0-12.8	147	85.4 ± 2.4	51.0 ± 1.2	16.1 ± 3.6	38.7 ± 5.9	22.8 ± 2.3	18.2 ± 1.3	18.8 ± 2.9	12.8 ± 1.3	8.7 ± 1.1	
13.0-15.9	16	82.7 ± 0.4	50.5 ± 1.2	19.0 ± 1.5	28.7 ± 2.3	24.8 ± 1.7	16.7 ± 1.8	18.6 ± 1.7	14.0 ± 0.9	9.9 ± 0.7	
16.0-18.8	4	84.1 ± 1.0	50.1 ± 0.5	19.9 ± 0.7	23.4 ± 1.3	23.2 ± 1.1	12.6 ± 0.9	16.6 ± 0.4	14.6 ± 1.1	10.0 ± 0.8	
19.1-21.6	4	85.0 ± 0.7	50.4 ± 0.9	19.8 ± 0.3	23.3 ± 1.3	24.2 ± 1.3	13.0 ± 0.3	17.3 ± 0.8	14.6 ± 0.7	9.4 ± 0.9	
22.2-26.1	5	86.1 ± 3.5	50.3 ± 0.9	20.6 ± 1.9	23.2 ± 2.3	24.3 ± 0.1	13.4 ± 0.8	16.4 ± 1.2	13.8 ± 1.0	9.5 ± 1.1	

toral fins without incipient fin rays; yolk sac extremely large, spherical (ca. 42% TL); yolk amber, with a single anterior oil globule; vitelline vein forming a plexus on mid-ventral yolk sac; head not deflected over the yolk sac; jaws developed; eye diameter oval. Notochord flexion occurs 4.9-6.0 mm; first rays form in pectoral fin (5.0-5.6 mm); anal fin rays (5.3-5.9 mm); first caudal fin ray form (5.3-5.9 mm); soft dorsal rays form (5.4-6.2 mm); and spinous dorsal rays form (5.9-6.2 mm). Caudal fin round (5.8-6.2 mm); incipient dorsal and anal fin margins partially differentiate (7.1-8.0 mm); spinous dorsal fin origin situated over preanal myomere 4, soft dorsal origin over preanal myomere 15-16 (7.8 mm); predorsal length 36.3% SL (range: 33-39.7% SL); anal fin margin completely differentiate (7.9 mm); pelvic fin buds form anterior to dorsal fin origin prior to complete absorption of yolk sac (7.9-8.5 mm); yolk absorbed (7.8-8.0 mm). Entire finfold absorbed by 8.2 mm; first pelvic fin rays form (8.8-9.2 mm); no swim bladder forms, remains rudimentary (trace presence); gut straight; squamation initiated at 10.0 mm. Preoperculomandibular canals form (10.8-11.8); supraorbital, infraorbital, and lateral head canals form (11.0-12.6 mm); supraorbital completely form (11.7 mm); preoperculomandibular pores 10, completely form (12.6-13.8 mm); infraorbital canal form with 8 pores (10.8 mm), completely form with retrogression to interrupted condition of 4 pores anteriorly and 2 pores posteriorly (14.4-14.6 mm). Lateral line forms (14.0-14.2 mm); squamation complete (14.7 mm); scales absent on the nape, cheek, opercle, breast, and prepectoral areas.

Meristics.—Myomere number in *E. f. lineolatum* is constant posthatching, preanal myomeres 15, postanal myomeres 19-21 (\bar{x} = 19.5; n = 155), with 34-36 total myomeres. Total vertebrae number 33-34 (\bar{x} = 33.8, n = 5), including one urostylar element (from cleared and stained specimens from Spring Coulee Creek and the Root River). Scales in the lateral series ranged from 42-57 (\bar{x} = 47.7; mode = 48; n = 13). Paired and median fin rays and length at appearance are summarized in Table 2.

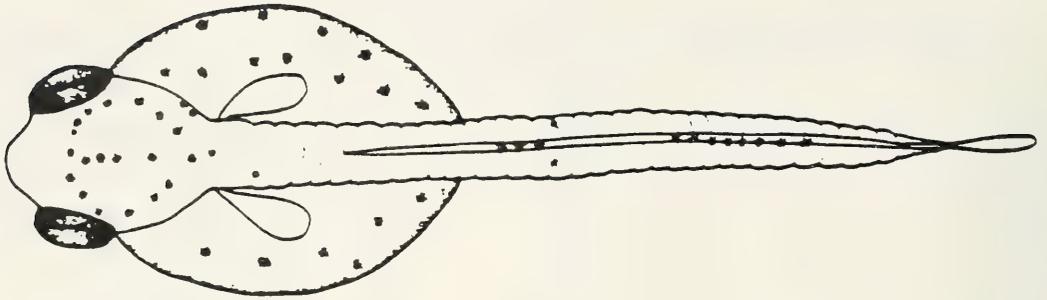
Pigmentation.—Newly hatched larvae with scattered melanophores on a large yolk sac with greatest concentration laterally. Stellate

TABLE 2. Selected meristic values and size (mm total length) at the apparent onset of development for *Etheostoma flabellare lineolatum* and *E. percnurum*. Mean values are underscored. The number of secondary rays of the median fins are in lowercase Roman numerals.

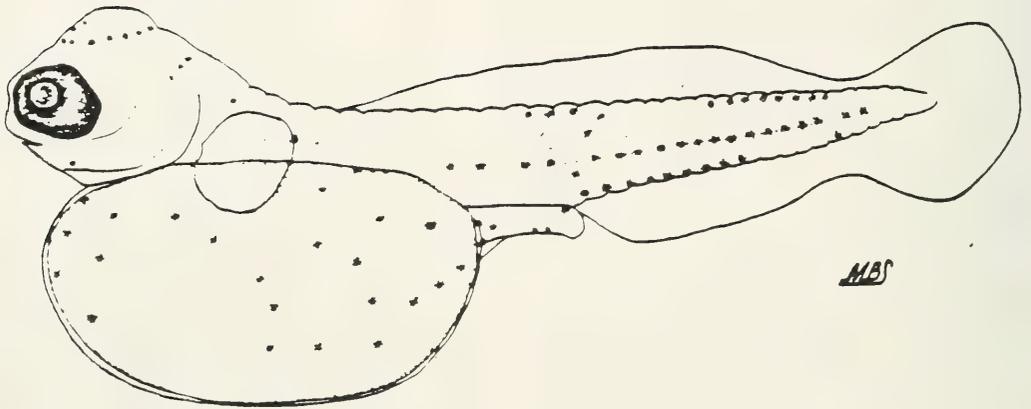
Attribute/Event	<i>E. f. lineolatum</i>	<i>E. percnurum</i>
Dorsal fin spines/rays	VIII-IX/12-13-14	VI-VII-VIII/10-12-13
First rays formed	5.9/5.7	5.8/5.8
Adult complement formed	7.6/7.2	6.1/7.4
Pectoral fin rays	12-13	12-13-14
First rays formed	5.0-5.6	5.8
Adult complement formed	7.0	6.1-6.2
Anal fin spines/rays	II/9-10-11	II/7-8-9
First rays formed	5.3-5.9	5.8
Adult complement formed	7.0-7.2	7.4
Pelvic fin spines/rays	I/5	I/5
Bud formed	7.9-8.5	>8.1
First rays formed	8.8-9.2	9.3
Adult complement formed	8.8-9.2	9.3
Caudal fin rays	ix-xiv, 8 + 7, viii-xiv	xi-xvi, 8 + 8-9, xi-xv
First rays formed	5.3-5.5	5.8
Adult complement formed	7.0-7.5	7.5
Lateral series—scales	42-48-57	38-40-45-48
Myomeres/vertebrae	34-36/33-34	34-36/33-34-35
Preanal myomeres	15	15
Postanal myomeres	19-21	19-21

melanophores encircle optic lobe. Several melanophores laterally, rising obliquely near nape; lateral pigmentation with a midlateral stripe from the yolk sac posterior to base of caudal peduncle. Ventral melanophores present on gut and from posterior anus to approximately postanal myomere 9. Dorsal melanophores in 2 blotches located just anterior the anus, and initiating near posterior of ventral postanal melanophores. Majority of preanal myomeres without pigmentation (4.5-5.9 mm; Fig. 1). Postorbital bar formed, with additional horizontal pigment on operculum. Yolk sac with stellate melanophores on distal half. Dorsally, pigmentation on nape and at the base of soft dorsal. Laterally, melanophores outlining preanal myosepta posterior of yolk sac, extending to middle of soft dorsal; a midlateral stripe formed from single melanophores at apex of preanal myomeres posterior of yolk sac. Ventrally, stellate melanophores present at almost every postanal myoseptum with several extending dorsally to midlateral; melanophores present at base of caudal fin (6.0-7.5 mm; Fig. 2A). Horizontal preorbital and post-orbital bars with additional pigment present on operculum, and dorsally on nape; melanophores outline lateral myosepta of all myo-

meres just posterior of soft dorsal. Melanophores extend onto rays of caudal, anal, and soft dorsal. Ventral pigmentation concentrated at midventral gut, and beneath operculum and branchiostegal rays (7.8-9.2 mm; Fig. 2B). Chevron shaped clusters present dorsal-anteriorly to orbit, and on the optic lobe. Dorsally 8 rectangular blotches extend from nape to base of caudal peduncle. Oval blotches become continuous anteriorly along the midlateral with scattered gut melanophores. Ventral pigmentation limited to 5 areas of concentration from just after anus to base of caudal peduncle. Spinous dorsal, pectoral, pelvic, and anal fins devoid of pigment (9.5-10.9 mm; Fig. 3A). An oblique bar extends towards nape laterally, posterior the orbit; cerebrum and optic lobe with clustered melanophores. Dorsally, 9-10 rectangular blotches with obliquely scattered melanophores connecting 12-13 midlateral blotches. Lateral epaxial scales outlined with scattered melanophores; pectoral girdle with a blotch near cleithrum. Lepidiotrichia of spinous and soft dorsal, anal, and base of caudal fins with melanophores. Mandible, maxilla, and interopercle with scattered melanophores; pectoral and pelvic fins devoid of pigment (11.0-13.9 mm). Juvenile pigmentation, cra-

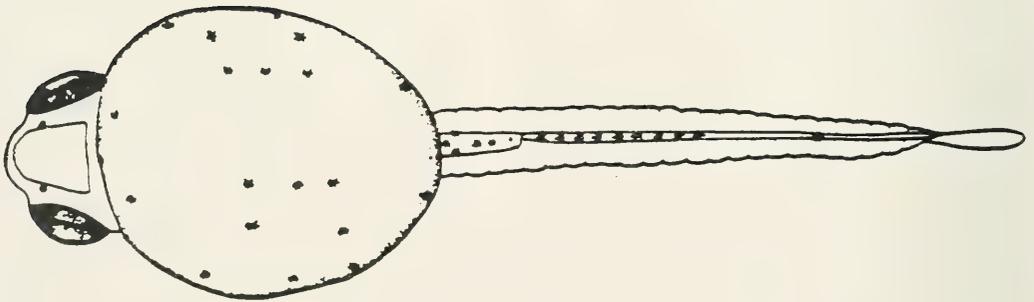


a.



b.

1 mm



c.

FIG. 1. *Etheostoma flabellare lineolatum*, striped fantail darter, newly hatched yolk sac larva, 4.8 mm TL, Coon Creek, Wisconsin. a. dorsal, b. lateral, c. ventral views.

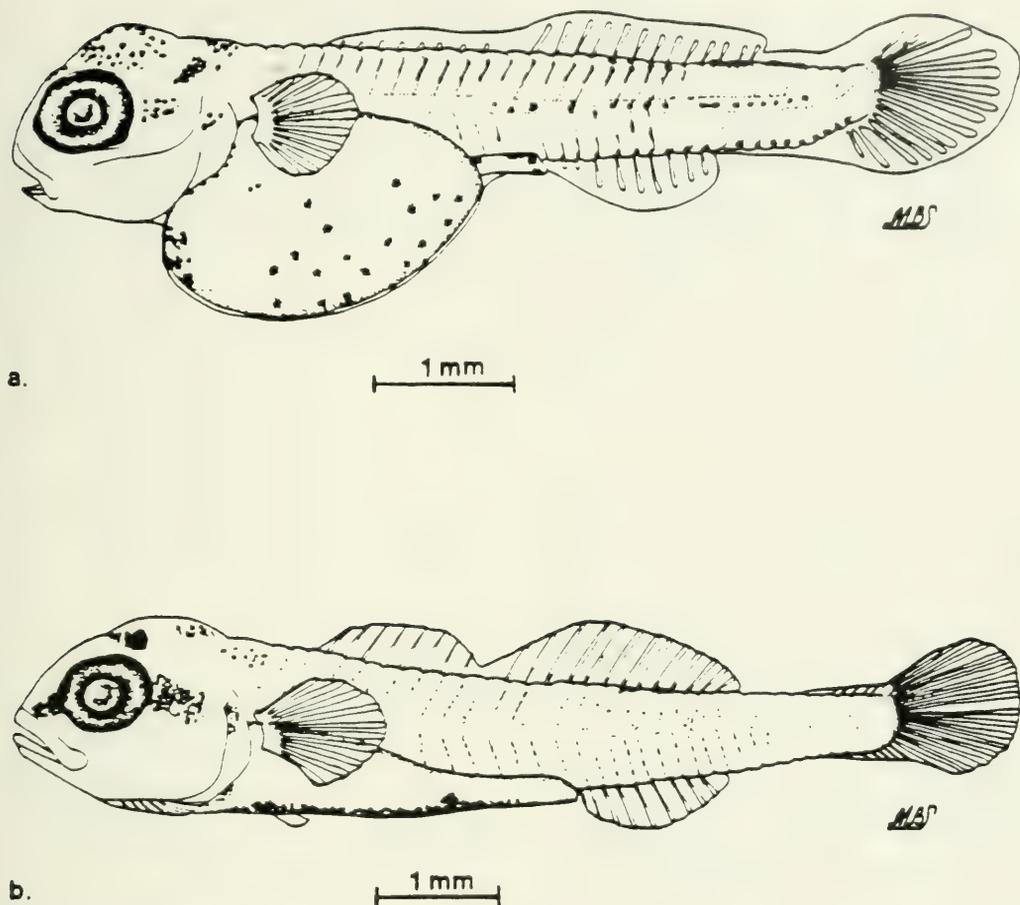


FIG. 2. *Etheostoma flabellare lineolatum*, striped fantail darter, Spring Coulee Creek, Wisconsin. a. 7.1 mm TL larva, lateral view, b. 8.2 mm TL larva, lateral view.

nium with concentrated melanophores over optic lobe and cerebrum; distinct preorbital and postorbital bars formed, no suborbital tear drop; a chevron shaped cluster of melanophores parallel to postorbital bar, scattered melanophores on cheek. Lateral pigmentation including 11–13 rectangular blotches connecting to 8 dorsal bands; the final lateral blotch may be divided to form 2 spots near midlateral caudal peduncle base. Horizontal lines of melanophores extend from the head to caudal peduncle, formed from individual melanophores on outer margins of scales; distinct humeral spot formed near posterior of opercular spine. Spinous dorsal, pectoral, and anal fins with scattered pigmentation on rays; 4–5 horizontal stripes on soft dorsal distributed on fin rays; caudal fin with 6–8 vertical stripes formed on

interstitial membranes; pelvic fins without pigmentation (Fig. 3B).

Duskytail darter,
Etheostoma percnurum

Eggs.—Nests of the duskytail darter in Little River consisted of single-layer clusters of 23 to 200 eggs ($n = 22$; $\bar{x} = 79$; $SD = 46$) attached to the undersides of slab-shaped stones (4, 21). Fertilized eggs were translucent and spherical, and averaged 2.8 mm diameter from wild nests (range: 2.6–3.3 mm; $n = 35$; $SD = 0.2$ mm) and 2.9 mm from aquarium nests (range: 2.6–3.3 mm; $n = 25$; $SD = 0.1$ mm; 4). The chorion was clear, adhesive, and flattened at the point of attachment to the nest stone. The yolk was translucent, and a large translucent amber oil globule, surrounded by

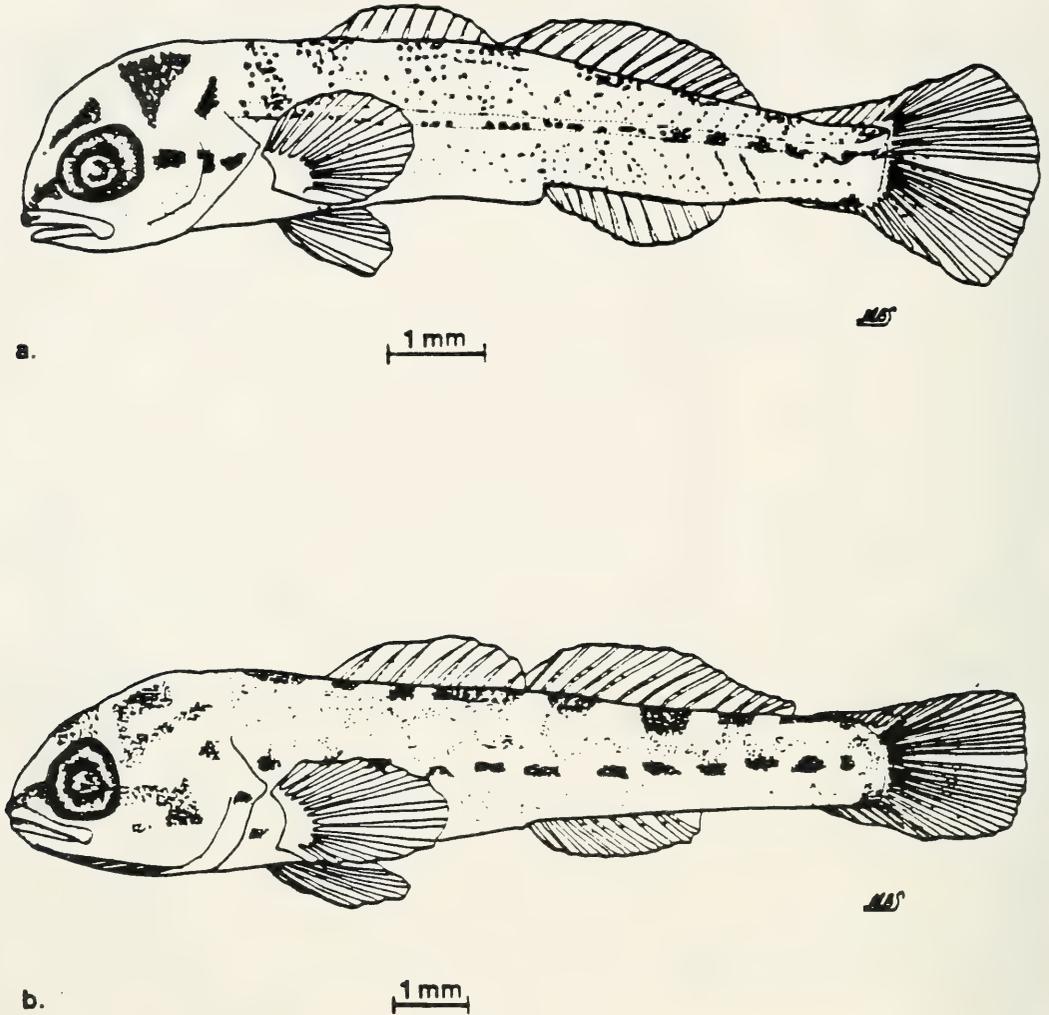


FIG. 3. *Etheostoma flabellare lineolatum*, striped fantail darter, Root River, Minnesota. a. 10.3 mm TL early juvenile, lateral view. b. 14.4 mm TL juvenile lateral view.

several much smaller ones, imparted an amber color to the egg.

Embryonic Development.—The morula was round and measured 0.8–0.9 mm in diameter; yolk was 2.2–2.3 mm in diameter, with a single large oil globule 0.6–0.7 mm in diameter; the perivitelline space ranged 0.1–0.4 mm (5 hr embryos). The blastoderm of the early embryo covered 67% of the yolk, the embryonic axis was forming, and the germ ring was visible (26 hr embryo). In the tail-bud stage, the optic vesicles were forming, and 9 somites were visible (ca. 28–47 hr embryos). In early tail-free embryos, dorsal and ventral finfolds were de-

veloping; auditory vesicles were visible; lenses were forming in the unpigmented eyes; and about 30 somites were discernible; the heart was clearly beating, and the embryo occasionally twitched. Melanophores were widely scattered over the yolk membrane and were most concentrated on the vent and body-yolk juncture (50 hr embryos). By the late tail-free stage, the head was highly elevated; the eyes were grayish-black; and the pectoral fin buds were present. Melanophores were more concentrated over the yolk membrane and had developed ventrally along the body to the tail (72–96 hr embryos). In late embryos, the eyes

TABLE 3. Morphometry of *Etheostoma percnum* larvae and early juveniles grouped by selected intervals of total length (N = sample size). Characters expressed as percent total length or head length^(a) with a single standard deviation.

Total length	N	Length (% TL)				Depth (% TL)				
		Standard	Preal	Snout ^(a)	Eye ^(a)	Head	Head	Shoulder	Anus	Caudal peduncle
4.5-6.2	6	96.3 ± 0.4	56.8 ± 4.6	9.5 ± 1.5	41.1 ± 5.2	21.4 ± 2.0	18.9 ± 1.3	37.7 ± 7.0	9.7 ± 1.3	4.3 ± 0.9
7.4-11.8	16	86.3 ± 2.7	50.3 ± 1.7	15.0 ± 2.4	37.6 ± 4.1	23.2 ± 2.3	18.6 ± 1.3	21.4 ± 3.7	12.3 ± 1.0	7.0 ± 0.5
12.3-15.5	2	82.8 ± 0.9	52.4 ± 1.6	17.8 ± 0.1	30.8 ± 5.1	26.6 ± 0.1	16.8 ± 0.8	17.0 ± 1.1	11.6 ± 1.1	6.8 ± 1.3

were pigmented black; the newly formed mouth opened and closed; opercles moved; and the well-developed pectoral fin buds fluttered. A highly branched vitelline plexus covered the antero-ventral portion of the yolk sac, very similar to that described by Paine (15) for embryos of *E. f. flabellare*. Melanophores were present on the yolk sac, dorsally and ventrally along the bases of the finfolds, mid-laterally toward the tail, and on top of the head. Late embryos wiggled frequently and vigorously, and the chorion was soft and delicate (>125 hr embryos). Eggs hatched in 264-336 hr (11-14 days) at 18°-27°C (5).

Larvae

Morphology.—The size of initial formation for selected structures is summarized for larval and early juvenile *E. percnum* with morphometric features shown in Table 3. At 4.5-5.4 mm TL, newly hatched, pectoral buds were present with 6 incipient rays; first pectoral rays form (5.8 mm); yolk sac robust, spherical ca. 47.9% TL, yolk translucent, single oil globule 0.7 mm diameter; vitelline vein form a plexus on midventral yolk sac; head not deflected over yolk sac; jaws developed; eye diameter spherical. Median fin rays in the spinous and soft dorsal, anal, and caudal fins form simultaneously with notochord flexion (5.8 mm); branchiostegal rays form and caudal fin round (6.2 mm); incipient anal fin margin partially differentiated (7.4 mm); incipient dorsal fin margin partially differentiated (7.5 mm); spinous dorsal fin origin situated over preanal myomere 3-4, soft dorsal origin situated over postanal myomere 16 (7.5 mm); predorsal length 32.3% TL (range: 29.0-44.2% TL); 38.3% SL (range 31.2-53.3% SL; 7.5 mm); incipient anal fin margin completely differentiated (7.8 mm); pelvic buds form anterior to spinous dorsal fin origin (>8.1 mm); yolk completely absorbed and first pelvic fin rays form (9.3 mm); no swim bladder forms; gut straight; entire finfold absorbed (8.1 mm). Scale formation initiated in 15.5 mm juvenile at base of caudal peduncle. Squamation nearly complete by 18 mm SL. Scales absent from the cheeks, opercles, nape, breast, prepectoral area, and middle of abdomen.

Meristics.—Myomere number in *E. percnum* constant posthatching, preanal myomeres 15, postanal 19-21 (\bar{x} = 19.9, n = 20),

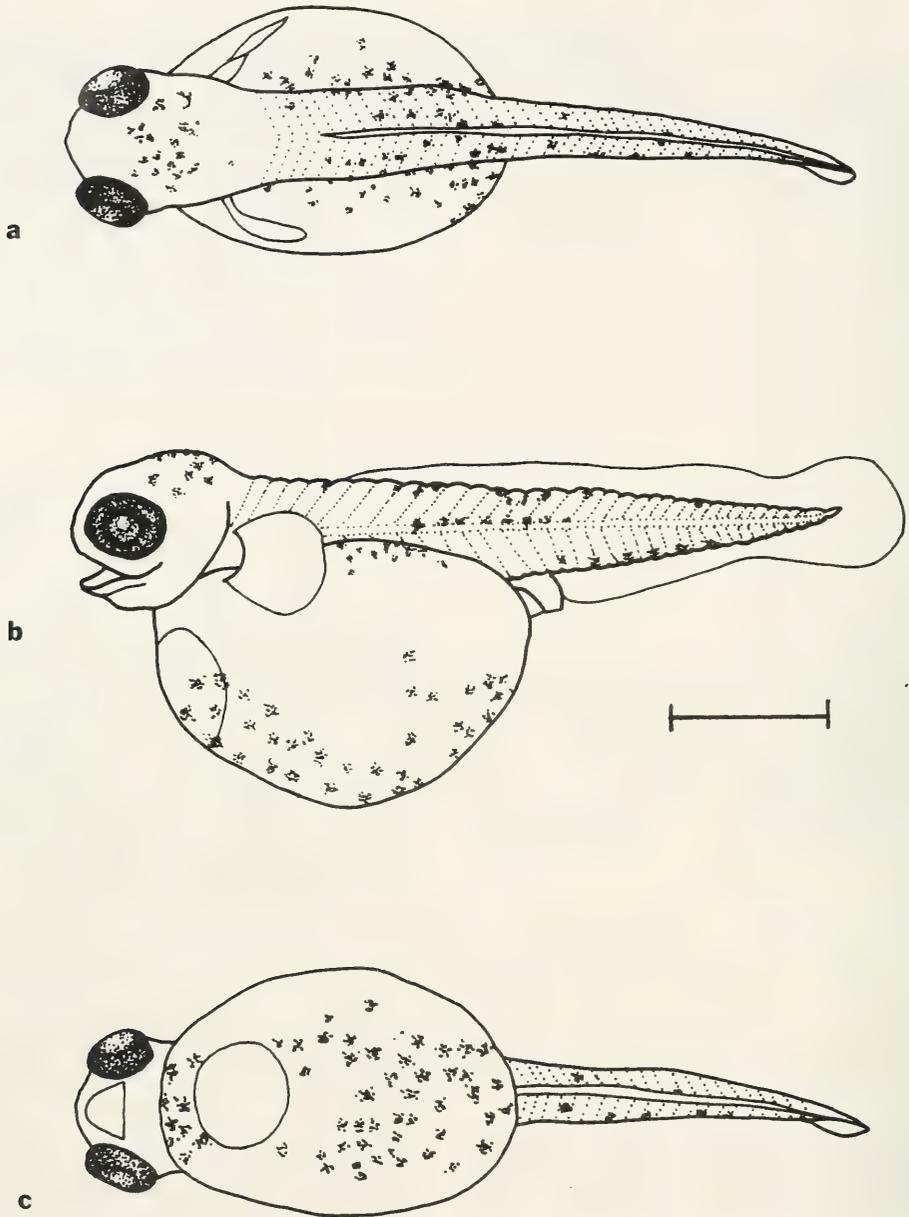


FIG. 4. *Etheostoma percnurum*, duskytail darter, newly hatched yolk sac larva, 5.1 mm TL, Little River, Tennessee. a. dorsal, b. lateral views, c. ventral views.

with 34–36 total myomeres ($\bar{x} = 34.8$, $n = 20$). Total vertebrae number 33–35 ($\bar{x} = 34.0$, $n = 5$), including one urostylar element (from cleared and stained specimens from Little River). Scales in the lateral series ranged from 38–48 ($\bar{x} = 43.3$; mode = 45; $n = 5$). Paired

and median fin ray values and lengths at appearance are presented in Table 2.

Pigmentation.—At 4.9–5.7 mm, newly hatched larvae: body translucent; amber oil globule flattened, located anteriorly in yolk sac; highly branched vitelline plexus (red in

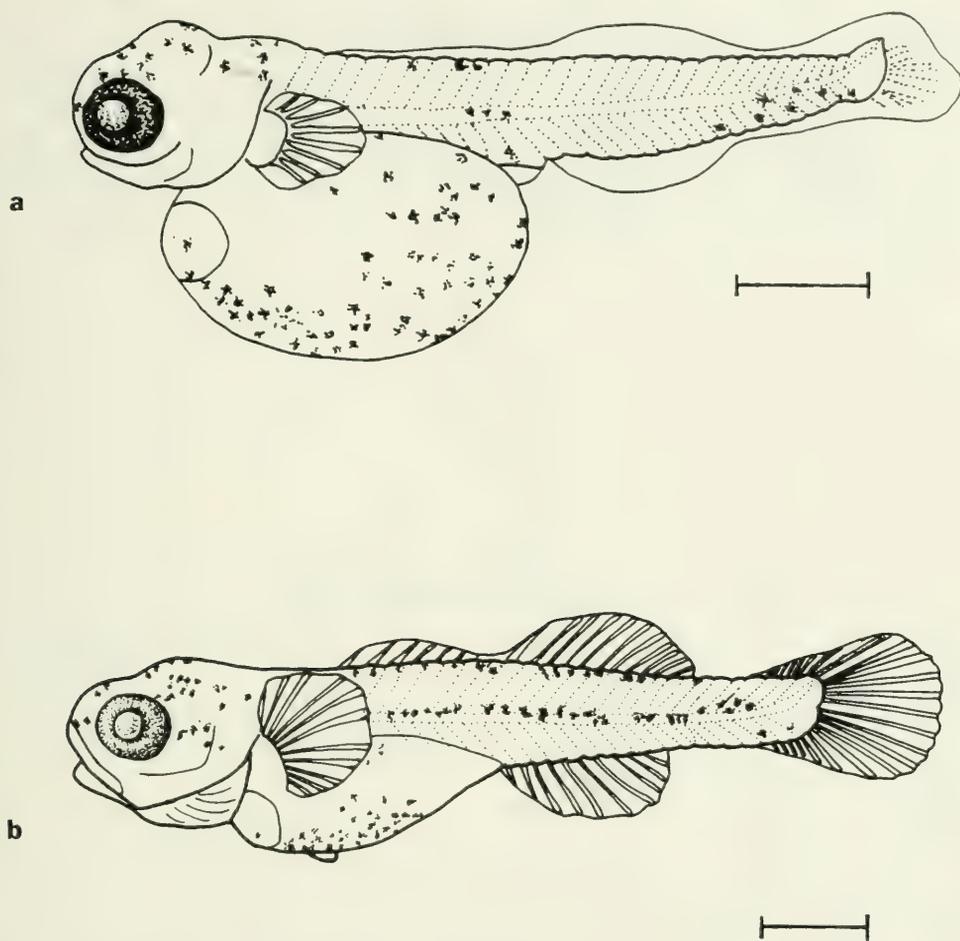


FIG. 5. *Etheostoma percnum*, duskytail darter, Little River, Tennessee. a. 6.9 mm TL larva lateral view, b. 8.2 mm TL larva lateral view.

life) over oil globule and antero-ventral portion of yolk sac; melanophores on yolk sac most concentrated ventrally, postero-laterally, and at body-yolk juncture; prominent patch of melanophores on top of head; melanophores irregularly distributed along dorsum, mid-laterally along myosepta, and ventrally toward the tail (Fig. 4A, B). Melanophores developing anteriorly on top of head toward snout (6.1–6.2 mm; Fig. 4C). Body straw-colored, less translucent; dense patch of stellate melanophores on top of cranium; orbital bar of melanophores developing from opercle to snout; melanophores concentrated medially along dorsum and mid-laterally along horizontal septum; melanophores beginning to develop on soft dorsal, caudal, and anal fin rays; subcuta-

neous melanophores dorsally on gut (7.4–8.3 mm; Fig. 5). Pre- and post-orbital bars distinct; melanophores forming indistinct blotches dorsally (7–8) and mid-laterally; light scattering of melanophores on soft dorsal, caudal, and anal rays, and to lesser extent on spinous dorsal fin; subcutaneous melanophores on back of head and opercles; gold iridescent pigment in eyes (9.2–9.8 mm). Juveniles pigmentation, body opaque, straw-colored, heavily pigmented with melanophores; melanophores on top of head concentrated and confluent; dorsal and lateral blotches wider, diffuse, with indistinct dorso-lateral connections between them; soft dorsal, anal, and caudal fin rays lined with melanophores; few melanophores on pectoral and pelvic fin rays, spinous dorsal

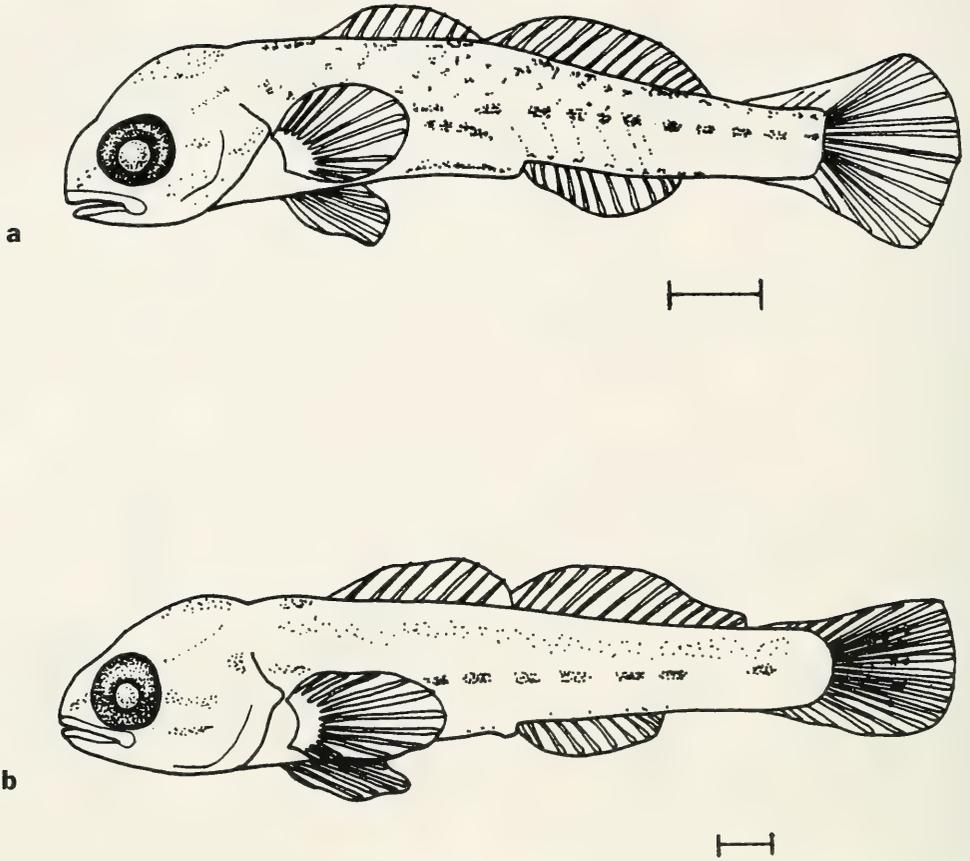


FIG. 6. *Etheostoma percnurum*, duskytail darter, Little River, Tennessee. a. 9.5 mm TL early juvenile lateral view, b. 15.5 mm TL juvenile lateral view.

fin, underside of head, breast, and belly; gold iridescence in eyes more pronounced (10.1–15.5 mm; Fig. 6). A wild juvenile at 18 mm SL: squamation nearly complete; pigmentation approaching that of adult: body straw-colored, 12 lateral vertical brown bars connected dorsolaterally to 6–7 irregular brown dorsal saddles, dark humeral spot, and cheeks speckled with melanophores.

DISCUSSION

Species of *Catnotus* are well known for their derived spawning habit of clustering eggs on the undersides of stones (8, 22). *Etheostoma flabellare* spawns beneath large stones in riffles, runs, or pools with slow to moderate current (12, 18). Spawning of *E. f. lineolatum* occurs in early April until May (23, 24, 25). In Wisconsin and Minnesota, spawning initiated

in early May and continued until June at temperatures between 13°–17°C, but continued until mid-July when temperatures approached 25°C in Iowa and Illinois (17). The duskytail darter spawns in Little River from late April through June in pools and moderate runs beneath slab-shaped cobbles (5).

Etheostoma f. lineolatum and *E. percnurum* are sympatric, however, the former species is referred to as *E. f. flabellare* by McGeehan (2). Duskytail darter larvae are more precocious, developing incipient rays in the pectoral fins and complete rays in the median fins earlier than striped fantail darters; however, squamation begins later, at lengths >15 mm TL. These two taxa can be distinguished from other described members of the *E. flabellare* species group, *E. f. flabellare* and *E. kennicotti*, based on myomere number, pigmentation and

TABLE 4. Summary comparison of meristic, pigmentation, and ontogenetic event characteristics for four taxa of the *Etheostoma flabellare* species group.

Characteristic	<i>E. flabellare flabellare</i>	<i>E. flabellare lineolatum</i>	<i>E. kennicotti</i>	<i>E. percnurum</i>
Size and shape				
Egg diameter	2.2–2.7 mm	2.0–2.8 mm	1.9–2.5 mm	2.6–3.3 mm
Hatching length	4.7–6.2 mm	4.5–5.9 mm	4.1–4.7 mm	4.5–5.4 mm
Yolk sac diameter	31% TL	42% TL	32% TL	48% TL
Yolk sac absorbed	9–10 mm	7.8–8.0 mm	7.5 mm	9.3 mm
Yolk color	pale yellow	amber	orange	amber
Eye shape	oval	oval	spherical	spherical
Fin ray formation				
First pectoral ray	7.2–7.5 mm	5.0–5.6 mm	5.1–5.2 mm	5.8 mm
First dorsal spine	7.2–7.5 mm	5.9–6.2 mm	5.1–5.2 mm	5.8 mm
First soft dorsal ray	7.2–7.5 mm	5.3–5.9 mm	5.1 mm	5.8 mm
First pelvic fin ray	8.8 mm	8.8–9.2 mm	<12.1 mm	9.3 mm
First anal ray	7.2–7.5 mm	5.3–5.9 mm	6.9 mm	5.8 mm
First caudal ray	7.2–7.5 mm	5.3–5.9 mm	7.5 mm	5.8 mm
Morphological event				
Notochord flexion	7.2 mm	4.9–6.0 mm	6.9 mm	5.8 mm
Squamation initiated	13.0 mm	10.0 mm	13.1 mm	15.5 mm
Meristics				
Total myomeres	34–36	34–36	34–35	34–36
Preanal myomeres	15	15	16	15
Postanal myomeres	19–21 (21)	19–21 (19.5)	18–19 (18.5)	19–21 (19.9)

fin ray development relative to size (Table 4). *Etheostoma kennicotti* can be separated from all other members of the *E. flabellare* species group since it possesses 16 preanal and 18–19 postanal myomeres. All other taxa have 15 preanal and 19–21 postanal myomeres. *E. kennicotti* has melanophores scattered across the yolk-sac similar to *E. flabellare*, while it differs in possessing a dorsal and ventral cluster posterior to the anus. The other three taxa can be separated based on yolk-sac diameter, eye shape, and ontogenetic development of fin rays. The duskytail darter has a spherical eye shape while both subspecies of *E. flabellare* have an oval eye shape. Significant differences exist in the ontogenetic development of fin rays between *E. f. flabellare* and *E. f. lineolatum*. Development of fin rays is more precocious in *E. f. lineolatum*, occurring at smaller length intervals for all but the formation of the first pelvic fin ray. Yolk sac diameter is greatest in duskytail darter (48% TL), followed by *E. f. lineolatum* (42% TL), and *E. f. flabellare* (31% TL), while the yolk sac is absorbed at smaller length intervals in *E. f. lineolatum* and at similar sizes for *E. f. flabellare* and duskytail darter.

Sympatric species of *Catonotus* are likely to

utilize similar slab rock habitat for spawning (3), and thus, their larvae and juveniles may often be collected in the same habitat. Differences between *E. f. lineolatum* and other described *Catonotus*, *E. squamiceps* and *E. smithi*, enable accurate identification based on myomere counts and pigmentation. *Etheostoma squamiceps* can be separated from *E. f. lineolatum* because the former possesses 16 preanal and 18–19 postanal myomeres (9). *Etheostoma smithi* has similar myomere counts to *E. f. lineolatum* but differs in yolk sac diameter, pigmentation, and formation of the rays (10). Yolk sac diameter is smaller (33.5%) than *E. f. lineolatum*; pigmentation is restricted to the nape, ventral yolk-sac, and mid-ventral postanal myosepta; and fin ray formation occurs later than in *E. f. lineolatum*.

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Aspects of the Dragonfly and Damselfly (Odonata) Community of Buck Creek, Pulaski County, Kentucky

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ABSTRACT

Buck Creek is a fifth-order tributary of the upper Cumberland River, in southcentral Kentucky. Thirty two species of Odonata were found to inhabit this stream. Ecological data, particularly seasonality, of 31 species are presented, along with behavioral observations. Published flight seasons were extended for 5 species of Anisoptera and 6 species of Zygoptera for Kentucky. Seasonal life histories are potentially important in understanding the dynamics involved in niche segregation of a diverse community of general predators.

INTRODUCTION

A review of the literature concerning the Odonata of Kentucky revealed a paucity of ecological data. Resner (1) presented the latest distributional list with seasonality data for most species. The literature review showed that the geographical area of this study had been given little attention in surveys of Odonata (1). Additionally, few seasonality studies of communities of lotic Odonata have been published (2-4).

We undertook this study to characterize the community of Odonata of Buck Creek, Pulaski County, Kentucky. Because of the desire to observe as many species as possible and offer a complete documentation of seasonality, 2 flight seasons were incorporated in this study (1991 and 1992). Specifically, we wanted to (1) determine the odonate community composition; (2) document specific flight seasons; (3) make ecological observations; and (4) observe behavior. In this paper, data are presented for 31 of the 32 species (5) known to inhabit Buck Creek.

STUDY AREA

Buck Creek, a fifth-order tributary of the upper Cumberland River, flows southward for 107.2 km, draining 767 km². This stream is located in southcentral Kentucky (37°10'N, 84°30'W) and flows primarily within the Eastern Highland Rim subsection of the Interior Low Plateaus Physiographic Province (6). The

surface geology is composed principally of Mississippian age limestone (7).

The lower 19% of Buck Creek is inundated by the back waters of Lake Cumberland. This occurred with the completion of Wolf Creek Dam on the Cumberland River in 1951. Buck Creek averaged less than 20 m wide and 2 m deep, but had a maximum width of 150 m and a maximum depth greater than 25 m near its mouth (8). Its gradient was 1.25 m/km (8) with an estimated mean flow of 11.7 km³/m (9).

METHODS

Six collecting sites (5) were chosen on the mainstem of Buck Creek. Two sites were visited per week, and a collecting circuit of all sites was completed every 3 weeks. Adult collections began in June and continued through October 1991, and from April to mid-September 1992. At each site, extensive searches for and observations of adults were made by wading upstream and downstream several 100 m. Physical and biological characteristics of each collection and observation site were made.

RESULTS

Figures 1 and 2 show the flight seasons of 31 of 32 species of Anisoptera and Zygoptera, respectively. The dragonfly species not included here, *Somatochlora linearis*, was collected as a larva. The Anisoptera flight season began in mid-April with *Basiaeschna janata* and concluded by end of October with *Boyeria vinosa* (Fig. 1). However, the Zygoptera flight season began in early May with *Calopteryx maculata* and concluded with *Hetaerina americana* and *Argia translata* in October (Fig. 2). Thus, the

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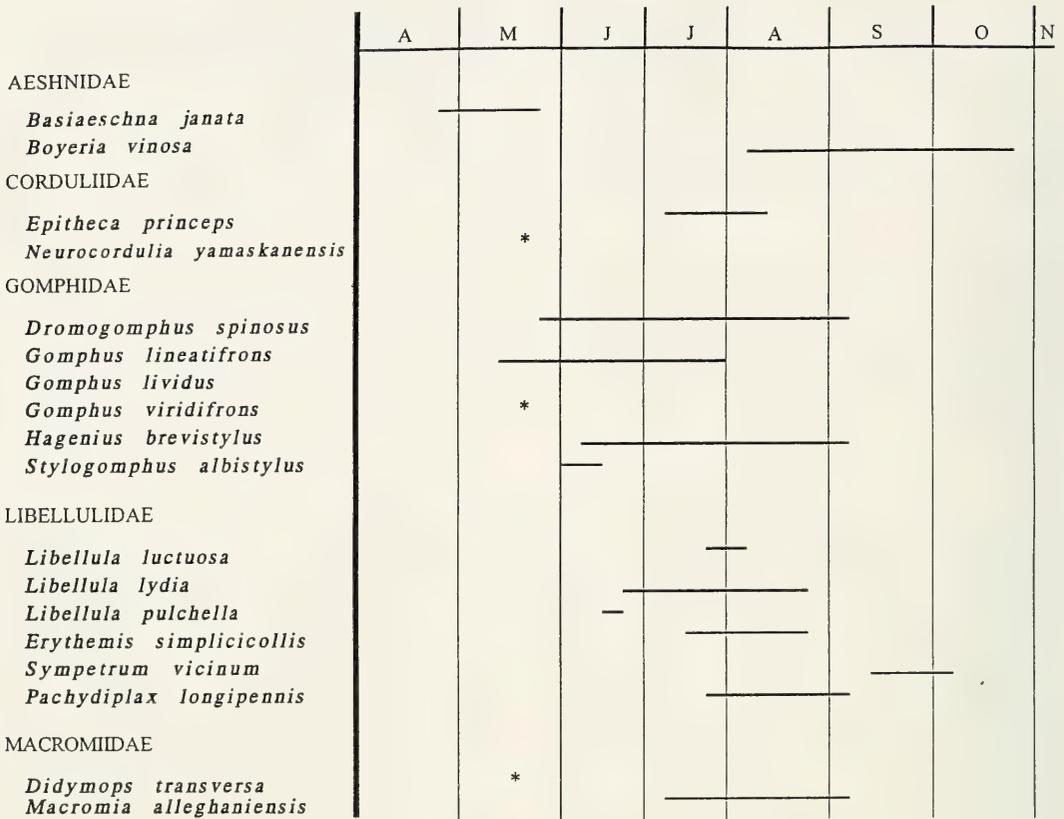


FIG. 1. Adult flight seasons of Anisoptera collected and observed (June–November 1991; April–mid-September 1992) at Buck Creek, Pulaski County, Kentucky. Asterisks (*) indicate single date record.

adult odonate flight season persisted for 190 known days (Tables 1 and 2). Of the 2 suborders, anisopterans had a shorter flight season (Figs. 1, 2). Student's *t* test was employed to compare the flight season between the 2 suborders (excluding those with less than 7 days recorded flight period). To compute the value of *t*, the flight season of each species was determined by taking the 2 seasons and averaging their flight period. This resulted in a statistically significant ($P < 0.05$) difference in flight season length. Kentucky flight season range extensions were recorded for 5 species of Anisoptera (Table 1) and 6 species of Zygoptera (Table 2).

DISCUSSION

Flight season in this study began with the first observation or collection of a reproductively mature adult. Corbet (10) reported that most reproductively mature Zygoptera live ap-

proximately 1 to 2 weeks and may extend to 5 to 8 weeks. Reproductively mature Anisoptera live 2 to 3 weeks and may extend to 3 to 6 weeks (10). Thus, synchrony of emergence was applied to those species with a flight season of approximately 6 weeks or less.

Most species collected were typical of lotic habitats; however, the Libellulidae characteristically breed in lentic waters. Westfall (11) reported the large genus *Libellula* and *Pachydiplax* from lotic waters. The upper one-half of Buck Creek had many braids that became isolated and thus lentic for a significant amount of time during both years of this study. This type of environment was previously reported in Buck Creek (7, 12). *Pachydiplax longipennis* was observed in large numbers at the most upstream site (State Route 70) (5); *Libellula lydia*, *L. pulchella* and *L. luctuosa* also were associated with the ponded water at this site. Of the 2 major isolated braids here, *P.*

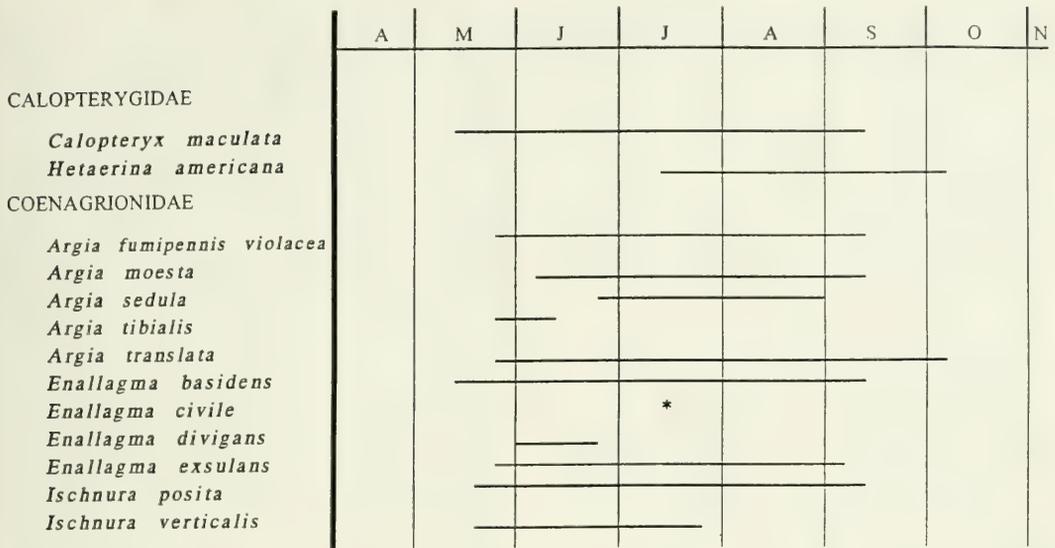


FIG. 2. Adult flight seasons of Zygoptera collected and observed (June–November 1991; April–mid-September 1992) at Buck Creek, Pulaski County, Kentucky. Asterisk (*) indicates single date record.

TABLE 1. Flight seasons for adult Anisoptera based on collections and sightings (June–November 1991; April–mid-September 1992) at Buck Creek, Pulaski County, Kentucky. Addition signs (+) indicate flight season extensions for Kentucky.

Aeshnidae	
<i>Basiaeschna janata</i> (Say)	20 April +–18 May +
<i>Boyeria vinosa</i> (Say)	3 Aug.–26 Oct.
Corduliidae	
<i>Epithea princeps</i> (Hagen)	5 July–10 Aug.
<i>Neurocordulia yamaskanensis</i> Provancher	18 May
Gomphidae	
<i>Dromogomphus spinosus</i> Selys	31 May–1 Sept.
<i>Gomphus (Arigomphus) lentulus</i> Needham	1 June +
<i>Gomphus (Gomphurus) lineatifrons</i> Calvert	12 May–31 July
<i>Gomphus (Hylogomphus) viridifrons</i> Hine	18 May
<i>Hagenius brevistylus</i> Selys	9 June–7 Sept. +
<i>Stylogomphus albistylus</i> (Hagen)	1 June–9 June
Libellulidae	
<i>Libellula luctuosa</i> Burmeister	19 July–3 Aug.
<i>Libellula lydia</i> (Drury)	22 June–21 Aug.
<i>Libellula pulchella</i> Drury	21 June–22 June
<i>Erythemis simplicicollis</i> (Say)	14 July–31 Aug.
<i>Sympetrum vicinum</i> (Hagen)	13 Sept.–4 Oct.
<i>Pachydiplax longipennis</i> (Burmeister)	19 July–7 Sept.
Macromiidae	
<i>Didymops transversa</i> (Say)	18 May +
<i>Macromia alleghaniensis</i> (Williamson)	5 July–7 Sept. +

TABLE 2. Flight seasons for adult Zygoptera based on collections and sightings (June–November 1991; April–mid-September 1992) at Buck Creek, Pulaski County, Kentucky. Addition signs (+) indicate flight season extensions for Kentucky.

Calopterygidae	
<i>Calopteryx maculata</i> (Beauvois)	
<i>Hetaerina americana</i> (Fabricius)	11 May–13 Sept. + 12 July–4 Oct.
Coenagrionidae	
<i>Argia fumipennis violacea</i> Hagen)	
<i>Argia moesta</i> (Hagen)	31 May–13 Sept. +
<i>Argia sedula</i> (Hagen)	21 June–13 Sept. +
<i>Argia tibialis</i> Rambur	21 June–31 Aug.
<i>Argia translata</i> Hagen	31 May–7 June
<i>Enallagma basidens</i> Calvert	31 May +–4 Oct. +
<i>Enallagma civile</i> (Hagen)	12 May +–13 Sept.
<i>Enallagma divagans</i> Selys	13 July
<i>Enallagma exsulans</i> (Hagen)	11 June–22 June
<i>Ischnura posita</i> (Hagen)	18 May–1 Sept.
<i>Ischnura verticalis</i> (Say)	11 May–13 Sept. + 12 May–14 July

longipennis was associated with the smaller one that flowed during high-water conditions.

The aeshmids, *Basiaeschna janata* and *Boyeria vinosa*, had dramatically different flight seasons (Fig. 1). Thus, these 2 similarly adapted predators demonstrated temporal segregation in this study. Paulson and Jenner (4) also found *B. janata* to be synchronized “spring” species (13), while *B. vinosa* was asynchronous and overwintered in many instars.

There was a marked segregation of seasonality among the 2 species of Macromiidae, *Didymops transversa* and *Macromia alleghaniensis* (Fig. 1). Although *D. transversa* was collected on one date only in this study (Table 1), it is known to be a synchronous spring flyer (1, 4). *Macromia alleghaniensis* had an asynchronous emergence and flew from mid- to late summer (Fig. 1). Both these species were found in the lower two-thirds of the stream, becoming more common downstream.

The family Libellulidae was represented by 6 species; 3 were congeneric (Table 1). While *Libellula lydia* and *Erythemis simplicicollis* were collected along most of Buck Creek, the other species had more restricted distributions. Only *Sympetrum vicinum* had a segregated flight season from other libellulids (Fig. 1). Boehms (14) studied the ecology and development of this species. Observations indi-

cated that *Pachydiplax longipennis* tolerated greater flow velocity than other libellulids. Thus, it occupied a different habitat than the other species. *Libellula lydia* and *Libellula pulchella* exhibited interspecific aggression. Both species occurred commonly around a lentic habitat created for flood control. Spatial segregation was observed for *L. lydia*, which perched on marginal vegetation, and *L. pulchella*, which preferred fallen trees and limbs in or near the water. *Erythemis simplicicollis* was collected or observed primarily along the fifth-order section of the stream. Adult *E. simplicicollis* utilized dense beds of *Justicia americana* or nearby rocks on which to perch.

While the gomphids represented the greatest numbers of individuals collected and observed, their flight season was especially interesting as there was no segregation between species (Fig. 1). Most members of this family were primarily stream dwellers and relatively little was known concerning the factors governing niche segregation. With many species exhibiting asynchronous emergence, larvae exhibited a great spread of instars. The mechanism(s) controlling their niche segregation must occur during the larval life history. Morphological variations in the design of their labium may serve significantly in niche segregation (15). Also, as sprawlers in leafmats and burrowers, this group occupied the majority of available habitat in the stream (horizontal distribution). Another strategy allowing these cryptic, sedentary odonates to withstand niche overlap may result from slow development which requires long generation times (16).

The family Calopterygidae had 2 representatives at Buck Creek, *Calopteryx maculata* and *Hetaerina americana*. *Calopteryx maculata* had the earliest flight season of the 2 species (Table 2). This species demonstrated asynchronous emergence, as evidenced by its long flight season (Fig. 2). *Calopteryx maculata* preferred the more heavily forested sections of the stream, utilizing the dense forest for perches. *Hetaerina americana* emerged 2 months later and flew until early October (Table 2). The latter species also emerged asynchronously; however, it was observed to occupy a different habitat, principally boulders and exposed gravel associated with riffles. These damselflies were always observed in

open, sunny situations, as opposed to *C. maculata*.

The difference in flight season between the 2 species may be due to developmental patterns as larvae. Paulson and Jenner (4) found *Calopteryx* sp. to overwinter in prefinal instars, final (F) F-2 and F-1; *Hetaerina* sp. overwintered in F-4 to F-2 stages. Developmental segregation in these 2 morphologically similar species may be a primary mechanism in controlling interspecific competition. Thus, flight-season variations between these species did indicate that larval-size variation is employed as a competitive strategy.

Within the large family Coenagrionidae, long, overlapping flight seasons predominated among the species (Fig. 2). An exception, *Enallagma divigans* flew from 1 June through 22 June. This species showed synchronous emergence associated with "spring" species (13). Very similar flight-season observations for this species have been previously demonstrated (4, 17). Paulson and Jenner (4) found *E. divigans* larvae to overwinter predominantly in F-1 stages in North Carolina.

Many studies have reported diverse odonate communities coexisting in relatively small geographic areas (2, 17–19). As general predators, odonates must avoid niche overlap by some mechanism(s). It has been suggested that the seasonal segregation of odonates is an important mechanism to reduce niche overlap of similarly adapted species (15, 16, 20). Van Noordwijk (20) also found spatial separation between 7 genera of an 8-species zygopteran community. Michiels and Dhondt (21) demonstrated that 3 species of *Sympetrum* distinctly partitioned resources both spatially and temporally. Interestingly, Zygoptera had a statistically significant longer flight season than Anisoptera. However, the placement of the longer flight season was not expected. Anisoptera began flight in early spring, while Zygoptera did not begin flight until mid-spring. One might expect a competitive advantage gained by damselflies if they had a flight season prior to dragonflies because of fewer potential large predators and the large emergences of potential prey (Chironomidae). This pattern of flight season had been reported by other studies (17, 22).

Detailed life-history studies are needed for many odonates. In addition, more studies are

needed to determine the dynamics controlling niche segregation in diverse communities of odonates.

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Serological Evidence for *Borrelia* sp. in *Peromyscus leucopus* from Western Kentucky

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ABSTRACT

During this study, 312 *Peromyscus leucopus* were surveyed for the presence of *Borrelia* sp. Mouse serum was screened for antibodies against *Borrelia* sp. by an immunoblot technique using whole spirochete antigen. By this technique, 21 of 312 (6.7%) of the samples appeared to be serologically positive. Each positive sample was further tested by western blotting against *B. burgdorferi* whole-cell proteins. Eighteen (5.7%) of the 21 samples were confirmed to possess antibodies which bound to the 39/41 kDa protein band on western blots. Equal numbers of positive samples (9 and 9) were obtained from the eastern and western shores of Kentucky Lake. A total of 40 ticks were removed from 24 of 312 mice and examined by the indirect fluorescent antibody (IFA) technique for the presence of spirochetes. All samples were negative by this method. Mouse ear biopsies were collected and incubated in BSK medium, however *B. burgdorferi* was not recovered by this culturing technique.

INTRODUCTION

Borrelia burgdorferi is a bacterial spirochete which is the etiological agent of Lyme disease. This agent has become the most common vector-borne pathogen in the United States (7) and is transmitted by ticks.

The primary tick vector of *B. burgdorferi* in the northeastern portion of the United States is *Ixodes scapularis* (6, 7, 11), whereas *I. pacificus* is most common in the western U.S. While neither of these vectors have been reported from Kentucky, it does appear that Lyme disease transmission is occurring in the state. Pelletier et al. (7) reported that 51 Kentucky cases of Lyme disease, based upon CDC criteria, met the case definition between 1985 and 1990.

This information suggests that either *I. scapularis* is present, but not yet identified, or another vector is transmitting the disease. Recent evidence by Teltow et al. (12) in Texas and Luckhart et al. (3) in Alabama indicated that other species of ticks harbor *B. burgdorferi*. Among the species noted was the lone star tick, *Amblyomma americanum*, a common tick in western Kentucky.

Levine et al. (2) reported that in the north-eastern United States the white-footed mouse,

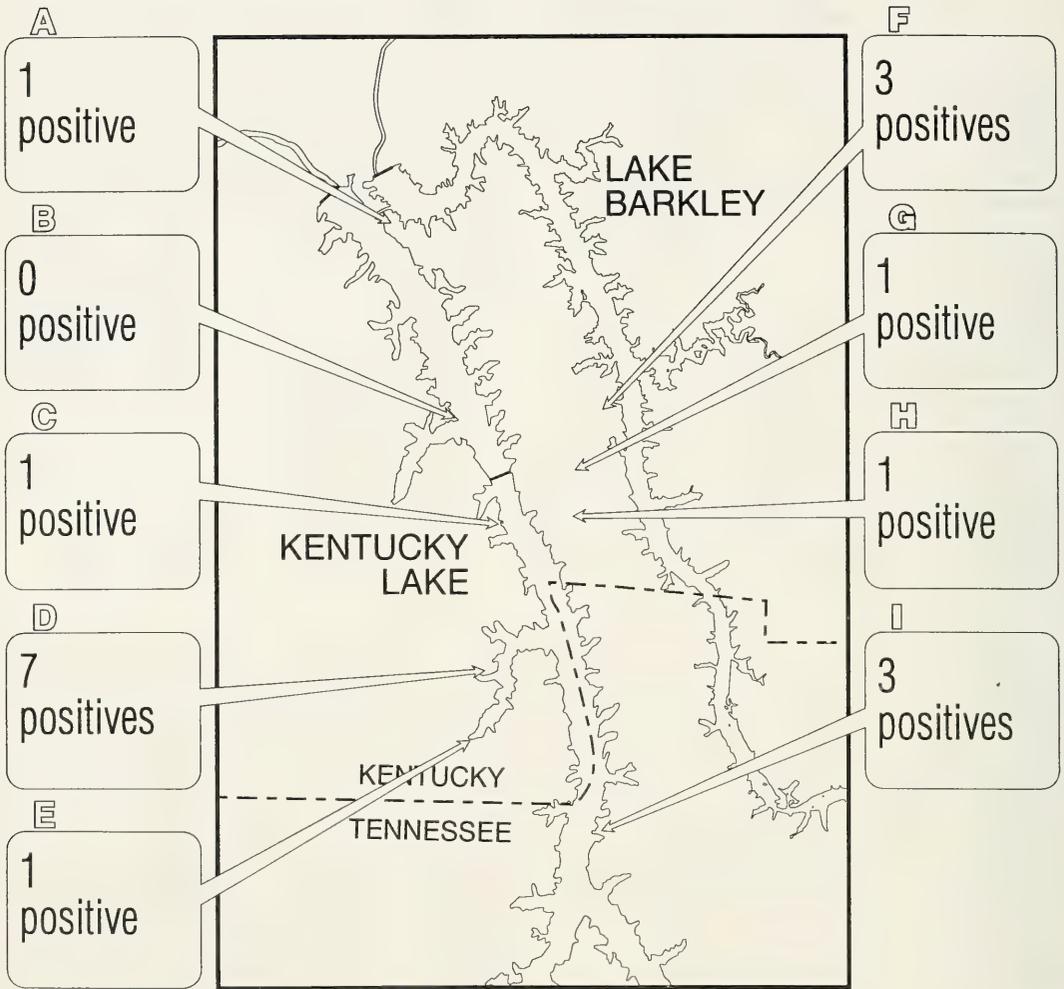
Peromyscus leucopus, serves as the primary vertebrate host for *I. scapularis* larvae and nymphs, thus it is the primary reservoir host for *B. burgdorferi*. In the southeastern U.S., *Dermacentor variabilis* larvae and nymphs infest *P. leucopus* most often. Schwan et al. (8) reported that white-footed mice remain persistently infected with *B. burgdorferi*. This information indicated that infected mice should be seropositive for antibodies against *B. burgdorferi* long after initial exposure and elevated antibody titers should be detectable weeks or even months after infection.

In order to ascertain whether *B. burgdorferi* possibly exists in western Kentucky, a study was initiated to examine the serum of white-footed mice for antibodies against *Borrelia* sp. The study was also designed to attempt culture of spirochetes from tissue samples of white-footed mice. Additionally, ticks removed from mice were analyzed by indirect fluorescent antibody technique (IFA) for the presence of *Borrelia* sp.

MATERIALS AND METHODS

Bacterial Strain.—The *B. burgdorferi*-Guilford strain used in this study was obtained from Dr. Gary Mullin, Dept. of Entomology, Auburn University, Alabama 36849. The Guilford strain is a high-passage isolate of *B. burg-*

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Localities of *P. leucopus* from LBL and the western shore of Kentucky Lake.

FIG. 1. Sample localities from LBL and TVA recreation areas: a total of 200 *P. leucopus* were collected from TVA public use areas on the western shore of Kentucky Lake: B (Jonathan Creek), C (White Sands Beach), D (Wildcat Creek), and E (Blood River). The positive samples were found in localities C, D, and E. A total of 112 *P. leucopus* were collected from the eastern shore of Kentucky Lake (LBL areas A, F, G, H, and I). Mice with positive sera were found in each of these five localities.

dorferi originally isolated by Alan Steere from *Ixodes* in Guilford, Connecticut.

Animal Capture.—Between 30 June 1990, and 29 August 1991, 185 *P. leucopus* were live-trapped at 4 sites along the western shore of Kentucky Lake in Calloway and Marshall counties, Kentucky. The 4 sites (Fig. 1), Blood River (BR), Wildcat Beach (WC), White

Sands Beach (WS), and the Boy Scout Camp on Jonathan Creek (JC) were selected as areas of high human activity and potential human exposure to *B. burgdorferi*. Each site included campgrounds, beaches, picnic areas, and/or natural areas and trails that concentrate recreational activities on TVA-owned lands. Additionally, 127 mice from the eastern shore of

Kentucky lake at Land Between the Lakes (LBL) recreation area were examined (Fig. 1).

Sherman live traps baited with peanut butter or bird seed were set and checked daily. Captured animals were transported to laboratory facilities at Murray State University.

Animal Handling.—Following transport, mice were held for a period of at least 3 days over water-filled containers lined with double-sided tape in order to collect engorged ticks. Any ticks collected were placed into vials and held at 4°C for analysis by IFA (see below). At the end of the holding period, animals were sacrificed by etherizing, bled, and ears removed. Blood was allowed to clot, and serum was collected, frozen at -80°C, and analyzed by immunoblotting. Ear punch biopsies were performed on each mouse as described below. Ear tissue remaining from the mice was stored frozen at -80°C.

Cultivation of Borrelia burgdorferi.—The medium for cultivation was the standard Barbour-Stoenner-Kelly (BSK) described by Barbour (1). The ear-punch biopsy method of Sinsky and Piesman (10) was initially used to culture spirochetes from captured wild mice. During the study this method was modified as follows. A pie-shaped wedge was clipped from the ear and soaked in 10% bleach for 10 min followed by a 10-min soak in 70% ethanol. The tissue was minced, added to 100 µl of BSK in a microcentrifuge tube and incubated at 32°C for 2 weeks in a candle jar. The culture was checked twice (7 days and 14 days) under a phase contrast microscope for the presence of spirochetes. With each new batch of media, positive controls were cultured by inoculating fresh media with a 10 µl aliquot of viable spirochetes (Guilford) and incubating at 32°C.

Indirect Fluorescent Antibody (IFA) Testing.—This test was modified from a procedure described by Luckhart et al. (3). Ticks were dissected, and the contents mixed with one drop of sterile water on the surface of a clean slide. This was allowed to air dry, and fixed by mild heating. Fifty µl of polyclonal rabbit anti-*B. burgdorferi* serum (1/50) was added to the tick smear, and the slide was incubated for 30 min at 37°C in a moist chamber. The slide was washed once in phosphate buffered saline (PBS) for 10 min and air dried. To the dried slide was added 1 drop of goat anti-rabbit gamma globulin (1:50 dilution) labeled with

fluorescein isothiocyanate (Sigma, St. Louis, Missouri). Incubation proceeded for 30 min at 37°C in a moist chamber. The slide was washed once with PBS as above, and rinsed with distilled water. The slide was air dried and observed using a fluorescence microscope. Positive controls consisted of placing a drop of live spirochetes from BSK culture on a slide instead of a tick smear.

Immunoblot Screen.—*B. burgdorferi* cells were grown into late log phase in BSK medium, centrifuged, and washed 3 times in PBS. Cells were resuspended in PBS at approximately 10⁸ cells/ml, and 100 µl aliquots were spotted onto a nitrocellulose sheet held by a Vacudot (American Bionetics; Hayward, California) apparatus. The nitrocellulose was dried, and each spot was cut from the sheet and stored at -80°C. The immunoblot procedure was adapted from Towbin (13). Each dot blot was blocked by incubating for 1 hr at room temperature (RT) in a solution of 3% bovine serum albumin (BSA, Sigma) suspended in PBS. The blots were then probed with a 1:200 dilution of serum from each experimental mouse for 1 hr at RT. Blots were subjected to 2 10 min washes with 1% BSA in PBS, then incubated with a 1:1,000 dilution of peroxidase conjugated goat anti-mouse IgG (H + L) (Jackson Immunoresearch; West Grove, Pennsylvania) suspended in 3% BSA-PBS for 1 hr at RT. Each dot blot was washed 3 times (10 min each) in 3% BSA-PBS then developed with a solution containing o-dianisidine (25 µg/ml) and 0.01% H₂O₂ in 10 mM Tris (pH 7.4) for 10–15 min. Positive and negative control sera were included with each test run. Positive serum was obtained by injecting each mouse in a group of *P. leucopus* twice with approximately 10⁸ freshly grown spirochetes. One week following the second injection, mice were bled and the serum collected and titrated by the above method. Positive control mouse serum attained a titer in excess of 1:3,200 with the immunoblot method. Negative controls were selected from experimental animals which appeared negative on the initial immunoblot screening. Further titrations of these sera were made at dilutions of less than 1:200 to insure that no antibody was present.

Western Blotting.—Western blotting was used to confirm each positive serum sample from the immunoblotting screen. Whole cell

Immunoblot

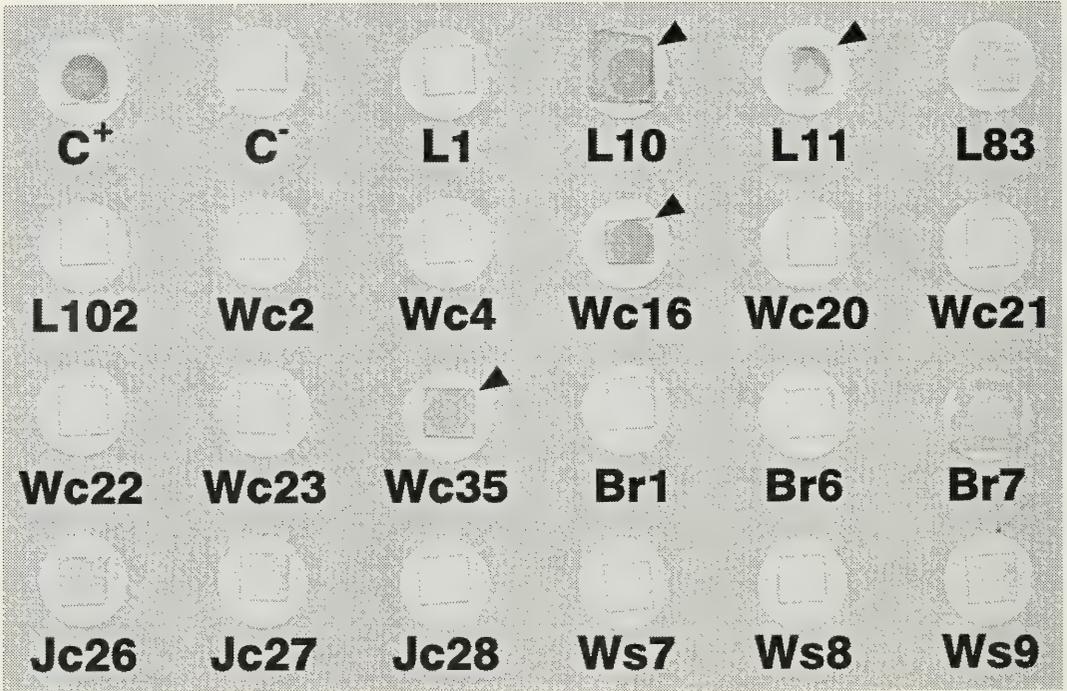


FIG. 2. Immunoblot screen: each serum sample was diluted 1/200 and used to probe whole spirochete antigen spotted on nitrocellulose. Arrows indicate positive reactivity of experimental serum with *B. burgdorferi* antigen. C+ and C- = positive and negative serum samples; L1, 10, 11, 83, 102 = experimental serum taken from mice captured from Land Between the Lakes; WC 2, 4, 16, 20, 21, 22, 23, 35 = experimental serum taken from mice captured from Wildcat Beach; Br 1, 6, 7 = experimental serum taken from mice captured from Blood River; Jc 26, 27, 28 = experimental serum taken from mice captured at Jonathan Creek; Ws 7, 8, 9 = experimental serum taken from mice captured at White Sands Beach.

proteins were extracted from *B. burgdorferi* using the boiling water bath method described by Simpson et al. (9). An aliquot containing 275 μg of protein was added to an SDS gel and subjected to polyacrylamide gel electrophoresis for 4 hr at a constant current of 40 ma. An aliquot containing 50 μg of marker protein (SDS-6; Sigma) was loaded adjacent to bacterial proteins in order to obtain a reference of molecular masses. Following resolution by SDS-PAGE, proteins were electroblotted onto nitrocellulose membranes (0.45 μ) (MFS; Dublin, California) using a Bio-Rad (Richmond, California) *Transblot Electrophoretic Cell* according to manufacturer's instructions. The transferred membranes were dried

and cut into 3 mm strips for probing with experimental mouse serum. The probing procedure was the same as described above in the immunoblot technique, again including positive and negative control sera.

RESULTS

Cultivation of Borrelia from Wild Animal Hosts.—All attempts to cultivate *B. burgdorferi* from *Peromyscus* tissue were negative, whereas positive control cultures produced high concentrations of viable spirochetes.

Indirect Fluorescent Antibody Testing.—A total of 40 ticks (all *D. variabilis* larvae or nymphs) were collected from 24 *P. leucopus*

TABLE 1. Immunoblot screen of *Peromyscus* serum.

Sample site	Number positive/total number			Subtotal
	Month* collected			
	1-4	5-8	9-12	
LBL ^a	0/5	8/109	2/13	10/127
BR ^b	1/27	0/10	0/2	1/39
JC ^c	0/15	0/20	0/15	0/50
WC ^d	0/5	9/43	—	9/48
WS ^e	1/5	0/43	—	1/48
Subtotal	2/57	17/227	2/30	
Total				21/312 (6.7%)

* Numbers indicate calendar month

^a Land Between the Lakes^b Blood River.^c Jonathan Creek.^d Wildcat Beach.^e White Sands

during the course of the study. IFA tests were considered negative for all ticks tested.

Immunoblot Screen.—Using an immunoblot technique, serum from each mouse was diluted 1:200 and used to probe a whole spirochete antigen preparation spotted on nitrocellulose. Figure 2 illustrates a typical test run with a positive and negative serum control. The positive experimental sera are marked with arrows. Table 1 represents the preliminary results of immunoblot screening by sample site, and approximate time period of the year.

Western Blotting.—Each positive sample from the immunoblot screen was subjected to further analysis by western blotting. Serum samples which possessed specific antibodies to the 39/41 kDa protein of *B. burgdorferi* were considered confirmed positives, since these proteins have been shown to occur in all varieties of *B. burgdorferi* (8, 9, 14) and to specifically mark this species. Figure 3 shows a selection of the confirmation experiments. Each of the 9 experimental serum samples shown in Figure 3 as well as 9 others (not shown) were found to contain antibodies which reacted with the 39/41 kDa protein band. Some additional reactive antibodies were noted, for example: serum sample WC19 (D) reacted with a 56 kDa protein band and LBL100 (H) reacted with 49, 56, and 72 kDa protein bands. The positive serum control possessed antibodies which recognized protein bands at 21, 32, 36, 41, 49, 56, and 72 kDa, whereas the negative serum control failed to

bind any *Borrelia* proteins. The geographic locality of each confirmed positive animal from all localities sampled is illustrated on Figure 1.

DISCUSSION

The inability to cultivate infectious spirochetes in this study was disappointing, but not unusual. Luckhart et al. (3) presented evidence of *Borrelia* sp. in tick populations by the indirect fluorescent antibody technique, but were unable to culture the bacterial agent from a large sample of wild rodents. Also, the inability to demonstrate ticks infected with *Borrelia* in our study is not unusual given the small sample size, and the fact that we were unable to collect ticks from any animals which were seropositive for this bacterium.

The dot blot rapid screen test (Fig. 2) proved to be a reliable indicator of the presence of antibodies versus *B. burgdorferi*, since 18 of 21 samples were confirmed by western blot analysis. From Table 1 it is notable that most seropositive animals were collected during the summer months from Wildcat Beach and 2 localities within LBL (Fig. 1). The western blot test (Fig. 3) showed 18 of 21 positive sera which contained antibodies reactive with the 39/41 kDa band. The 41 kDa protein band has been described by other investigators (8, 14), and corresponds to the flagellin antigen of *B. burgdorferi*. The 39 kDa protein band has more recently been described by Simpson et al. (9), and was described by these authors as a marker for infection of animals naturally inoculated with *B. burgdorferi*. It should be noted that these protein bands were resolved on PAGE gels, but the resolution did not extend to nitrocellulose blots. The positive serum control lane (C) in Figure 3 displays a broad band of reactivity in the 40 kDa region which includes both 39/41 kDa bands in the PAGE control (lane A).

All of the confirmed positives showed this broad band of reactivity which is especially evident from samples in lanes F, G, and L. These findings are in accord with other researchers in the southeastern U.S. who have performed serological surveys of wild animal populations to detect antibodies versus *B. burgdorferi*. Magnerelli et al. (4) reported that in Connecticut and other states on the eastern seaboard 51% and 20% of the sera examined (by ELISA) from *Odocoileus virginianus* and *Pro-*

Westernblot

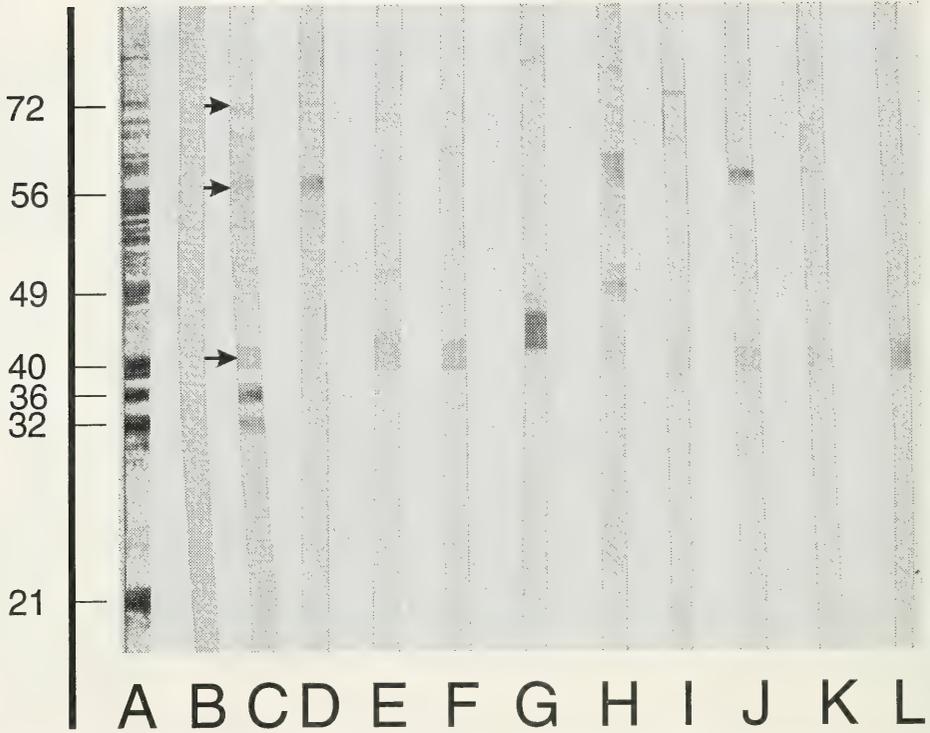


FIG. 3. Western blotting; selected positive serum samples from the immunoblot screen were used to probe whole cell proteins of *B. burgdorferi* subjected to PAGE and blotted to nitrocellulose. Lane A—whole cell *B. burgdorferi* proteins resolved by SDS-PAGE; B—negative *P. leucopus* control serum; C—positive *P. leucopus* control serum; D—L—immunoblot strips probed with serum collected from mice WC19 (D), WS1 (E), WC35 (F), BR16 (G), LBL100 (H), WC45 (I), WC47 (J), LBL6 (K), and LBL7 (L). Arrows indicate reactive proteins of 39/41 kDa, 56 kDa, and 72 kDa in the control lane.

cyon lotor, respectively, contained antibodies to *B. burgdorferi*. In another ELISA study by Magnarelli et al. (5), several localities in the eastern and southeastern U.S. were surveyed for anti-*Borrelia* antibodies in the serum of rodents. Positive *Peromyscus gossypinus* sera were obtained from each of the 7 southeastern states surveyed. The rates of positivity ranged from a high of 38% in South Carolina to a low of 15% in Alabama. From a total of 535 *P. gossypinus* serum samples tested, 27% showed antibodies to *B. burgdorferi*. We conclude that preliminary evidence from our study indicates a low level of infectivity by *Borrelia* sp. exists among *P. leucopus* populations located in western Kentucky. Further

studies will focus on identification of which tick species may harbor *B. burgdorferi* in western Kentucky and document the prevalence of the bacterium within potential vector populations.

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Pennsylvanian Sharks from Kentucky

JAMES X. CORGAN

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ABSTRACT

There are published records of 2 Pennsylvanian sharks from Kentucky that have been identified to species. *Edestus minor* Newberry, 1866 occurs in the Carbondale Formation of Muhlenberg County and *Symmorium reniforme* Cope, 1893 is known from the Kendrick Shale of Floyd County. This report adds a third record. *Edestus heinrichii* Newberry and Worthen, 1870 is reported from the Carbondale Formation of Union County.

INTRODUCTION

Part of the lower jaw of a shark, with 7 teeth in place, was found during mining in the Hamilton #2 mine of the Island Creek Coal Company, near Uniontown, Union County, Kentucky (Fig. 1). It occurred, at a depth of 350 feet, on a bedding plane that separates the Number 9 Coal from an overlying shale horizon within the Carbondale Formation of the Allegheny Series. The jaw fell from the mine roof while a crew was working on shaft maintenance. Because it fell, some teeth are chipped. Breakage does not obscure basic morphology.

The discovery was made in the fall of 1991. Some 2 years later the fossil was taken to the Evansville Museum of Arts and Sciences. At present the specimen belongs to the miner who made the find, Mr. Thomas Nass. He may donate it to the museum. For access to this specimen, I am indebted to Mr. Nass and to Mitchell Lumen, Curator of Collections in the Evansville museum.

Edestid Dentition and Classification

The arched tooth row, the morphology of teeth, and the appearance of the preserved cartilage identify this Union County fossil as part of the lower dentition of an edestid shark. Sharks of the Order Edestida are reasonably well-known (1). In life, the teeth of the lower jaw were supported by a massive cartilage complex formed by a fusion of the left and right mandibles (2). Mandibles fuse at the symphysis, the zone of juncture between the halves of the jaw. As the shark grew, the symphysis was produced into a long tooth-bearing rod. Curvature of this cartilaginous support is almost universal in edestids.

The symphysial dental battery of the lower jaw matched a comparable zone, the rostrum, in the upper jaw. The rostrum also bore teeth, occluding with those in the lower jaw. The skeletal support for rostral teeth was not sturdy. Other portions of the skeleton are little mineralized. Thus, edestid species are primarily known from symphysial teeth.

Shape, size, and dental sculpture place the specimen illustrated in Figure 1 within the genus *Edestus* Leidy. *Edestus* ranges from Mississippian to Pennsylvanian (1) and occurs throughout the world. Branson (3) clarified the distribution and synonymy of the type species. He also evaluated related genera and provided an inventory of species. About 6 valid species of Pennsylvanian *Edestus* are known from North America. Symphysial dentition is an adequate base for recognizing species.

Kentucky Shark Species

The shark shown in Figure 1 is *Edestus heinrichii* Newberry and Worthen (1870). In the plate explanation which was part of the original description, the name was given as *E. henreichsii*. This incorrect spelling is just a slip of the pen. It has never caused confusion. When the species was redescribed by Newberry (4), only the name *E. heinrichii* was used. The name honors a coal mine owner, John P. Heinrich. The type specimen came from his mine in the Pennsylvanian of Bellville, Illinois. The original description and the redescription were influenced by other specimens, collected in Illinois and Indiana.

The only prior record of *Edestus* in Kentucky is from a coal mine near Beech Creek, Muhlenberg County. Jillson (5) provides a well-illustrated, lengthy discussion. The species is *Edestus minor* Newberry, 1866.

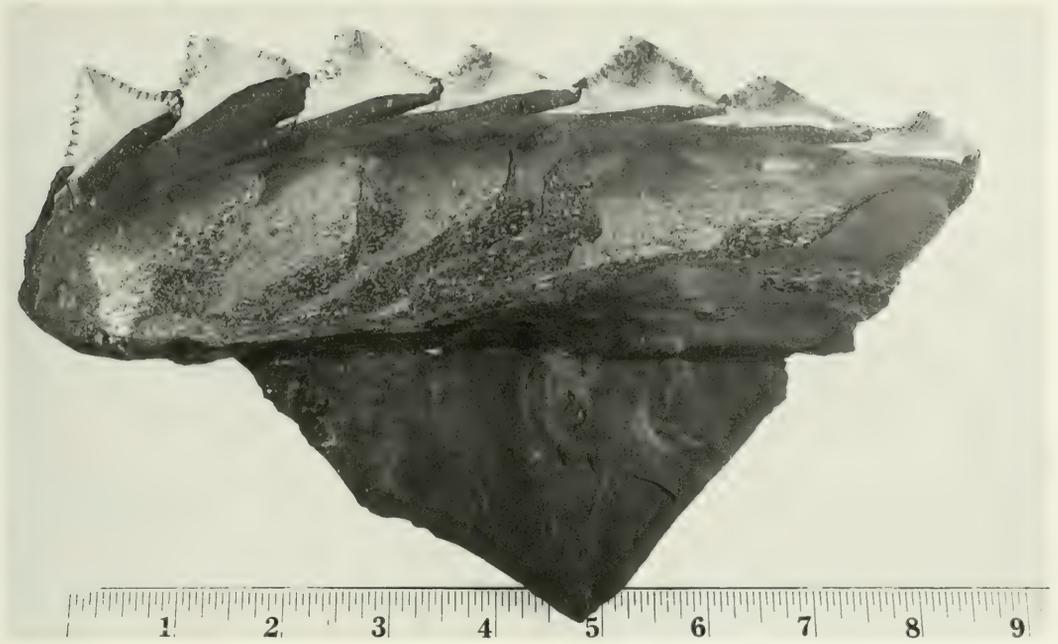


FIG. 1. *Edestus heinrichii* Newberry and Worton, 1870, from the Carbondale Formation at the 350-foot level within the Hamilton #2 Mine of the Island Creek Coal Company, near Uniontown, Union County, Kentucky. The specimen is 23.2 cm long, with a maximum height of 6.0 cm. Length of teeth at the junction with cartilage ranges from 3.5 to 4.5 cm. Well-preserved teeth have 11 anterior denticulations and exceeded 2.1 cm in height. Breakage prevents denticulation counts and height measurement for most teeth. The anterior direction is to the right. Massive tissue below the teeth is poorly preserved cartilage of the fused mandibles.

Kentucky occurrences of *E. heinrichii* and *E. minor* mark distributional limits. In the last century, Newberry (4) realized that no species of *Edestus* was known from Ohio, Pennsylvania, or areas to the east. He did not know why and did not comment on distribution to the south, probably because few Pennsylvanian sharks were known from the southern states.

More than a century later, there are still no records of edestiids from Ohio, the eastern United States, or adjoining Canadian provinces. Today, the southern distribution of Late Paleozoic vertebrates is better known. There is reasonable data on vertebrates from extensive Paleozoic outcrops in Tennessee and Alabama (6, 7). A few vertebrates are known from a relatively small region of Paleozoic outcrops in Mississippi. Those described during the Twentieth Century are well-known (8). Like Ohio and regions to the east, the area south of Kentucky seems to lack edestiids. As in Newberry's day, the absence remains unexplained.

There is one other record of a specifically identified Pennsylvanian shark from Kentucky. What is preserved is a series of punctures within the shell of a marine invertebrate, a nautiloid cephalopod. Punctures are clearly bite marks left by a large marine predator. Mapes and Hansen (9) attributed the marks to *Symmorium reniforme* Cope, 1893, a shark of the Order Cladodontida.

Through the work of Williams (10), the anatomy of this shark is almost singularly well-known. Thus species-level bite-mark identification seems reasonable. The cephalopod that preserves the marks of *S. reniforme* is from the Kendrick Shale of the Kanawha Series of Eastern Kentucky. It was collected near Ligon, Floyd County. The Kendrick Shale is slightly older than beds that yield *Edestus* in Western Kentucky. Like Kentucky species of *Edestus*, *S. reniforme* occurs in Illinois but not in states to the south. The eastern distribution of *S. reniforme* was not investigated in this study.

SUMMARY

Two species of the shark genus *Edestus* occur in Pennsylvanian strata of Western Kentucky. Identification of both *E. minor* and *E. heinrichii* is based on symphysial dentition with attached cartilage. Each species is known from a single specimen. By coincidence, in each specimen seven teeth are present. Both species occur in the same stratigraphic setting. They were found at the interface between the Number 9 Coal and overlying shales of the Carbondale Formation. One species of the Pennsylvanian shark genus *Symmorium* is known from the Kendrick Shale of Eastern Kentucky. Identification of *Symmorium reniforme* is based upon an interpretation of bite marks in a marine invertebrate shell. All species of Pennsylvanian sharks known from Kentucky also occur in states to the north of Kentucky. None occurs in reasonably diverse faunas from Tennessee and Alabama or in faunas from Mississippi that were described in the Twentieth Century. The eastern distribution of *Symmorium* was not examined in this study. Species of *Edestus* do not occur to the east.

The cause of this distributional pattern in Paleozoic sharks is not known.

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NOTE

New records of slender madtom, *Noturus exilis* Nelson, South Fork Licking River, Pendleton County, Kentucky.—As part of a stream investigation by the Kentucky Department of Fish and Wildlife Resources, Division of Fisheries, seine sampling a limestone rubble-riffle area, intermixed with gravel and numerous weathered freshwater mussel shells, in the South Fork Licking River below Hayes Station Rd. bridge crossing, 1.8 air km SW of Falmouth (6.9 stream km upstream of confluence with Licking River) 19 August 91, revealed several species of fish; 3 of which were *Noturus*. One was identified as *Noturus flavus*, the other 2 being problematical, having equal jaws and tooth pads on the upper jaw without long distinct backward extensions, were sent to R. R. Cicerello (Kentucky State Nature Preserves Commission) who tentatively identified them as *Noturus exilis*, and subsequently sent the specimens to B. M. Burr (Southern Illinois University at Carbondale). In return correspondence, Burr stated "I counted 17-18 anal rays, about 45 caudal rays, 10 POM pores, 1 internasal pore, 9 pelvic rays, and equal jaws. All of the counts fit *N. exilis* and there are very few madtoms with equal jaws. Even in weakly pigmented *N. exilis* there

is usually a black marginal band left in the dorsal fin. Unfortunately neither of these specimens have the band. Other than what I have noted as discrepancies I see no compelling reason not to call them *N. exilis*."

Based on characters examined, these specimens appear to be more closely allied to *N. exilis* than any other species, with noted exceptions, but could possibly be an undescribed form. The author wishes to advise collectors to look closely at any suspect *N. flavus* collected. *Noturus exilis* is listed as endangered in Kentucky (Warren et al., Trans. Ky Acad. Sci. 47:83-98, 1986) and is known from ten localities only in the state (Burr and Warren, Ky Nat. Pres. Comm. Sci. Tech. Ser. 4, 1986). Voucher specimens of *N. exilis* are housed at Southern Illinois University at Carbondale (SIUC 19120).

I thank Ronald R. Cicerello, Kentucky State Nature Preserves Commission; Brooks M. Burr, Southern Illinois University at Carbondale; and Al Surmont and Fred Howes, Kentucky Department of Fish and Wildlife Resources.—**Lewis E. Kornman**, Kentucky Department of Fish and Wildlife Resources, Division of Fisheries, Morehead, Kentucky 40351.

ACADEMY AFFAIRS

THE EIGHTIETH ANNUAL MEETING OF THE KENTUCKY ACADEMY OF SCIENCE

The Executive Inn, Paducah, Kentucky
3-5 November 1994

MINUTES OF THE GOVERNING BOARD MEETING KENTUCKY ACADEMY OF SCIENCE

Present: Presiding—Larry P. Elliott; Other Governing Board Members: Branley A. Branson, William Bryant, Julia Carter, Robert Creek, Gerald DeMoss, Vincent A. DiNoto, Jr., Patricia K. Doolin, Val Dunham, Blaine R. Ferrell, Valena Hurt, J. G. Rodriguez.

Guests in attendance: Jim Meeks, Local Arrangements Committee Chairperson, Andy Aliger, Tennessee Academy of Science representative, Chuck Cox, Laura Walker, and Jon Wells, representatives of Citizens Bank and Trust.

- The meeting was opened at 1:08 P.M., CST.
- President Larry Elliott welcomed participants to the meeting. The meeting was turned over to the representatives from Citizens Bank and Trust for an update on the Marcia Athey Trust Fund (Appendix A). They also discussed their investment strategy. A short discussion followed. It was learned that the bank charges 0.3% of the principal and 6% of the interest income to manage the money in accordance with Kentucky revised statutes. Larry Elliott thanked them for their report and work on the trust fund.
- The minutes of the 27 August 1994 meeting of the Governing Board held in Paducah at the Executive Inn were approved with one correction. Under the Vice President's report, it should read "William Bryant" not "Larry Elliott."
- Robert Creek presented a short report concerning the 1994 annual meeting (Appendix B). He noted several changes in room assignments and indicated that he had been in contact with the sectional chairs. Robert also discussed a correspondence from the West Virginia Academy of Science regarding a compendium of all state academies publications to be distributed on CD-ROM. It was decided that this correspondence, received by a representative of each university, should be forwarded to each school's librarian.

President's Report

- Larry Elliott summarized the president's report (Appendix C).

Awards

The winners of the 1994 KAS awards were:

- Distinguished Scientist—Dr. Donald T. Frazier, Department of Physiology and Biophysics, University of Kentucky.

- Industrial Scientist—Dr. Fred L. Tungate, United Catalysts, Inc., Louisville.
- Outstanding College—University Science Teacher—Dr. Barbara A. Ramey, Department of Biological Sciences, Eastern Kentucky University.
- Outstanding Secondary Science Teacher—Beverly Lynn White, North Laurel High School.

Future Meetings

- The dates for the joint KAS-TAS meeting is tentatively set for the dates 15-17 November and to be held at Western Kentucky University.

Treasurer's Report

Julia Carter presented the financial status of the KAS (Appendix D).

Elections

The results of the election of KAS officers are as follows:

- Vice-President—Marcus T. McEllistrem, Physics, University of Kentucky.
- Secretary—Peter X. Armendarez, Chemistry, Brescia College.
- Representative to the Governing Board—Social Science—James F. Hopwood, Anthropology, Northern Kentucky University.
- Representative to the Governing Board—Physical Sciences—Chemistry, Ashland Petroleum Research and Development.

Newsletter Editor

- Vince DiNoto indicated that he would obtain back issues of the *Transactions of the Kentucky Academy of Science* at the University of Louisville, store them in the Jefferson Community College, SW library on a short term basis in return for one copy of each issue being placed in this library permanently. Robert Creek indicated that ten copies of each issue stored at Eastern Kentucky University (nine year period) would be given to Rod Rodriguez and the remainder would go to Vince DiNoto to be discarded.

Transactions Editor

- Branley Branson pleaded for cooperation in getting the records of Academy affairs (e.g., meeting minutes, various reports, etc.) to him in a timely manner. He indicated that he and Varley Wiedemann, who prepares the

annual index, would be resigning from their positions within a year or two.

KJAS Director

- Val Dunham indicated that he was searching for a professional person to design a color brochure for the KJAS. The KJAS will be sending two students and a chaperon to the AAAS meeting in Atlanta. He mentioned that there has been a problem with KJAS student paper winners' attendance at the annual KAS meeting.

Executive Secretary's Report

- Rod Rodriguez commented that the KAS should have the *Transactions* put on microfiche. Robert Creek asked Rod whether or not copies of the *Transactions* were still being sent to the archivist, Charles Hayes, at Eastern Kentucky University. Rod said no.
- Rod commented that something should be done about the library subscription at the University of Louisville. Their request was down from 90 to 70 copies. Rod suggested that KAS should terminate its arrangement with the University of Louisville library. Robert Creek asked Rod to bring a specific recommendation before the board at its January meeting.
- He indicated that the KSTC membership was up for renewal. Last year KAS paid the KSTC \$250.00 and they paid KAS \$100.00. William Bryant moved that KAS join KSTC again this year, and Pat Doolin seconded. The motion passed.

Executive Committee Meeting

- The meeting came to order at 4:15 P.M., CST.
- Dates for the joint KAS-TAS meeting were discussed. The 2-4 November date had a conflict with the KSTA meeting, and 15-17 November had a conflict with the NABT meeting.
- Estimates for the number of papers ran approximately equal to 150 for TOS and 300 for KAS.

Business Meeting

November 5, 1994 10:00 A.M.

- President Larry Elliott spoke about the plans for next year's joint meeting with TAS. The meeting will be held at Bowling Green.
- Treasurer Julia Carter presented a brief financial report and reported on the start of an investment bond ladder in August. The interest rate will be a lot better than investment in CDs.
- Two resolutions were passed:
 1. Resolution thanking individuals of Paducah Community College and Murray State University for their hosting of the annual KAS Meeting.
 2. Resolution by KAS support for KERA.
 Both resolutions were passed by the membership.
- The constitutional changes proposed by Larry Giesman were presented to the membership and passed.
- Larry Elliott passed on the gavel to President-elect Robert Creek.

APPENDIX A

Raymond H. Athey Kentucky Academy of Science Trust

Asset Allocation Profile

Current (Includes Pending distributions and trades)

	11/02/94	Weight	Estimated	
			Income	Yield
Income				
Money Market.....	\$ 49,653		\$ 2,259	4.55%
Principal				
Money Market.....	\$ 83,712	17.64%	\$ 3,809	4.55%
Income Investments.....	\$297,669	62.71%	\$19,914	6.69%
Growth Investments.....	\$ 93,284	19.65%	\$ 2,316	2.48%
	\$474,665	100.00%	\$26,038	5.49%
Other Principal				
Real Estate.....	\$ 25,000			
Mineral Interest.....	\$ 6,584		N/A	N/A
Escrow Account.....	\$ 26,257		N/A	N/A
Total.....	\$ 57,840		N/A	N/A
Total.....	\$582,158		\$28,298	4.96%

APPENDIX B

Report from President-Elect
Robert Creek

Executive Board Meeting
Paducah, Kentucky
3 November 1994

1. Dr. Harry Horner from Iowa State University was invited and accepted our invitation to be the banquet speaker. The title of his talk will be *Plants, Fungi and Their "Kidney" Stones*.

2. The KERA Symposium will be on Friday at 9:15 and will be moderated by Curtis Wilkins of Western Kentucky University. The speakers will be Dan Ochs, Shirley Wrinkle, Nelda Freeman, Ben Malphrus and Mike Howard.

3. The Plenary Session was set up by Joe Winstead, who will also moderate the session. The presenters will be Ken Carsten, John MacGregor, Scott Franklin and William Bryant.

4. The Agriculture Symposium, *Agriculture, Environmental Stewardship, and the Role of the Kentucky Academy of Science*, will be Thursday at 6:30 P.M.

5. The program has 232 papers being presented with 28 posters. The breakdown of the presentations is listed below:

- a. Anthropology—15
- b. Botany and Microbiology—28
- c. Chemistry—17
- d. Geography—19
- e. Geology—15
- f. Physics—10
- g. Physiology and Biophysics—16
- h. Science Education—13
- i. Psychology—22
- j. Sociology—5
- k. Zoology and Entomology—22
- l. Computer Science—6
- m. Mathematics—8
- n. Agricultural Sciences—12
- o. Industrial Science—5
- p. Cell and Molecular Biology—19

6. I will meet with the sectional officers at 8:00 P.M. Thursday to discuss various topics to include:

- a. Program
- b. Abstracts
- c. Guidelines for student competition
- d. Duties of sectional officers

APPENDIX C

Memorandum

To: KAS Governing Board
From: Larry P. Elliott
Subject: President's Report
Date: November 3, 1994

On September 9, 1994, I met with Drs. Bob Creek, Val

Dunham, and Curtis Wilkins at Western Kentucky University concerning the symposium on KERA for our annual Kentucky Academy of Science (KAS) meeting in Paducah. I have met with Dr. Elmer Gray on several occasions to facilitate the planning of a symposium entitled "Agriculture: environmental stewardship, and the role of KAS."

From September 29 to October 1, 1994, I attended the 19th Annual Governor's Conference on the Environment in Louisville, KY. The conference focused on the importance of our forest lands and their interrelationship with the environment, as well as their importance to Kentucky's economic base. While in Louisville, I also attended another Kentucky Outlook 2000 meeting on which I serve as a Steering Committee member.

The KAS members that were appointed by the Governor to the Heritage Land Conservation Board were Drs. William S. Bryant and Charles Elliott. All candidates that ran for Vice President, Secretary, and Governing Board Member representatives for the Social Science and Physical Science Division were contacted and cordially thanked for being candidates. The winners were also congratulated. They were: Dr. Marcus T. McEllistrem, Vice President, Dr. Peter Z. Armendarez, Secretary, Dr. Jim Hoppood, Social Science, and Dr. Robert Wombles, Physical Science.

All committee chairs were contacted by phone and in writing. Reports that I received will be given under "Standing Committee Reports."

On October 24, 1994, I attended a town meeting at the Regional Conference Center in Bowling Green which was conducted by the Kentucky Long-Term Policy Research Center was created by the General Assembly in 1992 to explore the long-range implications of current policies, emerging issues and trends influencing the Commonwealth's future. This project is an effort to anticipate 21st Century needs and policy making responses. Hopefully, this total effort will not let Kentucky's future be determined by a select few.

TREASURER'S REPORT

Kentucky Academy of Science
1994

Starting Balance (January 1, 1994)	_____	\$91,361.48
(\$30,000.00 CD and \$61,361.48 divided into Money Market Savings [\$58,272.18] and Checking [\$3,089.30])		
Clearance of 1993 Checks	- 98.33	
Income	+30,377.30	
C.D.'s from Endowment, Athey, and Botany Funds		+81,000.00
		+111,278.97
Expenses	-20,973.26	
Ending Balance (September 30, 1994)	_____	\$181,667.19
(\$181,667.19 divided into Money Market Savings and Checking Account)		

Income—1994

Membership Dues	\$10,144.05
Regular ^o	\$9,794.05
Life (61) 1994	350.00
Institutional Memberships	\$12,376.00
Corporate Memberships ^o	4,845.00
Page Charges	690.00
Interest Income	2,231.74
Bank Account	\$1,655.83
Interest on Time Deposit	575.91
Griffith Memorial Trust	90.51
Total	\$30,377.30

^o Includes some Annual Meeting income (registration, banquet fees, Ashland Oil contribution). After Annual Meeting is completed, final report will be submitted.

Kentucky Academy of Science
Expenses—1994

KJAS	\$ 2,500.00
NAAS dues	90.00
KSTA	250.00
^o Awards (Grants—1994)	1,500.00
Georgetown College (Annual Meeting—1993)	2,904.21
Printing	9,404.95
Transactions	7,939.82
Newsletter	1,360.63
Anniversary Seals	104.50
Professional Services	583.00
Audit	575.00
Filing Fees	8.00
Insurance	107.30
Executive Secretary	3,633.80
Postage & P.O. Box Rent	1,130.24
Travel Expense	824.00
Book	13.45
Secretarial services	1,011.94
Misc	654.17
Total	\$20,973.26

^o Awards were made as follows: \$1,000 to Mr. Jeffrey Walck, T. H. Morgan School of Biological Sciences, University of Kentucky; \$500 to Mr. Randy Mears, Department of Biological Sciences, Eastern Kentucky University.

Endowment Fund—1994

Starting Balance (January 1, 1994)	\$26,784.79
Income	511.73
Interest	
Time Deposits	383.94
Bank Account	127.79
Ending Balance (September 30, 1994)	\$27,296.52
(\$20,000.00 CD and \$7,296.52 in Money Market Savings)	

Botany Fund—1994

Starting Balance (January 1, 1994)	\$14,229.20
Income	270.98
Interest	
Time Deposits	\$191.97
Bank Account	79.01
Ending Balance (September 30, 1994)	\$14,500.18
(\$10,000.00 CD and \$4,500.18 in Money Market Savings)	

Marcia Athey Fund—1994

Starting Balance (January 1, 1994)	\$57,664.89
Income	1,112.52
Interest	
Time Deposits	\$979.06
Bank Account	133.46
Ending Balance (September 30, 1994)	\$58,777.41
(\$51,000.00 CD and \$7,777.41 in Money Market Savings)	

Mentor Fund—1994

Starting Balance (January 1, 1994)	\$ 3,689.51
Interest	\$72.20
Ending Balance (September 30, 1994)	\$ 3,761.71

The above C.D.'s have been cashed and deposited into checking account ready for re-investment in Treasury Notes.

Investment in Treasury Notes

Two-year notes purchased 10/25/94	\$29,000-Rate: 6½%
Five-year notes purchased 10/25/94	\$29,000-Rate: 7½%

Order placed:

52-week T-Bill	\$58,000	Rate to be determined 11/10
3-year Treasury Note	\$29,000	Rate to be determined 11/8

PROGRAM, ANNUAL MEETING

KENTUCKY ACADEMY OF SCIENCE

80th ANNUAL MEETING, NOVEMBER 3-5, 1994
Paducah, Kentucky

Governing Board
Executive Committee

President	Larry P. Elliott Western Kentucky University
President-Elect	Robert O. Creek Eastern Kentucky University
Past President	Charles N. Boehms Georgetown College
Vice President	William S. Bryant Thomas More College
Secretary	Peter X. Armendarez Brescia College
Treasurer	Julia H. Carter Wood Hudson Cancer Research
Executive Secretary	J. G. Rodriguez University of Kentucky
Editor, <i>Transactions</i>	Branley A. Branson Eastern Kentucky University
Editor, <i>Newsletter</i>	Vincent A. DiNoto, Jr. Jefferson Community College

Division Representatives and At-Large Members

James E. Gotsick (1994)	Morehead State University
Kimberly W. Anderson (1995)	University of Kentucky
Blaine R. Ferrell (1995)	Western Kentucky University
Patricia K. Doolin (1996)	Ashland Petroleum Company
David Hogan (1996)	Northern Kentucky University
Valena Hurt (1996)	Hazard Community College
Gerald L. Demoss (1997)	Morehead State University
Wimberly C. Royster (1997)	Kentucky Science & Technology Council, Inc.
J. G. Rodriguez AAAS/NAAS Representative	University of Kentucky
Valgene L. Dunham (1995) Chairperson—KJAS	Western Kentucky University

HIGHLIGHTS OF THE 1994 ANNUAL MEETING

SYMPOSIUM

Agriculture, Environmental Stewardship, and the
Role of the Kentucky Academy of Science

6:30 P.M., 3 November 1994
Room—International B

Mr. Ed Logsdon—Commissioner of Agriculture

Topic: How do current agricultural practices in Kentucky affect the environment? How could the De-

partment of Agriculture and the Kentucky Academy of Science be mutually supportive?

Mr. Phillip Shepherd—Secretary Natural Resources and Environmental Protection

Topic: How does the current policy of the Natural Resources and Environmental Protection Cabinet affect Kentucky's agriculture? How does your Cabinet view the role of the Kentucky Academy of Science in environmental protection?

Mr. Bill Millikan—State Soil Conservationist, USDA Soil Conservation Service

Topic: How do SCS policies benefit production agriculture and ensure sustained productivity through environmental stewardship in Kentucky? How could the Kentucky Academy of Science be of assistance to your program?

Dr. Luther Hughes—Head, Department of Agriculture, Western Kentucky University

Topic: Practically speaking, how does the AG-2000 project support Kentucky farmers and contribute to environmental stewardship? Does the Kentucky Academy of Science have a role in AG-2000?

Dr. Bill Thom—Department of Agronomy, University of Kentucky

Topic: How have university researchers responded to needs of Kentucky farmers with respect to ensuring their sustained viability? How has research contributed to a greater level of stewardship with respect to the environment? What role do you see for the Kentucky Academy of Science in addressing these issues?

SYMPOSIUM

How Will KERA Change Science Education????

9:15 A.M., 4 November 1994

Room—Kennedy

Dr. Dan Ochs—Kentucky Department of Education

A brief overview of KERA followed by a discussion of how KERA has changed the approach to science in the high school classroom.

Ms. Shirley Winkle—Chemistry Teacher at Reidland High School, Paducah, KY

The impact KERA has had on teaching chemistry and the benefits the students have gained from it.

Ms. Nelda Freeman—Biology Teacher at Paducah Tilghman High School, Paducah, KY

How KERA has changed the teaching of biology and the perceptions that students now have of biology.

Dr. Ben Malphrus—Morehead State University

How science education is preparing future teachers for KERA.

Mr. Mike Howard—Kentucky Science & Technology Council

What will be the impact of KERA on college/university faculty and the changes that will be necessary as students enter college after having been education under KERA?

The Jackson Purchase Area

A Tribute to Raymond H. Athey

Kentucky Academy of Science Plenary Session

1:00–2:15 P.M., 4 November 1994

Room—International B

Moderator: Joe Winstead—Western Kentucky University

“Raymond H. Athey and the Kentucky Academy of Science”

Presenter: Ken Carstens—Murray State University
“Archaeological Highlights of Western Kentucky”

Presenter: John McGregor—United States Forest Service

“The Fauna of the Jackson Purchase Region: Some Zoogeographic Considerations”

Presenter: Scott Franklin—Southern Illinois University
“Vegetation History of the Jackson Purchase Area Prior to Settlement”

Presenter: William S. Bryant—Thomas More College
“Jackson Purchase Vegetation from 1820 to the Present”

Speaker for the Annual Awards Banquet

The speaker for the Annual Awards Banquet will be Dr. Harry T. Horner, Professor and Director of the Department of Botany and Bessey Microscopy Facility at Iowa State University. The title of his presentation is *Plants, Fungi and Their “Kidney” Stones*. In his abstract he states that the biomineralization consists of a series of complex cellular processes by which plants, animals and microorganisms convert solution ions into solid minerals. The more common bio-minerals are the calcium salts, silica and iron oxides. Some of the calcium salts and silica serve as major skeletal or shell minerals, whereas others form teeth and serve as ion storage depots for specific physiological functions. One calcium salt which is widespread throughout the Plant and Animal Kingdoms that has eluded being associated with these and other important structural and physiological functions, is crystalline calcium oxalate. This mineral can form aggregates or “stones” that are considered pathological in the kidneys and urinary tracts of animals and humans. In the higher vascular plants, algae, lichens and fungi, calcium oxalate does not seem to be detrimental. The specialized cells, cellular processes, and structures associated with calcium oxalate crystal mineralization in all of these latter organisms are unique. Two long-standing ideas are that the crystals may protect the plants against foraging animals and/or humans and, secondly, crystal formation ties up the toxic, metabolic by-product oxalate formed by the plant. Plant crystals have been observed and felt by humans for hundreds and thousands of years and, yet, they are still poorly understood and superficially studied. However, recent studies suggest some interesting possibilities for how this particular fungal and plant biomineralization process may be of benefit to the ecosystem and to humankind, in general.

For those that do not attend the banquet but would like to hear Dr. Horner’s presentation, I encourage you to come by after the banquet. There will be chairs available for anyone wishing to hear this interesting presentation.

80th Annual Kentucky Academy of Science Meeting

3–5 November 1994

Paducah Community College

Paducah, KY

Paducah Community College and Murray State University are co-hosting this year’s meeting. The Executive

Inn will be the headquarters hotel and all activities will be held there.

PROGRAM SUMMARY

Thursday, 3 November 1994

1:00 P.M.—4:00 P.M.

KAS Governing Board Meeting, Executive Board Room

4:00 P.M.—8:00 P.M.

Registration, Main Lobby

6:30 P.M.—8:00 P.M.

Symposium—Agriculture, Environmental Stewardship, and the Role of the KAS, International B

7:00 P.M.—8:00 P.M.

Meeting of Sectional Officers, Lincoln Room

8:00 P.M.—10:00 P.M.

Reception, International D

Friday, 4 November 1994

7:30 A.M.—5:00 P.M.

Registration, Main Lobby

8:00 A.M.—5:00 P.M.

Vendor Exhibits, International D

8:00 A.M.—5:00 P.M.

Scientific Poster Sessions, International D

9:15 A.M.—10:45 A.M.

Symposium: How will KERA Change Science Education???, Kennedy Room

11:00 A.M.—12:30 P.M.

Community College Biology Faculty, Jefferson Room

11:00 A.M.—12:30 P.M.

Community College Chemistry Faculty, Washington Room

11:00 A.M.—12:30 P.M.

Community College Physics Faculty, Truman Room

8:00 A.M.—9:00 A.M.

Sectional Meetings

11:00 A.M.—12:00 noon

Sectional Meetings

Section B—Botany & Microbiology (8:00), McKinley Room; Section C—Chemistry (11:00), Adams II Room; Section D—Geography (8:00), Roosevelt I Room; Section E—Geology (8:15), Roosevelt II Room; Section G—Physiology and Biophysics (11:15), Van Buren Room; Section K—Zoology and Entomology (8:15), Madison Room; Section Q—Agricultural Sciences (8:15), Lincoln Room; Section R—Industrial Sciences (9:25), Eisenhower Room

9:00—9:15

10:45—11:00

Refreshments, International D

12:00—1:00

Lunch, On your own

1:00 P.M.—2:15 P.M.

Plenary Session, International B

Presiding: Dr. Larry P. Elliott—President, Kentucky Academy of Science

Welcome: Dr. Len O'Hara—President, Paducah Community College

Announcements: Dr. James Meeks, Chairperson, Local Arrangement Committee

Plenary Session: Jackson Purchase Area A Tribute to Raymond H. Athey

Moderator: Joe Winstead—Western Kentucky University

2:15 P.M.—2:45 P.M.

Refreshments, International D

2:45 P.M.—5:00 P.M.

Sectional Meetings

Section A—Anthropology (2:45), Adams I Room; Section B—Botany and Microbiology (2:45), McKinley Room; Section C—Chemistry (2:45), Adams II Room; Section D—Geography (2:45), Roosevelt I Room; Section E—Geology (2:45), Roosevelt II Room; Section G—Physiology and Biophysics (2:45), Van Buren Room; Section H—Science Education (2:45), Washington Room; Section I—Psychology (2:45), Kennedy Room; Section J—Sociology (2:45), Jefferson Room; Section K—Zoology and Entomology (2:45), Madison Room; Section M—Mathematics (2:45), Truman Room; Section Q—Agricultural Sciences (2:45), Lincoln Room

2:30—5:00 P.M.

Student Reception—sponsored by Murray State University Sigma Xi, International B

6:30 P.M.—6:45 P.M.

President's Reception, International B

7:00 P.M.—9:30 P.M.

Annual Awards Banquet, International A

PLANTS, FUNGI AND THEIR "KIDNEY" STONES, Dr.

Harry T. Horner, Professor and Director, Department of Botany and Bessey Microscopy Facility, Iowa State University

Saturday, 5 November 1994

7:30 A.M.—12:00 noon

Registration, Main Lobby

8:00 A.M.—12:00 noon

Scientific Poster Exhibits, International D

8:00 A.M.—12:00 noon

Vendor Exhibits, International D

8:00 A.M.—9:30 A.M.

Sectional Meetings

11:00 A.M.—12:00 noon

Sectional Meetings

Section A—Anthropology (8:00), Adams I Room; Section B—Botany and Microbiology (8:00), McKinley Room; Section C—Chemistry (8:00), Adams II Room; Section F—Physics (8:15), Roosevelt I Room; Section G—Physiology and Biophysics (8:15), Van Buren Room; Section H—Science Education (8:00), Washington Room; Section I—Psychology (11:00), Kennedy Room; Section K—Zoology and Entomology (8:00), Madison Room; Section L—Computer Science (8:00),

Roosevelt II Room; Section M—Mathematics (8:15), Truman Room; Section S—Cell and Molecular Biology (8:00), Eisenhower Room

9:30 A.M.—10:00 A.M.

Refreshments, International D

10:00 A.M.—11:00 A.M.

Annual Business Meeting, International B

11:00 A.M.—12:00 noon

Sectional Meeting Continued

12:00 noon—1:00 P.M.

Lunch, On your own

1:00 P.M.—end

Sectional Meetings

Section I—Psychology, Kennedy Room

Section S—Cell and Molecular Biology, Eisenhower Room

Note: KJAS

Each spring the Kentucky Junior Academy of Science holds an Annual Spring Symposium. The 9th Symposium was held at Western Kentucky University in April, 1994. Activities at this meeting include the presentation of Science Projects by KJAS members as well as Science Bowl competition and Lab Skills competition. The winners of each division of the Science Projects presentations are invited to present their work at the annual meeting of the Kentucky Academy of Science. A KJAS precedes the title of each of the papers given by these young scientists.

Local Arrangements Committee
Paducah Community College

Exhibitors

Smith & Schaefer, Inc. (Fisher Scientific Co)
University of Kentucky
Woodson-Tenet Laboratories, Inc.
Parco Scientific Co.
Ace Glass Incorporated
IKA-Works, Inc.
Galbraith Laboratories, Inc.
Swift Instruments, Inc.
Thomas Scientific, Inc.

ANTHROPOLOGY

James Hopgood—Chairperson
Cara Richards—Secretary
Room—Adams I

Friday, 4 November 1994

James F. Hopgood—Presiding

2:45 P.M.

Japan: Impressions, Expectations, and Realities

James F. Hopgood—Northern Kentucky University

3:00 P.M.

A Site Catchment Analysis of a Prehistoric Site in Wickliffe, KY

April K. Haneline—Murray State University (Sponsored by Ken Carstens)

3:15 P.M.

The Role of Islamic Women in Pondok Pesantrens in East Java

Gina Meyer—Northern Kentucky University (Sponsored by James Hopgood)

3:30 P.M.

The POW Uprising of 1952: A Participant/Observer's Perspective

Raymond J. Lewis—Eastern Kentucky University

3:45 P.M.

Culinary Syncretism: An Analysis of Yucatecan Bakeries
Erin Roberts—Centre College (Sponsored by Cara Richards)

4:00 P.M.

A Preliminary Analysis of Nail Patterns at Feature 1, 15Lv207, A Civil War Gun Emplacement in Smithland, Kentucky

Kathleen Tucker—Murray State University (Sponsored by Ken Carstens)

4:15 P.M.

Underutilization of Anthropology in Third World Projects: The Developmental Handicap

Julie M. Pelle—Northern Kentucky University (Sponsored by James Hopgood)

4:30 P.M.

The Origins of Australia

Dianna Robinson—Murray State University (Sponsored by Ken Carstens)

4:45 P.M.

Groundhogs No More: The Joys and Perils of High-Rise Condominium Living

James Murray Walker—Eastern Kentucky University

Saturday, 5 November 1994

Cara Richards—Presiding

8:00 A.M.

Making Kinship: Ceremonial Exchange of Food, Liquor, and Words at Weddings in a Mexican Peasant Village

T. D. Murphy—Northern Kentucky University

8:15 A.M.

Redistribution and Entrepreneurship in Rural Kentucky: A 'Big woman's' Political Strategy

Randall Chalk—Northern Kentucky University (Sponsored by T. D. Murphy)

8:30 A.M.

What Function the Saddle-Shaped Mound at the Adams Site in Fulton County, Kentucky?

Charles Stout—Murray State University

8:45 A.M.

Counterintuitive Findings in a Child Fatalities Study

Cara Richards—Transylvania University

9:00 A.M.

An Ethnographic Investigation of Antazya's (Turkey) Street Children: A Preliminary Report

C. Robert Welch—Eastern Kentucky University

9:15 A.M.

Teaching Anthropology at the Community College: An Applied Approach

Iain Barksdale—Madisonville Community College

9:30 A.M.

Anthropology Section Business Meeting

9:45 A.M.

Refreshments, International D

10:00 A.M.

Kentucky Academy of Science Annual Business Meeting, International B

Botany and Microbiology Section

Barbara Rafaill—Chairperson
Room—McKinley

Friday, 4 November 1994

Barbara Rafaill—Presiding

8:00 A.M.

Synopsis of North American Hollies (*Aquifoliaceae*)

Ross C. Clark—Eastern Kentucky University

8:15 A.M.

Effects of the Herbicide 2,4-Dichlorophenoxyacetic Acid on Algal Communities in Kentucky Lake: Laboratory and Field Approaches

Minoo E. Kobraei and David S. White—Murray State University

8:30 A.M.

Preliminary Report on the Status of the Genus *Viburnum* in Kentucky

Timothy J. Weckman and Ronald L. Jones—Eastern Kentucky University

8:45 A.M.

Cryptococcus neoformans Serotype Groups Found in Clinical and Environmental Isolates

John M. Clauson and Larry Elliott—Western Kentucky University

9:00 A.M.

Refreshments, International D

9:15 A.M.

Symposium: How Will KERA Change Science Education, Kennedy Room

Moderator: Curtis Wilkins—Western Kentucky University

10:45 A.M.

Refreshments, International D

11:00 A.M.

Erythronium: The Effect of Mating System on Seed Set, Relationship Between Density and Flowering, and Pollinator Service

Judith Rozeman—Berea College; Tim Weckman and Ron Jones—Eastern Kentucky University

11:15 A.M.

Preliminary Flora of Shaker Village at Pleasant Hill, Mercer County, Kentucky

David Taylor—USDA Forest Service

11:30 A.M.

Cyperus of Kentucky

Randy Mears and Ronald Jones—Eastern Kentucky University

11:45 A.M.

Biomonitoring of *Leavenworthia exigua* var *laciniata* (Shepherdsville Glade Cress): A Cedar Glade Endemic

Gary Libby, Randy Mears and J. Danny Husband—Eastern Kentucky University

12:00 noon

Lunch, On your own

1:00 P.M.

Plenary Session: The Jackson Purchase Area—A Tribute to Raymond H. Athey

Moderator: Joe Winstead—Western Kentucky University, International B

2:15 P.M.

Refreshments, International D

Ron Jones—Presiding

2:45 P.M.

Morphological Responses of Amur Honeysuckle to Different Light Environments

Tim Tholemeier—Northern Kentucky University (Sponsored by James O. Luken)

3:00 P.M.

On the Road with Amur Honeysuckle

James O. Luken—Northern Kentucky University

3:15 P.M.

Micropropagation of American Chestnut

Larry A. Giesmann—Northern Kentucky University; David Beanson—Jacob Center, Cincinnati Public Schools

3:30 P.M.

Effect of Pitcher Size on Fly Symbionts of the New World Pitcher Plants (*Sarraceniaceae*)

Robert Naczi—Northern Kentucky University

3:45 P.M.

Photosynthetic Response to Enhanced Light in Amur Honeysuckle

Brian Kunkel—Northern Kentucky University (Sponsored by James Luken)

4:00 P.M.

Seedling Responses to Enhanced Light in Amur Honeysuckle

Linda Kuddes—Northern Kentucky University (Sponsored by James Luken)

4:15 P.M.

Analysis of an Old-Growth Upland Wet Woods in Christian County, Kentucky

E. W. Chesnut and S. M. Noel—Austin Peay State University; J. M. Baskins and C. C. Baskins—University of Kentucky; M. L. McReynolds—Hopkinsville Community College

4:30 P.M.

Kentucky Herbaria: An Update and Outlook

Ronald Jones—Eastern Kentucky University

- 4:45 P.M.
Curator's Meeting: Discussion of the Future of Kentucky Herbaria and Possible Cooperative Efforts
- Saturday, 5 November 1994
Barbara Rafaill—Presiding
- 8:00 A.M.
Dogwoods: Molecules, Morphology and Relationships
Zack E. Murrell—Western Kentucky University
- 8:15 A.M.
Fossil Diatoms from the Galapagos Islands: Their Use in Reconstructing the History of El Nino Events
Miriam Steinitz Kannan—Northern Kentucky University
- 8:30 A.M.
Protein and Polysaccharide Degradation and Nitrogen Fixation by the Microbial Communities of Pitcher Plants
Carrie Gillen—Northern Kentucky University (Sponsored by Miriam Kannan)
- 8:45 A.M.
Geographic Variation in the Purple Pitcher Plant (*Sarracenia purpurea*, Sarraceniaceae)
Brenda Racke—Northern Kentucky University (Sponsored by Robert Naczi)
- 9:00 A.M.
Trees of Paducah
William F. Beasley, Jr.—Paducah Community College
- 9:15 A.M.
Phytoplankton Production in a Limestone Quarry in Warren County, Kentucky
Robert C. Molloy and Joe Winstead—Western Kentucky University
- 9:30 A.M.
Refreshments, International D
- 10:00 A.M.
Kentucky Academy of Science Annual Business Meeting, International B
- 11:00 A.M.
RNA Biodiversity: Is Canopy Diversity an Adequate Measure?
David D. Taylor—USDA Forest Service; James D. Kiser—Eastern Kentucky University
- 11:15 A.M.
Influence of Carbon Source on Growth of Embryogenic Tissue Cultures of *Pinus strobus* L.
Karan Kaul and C. Mahl—Kentucky State University
- 11:30 A.M.
The Isoetes of Kentucky
Joe Winstead—Western Kentucky University; Lytton J. Musselman—Old Dominion University
- 11:45 A.M.
KJAS
Preventing Ultraviolet Radiation Induced DNA Damage
Kate Niehoff—duPont Manual High School (Sponsored by Barbara Fendley)
- 12:00 noon
Botany and Microbiology Section Business Meeting
- CHEMISTRY SECTION
Robert Berry—Chairperson
Larry Bigham—Secretary
Room—Adams II
- Friday, 4 November 1994
Robert Berry—Presiding
- 11:00 A.M.
Curing Kinetics Study of Epoxy Resins by Thermal Analysis
Charles W. M. Lee, Wei-Ping Pan and Jack Li—Western Kentucky University
- 11:15 A.M.
The Effect of the Electric Dipole of Short Peptides on Chemical and Physical Processes
Mark Meier and Hemant V. Toshi—University of Kentucky
- 11:30 A.M.
The Behavior of Chlorine During Coal Combustion
Bryan Travis and Wei-Ping Pan—Western Kentucky University
- 11:45 A.M.
Production of Activated Carbon from Used Automobile Tires
Susan Hodyclon and Wei-Ping Pan—Western Kentucky University
- 12:00 noon
Lunch, On your own
- 1:00 P.M.
Plenary Session: The Jackson Purchase Area—A Tribute to Raymond H. Athey
Moderator: Joe Winstead—Western Kentucky University, International B
- 2:15 P.M.
Refreshments, International C
- 2:45 P.M.
Reduced-Pressure ICP Detection for Gas Chromatography
M. R. Dunn and H. B. Fannin—Murray State University
- 3:00 P.M.
Prediction of Ash Fusion Temperatures from Elemental Composition
Kara Kleeman, Min Guo, W. G. Lloyd and John T. Riley—Western Kentucky University
- 3:15 P.M.
Modelling of Excited States Found in the ICP
R. S. Perry and J. M. Russell—Murray State University (Sponsored by H. B. Fannin)
- 3:30 P.M.
Design and Characterization of Processable High Temperature Thermosetting Polyimide Systems
Jenny Heidbrink—Western Kentucky University; Gerry

Meyer and James McGrath—Virginia Tech; Wei-Ping Pan—Western Kentucky University

3:45 P.M.

ICP-AES Analysis of Solids/Water Slurries

Matthew Renfrow, Jody Riley, Bugian Wang and John Riley—Western Kentucky University

4:00 P.M.

The Real Route to Solid Deposits in Aircraft Turbine Engines from Fuel

William D. Schulz—Eastern Kentucky University

4:15 P.M.

Using Near Infrared Spectroscopy for Reformer Unit Process Control

Roy R. Bledsoe—Ashland Petroleum Co. (Sponsored by Pat Doolin)

4:30 P.M.

College Chemistry—Can We Predict Which Students Will be Successful?

L. C. Byrd and *David R. Hartman*—Western Kentucky University

Saturday, 5 November 1994

Larry D. Bigham—Presiding

8:00 A.M.

A Route to Transition Metal-Containing Liquid Crystals

Jason D. May and Jose M. Workman—Centre College

8:15 A.M.

Oxidized Dextran as an Inhibitor of Tooth Decay

Timothy E. Davis and Christopher Fletcher—University of Louisville; Karl D. Pryor—du Pont Manual H.S.; Jujan Singh, K. G. Taylor and R. J. Doyle—University of Louisville

8:30 A.M.

Novel Synthesis of Anti-Cancer Drugs

Levi Harper and Gerald Rosenthal—University of Kentucky (Sponsored by John Rawls)

8:45 A.M.

Modern Technologies Available for the Chemistry Classroom and Laboratory

Nedah Rose—John Wiley & Sons, Inc. (Sponsored by Jim Niewahner)

9:00 A.M.

KJAS

Signal Transduction in Pulmonary Artery Smooth Muscle Cells: The Role of Nitric Oxide

Raj Mankad—Paul Lawrence Dunbar (Sponsored by Elizabeth Kikuchi)

9:15 A.M.

Chemistry Section Business Meeting

9:30 A.M.

Refreshments, International D

10:00 A.M.

Kentucky Academy of Science Annual Business Meeting, International B

GEOGRAPHY SECTION

Stuart Foster—Chairperson

Wayne Hoffman—Secretary

Room—Roosevelt I

Friday, 4 November 1994

Stuart Foster—Presiding

8:00 A.M.

Sides Cave: The Route to Pike Spring?

Christopher Groves—Western Kentucky University

8:15 A.M.

Central North American Grassland Migration: Stochastic Processes or Climatic Influence?

Conrad T. Moore—Western Kentucky University

8:30 A.M.

Vegetation Stress Analysis Utilizing Remotely Sensed Data—EPA Multi-Media Project—Kentucky

Neil Weber—Murray State University

8:45 A.M.

The Solstices and Daily Normal Maximum and Minimum Temperature Lags

Richard K. Snow—Western Kentucky University

9:00 A.M.

Refreshments, International D

9:15 A.M.

Symposium: How Will KERA Change Science Education, Kennedy Room

Moderator: Curtis Wilkins—Western Kentucky University

10:45 A.M.

Refreshments, International D

11:00 A.M.

Deaths and Injuries from Lightning in Kentucky: Analysis of Location

Mary M. Snow—Western Kentucky University

11:15 A.M.

Household Filtering and Motive for Household Location in Bowling Green, Kentucky

Kendra Vanhooser—Western Kentucky University

11:30 A.M.

Sinkhole Collapse Subsurface Investigation Using Microgravity

Nicholas Crawford and *Tim Slattery*—Western Kentucky University

11:45 A.M.

Inner City Revitalization: Bowling Green, Kentucky

Kenneth W. McDonald—Western Kentucky University

12:00 noon

The History of Kentucky Climate

Glen Conner—Western Kentucky University

12:15 P.M.

Lunch, On your own

1:00 P.M.

Plenary Session: The Jackson Purchase Area—A Tribute to Raymond H. Athey

Moderator: Joe Winstead—Western Kentucky University, International B

- 2:15 P.M.
Refreshments, International D
- Wayne Hoffman—Presiding
- 2:45 P.M.
The Geography of Golf in Kentucky
R. L. Marionneaux—Eastern Kentucky University
- 3:00 P.M.
Groundwater Monitoring of Carbonate Aquifers
Nicholas C. Crawford—Western Kentucky University
- 3:15 P.M.
Application of GIS in the Power Tool Repair Industry
Robin Diederich—Western Kentucky University
- 3:30 P.M.
Transportation Problems and Solutions: An Analysis of Package Transport with United Parcel Service
Blake R. Bunner—Western Kentucky University (Sponsored by James L. Davis)
- 3:45 P.M.
Transportation Analysis of Traffic Accidents in Kentucky in 1991
David Murrell—Western Kentucky University (Sponsored by James L. Davis)
- 4:00 P.M.
Retail Stores in Philadelphia: From the City to the Suburbs
Matthew C. Grecco—Western Kentucky University (Sponsored by James L. Davis)
- 4:15 P.M.
Interregional Migration in Peru
Patricia Villalobos—Western Kentucky University
- 4:30 P.M.
Spatial Influences in a Literary Context: A Case Study
James L. Davis and Nancy H. Davis—Western Kentucky University
- 4:45 P.M.
Urban Environments, Sense of Place, and the Genesis of Popular Music
David J. Keeling—Western Kentucky University
- 5:00 P.M.
The Persistence of Poverty in Kentucky: A Spatial Analysis
James M. Bingham and *Wayle L. Hoffman*—Western Kentucky University
- GEOLOGY SECTION
- Deborah Kuehn—Chairperson
Kenneth Kuehn—Secretary
Room—Roosevelt II
- Friday, 4 November 1994
Deborah Kuehn—Presiding
- 8:15 A.M.
Computer Aided Instruction in Geology Using Photographs Transferred to CD ROM
David R. Dockstader—Jefferson Community College
- 8:30 A.M.
A Conceptual Model for the Occurrence of Hydrochemical Facies in an Unmined Area of the Eastern Kentucky Coal Field
David R. Wunsch—Kentucky Geological Survey
- 8:45 A.M.
Petrology of the Elliott County, Kentucky—Kimberlite Intrusion
Brad Maynard—Morehead State University (Sponsored by Robert Boram)
- 9:00 A.M.
Refreshments, International D
- 9:15 A.M.
Symposium: How Will KERA Change Science Education, Kennedy Room
Moderator: Curtis Wilkins—Western Kentucky University
- 10:45 A.M.
Refreshments, International D
- 11:00 A.M.
Effect of Fractures on the Availability of Ground Water and the Occurrence of Pesticides and Nitrate in the Epikarst of the Inner Blue Grass Region, Bourbon County, Kentucky
Dwayne Keagy and *James Dinger*—Kentucky Geological Survey; *Steven Hampton* and *Lyle Sendlein*—Kentucky Water Resource Research Inst.
- 11:15 A.M.
The Kentucky Ground-Water Data Repository
Bart Davidson—Kentucky Geological Survey
- 11:30 A.M.
Characterization and Quantification of Nonpoint-Source Pollutant Loads in a Conduit-Flow-Dominated Karst Aquifer Underlying an Intensive-Use Agricultural Region, Kentucky
James Currens—Kentucky Geological Survey
- 11:15 A.M.
Regional Variation of Disconformities in the Mississippian Slade Formation, Northeastern, Kentucky
Matthew Vest—Morehead State University (Sponsored by Robert Boram)
- 12:00 noon
Lunch, On your own
- 1:00 P.M.
Plenary Session: The Jackson Purchase Area—A Tribute to Raymond H. Athey
Moderator: Joe Winstead—Western Kentucky University, International B
- 2:15 P.M.
Refreshments, International D
- Kenneth Kuehn—Presiding
- 2:45 P.M.
Shear-Related Folds in the Scandinavian Caledonides, Norway
Elizabeth A. McClellan—Western Kentucky University
- 3:00 P.M.
Sand and Gravel Resources of the Ohio River Valley
Warren Anderson—University of Kentucky (Sponsored by Don Chesnut)

3:15 P.M.

Implications of a Holocene Mangrove Peat from San Salvador, Bahamas

Deborah W. Kuehn—Western Kentucky University; Margaret Chai—University of Florida

3:30 P.M.

Pennsylvanian Sharks from Western Kentucky Coal Area

James X. Corgan—Austin Peay State University

3:45 P.M.

Sand and Gravel: Ohio River Mile 605 Area, Kentucky-Indiana

Graham Hunt—University of Louisville

4:00 P.M.

Prospects for Ultra-Clean Kentucky Coals

Kenneth W. Kuehn—Western Kentucky University

4:15 P.M.

The Geology of Roof Falls in Underground Coal Mines—A Comparison Between Eastern and Western Kentucky

David K. Hylbert—Morehead State University

4:30 P.M.

Investigation of Biochemical Oxygen Demand Levels on the Campus of the Nicholas County School System and Their Response to *Clostridium sporogenes*

Amanda Abnee—Nicholas County High School (Sponsored by Kathy Green)

4:45 P.M.

Geology Section Business Meeting

PHYSICS SECTION/FALL KAPT MEETING

Rico Tyler—Chairperson

Vince DiNoto—Secretary

Room—Roosevelt I

Saturday, 5 November 1994

Rico Tyler—Presiding

9:00 A.M.

Extension of I.M.C. Model to Nucleation of Binary System

H. R. Kobraei and B. R. Anderson—Murray State University

9:15 A.M.

The Kinematics and HT Structures of Interacting Galaxies: Implications for the Formation of Blue Dwarf Irregulars

Benjamin K. Malphrus—Morehead State University

9:30 A.M.

A Comparison of the Ultraviolet Spectral Morphology and MK Classifications of B Supergiants in the Small Magellanic Cloud

Anthony L. Borchers and Raymond C. McNeil—Northern Kentucky University

9:45 A.M.

A Remarkable Comet: Shoemaker-Levy 9

Roger Scott, Richard Hackney, Karen Hackney and Jim Parvin—Western Kentucky University

10:00 A.M.

Kentucky Academy of Science Annual Business Meeting, International B

11:00 A.M.

The Kentucky Space Grant Consortium Program into the Year 2000

Karen Hackney, Richard Hackney, Roger Scott and Tom Bohuski—Western Kentucky University

11:15 A.M.

OHMIC Contacts to SiC

John Crofton—Murray State University

11:30 A.M.

Physics Section/KAPT Business Meeting

Reports: President—Rico Tyler; Treasurer—Vince DiNoto; Survey—Chris Graney;

Other reports

11:45 A.M.

Effects of Ion Damage on the Superconductors Current-Voltage Relationships in Strong Magnetic Fields in NbSe₂.

J. G. Childers and W. F. Henshaw—Union College; Junqing Zhang and L. E. DeLong—University of Kentucky; R. C. Budhani—Brookhaven National Laboratory

12:00 noon

Analysis of Laser Induced Fluorescence Data from Rocket Motor Exhausts

Stephen M. Fuqua and Stephen H. Cobb—Murray State University

12:15 P.M.

Post-Impact Vehicle Lamp Filament Examination as an Accident Reconstruction Technique

Alan A. Johnson and David Harmeling—University of Louisville

12:30 P.M.

KJAS

Photoelectrochemical Cells

Sallie Rademacher—Notre Dame Academy (Sponsored by Sisters Averbeck and Parrott)

PHYSIOLOGY, BIOPHYSICS, BIOCHEMISTRY, AND PHARMACOLOGY SECTION

Suzanne Byrd—Chairperson

Chang Wang—Secretary

Room—Van Buren

Friday, 4 November 1994

Suzanne Byrd—Presider

11:15 A.M.

Mathematical Modeling of Primary Production in Kentucky Lake

B. R. Anderson, H. R. Kobraei and Mary C. Child—Murray State University

11:30 A.M.

Glycoconjugate Expression in Normal and Regenerating Lateral Line Sensory Epithelia of the Axolotl Salamander, *Ambystoma mexicanum*

Richmond J. Miller and K. J. Balak—Western Kentucky University

11:45 A.M.

Experimental Approaches to Investigate Neuromast Regeneration of the Axolotl Lateral Line

R. R. Speck and K. J. Balak—Western Kentucky University

12:00 noon

Lunch, On your own

1:00 P.M.

Plenary Session: The Jackson Purchase Area—A Tribute to Raymond H. Athey

Moderator: Joe Winstead—Western Kentucky University, International B

2:15 P.M.

Refreshments, International D

2:45 P.M.

The Steady-State Kinetics of Lithium (Li⁺) Inhibition of Yeast Enolase

William W. Farrar—Eastern Kentucky University

3:00 P.M.

Taste Comparison Between Smoked Paddlefish and Catfish Meat

Changzheng Wang and Steve D. Mims—Kentucky State University

3:15 P.M.

Transcutaneous Collection and Quantitative Measurement of Biological Hazards

Michael Timmons—Western Kentucky University; Steven Collier and Sorell Schwartz—Georgetown University; Claire Rinehart—Western Kentucky University

3:30 P.M.

Isozymes of Enolase in Pig Tissue

Vince Barrows and William Farrar—Eastern Kentucky University

3:45 P.M.

Consumer Factors Related to the Frequency of Fish Consumption

Changzheng Wang and Steve D. Mims—Kentucky State University

4:00 P.M.

Purification and Mechanism Studies of Pyruvate Kinase from the Flight Muscle of the House Sparrow (*Passer domesticus*)

William W. Farrar—Eastern Kentucky University

4:15 P.M.

Effect of Protein and Bicarbonate Intake on Kidney Mineral Contents of Fisher 344 Rats

Changzheng Wang, C. J. Lee and Abbas Babalmoradi—Kentucky State University

4:30 P.M.

Steady-State Mechanism of Lactate Dehydrogenase from House Sparrow Flight Muscle and Heart

William Farrar and Young Jo Farrar—Eastern Kentucky University

4:45 P.M.

Effects of Nutrients and Retention Time on the Primary Production of Kentucky Lake

Mary C. Child, H. R. Kobraei and B. R. Anderson—Murray State University

Saturday, 5 November 1994

Chang Wang—Presider

8:15 A.M.

Apoptosis in Differentiated and Undifferentiated Murine F9 Teratocarcinoma Cells

E. Deaton, S. Vogelpohl, B. Baldwin, R. Snyder and J. Carter—Wood Hudson Cancer Research Laboratory

8:30 A.M.

The Role of Arginine in Pinene Synthases of *Salvia officinalis*

Steve Hume—Centre College; Thomas Savage and Rodney Croteau—Washington State University; Linda Roberts—Centre College

8:45 A.M.

Sclerotinia Shows Mating Type Homology to Both Heterothallic and Homothallic Fungi

Martin Brock—Eastern Kentucky University

9:00 A.M.

Investigation of the Effect of Methionine Oxidation on the Conformation of Soluble and Lipid-Bound Apolipoprotein A-1 by Limited Proteolysis

Michelle Reader—Centre College; Linda Roberts—Whitman College; Christie Brouillette—Southern Research Institute

9:15 A.M.

Physiology, Biophysics, Biochemistry, and Pharmacology Section Business Meeting

9:30 A.M.

Refreshments, International D

10:00 A.M.

Kentucky Academy of Science Annual Business Meeting, International B

SCIENCE EDUCATION SECTION

Robert Boram—Chairperson

Peter V. Lindeman—Secretary

Room—Washington

Friday, 4 November 1994

Robert Boram—Presiding

2:45 P.M.

Are Kentucky Students Sleeping Enough?

John G. Shiber—Prestonsburg Community College

3:00 P.M.

KERA, "Fast Plants," and Curriculum Integration: What do These Things Have in Common?

Kim Alexander and Robert Creek—Eastern Kentucky University

3:15 P.M.

The Gender Equitable Classroom—Instruction That Improves Learning for Male and Female Students

Robert Boram and *Joyce Saxon*—Morehead State University

3:30 P.M.

Conservation Biology for the College Freshman Non-Science Major

Peter V. Lindeman—Madisonville Community College

3:45 P.M.

Development of Performance Assessment Tasks for Future Science Teachers

Vince DiNoto—Jefferson Community College; *Valgene Dunham*—Western Kentucky University

4:00 P.M.

Scientists of Kentucky: An Oral History Project

Howard Powell and *Morris Taylor*—Eastern Kentucky University

4:15 P.M.

A Pilot Project Pairing an Introduction to College Science with Intermediate Algebra

David Dockstader and *Brita Dockstader*—Jefferson Community College

4:30 P.M.

Effect of Collaborative Activities on Grade Distribution in Biology

Veena Sallan—Owensboro Community College

Saturday, 5 November 1994

Peter V. Lindeman—Presiding

8:00 A.M.

Penny Experiments for General Chemistry

Harry Conley—Murray State University; *James Meeks*—Paducah Community College

8:15 A.M.

A Model Classroom Curriculum Integrating the Core Concepts of Biomedical Research and Societal Values to Achieve KERA Outcomes in the Secondary Science Classroom

Stephanie Wyatt and *Cloyd Bumgardner*—Calloway County High School; *Gregory Popken*—University of North Carolina

8:30 A.M.

Use of Gel Permeation Chromatography in the Undergraduate Polymer Laboratory

Jon Doyle, *Kevin Petal* and *Thomas Green*—Western Kentucky University

8:45 A.M.

Polymer Synthesis in the Undergraduate Polymer Laboratory

Kevin Petal, *Jon Doyle* and *Thomas Green*—Western Kentucky University

9:00 A.M.

Enhancing Student Perceptions of Science and Achieving Kentucky Education Reform Act Learner Outcomes Through a Creative Science Classroom Curriculum

Stephanie Wyatt and *Cloyd Bumgardner*—Calloway County High School

9:15 A.M.

Science Education Section Business Meeting

9:30 A.M.

Refreshments, International D

10:00 A.M.

Kentucky Academy of Science Annual Business Meeting, International B

PSYCHOLOGY SECTION

Terry Barrett—Chairperson

Jeff Smith—Secretary

Room—Kennedy

Friday, 4 November 1994

Terry Barrett—Presiding

2:45 P.M.

Gender Differences in Public Speaking Anxiety

Tina Lane—Murray State University (Sponsored by *Terry Barrett*)

3:00 P.M.

Longlasting Behavioral and Neurochemical Consequences of Chronic Apomorphine Treatments

Tracey Ellison—Morehead State University; *Patricia Robinet*—University of Kentucky; *Bruce Mattingly*—Morehead State University; *Mike Bardo*—University of Kentucky

3:15 P.M.

Reality Characteristics of Hallucinations Reported by Normal Adults and by Chronic Schizophrenics

Terry R. Barrett—Murray State University

3:30 P.M.

The Personality Characteristics Associated with Alienation in College Students

Valerie Haire—Murray State University (Sponsored by *Terry Barrett*)

3:45 P.M.

Mental Practices: An Undergraduate Sample

Danny R. Adamson—Murray State University (Sponsored by *Terry R. Barrett*)

4:00 P.M.

A Timely Look at the Fundamentals of Academic Procrastination

Christina E. Smith—Murray State University (Sponsored by *Terry R. Barrett*)

4:15 P.M.

Effects of Coaching Style on Competition Performance in Children

Beverly Lenicky—Northern Kentucky University (Sponsored by *David Hogan*)

4:30 P.M.

Emotion Affects Memory

Kathy Alexander—Murray State University (Sponsored by *Terry R. Barrett*)

4:45 P.M.

Women's Perceptions of Female Gender Roles

K. Eleanor Sheets—Murray State University (Sponsored by Terry R. Barrett)

5:00 P.M.

Effects of Response Prevention on the Development of Behavioral Sensitization to Apomorphine

Sonia Fields, Steve McDonald and Bruce Mattingly—Morehead State University

Saturday, 5 November 1994

Jeff Smith—Presiding

11:00 A.M.

Effects of Chronic Dopamine D3 Receptor Stimulation of Locomotor Activity and Subsequent Sensitivity to Apomorphine

Mike Cecil, Sonia Fields, Steve McDonald and Bruce Mattingly—Morehead State University

11:15 A.M.

Time Urgency Between Type A and B Coronary Prone Behavior

Michael S. Dykes—Murray State University (Sponsored by Terry R. Barrett)

11:30 A.M.

The Effects of a Newsletter on Increasing Familiarity Among Fast Food Restaurant Employees to Improve Teamwork

Kara J. Collins—Northern Kentucky University (Sponsored by David Hogan)

11:45 A.M.

Neurochemical and Behavioral Consequences of Chronic Dopamine D3 Receptor Stimulation

Mike Langfels—Morehead State University; Patricia Robinet—University of Kentucky; Bruce Mattingly—Morehead State University; Mike Bardo—University of Kentucky

12:00 noon

Lunch, On your own

1:00 P.M.

The Relationship Between High Versus Low Imagery Recall and Performance in an Introductory Psychology Course

Francis H. Osborne and Aimee C. Lawson—Morehead State University

1:15 P.M.

Alcohol Expectancies of College Students

Joe D. Littleton—Murray State University (Sponsored by Terry R. Barrett)

1:30 P.M.

A Cross Cultural Comparison of Posture Perception

Kim Kelly and Jack Thompson—Centre College

1:45 P.M.

Personality Correlates of Performance in an Introductory Psychology Course

Francis H. Osborne and Steven E. Dolan—Morehead State University

2:00 P.M.

Sex and Age Effects on Grouping Behavior and Interpersonal Touch

Kim Kelly, Phyllis Passariello and Jack Thompson—Centre College

2:15 P.M.

The Effect of Anxiety on Dysmenorrhea in College Students

Valeria S. Wagoner and Frank H. Osborne—Morehead State University

2:30 P.M.

Self-Esteem of Black, White, Male, and Female College Students

Laura Engle and Jack Thompson—Centre College

2:45 P.M.

KJAS

Transformation of a Personality: A Three Year Study

Phonesavane Liankehammy—Warren Central High School (Sponsored by Linda Walker)

2:45 P.M.

Psychology Section Business Meeting

SOCIOLOGY SECTION

J. Allen Singleton—Chairperson

Steve Savage—Secretary

Room—Jefferson

Friday, 4 November 1994

J. Allen Singleton—Presiding

2:45 P.M.

The Electoral College: History and Prospects

Tamatha Brewer—Eastern Kentucky University (sponsored by J. Allen Singleton)

3:00 P.M.

Environmental Consciousness and Awareness: A Quantitative Analysis of University Students

Molly B. Kerby—Western Kentucky University (sponsored by Joe Winstead)

3:15 P.M.

Single Male Parenting

Kelly Davis—Eastern Kentucky University (sponsored by Steve Savage)

3:30 P.M.

Beyond the Headlines: Examining Ethics and the Kentucky General Assembly

Amy Etmans—Eastern Kentucky University (sponsored by J. Allen Singleton)

3:45 P.M.

Urban-Rural Aspects of Kentucky Politics

J. Allen Singleton—Eastern Kentucky University

4:00 P.M.

Sociology Section Business Meeting

ZOOLOGY AND ENTOMOLOGY SECTION

Guenther Schuster—Chairperson

Gordon Weddle—Secretary

Room—Madison

Friday, 4 November 1994

Guenter Schuster—Presiding

8:15 A.M.

Daily Pattern of Brain GABA Levels in the Cockroach, *Leucophaea maderae*

Juli McCay, Kimberly Romero, Blaine Ferrell and Darwin Dahl—Western Kentucky University

8:30 A.M.

The Freshwater Unionids (Mussels) of Marsh Creek, McCreary County, Kentucky

Ronald R. Cicerello and Ellis Lauder milk—Kentucky Nature Preserve Commission

8:45 A.M.

Coccinellidae Predation of First and Second Generation European Corn Borer, *Ostrinia nubilalis*, Egg Masses Kentucky Field Corn

Donna Shanklin, Douglas Johnson, Lee Townsend, Jr. and Ric Bessin—University of Kentucky

9:00 A.M.

Refreshments, International D

9:15 A.M.

Symposium: How Will KERA Change Science Education, Kennedy Room

Moderator: Curtis Wilkins—Western Kentucky University

10:45 A.M.

Refreshments, International D

11:00 A.M.

Changes in Gut Capacity due to Increased Energy Demand in the Pine Vole (*Microtus pinetorum*)

Mitzi Austin and Terry Derting—Murray State University

11:15 A.M.

Development of Sawtoothed Grain Beetle and Red Flour Beetle on Corn Previously Infested by Angoumous Grain Moth

Patti Rattlingourd and Paul Weston—Kentucky State University

11:30 A.M.

A Comparison of the Seasonal Variation of Intestinal Villi Morphology of a Herbivorous Species (*Microtus pennsylvanicus*) and an Omnivorous Species (*Peromyscus leucopus*)

Arthur Scott and Terry Derting—Murray State University

11:45 A.M.

Potential Mode of Action on Host Development of a Secretory Product from a Parasite

D. L. Dahlman, E. J. Schepers, Z. Zhang and F. A. DiLuna—University of Kentucky

12:00 noon

Lunch, On your own

1:00 P.M.

Plenary Session: The Jackson Purchase Area—A Tribute to Raymond H. Athey

Moderator: Joe Winstead—Western Kentucky University, International B

2:15 P.M.

Refreshments, International D

2:45 P.M.

Similar Seasonal Changes in Gut Morphology in a Herbivore (*Microtus pennsylvanicus*) and a Granivore (*Peromyscus leucopus*)

Eduard Noakes and Terry Derting—Murray State University

3:00 P.M.

Exciting Discoveries in the Lepidoptera Fauna of the Jackson Purchase, Kentucky: A Review

Charles Covell, Jr. and William Black, Jr.—University of Louisville

3:15 P.M.

The Impact of Red-Cockaded Woodpecker Forest Management Practices on Populations of White-Footed Mice (*Peromyscus leucopus*)

Raymond D. Campbell—University of Kentucky; David Nusbaumer—Elizabethtown Community College

3:30 P.M.

Food Habits and Distribution of Larval Bluegill, *Lepomis macrochirus*, in Ledbetter Embayment, Kentucky Lake

M. J. Brouder and T. J. Timmons—Murray State University

3:45 P.M.

Density and Relative Abundance of the River Cooter (*Pseudemys concinna*) in Kentucky Lake and the Lower Tennessee River: A Spotting-Scope Survey

Peter V. Lindeman—Madisonville Community College

4:00 P.M.

Equine Protozoal Myeloencephalitis: A Review

Shelby Stamper and D. E. Granstrom—University of Kentucky

4:15 P.M.

Conservation Status and Systematics of the Palezone Shiner, *Notropis albizonatus* (Pisces: Cyprinidae), a Federally Endangered Species in Kentucky

Brooks Burr and Kenneth Cook—Southern Illinois University at Carbondale; Melvin Warren—Southern Forest Experiment Station, Oxford, Mississippi

4:30 P.M.

Distribution, Reproduction and Recovery of the Relict Darter, *Etheostoma chienense* (Percidae), in Western Kentucky

4:45 P.M.

Intraspecific Phylogeography of Five North American Highland Fishes: A Test of the Pleistocene Vicariance Hypothesis

Rex Meade Strange and Brooks M. Burr—Southern Illinois University; Kyle Piller and Brooks M. Burr—Southern Illinois University at Carbondale

Saturday, 5 November 1994

Gordon Weddle—Presiding

8:00 A.M.

Microhabitat Preferences of Larval Studfishes (*Fundulus catenatus*): Impact of Predator Presence

Will Sudduth and Mike Barton—Centre College

8:15 A.M.

The Identification, Distribution, and Habitat of *Anguipira rugoderma* Hubricht, A Land Snail Endemic to Southeastern Kentucky

John MacGregor—USDA Forest Service; James Kiser—Eastern Kentucky University

8:30 A.M.

The Influence of Salinity and pH on the Rate of Heart Beat and Locomotor Activity of *Daphnia magna*

Jeff Reynolds—Moss Middle School; Robert Hoyt—Western Kentucky University

8:45 A.M.

The Bluegrass and Western Coal Fields: Filter Barriers to the Dispersal of Kentucky Vertebrates

Les Meade—Morehead State University; John MacGregor—USDA Forest Service

9:00 A.M.

Factors Affecting Gas Exchange in Gila Woodpecker Nest-Cavities

David Nusbaumer—Elizabethtown Community College

9:15 A.M.

KJAS

Effectiveness of Alternative Insecticide Formulations on the German Cockroach, *Blatena germanica*

Burr Settles—Lafayette High School (Sponsored by James Gentry)

9:30 A.M.

Zoology and Entomology Section Business Meeting

9:45 A.M.

Refreshments, International D

10:00 A.M.

Kentucky Academy of Science Annual Business Meeting, International B

COMPUTER SCIENCE SECTION

Sylvia C. Pulliam—Chairperson

Richard A. Rink—Secretary
Room—Roosevelt II

Saturday, 5 November 1994

Sylvia Pulliam—Presiding

8:00 A.M.

Fuzzy Logic Controller Projects for Students
Art Shindhelm—Western Kentucky University

8:15 A.M.

Data Compression: Theory and Practice
John Crenshaw—Western Kentucky University

8:30 A.M.

Using Algorithm Animation in CS1 and CS2
Carol W. Wilson—Western Kentucky University

8:45 A.M.

Teaching Computer Science in Russia
Sylvia Clark Pulliam—Western Kentucky University

9:00 A.M.

Open Labs for CSC Using Graphical Displays of Algorithms for Large Integer Arithmetic

Kenny Napier and Bill Janeway—Eastern Kentucky University

9:15 A.M.

Cards/Permutations: Open Labs and Analysis of Algorithms

Bill Janeway and Donnie Grimes—Eastern Kentucky University

9:30 A.M.

Refreshments, International D

10:00 A.M.

Kentucky Academy of Science Annual Business Meeting

MATHEMATICS SECTION

Carroll Wells—Chairperson

Russell Brengelman—Secretary
Room—Truman

Friday, 4 November 1994

Carroll Wells—Presiding

2:45 P.M.

Common Threads of Math Reform Movements
Joseph F. Stokes—Western Kentucky University

3:00 P.M.

Using "Derive" in Calculus

Kathy Kepner—Paducah Community College; Maura Corley—Henderson Community College

3:15 P.M.

"Hands on" Geometry Activities

Carroll G. Wells—Western Kentucky University

3:30 P.M.

Using the Genetic Algorithms

James Porter—Western Kentucky University

Saturday, 5 November 1994

Russell Brengelman—Presiding

8:15 A.M.

A Pilot Project Pairing Intermediate Algebra with an Introductory Science Course

Brita Dockstader—Jefferson Community College

8:30 A.M.

Parts from Dirichlet

J. B. Barksdale, Jr.—Western Kentucky University

8:45 A.M.

Developing Actuarial Science and Operations/Quality Control Curricula in Applied Mathematics

Alan D. Smith—Robert Morris College

9:00 A.M.

An Examination of a Partial Differential Equation from Underwater Acoustics

Mark P. Robinson—Western Kentucky University

9:15 A.M.

Mathematics Section Business Meeting

9:30 A.M.

Refreshments, International D

10:00 A.M.

Kentucky Academy of Science Annual Business Meeting,
International B

AGRICULTURAL SCIENCES SECTION

Matthew E. Byers—Chairperson
Elmer Gray—Secretary
Room—Lincoln

Friday, 4 November 1994

Matthew E. Byers—Presiding

8:15 A.M.

Ecology of Insects in Two-Year-Old On-Farm Stored
Shelled Corn

J. D. Sedlacek, P. A. Weston, B. D. Price and P. L. Rat-
tlingourd—Kentucky State University

8:30 A.M.

Effects of Soybean Cultivars and Planting Dates on Bio-
mass Production

Aslam Tawhid and Elmer Gray—Western Kentucky Uni-
versity

8:45 A.M.

Effects of Winter-Cropped Hairy Vetch and Tillage on Ni-
trogen Nutrition of Sweet Corn and Watermelon

Anthony Silvernail and Gary Cline—Kentucky State Uni-
versity

9:00 A.M.

Refreshments, International D

9:15 A.M.

Symposium: How Will KERA Change Science Education,
Kennedy Room

Moderator: Curtis Wilkins—Western Kentucky University

10:45 A.M.

Refreshments, International D

11:00 A.M.

A Standardized Comparison of Population Characteristics
of Freshwater Prawns, (*Macrobrachium rosenbergii*),
Raised in Two Different Geographical Regions (Ken-
tucky and Mississippi)

James Tidwell and *Shawn Coyle*—Kentucky State Uni-
versity; Louis R. D'Abramo—Mississippi State Uni-
versity

11:15 A.M.

Dacthal Persistence in Soil

Nekiya Baker, Matt Byers, George Antonious and Debra
Hilborn—Kentucky State University

11:30 A.M.

Analysis of Esfenvalerate on Vegetables

Dawn Greene, George Antonious and Matthew E.
Byers—Kentucky State University

11:45 A.M.

Vegetable Culture on Erodible Land

Debra Hilborn, Matthew Byers and George Antonious—
Kentucky State University

12:00 noon

Lunch, On your own

1:00 A.M.

Plenary Session: The Jackson Purchase Area—A Tribute
to Raymond H. Athey

Moderator: Joe Winstead—Western Kentucky University,
International B

2:15 P.M.

Refreshments, International D

Elmer Gray—Presiding

2:45 P.M.

Sustainable Agronomic Research Projects at Western
Kentucky University

Elmer Gray, Aslam Tawhid, Tena Wright and Brian Lace-
field—Western Kentucky University

3:00 P.M.

The Influence of Incident Light Intensity and Fertilization
on Growth and Development of Pawpaw (*Asimina tri-
loba* Dunal) Seedlings

Lakeasha Jones, Desmond Layne and Michael Kwantes—
Kentucky State University

3:15 P.M.

Influence of Mulch Color on Yield of Sweet Basil

E. Greer, K. Kaul and C. L. *Mahl*—Kentucky State Uni-
versity

3:30 P.M.

Environmental Effects on Indian Mock Strawberry

Brian D. Lacefield and Elmer Gray—Western Kentucky
University

3:45 P.M.

The Street Tree Inventory—Putting the Data to Work

James M. Martin—Western Kentucky University

4:00 P.M.

Agricultural Sciences Section Business Meeting

INDUSTRIAL SCIENCE SECTION

Burton H. Davis/Patricia K. Doolin—
Co-Chairpersons
Room—Eisenhower

Friday, 4 November 1994

9:25 A.M.

Introduction

9:30 A.M.

Biological Research in the Electric Utility Industry: The
Search for an Answer to the Electromagnetic Field Di-
lemma

Jeff West—East Kentucky Power Corporation

10:00 A.M.

Method to Reduce Diesel Engine Exhaust Emissions

Greg Garr—I.C.T. Incorporated

10:30 A.M.

Petroleum Products in Your Everyday Life

Robert Wombles—Ashland Petroleum Company

11:00 A.M.

Ground Water Investigation and Clean Up

Colleen Winker, P.E.—Martin Marietta

11:30 A.M.

Trans-Isomers Minimization During the Catalytic Hydrogenation of Vegetable Oils

John H. Hasman and Patrick McLaughlin—United Catalysts Inc.

12:00 noon

Closing Remarks

CELL AND MOLECULAR BIOLOGY

John Rawls—Chairperson

Claire Rinehart—Secretary

Room—Eisenhower

Saturday, 5 November 1994

John Rawls—Presiding

8:00 A.M.

A Cytokinin-Responsive mRNA in *Phaseolus vulgaris* L.

Kerrie L. McDaniel—Western Kentucky University; David Lightfoot—Southern Illinois University

8:15 A.M.

Molecular Dissection of the Mouse Alpha-Fetoprotein Repressor Region

Cassandra Backer, Amy Ellis and Brett Spear (Sponsored by John Rawls)—University of Kentucky

8:30 A.M.

Mapping the Location of Somatostatin-Like Immunoreactive Cells in Tadpole Tectum

Angie Baker and Elizabeth Debski (Sponsored by John Rawls)—University of Kentucky

8:45 A.M.

Studies of Non-Protein Amino Acid Disruption in Proteins

Michael Bass and Gerald Rosenthal (Sponsored by John Rawls)—University of Kentucky

9:00 A.M.

Intracellular Niacin Status and Mechanisms of Carcinogenesis

Christopher Watt and Elaine Jacobson (Sponsored by John Rawls)—University of Kentucky

9:15 A.M.

Influence of Antioxidant and Temperature Treatment on Lipid Peroxidation and Senescence in Broccoli Florets

Sau-Min Tam, Hong Zhuang and Margret Barth (Sponsored by John Rawls)—University of Kentucky

9:30 A.M.

Refreshments, International D

10:00 A.M.

Kentucky Academy of Science Annual Business Meeting, International B

11:00 A.M.

Studies of the Variation of a Surface Protein of *Streptococcus equi* Subspecies *zoepidemicus*

Matt Blair, John Walker and John Timoney (Sponsored by John Rawls)—University of Kentucky

11:15 A.M.

Investigation of Repressor Dependent Segregation in *Escherichia coli*

Michael Zgoda and Don Bick (sponsored by John Rawls)—University of Kentucky

11:30 A.M.

Modulation of TNF Cytotoxicity by NAD.

Mandy Wilson, David Hiestand, Boyd Haley, Elaine Jacobson and John Rawls—University of Kentucky

11:45 A.M.

The Influence of Methyl Jasmonate on Lipid Peroxidation and Deterioration in Broccoli

D. Gueorguieva, H. Zhuang and M. Barth (Sponsored by John Rawls)—University of Kentucky

12:00 noon

Lunch, On your own

Claire R. Rinehart—Presiding

1:00 P.M.

Roles of Polyunsaturated Fatty Acids in Neuronal Death: Lipid Peroxidation and Arachidonic Acid Metabolism

Irene Hong and Marion Steiner (Sponsored by John Rawls)—University of Kentucky

1:15 P.M.

Effect of Ozone Exposure on Antioxidant Vitamins in Fruit Tissue

Kellie Kute, M. Margaret Barth and Zhou Chen (Sponsored by John Rawls)—University of Kentucky

1:30 P.M.

Regulation of Expression of Protease Nexin 1 in Neural Tissue: Relationship to Alzheimer's Disease

Paul Jett, John Rawls and Stephen Zimmer—University of Kentucky

1:45 P.M.

Interactions of the Tobacco Vein Mottling Virus' Cylindrical Inclusion Protein with Other Virus Proteins

Beth Smith and Arthur Hunt (Sponsored by John Rawls)—University of Kentucky

2:00 P.M.

The Organelle Specific Role of the Delta-9 Fatty Acid Desaturase

Michael Scouby, Sergei Avdiushko and David Hildebrand (Sponsored by John Rawls)—University of Kentucky

2:15 P.M.

Fatty Acid Effects on Vitamin Levels in Cultured Endothelial Cells

Brad Middendorf and Bernhard Hemig (Sponsored by John Rawls)—University of Kentucky

2:30 P.M.

The Effects of Teratocyte Secretory Products on Protein Synthesis by Fat Body in Various Larval Stages of *Heliothis virescens*

Lisa McGraw and Douglas L. Dahlman—University of Kentucky

2:45 P.M.

How Do Lymphocytes Recognize Antigens in Immune Privileged Sites?

Loh, Wai Khan (Sponsored by John Rawls)—University of Kentucky

3:00 P.M.

The Synthesis of Insulin-Like Growth Factor Binding Protein-2 mRNA in Human T-Cells

Sandhya Venugopal, Lorri Ann Morford and Thomas Roszman (Sponsored by John Rawls)—University of Kentucky

3:15 P.M.

Cell and Molecular Biology Section Business Meeting

SECTIONAL POSTERS

Posters will be available for viewing for the duration of the meeting. Presenters are requested to be at their posters to facilitate discussion of their research at the following times: Friday, November 4 from 2:15 P.M. to 3:00 P.M.; Saturday, November 5 from 9:00 A.M. to 10:00 A.M.; Saturday, November 5 from 11:00 A.M. to 12:00 noon.

1. The Effect of Agricultural Chemicals on Ground-Water Quality in Lacustrine Deposits of the Western Kentucky Coal Field
Philip Conrad and James Dinger—Kentucky Geological Survey; Lyle Sendllein—Water Resources Research Institute
2. A Study on Dental Health Professionals Who Provide Care to the Homeless
Arthur Van Steward and Eric Veal—University of Louisville Dental School
3. Decomposition of Nerve Gas and Mustard Gas Analogs Using Nicotine and Ultrasound
John Meisenheimer, Lawrence Miller and William Schulz—Eastern Kentucky University
4. Influence of Constructed Wetlands on Pathogen and Nutrient Removal
Frank Young III, Matt Byers, George Antonious and Arla Burks—Kentucky State University
5. Elkhorn Creek Monitoring Program: Influence of Livestock on Water Quality
Arla Burks, Frank Young, Dan Logan and Matt Byers—Kentucky State University
6. Biological Control Using Interplantings
Patty Lucas and Doug Johnson—University of Kentucky
7. Fullerenes and Fullerene Derivatives: Preparative Chromatography of Higher Fullerenes and Synthesis of Fullerene Derivatives
Rebecca Massie and B. Henshaw—Union College; J. Selegue—University of Kentucky
8. Effects of Acidified Field Soil on *Sericea Lespedeza* Inoculated with *Bradyrhizobium*
G. R. Cline and A. F. Silvermail—Kentucky State University
9. A Theoretical Study of Molecular Geometries and Harmonic Vibrational Frequencies: Ge₂C and GeC₂
Regina Rao—Gannon University; Roger Grey (Mentor)—University of Kentucky (Sponsored by Sylvia Daunert)
10. Comparisons of Composts for Growing Vegetables in Containers
Aslam Tawhid and Elmer Gray—Western Kentucky University
11. Container Herb Production Using Compost Mixtures
Tena Wright and Elmer Gray—Western Kentucky University
12. Treating Livestock Waste Waters with Constructed Wetlands
D. A. Stiles, B. Kessler, O. W. Dotson III, B. Basham, R. Johnson and A. Bedel—Western Kentucky University
13. NMR Spectra of Sulfonium Salts in Presence of Lanthanide Shift Reagents
Bijan Radmard, Thomas Green and Lester Pesterfield—Western Kentucky University
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26. Thermal 1,3-Dipolar Cycloreversion of Fullerene Isoxazolines

Vahid Majidi and Robert Pogue—University of Kentucky; Magdalena Poplawska—Warsaw Technical University; Craig Thomas—University of Indiana (Indianapolis); Mark Meier—University of Kentucky

ABSTRACTS OF SOME PAPERS PRESENTED AT THE ANNUAL MEETING, 1994

AGRICULTURAL SCIENCES

Analysis of esfenvalerate on vegetables. D. GREENE,* G. ANTONIOUS, and M. E. BYERS, Kentucky State University, Frankfort, KY 40601.

Esfenvalerate, [(s)-cyano (3-phenoxyphenyl) methyl (s)-4-chloro-alpha-(1-methyl ethyl) benzene acetate], is the S isomer of the pyrethroid fenvalerate (70% or more active isomer of fenvalerate). An experiment was conducted in 1992, at Kentucky State University Research Farm (Franklin County, Kentucky), to study dissipation of esfenvalerate on pepper and pumpkin plants following spraying. Field plots (22.0 × 3.7 m, n = 160) were arranged on a 10% grade in a randomized complete block design and were planted with green bell pepper and pumpkin. Plots contained 10 rows oriented along the contour of the slope. Esfenvalerate was extracted using n-hexane from representative plant samples collected at different time intervals for residue analysis. The samples indicated initial deposits of 3.34 and 1.18 ppm on pumpkin and pepper leaves, respectively. Only trace levels were detected on pepper fruits on day 21 (0.0001 ppm). Half-life values were 1.11 and 2.79 d on pumpkin and pepper fruits, respectively, whereas the values were 1.92 and 3.38 on pumpkin and pepper leaves, respectively. Generally, residues of esfenvalerate on the treated vegetables were low. These low residues are encouraging because risk of exposure to the consumer is low.

Dacthal persistence in soil. N. BAKER,* M. E. BYERS, G. F. ANTONIOUS, and D. HILBORN, Kentucky State University, CRS, Frankfort, KY 40601.

The use of herbicides to control weeds on erodible lands may reduce the need for tillage and contribute to a sustainable agricultural system. But soil applied herbicides are perceived to potentially affect surface water and groundwater. The fate of a compound in soil is directly related to its potential fate in water. Dacthal (DCPA, dimethyl 2,3,5,6 tetrachloro-1,4-benzenedicarboxylate), a selective herbicide, was applied on 21 April 1994 at 10.1 kg/ha to plots (3.7 × 22 m) on a 10% slope, with Lowell silt loam soil, to which broccoli transplants were planted. Plots in 1994 had fescue strips every row (F1), fescue strips every other row (F2), or conventional tillage (CT) as soil treatments. Dacthal extraction methods, extraction efficiency, and persistence over time in soil were determined. The most effective extraction procedure was using soxhlet and hexane with 2-h refluxing. Analysis was by GC-NPD and GC-MS (301 and 332 m/e). Although applied at relatively high rates, Dacthal was found in soil at concentrations of 0.5, 0.29, and 0.08 ppm on 6 June 1994 in CT, F2, and F1 treatments, respectively.

Effects of cultivars and planting dates on biomass production in soybeans. ASLAM TAWHID* and ELMER GRAY, Department of Agriculture, Western Kentucky University, Bowling Green, KY 42101.

Recent concern about the environment has resulted in renewed interest in the use of organic matter in agriculture. The objective of the present investigation was to determine the effects of cultivar maturity differences and planting dates on biomass production of soybean, *Glycine max*. The research was conducted on the Western Kentucky University Farm in 1993. The experimental design was a split-split-plot with four replications. The three planting dates (2 June, 16 June, and 6 July) were main-plots, the three harvest dates were split-plots, and the five cultivars were split-split-plots. Cultivars and their maturity groupings and areas of adaption are McCall (00, Minnesota), A2506 (II, Iowa), FFR 561 (V, Kentucky), Perrin (VIII, South Carolina), and Laredo (undesignated maturity, forage cultivar). Each experimental unit was 45 m². Seeds were inoculated and broadcast by hand at a rate of 175 kg/ha and covered by disking. Average biomass production (oven-dry basis) decreased progressively (2,918, 2,450, and 2,088 kg/ha) for the later planting dates. For planting date 1 and 2, biomass yields increased for successive harvest dates. However, for planting date 3, biomass yields did not differ for harvests 2 and 3. When cultivars were compared at the early bloom stage, later maturing cultivars produced more biomass. Laredo was consistently the highest producer. There were significant interactions involving planting dates, harvest dates, and cultivar. Soybean stands and yields were reduced by inadequate coverage of the broadcast seed, by insufficient soil moisture at planting, and by competition from Johnson grass and pigweed. However, these results indicate that soybean is a good source of biomass production in summer.

Elkhorn Creek Monitoring Program: Influence of livestock on water quality. A. BURKS,* F. S. YOUNG III, and M. E. BYERS, Kentucky State University, CRS, Frankfort, KY 40601.

The presence of pathogenic bacteria in surface water is indicative of human and/or animal waste being present. In Kentucky, fecal coliforms are continually problematic for surface water. The Elkhorn Creek watershed was identified as being impaired by livestock-originated pollution and sediment. Animals given direct access to surface water bodies or the application of generally improper grazing practices may cause stream-bank degradation and erosion. The current study was originally conceived as an interdisciplinary demonstration and research project. It was designed to show growers useful technology for precluding or reducing animal access to the Elkhorn Creek, and then to provide the grazers with water through novel alternative sources through best management practices (BMPs). Pre- and post-BMP monitoring have been planned. Results thus far indicate pre-BMP BOD (5-day) values of less than 10 mg for both upstream and downstream, fecal coliforms ranging from 0 to 1,900 col/dl and generally less than 200 col/dl, average nitrate values near 5 ppm, and

dissolved oxygen values ranging from 4 to 14 mg/liter. The precaution of cattle in the future should increase water quality.

Environmental effects on Indian mock strawberry. BRIAN D. LACEFIELD* and ELMER GRAY, Department of Agriculture, Western Kentucky University, Bowling Green, KY 42101.

Indian mock strawberry, *Duchesnea indica*, is a common lawn weed favored by relaxed lawn management. Research was conducted to characterize the growth and reproduction of individual Indian mock strawberry plants at Bowling Green, Kentucky. The plants were transplanted from 10 different source locations in the experimental area (60 × 60 m) to six test plots in the same experimental area. Each test plot included 10 plants from each source, resulting in 600 plants for the experiment. A test plot consisted of a 10 × 10 Latin square design with plants spaced 30 cm apart within and between rows. Individual-plant data were collected on petiole length, leaflet length, and leaflet serrations at transplanting; and on plant height, number of leaves, number and length of stolons, number of rooting plantlets, and number of fruits at intervals during the growing seasons of 1992 and 1993. Plants from the different sources varied significantly for length of petioles and leaflets and for numbers of serrations. Plant proliferation, as measured through the various indexes, varied significantly among test plots. Maximum expressions of the characters for the different test plots included 100% of plants producing stolons, an average of 22.8 stolons/plant, an average of 491.4 cm stolon length/plant, 99% of plants producing plantlets, an average of 27.7 plantlets/plant, 98% of plants producing fruits, and an average 11.3 fruits/plant. Indian mock strawberry is a prolific weed through both asexual and sexual reproduction.

Growing container herbs in composted materials. TENA M. WRIGHT* and ELMER GRAY, Department of Agriculture, Western Kentucky University, Bowling Green, KY 42101.

An increased interest in substituting recycled nutrients for chemical additives in gardening was the basis for this research. Growth media consisted of four materials (local soil, brush compost, leaf compost, and N-Viro soil) and 1:1 mixtures of the materials, resulting in 10 growth-media treatments. Each treatment was repeated four times for a total of 40 containers. Each plastic barrel container (58 cm diameter, 38 cm depth) contained ca. 60 liters of mixture. No pesticides or fertilizers were used. Results were reported on days to emergence for borage, summer savory, and thyme; plant material weight for sweet basil; and number of leaves on parsley. For days to emergence, mixtures had no influence on thyme. The range was 7–19 days for borage and savory. Of the mixtures, 50% soil:50% brush had the longest delay in emergence for borage; 100% soil resulted in the longest delay for savory. Total weight of sweet basil (three plants per container) ranged from 240 to 10,145 g per container. Its highest average

weight, 7,180 g, occurred in the mixture 50% leaves:50% brush; the lowest, 1,573, occurred in 100% brush. The number of leaves produced per parsley plant ranged from 41 to 349 and averaged 119. The highest number of leaves was found in the 100% N-Viro soil, the lowest was in 50% leaves:50% N-Viro soil. When the results were combined for all herbs, no mixture was superior. However, the mixtures containing brush produced the least favorable results.

Influence of constructed wetlands on nutrient and pathogen removal. F. S. YOUNG III,* M. E. BYERS, and G. F. ANTONIOUS, Kentucky State University, CRS, Frankfort, KY 40601.

In Kentucky, NPS pollution from on-site sources affects environmental and human health. In response to this problem, in excess of 1,400 on-site constructed wetlands exist in Kentucky. However, few have been monitored. These systems generally consist of a lined trench filled with rock, planted with emergent macrophytes, and with water level maintained below the rock surface. The inlet of the system is fed septic-tank effluent. Macrophytes, their symbionts, and other benthic micro-organisms contact the flowage and metabolize nutrients and consume pathogens. Effectiveness of treatment can be determined through monitoring. Acceptable level of treatment is determined by regulators. We have monitored four systems for 2 yr. Some of these systems are mature and seem to have reached their biotic potentials. Fecal coliform removal efficiencies often approach 99% but usually far exceed 200 col/dl. System's efficiency regarding nutrient removal is variable but generally not effective. System's loads seem to generally exceed capacity. Increasing real residence time and adequate maintenance will likely increase efficiency.

Influence of incident light intensity and fertilization on growth and development of pawpaw [*Asimina triloba* (L.) Dunal] seedlings. LAKEASHA JONES,* DESMOND R. LAYNE, and MICHAEL G. KWANTES, Atwood Research Facility, Kentucky State University, Frankfort, KY 40601.

At KSU we are conducting research to overcome the horticultural limitations to developing pawpaw as a new commercial fruit crop. Currently, no scientifically based recommendations exist for pawpaw seedling propagation. Pawpaw enthusiasts recommend growing seedlings in shade and providing fertilizer once transplanted. The purpose of our study was to determine whether supplemental illumination and fertilization could result in more robust seedlings for field transplanting. Stratified pawpaw seeds were planted in a greenhouse and grown until seedlings reached the 2–3 leaf stage. Uniform plants were selected and assigned one of the four following treatments for 5 wk: (1) ambient light plus fertilizer (Amb/+Fert), (2) ambient light without fertilizer (Amb/–Fert), (3) shade (80%) plus fertilizer (Shade/+Fert), and (4) shade without fertilizer (Shade/–Fert). Seedling height and leaf number

were recorded 3×/wk. Following 5 wk of treatment, plants were destructively harvested to determine biomass accumulation, and leaf samples were collected and analyzed for chlorophyll. Within 15 d of treatment, Amb/+Fert seedlings were significantly taller and had more leaves than those in the other three treatments. Following 5 wk, the Amb/+Fert seedlings had 40% more biomass than the Amb/-Fert and more than double the biomass of seedlings from either shaded treatment. Unfertilized plants had leaves with significantly less chlorophyll than fertilized plants. Seedlings provided with additional light and fertilizer were significantly larger, had a more developed root system, and were more suitable for transplanting than seedlings grown without these additions.

Plant (wheat) and soil analyses following high rates of manure applications on corn. RAY E. JOHNSON, BRENT BASHAM,* BRYAN KESSLER, O. W. DOTSON III, DAVID STILES, and ALVIN BEDEL, Department of Agriculture, Western Kentucky University, Bowling Green, KY 42101.

The study was conducted on a Pembroke silt loam (finesilty, mixed, mesic, Typic Paleudalf) on the Western Kentucky University Farm. Dairy cow solid manure was added at rates to supply 0, 168, 336, 672, and 1,344 kg/ha of total N, referred to as the 0, 1×, 2×, 4×, and 8× rates, respectively. Liquid swine-manure was added at the 0, 1×, and 2× rates. One plot in each replication received only urea to supply 168 kg/ha of N. Applications were prior to planting of corn. Corn had been planted on these plots and then the whole plant was removed. Wheat was planted in the fall without any further manure application. Wheat dry-matter yields on the cow manure treatments varied from 2,164 kg/ha to 4,842 kg/ha with an increased response of the 2×, 4×, and 8× manure rates ($P < 0.05$) over the 0, F, and 1× treatments which were not different. Nitrogen removal via the wheat plant followed the same pattern with the 4× and 8× treatments removing over 60 kg N/ha ($P < 0.05$) compared to the 27–36 kg N/ha removed by the wheat on the other treatments. The rates of nutrient uptake and removal tended to follow the same pattern for P (5–15 kg P/ha), K (19–92 kg/ha), S (2.8–7.3), Mg (3.4–13), and Ca (4.8–11.9 kg/ha) with a fairly consistent difference ($P < 0.05$) with the two higher applications being greater than the 0, F, 1×, and 2× treatments. Wheat dry-matter yields on the 0, F, 1×, and 2× swine manure application were 2,640^a kg/ha, 2,614^a, 2,774^a and 4,203^b kg/ha, respectively, for the four treatments. With the swine manure study, the following nutrient removal was observed: N, 31–43 kb/ha; P, 6 to 8; K, 37 to 53; and sulphur, 3 to 4 kg/ha. In the cow manure study, soil micronutrient elements varied, with the following average levels observed: S, 13.6 ppm; B, 0.82 ppm; Cu, 2.56 ppm; Fe 28.5 ppm; Mn 166 ppm; and Zn, 5.6–6.1 ppm for 4× and 8× treatments ($P < 0.05$) down to 3.5–2.5 ppm for lower levels of manure application. It would appear that the summer corn crop removed a considerable amount of animal waste nutrients on the F, 1×, and 2×

treatments as increased forage production and nutrient removal were noted mainly on the 4× and 8× treatments.

Street tree inventory: putting the data to work. JAMES M. MARTIN, Department of Agriculture, Western Kentucky University, Bowling Green, KY 42101-3576.

Data have been collected and evaluated from the inventory of ca. 6,000 street trees in Bowling Green, Kentucky. Now the data must be used to effect change in the city's street-tree care and planting program. Six species constituted 50% of the total. Three of these six are inferior species, not suitable for this use. There are 309 trees in need of immediate removal; most of the remaining population requires attention and periodic care. Currently no ordinance governs selection and planting of street trees in Bowling Green. To put these data to work, three necessary steps are being implemented. This first is the political/governmental support from the city to be accomplished in part by direct appeal to the City Commission. The second is the formulation of policy, including a street-tree ordinance based on these data to be prepared by the City Tree Board. The third step is a public education to make the citizens aware of the problems and the potential.

Sustainable agronomic research projects at Western Kentucky University. ELMER GRAY,* ASLAM TAWHID, TENA M. WRIGHT, and BRIAN LACÉFIELD, Department of Agriculture, Western Kentucky University, Bowling Green, KY 42101.

The recent sustainable agriculture movement has encouraged renewed interest in old agricultural practices and has enhanced the attention being given to selected new developments. Agronomic research at Western Kentucky University has involved several aspects of sustainable agriculture. Pre-emergence mulching of garden beans with leaf compost increases the percentage of emergence and pod yield. Reinstatement of more intensive lawn clipping management aided in control of Indian mock strawberry and common blue violet, thereby reducing the need for herbicide application. Vegetable and herb production using containers of various compost media has been successfully demonstrated. Through the use of selected crops (wheat, rye, spring oats, soybeans, and sorghum), it is possible to have a supply of organic matter throughout the year. The use of companion crops as living mulches has received preliminary investigation. Although such crops have shown promise for controlling selected weeds, they have been competitive with the main crop.

Treating livestock waste waters with constructed wetlands. DAVID STILES, BRYAN KESSLER,* O. W. DOTSON III, BRENT BASHAM, RAY JOHNSON, and ALVIN BEDEL, Department of Agriculture, Western Kentucky University, Bowling Green, KY 42101.

A commercial producer of swine in south central Kentucky has a constructed wetland system to handle part of the waste produced from a 1,500-sow (12,000–15,000 animals at any one time) operation. The waste is routed to

a two-cell-in-series lagoon system and then from there to land application or in summer to a 4 ha wetland system with 9 cells. The first three wetland cells served as mixing ponds where the effluent from the lagoon (2nd) is diluted so that the $\text{NH}_3\text{-N}$ level is ca. 100–150 ppm. The cells are predominantly cattail (*Typha latifolia*), bulrush (*Scirpus validus*), panicum (*Panicum* sp.), and reed (*Phragmites australis*). The following data, collected in summer 1994, represent the average of the first three cells and the % removal through wetland cell 9 in the closed system: ammonia-N 59, mg/liter, 93.7%; total P, 16.8 mg/liter, 72%; total suspended solids, 504 mg/liter, 21%; total dissolved solids, 715 mg/liter, 23%; fecal coliforms, 33,000 CFu, 100 ml, 85%; and BOP 92, mg/liter, 56%. Dissolved oxygen increased almost three-fold through the cells. Constructed wetlands appear to be an effective and reliable means for treatment of swine lagoon effluent. Further studies of management and monitoring are indicated to follow the start-up year of functioning of these wetlands.

Using composts for growing vegetables in containers. ASLAM TAWHID* and ELMER GRAY, Department of Agriculture, Western Kentucky University, Bowling Green, KY 42101.

Container gardening provides the urbanite the opportunity to grow vegetables in minimum space. The use of composted waste materials adds to the attractiveness of container gardening. Our study was conducted to evaluate common waste products as soil amendments for growing food crops in containers. The materials included soil (S), leaf mulch (L), wood mulch (W), N-viro soil (N), and chicken manure (C). Each of the 40 plastic barrel sections (58 cm diam, 38 cm depth) contained ca. 60 liters of one of the following mixtures (percent by volume): S(100); S(50) L(50); S(50) W(50); S(50) L(25) W(25); S(50) L(25) C(25); S(50) W(25) C(25); S(50) L(25) N(25); and S(50) W(25) N(25). The mixtures not including chicken manure (C) or N-viro soil (N) were supplemented with 56.0 N, 24.5 P, and 46.5 K kg/ha. The 100% soil was used as the standard for evaluating the mixtures. Fifteen different food crops were grown in these mixtures in 1993. Seedling emergence, seed or fruit yields, plant height, and biomass productions indicated that some mixtures were equal or superior to soil only. For example, bush snap beans had higher emergence and pod yield on SLN than on S. There were many examples of interactions between mixtures and crops and mixtures and cultivars within crops. These results indicated that gardening with waste compost has strong potential for urban gardeners.

Vegetable culture on erodible land. D. HILBORN,* M. BYERS, and G. ANTONIOUS, Kentucky State University, Frankfort, KY 40601.

Limited-resource farmers are looking for profitable alternative crops. Broccoli is a possible alternative. Past studies, with other vegetables on erodible land (10% slope), have shown that fescue strips are effective in reducing water and soil runoff. Our study involved three soil

treatments for comparison: (1) no mulch or conventional till (CT), (2) fescue every other row (FEOR) of crop, and (3) fescue every row (FER) of crop with respect to effect on broccoli yield. Spring and fall broccoli were transplanted (17,857 plants per hectare) and eventually harvested; results for the year were combined. Though head mass and diameter on any given harvest date were not significantly different, overall head mass and diameter were significantly higher in the conventional till plot. Head mass (g): CT = 158.7, FEOR = 140.6, and FER = 144.0 with head diameter (mm): CT = 98.2, FEOR = 91.3, and FER = 90.2. With respect to total annual harvest, the conventional till plot produced the highest annual yield of 2,700 kg/ha compared with 2,497 kg/ha for the fescue-every-row plot and 2,434 kg/ha for the fescue-every-other-row plot. Therefore, the conventional till plot yielded a better overall harvest in kg/ha, but not significantly enough to offset the benefits from fescue, which helped to retard loss of water and soil. This study bears repeating at a higher planting density.

BIOCHEMISTRY AND PHARMACOLOGY

Apoptosis in differentiated and undifferentiated murine F9 teratocarcinoma cells. E. DEATON,* S. VOGEL-POHL, B. BALDWIN, J. CARTER, and R. SNYDER, Wood Hudson Cancer Research Laboratory, Newport, KY 41071.

Apoptosis, a genetically driven process wherein cells commit suicide in response to internal and external stimuli, is fundamental in embryological development and in maintenance of organ homeostasis in multicellular organisms from nematodes to man. Aberrant modulation of the apoptotic process is associated with tumor progression, neurodegenerative disease, and other human conditions. As part of our ongoing program aimed at understanding the molecular basis for, and possible modulation of, this process as it relates to cancer, we have investigated spontaneous and induced apoptosis in pluripotent murine F9 teratocarcinoma cells that had or had not been stimulated to differentiate into endodermal cells by 72 hr exposure to 1 μM retinoic acid (RA). Apoptosis was induced by the S-phase specific agents, ara-C and etoposide and the non S-phase agent, hydrogen peroxide. Background levels of apoptosis were taken to represent spontaneous rates. Apoptotic DNA fragmentation was measured by DNA/Dye binding assay and agarose gel electrophoresis. Morphometric analysis of stained cells was also used both to verify the apoptotic nature of the event and to analyze death on a per cell basis. We have determined that F9 cells can be induced to undergo apoptosis by all three agents and that this cannot be antagonized by agents such as cycloheximide or zinc, which are classical inhibitors of the process in most cell types. RA-differentiated F9 cells display a level of spontaneous apoptosis similar to that seen in undifferentiated cells. However, differentiated cells exhibit clearly different responses to agent-induced apoptosis than do undifferentiated cells. These findings

were discussed with regard to their implications in cancer therapy.

BOTANY AND MICROBIOLOGY

Cryptococcus neoformans serotype groups found in clinical and environmental isolates. JOHN M. CLAUSSON* and LARRY P. ELLIOTT, Department of Biology, Western Kentucky University, Bowling Green, KY 42101-3576.

Cryptococcus neoformans is the most life-threatening fungal pathogen that infects patients with the acquired immunodeficiency syndrome (AIDS). Locally *C. neoformans* var. *neoformans* has been associated with pigeon feces in those months with an average temperature of 68.8°F and above. Clinical and environmental isolates of *C. neoformans* obtained from area hospitals and the environmental sampling, respectively, have been grouped into their variety status utilizing Canavanine Glycine Bromothymol Blue agar. Antisera against *C. neoformans* serotypes A, B, C, and D was produced in rabbits and utilized in serotypic characterization. Serological studies utilizing monoclonal antibody against each of the four serotypes have been employed. Comparing serotypes found in the environment to those isolated from patients in area hospitals will provide information leading to the origin of infection and ultimately inform immuno-compromised individuals on situations in which exposure to *C. neoformans* is likely.

Dogwoods: molecules, morphology, and relationships. ZACK E. MURRELL, Department of Biology, Western Kentucky University, Bowling Green, KY 42101.

The dogwoods (*Cornus*) have been variously divided or segregated over the past 3 centuries, usually on the basis of misunderstood inflorescence structure or a disregard for natural groups. A morphological analysis of the genus, using macro-morphology, micro-morphology, anatomy, and chemistry, supports the recognition of all nine subgenera as a single genus. Molecular sequence data derived from the internal transcribed spacer (ITS) region of 18-26S nuclear ribosomal DNA are difficult to align at the subgeneric level, indicating that splits between the lineages are ancient. The blue- and white-fruited dogwoods comprise two thirds of the genus but have been poorly represented in analyses of relationships. Although these dogwoods have generally been considered shrubs, there are four architectural types represented; molecular data from the ITS region support recognition of at least three lineages within the group. The blue- and white-fruited dogwoods have traditionally been segregated into taxa with opposite or alternate leaves, but the chromosome number and endocarp structure of the South American *C. peruviana* blur this distinction. Comparison of morphological and molecular data supports recognition of all of the blue- and white-fruited dogwoods, except *C. oblonga*, as subgenus *Thelycrania*.

CHEMISTRY

College chemistry—Can we predict which students will be successful? L. C. BYRD and D. R. HARTMAN,* Department of Chemistry, Western Kentucky University, Bowling Green, KY 42101.

Attempts to identify underprepared students encouraged investigations into correlation of success in college chemistry with high school: grade-point average, class rank or chemistry grade, ACT, SAT-math, aptitude test scores and/or customized tests to measure chemistry/math skills. Hovey and Krohn, leaders in the investigations, developed the Toledo Chemistry Placement Examination (TCPE). Several universities require TCPE raw scores above 35–50 for entrance into college chemistry. Correlations of success in college chemistry to various parameters found ranges from 0.21 to 0.51 with multivariate analysis giving the best correlations (0.59–0.63). The Department of Chemistry at Western Kentucky University (WKU) determined correlation coefficients for each ACT score, high school grade-point average or TCPE raw scores to success in college chemistry (grade of A, B, or C). The best correlation (0.55) was with the TCPE total. The next best correlation (0.49) was with the TCPE, part I (math section). To help students be more successful and to give them a better experience in chemistry at WKU, the TCPE is given prior to registration; the scores are used to place students in college chemistry + math, preparatory chemistry + math, or no chemistry—math only. In fall 1994, 514 students took the TCPE; 215 were enrolled in college chemistry, 85 in preparatory chemistry, and 214 took no chemistry. In fall 1993, 338 students enrolled in college chemistry with 175 remaining after mid-term, and 75 enrolled in preparatory chemistry.

GEOLOGY

A conceptual model for the occurrence of hydrochemical facies in an unmined area of the Eastern Kentucky Coal Field. DAVID R. WUNSCH, Kentucky Geological Survey, University of Kentucky, Lexington, KY 40506-0107.

A comprehensive hydrogeologic and hydrogeochemical study was conducted at an unmined site in the Eastern Kentucky Coal Field. Sixteen piezometers were installed to approximate a vertical grid in a ridge characteristic of the geologic and hydrologic conditions of the region. Piezometer placement was based on data collected from geologic core description, geophysical logs, water-injection packer tests, and down-hole camera investigations. Water levels were measured for a period of 1 yr on approximately 10-day intervals. Water samples were collected monthly to evaluate temporal variation in water chemistry. A one-time sampling for tritium analysis was performed to aid in determination of recharge zones. Piezometers monitoring shallow, fractured bedrock and coal beds showed the greatest temporal water-level fluctuation and hydrochemical variation. These effects decreased with depth below the surface. Coal beds of the Hazard series

apparently act to dewater the upper portion of the ridge by laterally transmitting water, which discharges as springs or seeps. Interpretation of the tritium data indicates that most of the ground-water recharge enters the ridge along the hillslope where fractures, along with a reduction in the degree of slope, allow for the greatest infiltration of ground water. Temporal variation in ground-water chemistry was minimal at the site except for a few specific cases. Ground water derived from coal seams contained the lowest pH and was predominantly a Ca-Mg-HCO₃ water type. Water derived from fractured zones varied between Ca-HCO₃ and Mg-SO₄ water types. Ground water from the ridge interior was a Na-HCO₃ type, which contained a high pH and characteristically high fluoride. Barium was found in anomalously high concentrations (>1.0 mg/L) in piezometers near the discharge area (valley bottom) where Na-Cl water types were encountered, although ground water with elevated barium concentrations was also shown to exist in other locations. Sulfate reduction and cation exchange appear to control the occurrence of barium. Reaction path modeling of the geochemical evolution of ground water at the site showed excellent agreement with observed trends. A set of plausible water-rock reactions is given for the occurrence of each hydrochemical facies zones within the ridge.

Computer-aided instruction in geology using photographs transferred to CD-ROM. DAVID R. DOCKSTADER, Division of Natural Sciences and Mathematics, Jefferson Community College, Louisville, KY 40202-2005.

Establishment of a standard for Photo CDs has allowed inexpensive commercial transfer of photographic negatives and slides to digital format on CDs. The standard Kodak Photo CD contains a photograph in five levels of resolution. The 384 × 256 files are adequate for computer-aided instruction. At this resolution, the file for each photograph occupies 232 kilobytes of memory and may be readily transferred to a disk drive for use on computers that do not have a CD ROM drive attached. Use of digitized images from CD ROM offers several advantages over use of original photographs. Among these advantages are preservation of original photographs, rapid random and repeat access, inexpensive duplication without loss of quality, and ease of incorporation into computer-driven instructional media. Using funding from a UKCCS Mini-Grant, I have transferred 500 slides to Photo CDs. They include photographs of the Falls of the Ohio, Clifty Falls State Park, Red River Gorge, Niagara Falls, Glacier National Park, Arches National Park, the effects of Hurricane Hugo, and many other geologically interesting areas. Copies of these photographs are available for the cost of duplicating and shipping the disks. Some of the photographs have been incorporated into illustrated computer instruction and simulated field trips for student use with HyperCard on the Macintosh computer. Copies of these HyperCard materials may also be obtained from me.

Effect of agricultural chemicals on ground-water quality

in lacustrine deposits of the Western Kentucky Coal Field. PHILIP G. CONRAD* and JAMES S. DINGER, Kentucky Geological Survey, University of Kentucky, Lexington, KY 40506-0107; LYLE V. A. SENDLEIN, Kentucky Water Resources Research Institute, Lexington, KY 40506-0107.

Ground-water quality at two farm sites in western Kentucky was monitored before and after application of agricultural chemicals. Both sites lie in wide, flat valley floors of the Western Kentucky Coal Field. Surface sediments at each site consist of Pleistocene lacustrine deposits and some alluvial deposits and are underlain by Pennsylvanian sandstone, shale, coal, and limestone of the southern Illinois Basin. Shallow ground-water flow above homogeneous clays was mostly lateral toward local and regional-scale drainage ditches at both sites. Nitrate-nitrogen concentrations at the Hopkins County site were periodically over 20 mg/liter in ground water less than 10 ft in depth. Conversely, nitrate-nitrogen concentrations at the Daviess County site were all below 1.5 mg/liter from the water table to over 60 ft in depth. The large difference is apparently caused by slower denitrification at the Hopkins County site due to lower concentrations of organic carbon below the water table than in Daviess County. Organic carbon and other nutrients are necessary for aggressive denitrification by bacteria. Near the Hopkins County wells, the addition of nitrogen fertilizer raises the concentration of nitrate-nitrogen in shallow ground water. The vertical movement of ground water and pesticides was impeded by argillaceous lacustrine deposits. Some downward flow of very shallow ground water and pesticides was noted where there is little lacustrine clay above bedrock and where the clay is perforated by macropores.

Effect of fractures on availability of ground water and occurrence of pesticides and nitrate in the epikarst at a site in the Inner Blue Grass Region, Kentucky. DWAYNE M. KEAGY* and JAMES S. DINGER, Kentucky Geological Survey, University of Kentucky, Lexington, KY 40506-0107; STEVEN K. HAMPSON, UK Federal Facilities Oversight Unit, Kentucky Water Resources Research Institute, University of Kentucky, Lexington, KY 40506-0107; LYLE V. A. SENDLEIN, Kentucky Water Resources Research Institute, University of Kentucky, Lexington, KY 40506-0107.

A study site in the Inner Blue Grass Region is characterized by gently rolling topography, sparse outcrops, and scattered, broad, shallow sinkholes. Bedrock consists of interbedded limestones, siltstones, and shales. Soils are generally clay-rich loams. Agricultural practices include growing corn, soybeans, and tobacco and pasturing beef cattle. Shallow ground water in the epikarstic zone was the major focus of this study. Two sites were chosen: a cattle pasture where no agricultural chemicals were applied and a corn field where atrazine, metolachlor, and nitrogen fertilizer were applied. The sites were then subdivided into fracture zones and interfracture zones. Wells located in fracture zones produced more shallow ground

water than those located in interfracture zones. Triazines and metolachlor were found only at the crop site, whereas nitrate, found in higher concentrations at the crop site, was detected at both sites. In fracture zones, triazines and nitrate concentrations showed little variation with depth and were lower than in shallow interfracture zone ground water. Triazines and metolachlor concentrations in both fracture and interfracture zones peaked ca. 2 wk after application and then fell to below 1 $\mu\text{g/L}$ less than 3 months later. Pesticides and electrical conductivity measurements indicate that shallow epikarstic ground water from interfracture zones discharges into solution conduits, fracture networks, and eventually from springs. Drilling wells in fracture zones in similar geologic settings should produce the best water quality and provide better ground-water yields.

Geology of roof falls in underground coal mines: comparison between eastern and western Kentucky. DAVID K. HYLBERT, Department of Physical Sciences, Morehead State University, Morehead, KY 40351.

The geology of rocks associated with coal beds is known to have a direct bearing on the stability of roof rocks in underground coal mines. Kentucky is unique in that the eastern Coal Field is part of the Appalachian Basin and the western Coal Field is part of the Illinois Basin. The kind and geometry of rocks in eastern Kentucky are associated with sediment deposition in a basin with relatively unstable and more rapidly subsiding platforms. The rate of sediment influx was also relatively greater. The resulting coal beds and associated rocks are typically more non-marine in their character. Roof falls in these rocks are most often attributed to sandstone channel-fills, non-tectonic faults, and slips caused by differential compaction, lateral facies changes, and geologic structures such as kettlebottoms and concretions. Coal beds and associated rocks deposited in the Illinois Basin typically reflect deposition on more stable platforms with relatively less sediment influx. The rocks show more of a marine influence. In addition to roof falls caused by sandstone channels and non-tectonic faults caused by differential compaction, an unstable roof may result from clay dikes or clay-dike faults. Also, tectonic faults in well-defined systems are much more numerous in western Kentucky.

Nonpoint-source pollutant loads in a karst aquifer underlying an intensive-use agricultural region, Kentucky. JAMES C. CURRENS, Kentucky Geological Survey, University of Kentucky, Lexington, KY 40506-0107.

The Pleasant Grove Spring Basin, southern Logan County, Kentucky (10,082 acres, 40,823 hc), was studied because it is largely free of non-agricultural pollution sources. Water samples were collected at six locations over a 14-month period. Pleasant Grove Spring was instrumented to monitor five water-quality parameters, stage, and discharge velocity, and to automatically collect samples. Stage recorders were installed at four upstream sites. Nitrate is widespread and persistent but concentrations do

not exceed drinking water standards (MCLs). Atrazine was detected year round; other pesticides occur. Triazines (including atrazine) exceeded MCLs during spring floods. Bacteria counts always exceeded MCLs. Suspended sediment was exceptionally high when storms occurred before cover crops were established.

Soil, sand, and gravel hydrostratigraphic units associated with glacial lake deposits, carbonates, and uranium-bearing black shales of Jefferson County area, Kentucky. GRAHAM HUNT, Department of Geography & Geosciences, University of Louisville, Louisville, KY 40292.

Because the terrain in and around Jefferson County, Kentucky, is estimated to have a high geologic radon potential of greater than 4 picocuries per liter, a geologic radon study may be warranted under three geologic investigations: (1) source or generation of radon, (2) migration or transmission of radon, and (3) concentration or trap of radon. During the past decade a random sampling of indoor radon was carried out by mainly government workers in scattered localities of Kentucky, including the study area. The counties were assigned to zones with predicted average radon indoor screening levels; Jefferson County is given a high rating of radon potential. These zone ratings were determined by assessing the following data: indoor radon measurements, geology, aerial radioactivity, soil parameters, and foundation types. Based on a preliminary geologic study it may be concluded that there are many important geologic and climate-controlled features that may have contributed to the source, migration, and concentration of radon in the study area. Investigated rock types that may produce anomalous radon levels are (1) black marine shales of mainly Devonian age, (2) karst carbonates, (3) soils, sands, and gravels of Recent to Quaternary age, and (4) glacial lake deposits. There may be localized concentrations of radioactive minerals in or near fractures of high permeability to allow migration and a trapping mechanism of radon.

The Kentucky Groundwater Data Repository. BART DAVIDSON, Kentucky Geological Survey, University of Kentucky, Lexington, KY 40506-0107.

The Kentucky Groundwater Data Repository, initiated in 1990 by the Kentucky Geological Survey under mandate from the Kentucky State Legislature (KRS 151:035), was created to archive and disseminate Kentucky groundwater data collected by various state agencies and other organizations. Data for 443 water wells drilled in McCracken County are currently available. In addition, data are also available for drainage areas of tributaries to the Ohio River. The complete data base contains information for over 27,000 water wells, 450 springs, 300 dye traces, and 17,000 water-quality analyses. Types of computerized data in the repository include general water-well information such as location, usage, total depth, and static water level; well-construction information; water-quality data such as major and minor ionic constituents, physical properties, isotopic analyses, trace-organic analyses, and bac-

terial analyses; spring data; discharge measurements; and groundwater dye-trace data. The repository also contains many reports on groundwater topics and maps showing various types of groundwater information. Products and services of the repository include well searches within a user-specified radius of a site location; overlay maps showing well locations (7.5-min quadrangle); hard-copy printouts of groundwater data; groundwater data downloaded to magnetic media (diskette, 9-track tape, 8 mm tape); and assistance with public service requests concerning groundwater.

GEOGRAPHY

Deaths and injuries from lightning in Kentucky: analysis by location. MARY M. SNOW, Department of Geography and Geology, Western Kentucky University, Bowling Green, KY 42101.

Analysis of locations of occurrences of deaths and injuries caused by lightning in Kentucky was conducted to determine whether a disproportionate number of events was farm related. Relevant data from the National Oceanic and Atmospheric Administration (NOAA) extend from 1959 to 1992 with the strikes organized by counties. NOAA's publication *Storm Data* provides details concerning specific locations. Each death and injury during the 33 yr was reviewed and further categorized to investigate possible patterns of locations in Kentucky. NOAA's classification of lightning-caused deaths and injuries in the U.S. does not include a category for farm-related events. However, my analysis reveals that 23% of the injuries and 41% of the deaths from lightning in Kentucky took place on farms.

Solstices and daily normal maximum and minimum temperature lags. RICHARD K. SNOW, Department of Geography and Geology, Western Kentucky University, Bowling Green, KY 42101.

Differences between dates of solstices and daily normal maximum and minimum temperatures were plotted as isochrones from 1885 to 1925 and from 1948 to 1992, comparing seasonal temperature lags as well as the relationship to elevation and latitude. Our study focused on Kentucky, including stations from 35 to 41°N and from 82 to 94°W. Scattergrams and a Pearson's product-moment correlation coefficient analysis indicated that there was no association between seasonal lag and elevation in Kentucky. However, a regression analysis determined that there was a positive relationship between latitude and lag in winter and an inverse relationship in summer. The mean number of days from the solstices to the daily normal minimum and maximum temperatures has decreased with daily normals slightly slower in winter and slightly higher in summer.

HEALTH SCIENCES

Effect of ag-chemicals and toxic compounds on human erythrocyte delta-aminolevulinic acid dehydratase. H. A.

DOWLA,* M. PANEMANGALORE, M. E. BYERS, and R. MUKHERJEE, Water Quality and Toxicology Program, Atwood Research Facility, Kentucky State University, Frankfort, KY 40601.

Delta-aminolevulinic acid dehydratase (ALAD), a sulfhydryl enzyme found in human erythrocytes (RBC), catalyzes condensation of two moles of aminolevulinic acid (ALA) to form one mole of porphobilinogen. This enzyme is sensitive to inhibition by cadmium (Cd) and some agriculture chemicals. We investigated the effect of chemicals used in tobacco fields on changes in human RBC ALAD activity in vitro. ALAD was assayed in RBC using ALA as substrate in the presence of dithiothreitol. The data show that % inhibition obtained varied for Cd, acetate, maleic hydrazide (MH) and nicotine. Dose response curves were developed for each chemical; maximum inhibitions obtained for these chemicals were (1) 57% for 2.3 μM of Cd, (2) 52% for 180 mM of acetate, (3) 90% for 6.7 mM of MH-30, and (4) 87.6% for 162.24 mM of nicotine. These data clearly illustrate that RBC ALAD is sensitive to and is inhibited to various degrees by these chemicals. ALAD thus has the potential to be a biomarker to evaluate exposure and extent of toxicity in occupationally exposed tobacco-farm workers. (USDA CBG #93-38814-8733).

MATHEMATICS

Parts from Dirichlet. JAMES B. BARKSDALE JR., Department of Mathematics, Western Kentucky University, Bowling Green, KY 42101.

This expository presentation highlights an observation by L. M. Graves [Amer. Math. Monthly 38:277-278] regarding a logical link between two well-known and fundamentally significant results that are central items in elementary calculus courses. The presenter of this paper suggested that such observation could serve as an element of instructional enrichment for beginning calculus courses. In the note by Graves, it was observed that the procedure of integration by parts is a special instance of the Dirichlet Formula for interchanging the order of integration in an iterated integral; more precisely, the formula of Dirichlet is given by

$$\int_a^b \int_a^x h(x, t) dt dx = \int_a^b \int_t^b h(x, t) dx dt. \quad (\text{DF})$$

In this paper, the implication of "parts" from "Dirichlet" was noted and detailed. The presenter then offered the following direct corollary which, regarding the observation by Graves, replaced the implication with a biconditional. More exactly stated: Let $h(x, t) = g(x)f'(t)$, and let g and f' each be Riemann integrable on $[a, b]$, and define $G(x) = G(b) + \int_b^x g(t) dt$; it then follows that

$$\int_a^b f(x)g(x) dx = f(x)G(x) \Big|_a^b - \int_a^b f'(x)G(x) dx. \quad (\text{DF})$$

MOLECULAR AND CELL BIOLOGY

Effect of ozone exposure on antioxidant vitamins in fruit tissue. K. M. KUTE,* C. ZHOU, and M. M. BARTH, Nutrition and Food Sciences Department, Biological Sciences, and Graduate Center for Toxicology, University of Kentucky, Lexington, KY 40506-00541.

Once strawberries are harvested, the length of time which they can be stored is limited by rapid fungal decay and increased rate of senescence. Ozone (O₃), a strong gaseous oxidant, has been demonstrated to reduce fungal growth and abate ethylene produced by the plant, thereby slowing the rate of senescence when used in conjunction with coldroom storage. Despite these benefits, ozone has the potential to react with important antioxidant vitamins in the fruit tissue, thus reducing the antioxidant vitamin content in the human diet. The purpose of this study was to assess the effects of ozone exposure on the antioxidant vitamin content in strawberries through evaluation and quantification of vitamin A and vitamin C activity using traditional spectrophotometric and fluorometric assays (respectively) and High Performance Liquid Chromatography (HPLC) techniques. Vitamin A activity in strawberries was measured using HPLC. Levels of activity were so low in the control group of this cultivar that no meaningful comparison could be made; focus was thus placed on measurement of vitamin C activity. Microfluorometric analysis of total ascorbic acid activity (TAA) revealed initial concentrations of 7.47 mg/g dry weight. At 24 hr, 0.3 ppm berries showed greater vitamin C content than the control, but by 1 wk no significant differences were observed. Although overall quality appeared greater in ozone treated samples, ozone storage resulted in no significant decrease in antioxidant vitamin content over 1 wk storage time.

Effects of teratocyte secretory products on protein synthesis by fat body in various larval stages of *Heliothis virescens*. LISA MCGRAW* and DOUGLAS L. DAHLMAN, Department of Entomology, University of Kentucky, Lexington, KY 40508.

The tobacco budworm, *Heliothis virescens*, is a major tobacco pest notoriously resistant to pesticides. Parasitism of *H. virescens* by *Microplitis croceipes*, a braconid wasp, interferes with development of the budworm and eventually kills it. As the parasitoid egg hatches within the host, the serosal membrane dissociates into individual cells called teratocytes. Teratocyte secretory products (TSP) have been shown to inhibit protein synthesis by fat body in *H. virescens*. The effects of TSP on various larval stages of *H. virescens* (D1, D2, BD, and CF1) are determined by using an in vitro fat-body assay. About 5 mg of fat body is taken from various staged larvae and labeled with ³⁵S Methionine. Four larval equivalents of TSP are then added and the samples are incubated for 4 hr. After incubation, the samples are washed in various amounts of trichloroacetic acid (TCA) to precipitate the protein. The TCA is removed through ethanol and ether washes. Finally, a SDS/sodium hydroxide wash is used to digest the proteins. The amount of protein secreted by the fat body

is measured through the use of scintillation spectroscopy. In assays run without the addition of TSP, early stages (D1 and D2) were shown to synthesize more proteins than later stages (BD and CF1). When TSP was added to the assay, it inhibited protein synthesis more in D2 stage larvae than in the other, later stages.

Fatty acid effects on vitamin E levels in cultured endothelial cells. BRAD MIDDENDORF* and BERNHARD HENNING, Department of Nutrition and Food Science, University of Kentucky, Lexington, KY 40506-0054.

One of the first steps in the atherosclerotic disease process is the formation of lesions within the vascular lining. Lesion formation may be initiated by the disruption of the vascular endothelium. Different nutrients can play a key role during this process, in both disruption and protection. Fatty acids, especially unsaturated fatty acids, can induce oxidative stress upon endothelial cells. Linoleic acid [18:2(n-6)] has been shown to be cytotoxic to endothelial cells. Oxidative stress can be lessened by the quenching effects of antioxidants such as vitamin E, the most significant lipid soluble antioxidant. Thus, a decrease or alteration in vitamin E levels should be expected in endothelial cells exposed to linoleic acid as opposed to cells left untreated. To test this hypothesis, an experimental group of cultured porcine pulmonary endothelial cells was exposed to media containing 90 μmol/liter linoleic acid for 24 hr. The control group was treated with the same media minus the linoleic acid. Results calculated from HPLC analysis showed no significant difference in overall vitamin E levels between the two groups. There were, however, significant differences in vitamin E profiles. Vitamin E (tocopherol) exists as several isomers, differing in degrees of antioxidant properties. The γ-tocopherol levels in the treated groups were significantly higher than those in the control group [0.667 to 0.360 nM/mg protein]. These results indicate that exposure of endothelial cells to fatty acids can alter the overall cellular vitamin E profile. This may have important implications in understanding the mechanisms by which dietary fat may influence the atherosclerotic disease process.

How do lymphocytes recognize antigens in immune privileged sites? WAI KHAN LOH* and JEROLD WOODWARD, Department of Microbiology and Immunology, University of Kentucky, Lexington, KY 40506.

The objective of this summer project was to determine how lymphocytes recognize antigens in immune privileged sites. Spleen cells from normal and transgenic mice were used. In a "naive" mouse that has not encountered an antigen, the frequency of T-cell precursor specific for the antigen is about 1/1,000. If the mouse is exposed to the antigen, the frequency of T-cell precursor will increase. T-cell recognition of the antigen on a target cell initiates differentiation of T-cell precursor to CTL (cytotoxic T-lymphocyte), which then kills the target cell. CTL-precursor Frequency Assay was carried out to measure CTL-

precursor frequencies of target cells from normal BALB/C mice and transgenic B6.D¹ mice that expressed class I MHC antigen, H-2D^d. If the T-cell precursors in the transgenic mice were exposed to the alloantigen, they would demonstrate a higher CTL-precursor frequency than that of the normal mice. The responding cells in the assay were taken from the normal C57BL6 mice, which did not carry the antigen. The experimental data were insufficient to answer the proposed question. However, they did indicate that the CTL-precursor Frequency Assay was a feasible method to be used to help achieve the objective of this project in the future.

Influence of antioxidant and temperature treatments on lipid peroxidation and senescence in broccoli florets. S. M. TAM,* H. ZHUANG, and M. M. BARTH, Biological Sciences and Nutrition and Food Science Departments, University of Kentucky, Lexington, KY 40506-0054.

As plant products senesce during the postharvest period, membrane lipids break down resulting in release of polyunsaturated fatty acids (PUFA). Peroxidation of PUFA by lipoxygenase (LOX) results in formation of hydroperoxides, which contribute to increased cell membrane deterioration, hence promoting loss of protein and chlorophyll. Studies have shown that application of antioxidants along with reduced temperature storage delay green color loss in broccoli florets. The purpose of our study was to assess the effect of an antioxidant (commercial product) and temperature storage treatments (2°C, 10°C, 20°C) on lipid peroxidation and senescence in broccoli tissue by measuring thiobarbituric acid reacting substances (TBARS), PUFA, C₆-aldehyde formation and total chlorophyll loss. There was an increase in TBA values and reduction in chlorophyll content and PUFA as storage temperature increased over time. By 96 hr, TBA values increased 50% in 20°C vs. 10°C samples and by 144 hr, TBA values increased 30% in 20°C vs. 10°C samples. A 3-fold loss in chlorophyll content as observed in 20°C samples by 96 hr and 5 fold loss by 144 hr compared to 2°C samples. Relative PUFA decreased by 30% in 20°C vs. 10°C samples by 144 hr, but no significant difference was observed in 10°C vs. 2°C samples. Application of the commercial antioxidant preparation inhibited C₆-aldehyde formation at the early stage of postharvest storage. Hexanal content in antioxidant-treated samples decreased dramatically by 24 hr at 2°C compared to control, whereas hexanal levels in antioxidant-treated broccoli decreased by 50% compared to control by 72 hr. By 144 hr, hexanal levels were similar among all treatments. These results indicate a direct relationship between lipid peroxidation and senescence.

Influence of methyl jasmonate on lipid peroxidation and deterioration in broccoli. D. S. GUEORGUIEVA,* H. ZHUANG, and M. M. BARTH, Biological Sciences Department and Nutrition and Food Sciences Department, University of Kentucky, Lexington, KY 40506-00541.

Methyl jasmonate (MJ) is a fatty-acid compound, nat-

urally occurring and widely distributed in plants. Research studies have shown that exposure of plant leaves to MJ results in increased lipoxygenase (LOX) gene expression, lipid peroxidation (LP) and hydroperoxides formation. Hydroperoxides can cause loss of cell membrane integrity, proteins, and chlorophyll. Thus LP is thought to be responsible for senescence of plant tissue. The aim of our project was to elucidate the mechanism for induction of senescence in broccoli following MJ exposure. The experimental procedure involved measuring LOX protein levels and thiobarbituric acid-reactive substances (TBARS) to indicate the level of LP and measuring chlorophyll to assess senescence. LOX protein levels were characterized by Western blot. Our results showed that broccoli LOX protein increased during storage. The MJ-treated samples showed higher LOX levels than the controls of the same storage time in all experiments. TBARS significantly increased during storage in both controls and MJ-treated broccoli. At 96 hr and especially at 48 hr of storage, the MJ samples demonstrated much greater formation of TBARS than the controls, while at 72 hr certain variation was observed as the MJ samples had slightly lower TBARS levels than controls. Chlorophyll a and chlorophyll b (Cb) substantially decreased during storage, particularly Cb, which decreased by 25% from 0 hr to 96 hr in the MJ-treated samples. Total chlorophyll exhibited the same trend, progressively decreasing with time. MJ-treated broccoli had significantly less chlorophyll than controls. These results suggest that MJ enhances LP and will contribute to further description of the senescence mechanism in broccoli.

Interactions of the tobacco vein mottling virus' cylindrical inclusion protein with other virus proteins. BETH SMITH* and ARTHUR HUNT, Agronomy Department, N122 Agricultural Science Center North, University of Kentucky, Lexington, KY 40506.

The single-stranded RNA potyvirus tobacco vein mottling virus contains six classified coding regions: the Helper Component, CI, NIa, NIb, Coat Protein, and P1. The CI gene expresses a protein approximately 70 kDa which has a putative function in viral replication. To better understand the viral protein interactions of the tobacco vein mottling virus the two hybrid system has been employed, which utilizes a unique property of the Gal4 protein. The Gal4 protein has two separable domains: the activation domain and the binding domain. The plasmid pMA424 was genetically engineered to contain the activation domain; the plasmid pGAD2F was engineered to contain the binding domain of the Gal4 protein. The gene of interest was cloned, creating an in-frame fusion protein with each of the separable domains. The CI was cloned into both plasmids, while clones containing the other genes of interest were obtained from the labs of Dr. Hunt and Dr. Pirone. Combinations of both plasmids were transformed into the yeast strain GGY1:171, which has incorporated into its genome the gall-lacZ gene. Beta-galactosidase was then assayed using the chromogenic substrate ortho-nitro-

phenyl beta-D galactoside at OD 421. From this assay, the activity of the gall-lacZ gene was determined. Results from this project will help to better understand the viral protein interactions of the tobacco vein mottling virus.

Intracellular niacin status and mechanisms of carcinogenesis. CHRISTOPHER D. WATT* and ELAINE L. JACOBSON, Department of Clinical Sciences, University of Kentucky, Lexington, KY 40536.

In the 1960s, researchers discovered that nicotinamide adenine dinucleotide (NAD), the major metabolite of the vitamin niacin, is utilized as a substrate in ADP-ribose transfer reactions. One particular type, the poly(ADP-ribose) transfer reaction, is involved in the repair of damaged DNA. Subsequent research has shown that a subnormal intracellular NAD concentration can limit the effectiveness of this DNA repair mechanism. This knowledge, plus the fact that DNA damage contributes significantly to cancer development, has encouraged researchers to re-examine the relationship between intracellular niacin status, mechanisms of carcinogenesis, and cancer incidence. The investigation of this relationship in humans has been assisted by the recent development of a method to assay intracellular niacin status. This technique involves isolation of oxidized forms of the pyridine nucleotides, NAD and NADP, from primarily erythrocytes. The ratio of [NAD] to [NADP] in erythrocytes is defined as niacin number, a specific measure of intracellular niacin status in humans. The unique metabolism of niacin precursors in humans relative to potential animal models dictates the use of humans in this investigation of niacin and carcinogenesis. Assessing the association of intracellular niacin status and cancer incidence was the overall objective of the studies reported here. The development of techniques to conduct this investigation and their application to the ongoing study of diet and cancer in Malmö, Sweden, will be reported. Understanding the contributions of niacin in limiting carcinogenic cellular events may lead to dietary interventions that could impact cancer prevention.

Investigation of repressor dependent segregation of plasmid F in *Escherichia coli*. MICHAEL A. ZGODA* and DONALD P. BIEK, Department of Microbiology and Immunology, University of Kentucky Medical Center, Lexington, KY 40513.

I am working with a plasmid that may give some insight into the mechanism by which chromosomes segregate during cell division in prokaryotes. pZC178 contains the partition region from plasmid F and is maintained at a very low copy number, 1-2 per chromosome. The partition region is believed to be an autoregulated operon that expresses two gene products (sopA and sopB) and contains a region (sopC) of 12 imperfect 43 base pair repeats that have been suggested to be a functional analog of the centromere of eukaryotic chromosomes. I found that proper partitioning of this plasmid is dependent on lactose repressor binding to a lactose operator site located close

to the sopC region. The interesting phenotype observed with this plasmid is that, when the lactose repressor is induced with IPTG, the plasmid becomes actively unstable and is lost at a greater rate than a sop mutant plasmid. My research has shown that some type of anchoring of the plasmid with another cellular component, via sopC, is probably allowing for proper DNA topology. This is essential for faithful plasmid maintenance, but the topological requirements can also be accomplished by transcription into sopC. The data suggest that, when the sopC region is not able to maintain a specific topology, an aggregation of plasmids occurs, accounting for the active instability observed with lactose repressor is induced.

Mapping the location of somatostatin-like immunoreactive cells in tadpole tectum. ANGIE BAKER* and ELIZABETH A. DEBSKI, Biology Department, University of Kentucky, Lexington, KY 40506.

Previous work in this laboratory has shown that somatostatin-like immunoreactive (Som-ir) tectal cells are distributed non-uniformly in the adult optic tectum: Som-ir cells are predominantly located in the caudal third of the tectum and virtually absent from the rostral third. We wanted to determine whether this non-uniform distribution of Som-ir cells was also found in the developing optic tectum of tadpoles. A stage XVIII *Rana pipiens* tadpole was anesthetized and then perfused through the heart with saline solution followed by 4% paraformaldehyde. The brain was removed, fixed overnight, and then sunk in a 30% sucrose solution. The tissue was cut sagittally at 16 μ m on a cryostat. The anti-somatostatin serum used was obtained from Chemicon and diluted eighty fold. Staining was visualized using a fluorescent secondary antibody. Sections of the optic tectum were divided into regions (caudal, middle and rostral) using a grid in the ocular of the microscope. The distribution of Som-ir cells was recorded according to the tectal region and layer in which the cells fell. Som-ir cells were found in all three regions of the tadpole optic tectum. However, most Som-ir cells (49.9%) were located in the caudal third of the tectum with the rest evenly divided between the rostral (26.8%) and middle (23.4%) third. Overall, the lateral, more mature tectal regions did not have any more Som-ir cells than the less mature medial regions. The tectum is a layered structure composed of alternating cellular and plexiform layers. Som-ir cells were distributed fairly evenly between the three cellular layers of the tectum; layer II had 24.0%; layer IV, 26.3%; and layer VI, 19.3% of the Som-ir cells. Many of the remaining Som-ir cells (16.4%) were located in the superficial tectal layers. While adult tectum contained an average of 231 Som-ir cells, 647 cells were found in the tadpole tectum. The number and distribution of Som-ir cells in the tadpole optic tectum suggest that somatostatin may be transiently expressed during development. Its function may be to help in the construction and assembly of the neuronal arrays that subserves visual processing.

Modulation of TNF cytotoxicity by NAD. MANDALA V. WILSON,* DAVID M. HIESTAND, BOYD E. HALEY, and ELAINE L. JACOBSON, Department of Clinical Sciences, University of Kentucky, Lexington, KY 40536.

Tumor Necrosis Factor (TNF) is a cytokine originally named for its ability to selectively kill tumors in several mouse cell lines. Since its discovery and early testing TNF has been documented to play a role in many cellular activities, with effects ranging from protective to toxic. Recently several cytokines, including TNF, have been shown to selectively bind NAD. Further, preliminary experiments showed that addition of NAD enhanced survival of tumor cells in the presence of TNF. To test the hypothesis that NAD can modulate TNF cytotoxicity an alternative assay was needed to measure survival of cells with NAD in the culture medium. Development of these assays will be reported. In addition, experiments designed to study the mechanism by which NAD, TNF, and tumor cells interact to modulate the cytotoxic response will be presented.

Molecular dissection of the mouse α -fetoprotein repressor region. CASSANDRA K. BACKER,* AMY W. ELLIS, and BRETT SPEAR, Departments of Microbiology & Immunology and Pathology, University of Kentucky College of Medicine, Lexington, KY 40536-0084.

The mouse α -fetoprotein gene (AFP) provides a system to study both positive and negative transcriptional regulation. AFP is controlled in a tissue-specific manner, being expressed at high levels in the visceral endoderm of the yolk sac and fetal liver and at low levels in the fetal gut. A dramatic postnatal decline in AFP transcription is seen and within 3 wk after birth the gene is fully repressed. This repression is due to a 550 base pair (bp) region upstream of the AFP structural gene. Studies in transgenic mice have shown that removing this 550 bp region results in continued transgene expression in the adult liver and gut. Our objective was to further define the region important for AFP shut-off. We hypothesized that negative regulation is controlled by a discrete regulatory motif rather than the entire 550 bp region. Standard recombinant DNA methods and the Polymerase Chain Reaction (PCR) were used to obtain three 200 bp subfragments that encompass the repressor region. These subfragments will be sequenced and subcloned into a eukaryotic expression vector currently being used in our lab. Our long-term goal is to test the repressor activity of these subfragments in transgenic mice. This will enable us to identify the subfragment containing the repressor region and further our understanding of AFP repression. These results will help elucidate mechanisms of mammalian gene regulation.

Novel synthesis of anti-cancer drugs. LEVI HARPER* and GERALD ROSENTHAL, School of Biological Sciences, University of Kentucky, Lexington, KY 40506.

Plants must have some way to defend themselves since they are not motile. Many use nonprotein amino acids,

amino acids not normally incorporated into proteins, as a means of chemical defense. Our laboratory focuses on the nonprotein amino acid L-canavanine. L-canavanine is the structural analog of L-arginine characterized by the replacement of the terminal methylene group with an oxygen atom. The terminal oxygen atom, which is more electronegative than carbon, causes canavanine to be more acidic than arginine. Canavanine incorporation into proteins in place of arginine causes changes in the protein's R-group interactions thus affecting structure and function. X-ray crystallographic study of canavanine and arginine shows that canavanine is shorter than arginine because oxygen takes up less space than the methylene group. When an extra methylene group is added to canavanine, canavanine should look more like arginine. This greater similarity should facilitate canavanine's incorporation into proteins at a greater rate. By adding an OH group to a terminal nitrogen or replacing O atom with a sulfur atom, we hope to make canavanine more acidic. Research in rats and nude mice has shown that canavanine causes significant reduction in tumor size in tumor-bearing rodents. Although canavanine has anti-tumor properties, the toxicity associated with canavanine caused the treated animals to lose body weight. To remedy this situation, many derivatives of canavanine have been proposed that have greater anti-tumor properties and decreased toxicity.

Organelle-specific role of the delta-9 fatty acid desaturase. MICHAEL S. SCOWBY,* SERGEI AVDIUSHKO, and DAVID F. HILDEBRAND, Department of Agronomy, University of Kentucky, Lexington, KY 40546.

Genetically engineering plants to produce lower levels of saturated and higher levels of monounsaturated fatty acids is significant for improving these plants' nutritional values. Such studies are also useful for analyzing the complex lipid biosynthesis pathways in plants. Transgenic plants containing a mammalian delta-9 desaturase gene demonstrated significant conversions of saturated to monounsaturated fatty acids in various tissues. Western blot analyses of chloroplasts, mitochondria, nuclei, and microsomes isolated from leaf tissue of the transgenic plants indicate that the foreign desaturase is associated with the endoplasmic reticulum. Most plant tissues, such as leaves, contain high levels of the 16-carbon saturated fatty acid palmitic acid (>10%), but very low levels of the delta-9 monounsaturated fatty acid palmitoleic acid (<0.1%). The introduced desaturase results in significant accumulation of palmitoleic acid, which provides a qualitative marker for the impact of the desaturase on specific organelle lipids and different lipid classes. Palmitoleic acid was found to be incorporated into most major lipid classes found in the isolated organelles, with the greatest amounts occurring in the monogalactosyldiacyl glycerol classes. Further organelle and lipid class separations were done to determine the positional specificity of fatty acids when they are incorporated into different lipid molecules.

Regulation of expression of protease nexin-1 in neural

tissue: relationship to Alzheimer's Disease. PAUL JETT* and STEPHEN ZIMMER, L. P. Markey Cancer Center, Department of Microbiology & Immunology, University of Kentucky Medical Center, Lexington, KY 40536.

The molecule protease nexin-1 (PN-1), is a 43,000-dalton protein belonging to a family of serine protease inhibitors known as serpins. PN-1 binds to thrombin and other proteases in glial tissue which may serve as a protective effect in neurodegenerative diseases like Alzheimer's. It has recently been found that the inflammatory mediators Interleukin-1 (IL-1) and tumor necrosis factor-alpha (TNF- α) are simulators of PN-1. Our laboratory has found that the genomic promoter for PN-1 is stimulated in cells containing ras or eukaryotic initiating factor 4E (eIF-4E). This suggests a potential signaling pathway involving ras, translational control through eIF-4E, and effector functions involving IL-1 and TNF- α . These points converge at phospholipase 2 (PLA2), which is the rate limiting enzyme in precursors of prostaglandins. Clinical studies using inhibitors of this pathway have shown a significant delay in the progression of Alzheimer's disease. These data suggest that PN-1 may be expressed in response to a process involving neurodegeneration in the brain. Our research is focused on testing the validity of this model through the use of techniques such as transient transfections with a PN-1 promoter driven chloramphenicol acetyltransferase (CAT) reporter assay in tissue culture and immunohistochemical analysis of Alzheimer tissues.

Roles of polyunsaturated fatty acids in neuronal death: arachidonic acid metabolism and lipid peroxidation. MARION STEINER and IRENE HONG,* Department of Microbiology and Immunology, University of Kentucky, Lexington, KY 40536.

Alzheimer's disease is a degenerative neurological disorder marked by dementia. The roles of polyunsaturated fatty acids in neuronal death were studied as a component in understanding neuronal degeneration in the disease. Classes of polyunsaturated fatty acids that have potent biological effects were examined, namely arachidonic acid metabolites and lipid peroxides. To study arachidonic acid metabolism, PC12 cells (a rat pheochromocytoma derived cell line) were radiolabeled with C-14 arachidonic acid, treated with test reagents such as indomethacin, and then analyzed for metabolites (e.g., prostaglandins) using thin-layer chromatography and paper chromatography. To detect products of lipid peroxidation, TBARS (thiobarbituric acid reactive substances) assays were performed. Such techniques were utilized in preparation for the determination of the effects of β amyloid peptide on lipid peroxidation and arachidonic acid metabolism. Analysis of PC12 cells under standard growth conditions (without β amyloid treatment) indicated (1) low levels of production of cyclooxygenase and lipoxygenase pathway products of arachidonic acid metabolism and (2) nondetectable lipid peroxidation.

Studies of non-protein amino acid disruption in pro-

teins. MICHAEL J. BASS* and G. A. ROSENTHAL, School of Biological Sciences, University of Kentucky, Lexington, KY 40506.

Plants must protect themselves from invasion by microorganisms. Many higher plants defend themselves from predation by synthesizing non-protein amino acids. L-canavanine, the 2-amino-4(guanidinoxy)butyric acid structural analog of L-arginine, is an example of a non-protein amino acid with demonstrated cytotoxic and insecticidal properties. Canavanine-containing proteins can exhibit an altered three-dimensional conformation. The female migratory locust, *Locusta migratoria-migratorioides* (Orthoptera), synthesizes the storage protein vitellogenin for use by developing oocytes. Injection of canavanine into the locust during vitellogenin synthesis results in a replacement of 18 of the 200 arginyl residues with canavanine. This replacement has been shown to alter dramatically the native three-dimensional conformation of this protein. Canavanine incorporation into an enzyme can also hinder function. Larvae of the tobacco hornworm, *Manduca sexta*, synthesize a protective antibacterial protein, lysozyme (EC 3.2.1.17), upon injection of cell wall fragments of *Micrococcus lutea*. If canavanine is injected into the larvae at the time of bacterial challenge, the arginine analog is incorporated into lysozyme. The newly formed canavanyl lysozyme exhibits a 50% loss in catalytic activity relative to the native protein. Data from the insectan proteins isolated from *L. migratoria* and *M. sexta* provide evidence that canavanine's incorporation into protein can alter protein conformation and catalytic activity. However, there needs to be a greater understanding of how canavanine and other non-protein amino acid anti-metabolites function to alter protein conformation.

Studies of variation of a surface protein (Szp) of *Streptococcus equi* subsp. *zooepidemicus*. MATT BLAIR,* JOHN WALKER, and JOHN TIMONEY, Gluck Equine Research Center, University of Kentucky, Lexington, KY 40506.

The DNA sequence of the *szp* genes from a series of 14 *S. zooepidemicus* strains characterized by Moore and Bryans (MB) has been previously determined; though highly homologous, three regions of variability were identified. The 5' end of the gene exhibits one of two motifs that were arbitrarily named class I and II. A second region of hypervariability lies 500 bases into the gene. The third region, located near the 3' end of the gene, translates into a variable number of PEPK repeats in the Szp polypeptides of the 14 MB strains. To determine whether this structure of the *szp* gene was present in more recent isolates we examined the genetic basis of Szp variation in four strains of *Z. zooepidemicus*—#54, 54-15, 54-16, and 54-19—isolated simultaneously from the tonsil tissue of a single horse. A Western blot of the surface protein revealed three different migration patterns in the four strains, #54 and #54-15 being identical. Using the Polymerase Chain Reaction (PCR) with specific amplification primers, the entire *szp* gene and the internal hypervari-

able region of the gene were amplified from each of the four strains. The DNA fragments were then cloned into a plasmid vector and maintained in *Escherichia coli*. The DNA sequence of the 5' and 3' ends of the *szp* gene as well as of the internal hypervariable region was determined. The results showed that the 5' region of #54, 54-15, and 54-19 was like class II and that #54-16 had a class I motif. The hypervariable regions of #54, 54-16, and 54-19 were different from each other but were identical to sequences of the *szp* gene found in the MB strains of *S. zooeptidemicus*. The 3' end of the four *szp* genes encoded for the same number of PEPK repeats. The DNA sequence of the *szp* genes of #54 and #54-15 were identical, which is consistent with the protein profiles. The similarity of DNA sequence of the *szp* genes of the four #54 strains with those of the Moore and Bryan's isolates indicated that there is significant conservation of structure in the Szp family of proteins. We also showed that a single horse can carry multiple strains of *S. zooeptidemicus* at the same time. This study may aid in future work of classifying *S. zooeptidemicus* strains.

Synthesis of insulin-like growth factor binding protein 2 mRNA in human T-Cells. SANDHYA VENUGOPAL,* LORRI ANN MORFORD, and THOMAS ROSZMAN, Department of Immunology and Microbiology, University of Kentucky, Lexington, KY 40502.

Patients with malignant gliomas exhibit a broad immunosuppression. One explanation for this is that a circulating glioma-derived suppressor factor (GSF) interacts with T-cells and renders them unresponsive to antigenic stimulation. Others in the laboratory have developed a monoclonal antibody that initially appeared to reverse GSF-induced suppression in human T-cells in vitro. Based on western analysis of glioma culture supernatants and protein sequencing, the protein recognized by this monoclonal antibody has been identified as insulin-like growth factor binding protein 2 (IGFBP-2). However, it is unclear what relationship (if any) exists between IGFBP-2 and GSF. If IGFBP-2 is inhibitory to T-cell proliferation, one would expect that IGFBP-2 would not be produced by T-cells. The monoclonal antibody to IGFBP-2 reacts with the surface of purified T-cells and can stimulate peripheral blood lymphocytes (PBL) to proliferate in culture. Recently it has been suggested that human PBL are capable of producing a number of IGFBPs. To date it is unclear which IGFBPs are actually produced by T-cells, why they are produced, and what role these proteins have in normal lymphocyte function. To examine the synthesis of IGFBPs in T-cells, we utilized Northern blot analysis to determine whether mRNA to IGFBP-2 was actively synthesized in stimulated T-cells. Our data indicate that IGFBP-2 mRNA is synthesized by T-cells. The results demonstrate that GSF and IGFBP-2 are not the same.

PHYSICS

Comparison of the ultraviolet spectral morphology and MK classifications of B supergiants in the Small Magel-

lanic Cloud. ANTHONY L. BORCHERS* and RAYMOND C. McNEIL, Department of Physics and Geology, Northern Kentucky University, Highland Heights, KY 41099.

Study of ultraviolet spectra of a sample of B supergiants in the Small Magellanic Cloud is being carried out in collaboration with Richard P. Fahey and George Sonneborn of NASA Goddard Space Flight Center. As a first step in this study, ultraviolet spectral morphology of program stars is being examined for consistency with published MK spectral classifications. The analysis includes a tabulation of ultraviolet spectral features and their expected variation with spectral type and luminosity class, preparation of spectral sequences of standard stars, and comparison of program spectra with the standard sequences. Standard stars selected for the comparison include B dwarfs and giants as well as supergiants. All spectra are either new or archival spectra obtained with the International Ultraviolet Explorer and processed using the VAX Cluster of the Laboratory for Astronomy and Solar Physics at Goddard. The support of NASA and Northern Kentucky University through the Joint Ventures (JOVE) program is gratefully acknowledged.

PHYSIOLOGY AND BIOPHYSICS

Effects of nutrients and retention time on the primary production of Kentucky Lake. MARY CHILD,* H. R. KOBRAEI, and B. ANDERSON, Department of Astronomy and Physics, Murray State University, Murray, KY 42071.

Data taken from the Kentucky Lake reservoir system for 1991, 1992, and 1993 were used to calculate experimental primary production. The data were also analyzed for possible physical, chemical, and biological factors that could contribute to development of a model for prediction of primary production. Possible correlations were investigated between nitrate/nitrite (NO_3/NO_2), silicon dioxide (SiO_2), soluble reactive phosphorus (SRP), light, temperature, carbon assimilation (CAS), and biomass (chlorophyll a + phaeophytin a). CAS and biomass have a linear relationship to each other and can therefore be used to obtain an experimental value for primary productivity. All available nutrients appear to have the same characteristic behavior and an inverse relationship with CAS and biomass. We also investigated the possible relation between retention time and primary production.

Experimental approaches to investigate neuromast regeneration of the axolotl lateral line. SPECK, R. R. and K. J. BALAK,* Department of Biology, Western Kentucky University, Bowling Green, KY 42101.

The lateral line system of amphibians consists of discrete organs called neuromasts distributed in lines within the epidermis. The posterior lateral line runs along the dorsal midline of the tail. If the tail tip of an axolotl is amputated it will regenerate. The posterior lateral line will also regenerate about the same number of neuromasts as were removed with the tail tip amputation. Vital-dye la-

bellings experiments have shown that the regenerated neuromasts are derived exclusively from cells of the last neuromast left on the tail after tail tip amputation. Single-cell labeling experiments have implicated the supporting cells as the source of cells for the regenerated neuromasts. Partial tail tip and neuromast amputations were performed and the effect of these manipulations on neuromast regeneration examined. Three types of experiments were performed: (1) removal of the neuromasts without tail amputation, (2) amputation of the dorsal tail tip and neuromasts, but not the spinal cord, and (3) amputation of the ventral tail tip and spinal cord, but not the neuromasts.

Glycoconjugate expression in normal and regenerating lateral line sensory epithelia of the axolotl salamander, *Ambystoma mexicanum*. MILLER, RICHMOND J. and K. J. BALAK,* Department of Biology, Western Kentucky University, Bowling Green, KY 42101.

The sensory epithelia of the lateral line system of amphibians and the inner ear of birds and mammals consists of two cell types: sensory hair cells and supporting cells. If the hair cells of mammals are destroyed, they are not replaced, resulting in permanent hearing impairment. This is not the case in amphibians and birds. If the hair cells of the cochlea are destroyed in young chicks they are replaced by regeneration. Experiments with the amphibian lateral line have shown that the regenerated hair cells are derived from progeny of supporting cell divisions. The hair cells of lateral line neuromasts specifically accumulate a fluorescent dye abbreviated DASPEI. When exposed to blue light the DASPEI labeled hair cells are killed by phototoxicity. These hair-cell-depleted neuromasts will regenerate new hair cells in a process similar to that of a chick cochlea. Lectins are plant proteins that bind to monosaccharides and short polysaccharide chains. Biotinylated lectins were used to compare glycoconjugates expressed in normal intact neuromasts and neuromasts depleted of hair cells.

SCIENCE EDUCATION

A model classroom curriculum integrating core concepts of biomedical research/societal values to achieve KERA learner outcomes in secondary science classrooms. STEPHANIE WYATT, Calloway County High School, Murray, KY 42071, GREGORY J. POPKEN, University of North Carolina, Chapel Hill, NC 27599, and CLOYD J. BUMGARDNER, Calloway County High School, Murray, KY 42071.

A basic tenet of school reform mandated by the Kentucky Education Reform Act of 1990 is integration of knowledge. This goal necessitates the development of district, building, and classroom-level curriculum. In this model classroom curriculum, high school students completed a written questionnaire designed to elicit their opinions of how biomedical research utilizing animal studies impacted their everyday lives. The students then received instruction on the importance of this research to themselves and society based on opinions of researchers

who are active in the field. The information was presented in audio-visual, lecture, and cooperative learning formats. The depth and breadth of instruction were based on prevailing societal concerns and a teacher assessment of student opinions noted on the questionnaire. Student completion of the questionnaire prior to instruction provided the instructor with a baseline of information from which to proceed when teaching. Completion of the same questionnaire after instruction indicated a broadening of student perceptions of the role biomedical research plays in our society's development.

Are Kentucky students sleeping enough? JOHN G. SHIBER, Division of Biological Sciences & Related Technologies, Prestonsburg Community College, Prestonsburg, KY 41653.

Over 3,000 western Kentucky students (K-College) were surveyed about their sleep habits. The majority of K-5 graders said they sleep 9-10 hr per night. Most 6-8 graders get 8-9 hr, but nearly a quarter of them get fewer than 8. Ninth to 12 graders generally get 7 or fewer hr (21% get 6 or less), and, although 99% of college students (18 yr->40) said adults should get 7-8 hr of sleep per night, only 40% of them get that amount. Except for the 4% who get more, the majority get 6 or fewer hr of sleep per night. Not surprisingly, 45-55% of all students surveyed have difficulty sleeping at night. Also, the older the student, the less rested he/she seems to feel upon waking, i.e., 29% of K-5, 39% of 6-8, 55% of 9-12, and 59% of college students do not feel well rested in the morning. Naps are a good idea, according to 71% of college students, but only 17% take them regularly. 41% of 9-12 graders take naps on days off, and 24% of them sleep in school, as opposed to only 8% of K-8 graders who do. After-school activities occupy 62% of all K-12 students surveyed. K-5 graders are out 1-2 evenings per week, usually getting home by 9 PM, and often before 7 PM; 6-8 graders are involved 4-5 nights and also get home before 9 PM; and 9-12 graders get home from their 3-5 nights of activities by 10 PM, except for 19% who get home later. To top it off, 66% of K-12 students watch TV until bedtime! About 14% watch only 1 hr or less, but 43% watch 2-3 hr and 44% watch 4 or more hr each night. An alarming statistic now exists that drowsy drivers cause more fatalities per vehicle accident than drunken drivers. In this survey, 56% of college students get sleepy while driving and most of them keep on driving anyway, doing various things to remain awake! Last, according to this survey, 18% of K-5, 24% of 6-8, and 45% of 9-12 graders do not eat any breakfast. This is very disturbing. How effective can educational reform in Kentucky (or anywhere else) be when children not only go to school poorly rested, but also without having eaten? Surely some of our reform efforts should be focused on instilling the importance and rewards of good eating/sleeping habits, the most basic of biological disciplines, in students and parents alike. After all, "A healthy mind is in healthy body!"

Enhancing student perceptions of science and achieving Kentucky Education Reform Act learner outcomes through a creative science classroom curriculum. STEPHANIE L. WYATT,* Calloway County High School, and CLOYD J. BUMGARDNER,* Calloway County Middle School, Murray, KY 42071.

Student opinions of science may often be determined by the extent to which they perceive science impacting their daily lives. Many factors, including but not limited to overwhelming curricular and social concerns, time constraints, and disciplinary requirements, may limit the extent to which students retain factual knowledge presented in a lecture format. A model classroom curriculum employing real life experiences as an instructional foundation was developed to address student perceptions of biotechnology and microbiology. Student opinions of their own level of awareness of biotechnological issues were determined by using a short questionnaire before and after instruction. In addition to enhancing student opinions and sensitivity to these areas, the curriculum was designed to address several of the learner goals specified by the Kentucky Education Reform Act of 1990. Success under the goal-centered focus of the Reform Act mandates a teaching methodology dissimilar to the more traditional lecture-oriented approaches previously used in many classrooms.

ZOOLOGY AND ENTOMOLOGY

Equine protozoal myeloencephalitis: a review. S. STAMPER* and D. E. GRANSTROM, Department of

Veterinary Science, Gluck Equine Research Center, University of Kentucky, Lexington, KY 40546-0099.

Equine protozoal myeloencephalitis (EPM) was first described in 1964 as segmental myelitis. In the mid-1970s, organisms were found to be associated with the lesions. S. W. Davis, a post-doc with J. P. Dubey, was the first to culture *Sarcocystis neurona*; Dubey later named the organism *S. neurona*. The disease occurs only in the western hemisphere, with cases reported from North, South, and Central America. All breeds of horses are affected. The life cycle of *S. neurona* is not known; however, it should be similar to other members of the genus *Sarcocystis*. The genus typically has a predator/prey or scavenger/carrion life cycle. Horses appear to be aberrant, dead-end hosts with no sarcocysts being found. Therefore, transmission to the definitive host is prevented. Many species have been suggested as the definitive host. However, only skunks have shown the presence of antibodies to *S. neurona*-specific proteins, but they, like horses, may have been exposed inadvertently. The response of horses to the parasite probably depends on infective dose, immune competency, and stress. Relapses can occur after treatment. Such relapses represent a latent stage or a small focus of infection or reinfection. Immunodiagnosis is possible with Western Blot, using cerebrospinal fluid (CSF) or serum. Negative serum is more conclusive than positive. Positive serum demonstrates exposure, while a positive CSF indicates the parasite has crossed the blood/brain barrier and has stimulated a local immune response.

DISTINGUISHED SCIENTIST AND OUTSTANDING TEACHER AWARDS, 1994

OUTSTANDING SECONDARY SCIENCE TEACHER

Beverly Lynn White—Instructor, North Laurel High School, London, Kentucky 40741
Native of Charleston, West Virginia
Education: Marshall University—B.A. Biology and Physical Science; Marshall University—M.S. Biology; Post graduate courses at UK, EKU, Marshall, Union College

Lynn White has taught at Paul Blazer H.S. in Ashland, KY; at Sue Bennett College from 1972–1989; at South Laurel H.S. 1989–1992; and at North Laurel H.S. since 1992.

She teaches Anatomy & Physiology; AP Biology; Biology; Introduction to Physics and Chemistry; and Ecology.

She has attended numerous workshops and conferences for teachers: Anatomy & Physiology for H.S. teachers—UK 1990; Rainforest Ecology workshop—Lima, Peru, 1992; Wildlife Management workshop—Cumberland Falls St. Pk, UK; DNA Science workshop—UK, 1994.

She has attended the Kentucky Science Teacher Association Conferences and the National Science Teacher Association Conferences.

She maintains many memberships in scientific organizations and has been a member of KAS since 1977.

Lynn has received the Outstanding Appalachian Teaching Award from the Appalachian Scholars Program in 1991. She was recognized for excellence in teaching by the Governor's Scholars Program. She was voted the Teacher of the Year at North Laurel H.S. for 1991–1992.

Lynn has been involved with the state's Water Watch Program at Levi Jackson State Park with her Ecology class. She received a grant to develop an outdoor classroom at North Laurel H.S. and this has become an interdisciplinary school-wide project.

She serves as a volunteer guide for wildflower tours of the Daniel Boone National Forest giving tours to campers and visitors. She gives talks about her experiences esp. Rainforest Ecology to schools and civic organizations.

As a teacher she motivates students and allows them to develop in the curriculum area of science. She excites children. She cares for students and stays after school to give extra help. Students stated that “she is never boring, and gives practical application to what she teaches.” She stresses “hands-on.”

Lynn White has a genuine interest in her students. She is the kind of teacher who is “often discussed and admired at our family dinner table.”

OUTSTANDING COLLEGE/UNIVERSITY SCIENCE TEACHER

Dr. Barbara A. Ramey—Professor of Biological Sciences, Eastern Kentucky University
Native of Freeport, Illinois
Education: Cornell College, Mt. Vernon, IA—B.A. Biology (1968); Miami University, Oxford, OH—M.S. Zoology (1971); University of Kentucky—Ph.D. Biology (1982)

Barbara Ramey is a versatile teacher who is prepared, highly competent, and very effective. She expends many hours counseling students. She is at home in a freshman Zoology course, an upper level Cellular Biology class, or in a graduate embryology or histology course.

She is highly involved with the Premedical Sciences program.

She is active in a number of professional organizations, including the Kentucky Academy of Science, where she has served as membership chairperson.

Barbara is her department's Coordinator of Graduate Programs.

She maintains an active research agenda involving the effects of acid pH on embryonic and juvenile stages in fish; teratogenic effects of aquatic pollutants on embryos of freshwater fish and amphibians; and biomonitoring a constructed wetland site. Her research has received funding from the U.S. Dept. of Interior and the U.S. Geological Survey via the KY Water Resources Research Institute.

She has presented many papers at professional meetings. She also has continued to

publish her research in regional and national journals.

Additionally, Barbara has been a member of numerous committees at Eastern Kentucky University.

In 1989, she received the ECU National Alumni Association Award for Teaching Excellence. That is a student nominated and student recommended award.

As a teacher, Barbara has the natural ability to make difficult concepts easier for her students to grasp. She loves teaching and students realize her dedication and compassion for them. She is sincerely concerned about her students and schedules review sessions for her classes. She even comes in on weekends to help her classes. She is an excellent counselor for students who have expressed an interest in science. She involves students in her research. She brings innovation to her classes. Her exams are challenging and require a sound understanding and critical analysis of the material. She is friendly and approachable. She communicates with her classes.

Dr. Ramey has chosen to make teaching her top priority.

DISTINGUISHED SCIENTIST AWARD

Dr. Donald T. Frazier—Professor of Biomedical Engineering, Department of Physiology and Biophysics, University of Kentucky College of Medicine

Native of Floyd County, Kentucky

Education: B.S. University of Kentucky (1958); M.S. University of Kentucky (1960); Ph.D. University of Kentucky (1964)

After receiving his Ph.D., Donald Frazier taught at the University of New Mexico School of Medicine where he remained until 1969 when he returned to the UK College of Medicine. He has remained there in the Department of Physiology since, first as Associate Professor and since 1974 as Professor.

From 1980–1992, he served as Chair of the Department of Physiology. He has been recognized as a creative scientist and outstanding teacher.

Professor Frazier has distinguished himself in three areas: research and scholarly activity, education, and out-reach educational programs.

Don ranks among the university's most dis-

tinguished scientists, whose research has been acknowledged nationally and internationally. He has maintained a well-funded research laboratory by the National Institutes of Health that focuses on the neural regulation of breathing, for the last 20 years.

In addition to Don's highly effective classroom teaching of medical, doctoral, and undergraduate students, he has utilized his research laboratory to train postdoctoral fellows in the intricacies of neurobiological research. He has also made contributions on the national level by being the Director of the Summer Fellowship Program at the renowned Marine Biological Laboratories in Woods Hole, Mass.

Most importantly, Don has provided leadership within the university to establish science out-reach programs for under-represented students including high school and undergraduate minority students, to bring them to the campus to provide an opportunity to obtain first hand experience in biomedical, health-related and scientific research. It is important to recognize that he has competed successfully on the national level to obtain extramural funding from the National Institutes of Health for this minority educational program.

He is a person who remembers his Appalachian roots and has worked hard to get the school systems involved in higher education.

He has maintained a long list of memberships in professional societies at the state, regional, and national level. He has directed doctoral research and his list of publications is extensive.

He has accepted his responsibilities very seriously. He was named to the Commission on Human Resources and Social Change of the National Association of State Universities and Land Grant Colleges.

INDUSTRIAL SCIENTIST OF THE YEAR 1994

Dr. Fred L. Tungate—Manager of Research and Development, United Catalysts, Inc., Louisville, KY

Native of Indiana

Education: Indiana University—A.B. Chemistry (1972); University of Tennessee—Ph.D. Chemistry, specializing in Inorganic Chemistry (1978)

Dr. Tungate has been an industrial re-

searcher since receiving his doctorate. Since 1977, he has worked exclusively in the area of catalyst and adsorbent research, development, and commercialization.

Dr. Tungate holds six patents and has authored a number of publications.

He is exceptionally effective in interacting with technical personnel of client companies. His contributions in research and in working with clients has contributed significantly to the major expansion of existing product lines and the construction of new plant facilities.

His work has broad applications including the edible oil hydrogenation to produce salad oils, frying oils, confectionery products. He invented the G-95 catalyst which has been the industry standard for 14 years. Additionally, he has worked with the highly advanced technology of zeolites.

Fred is a member of a number of professional organizations.

He has been a part-time lecturer at the University of Louisville, teaching a graduate course in Zeolite Chemistry. He has been a lecturer at the Mexican Institution of Petroleum International.

He is an excellent example of an Industrial Scientist at work, keeping abreast of the latest technology in his chosen field of catalysis, while also participating in programs to promote scientific interests in society and his community.

He is now the Director-at-Large to The Catalysis Society and has served in numerous capacities of the Tri-State Catalyst Club.

Dr. Tungate's career could be a model of what an industrial scientist should be.

NEWS

The annual meeting of the Kentucky Academy of science for 1995 will be a joint affair with the Tennessee Academy of Science, 16, 17, 18 November. All members should make an effort to attend this forum.

The 1996 meeting will be at Kentucky State University in Frankfort, and the 1997 meeting will be at Morehead State University.

Instructions for Contributors

Original papers based on research in any field of science will be considered for publication in the Transactions. Also, as the official publication of the Academy, news and announcements of interest to the membership will be included as received.

Manuscripts may be submitted at any time to the Editor. Each manuscript will be reviewed by one or more persons prior to its acceptance for publication, and once accepted, an attempt will be made to publish papers in the order of acceptance. Manuscripts should be typed double spaced throughout on good quality white paper 8½ × 11 inches. NOTE: For format of feature articles and notes see Volume 43(3-4) 1982. The original and one copy should be sent to the Editor and the author should retain a copy for use in correcting proof. Metric and Celsius units shall be used for all measurements. The basic pattern of presentation will be consistent for all manuscripts. The Style Manual of the Council of Biological Editors (CBE Style Manual), the Handbook for Authors of the American Institute of Physics, Webster's Third New International Dictionary, and a Manual of Style (Chicago University Press) are most useful guides in matters of style, form, and spelling. Only those words intended to be italicized in the final publication should be underlined. All authors must be members of the Academy.

The sequence of material in feature-length manuscripts should be: title page, abstract, body of the manuscript, acknowledgments, literature cited, tables with table headings, and figure legends and figures.

1. The title page should include the title of the paper, the authors' names and addresses, and any footnote material concerning credits, changes of address, and so forth.
2. The abstract should be concise and descriptive of the information contained in the paper. It should be complete in itself without reference to the paper.
3. The body of the manuscript should include the following sections: Introduction, Materials and Methods, Results, Discussion, Summary, Acknowledgments, and Literature Cited. All tables and figures, as well as all literature cited, must be referred to in the text.
4. All references in the Literature Cited must be typewritten, double spaced, and should provide complete information on the material referred to. See Volume 43(3-4) 1982 for style.
5. For style of abstract preparation for papers presented at annual meetings, see Volume 43(3-4) 1982.
6. Each table, together with its heading, must be double spaced, numbered in Arabic numerals, and set on a separate page. The heading of the table should be informative of its contents.

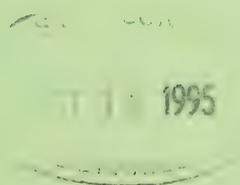
Each figure should be reproduced as a glossy print either 5 × 7 or 8 × 10 inches. Line drawings in India ink on white paper are acceptable, but should be no larger than 8½ × 11 inches. Photographs should have good contrast so they can be reproduced satisfactorily. All figures should be numbered in Arabic numerals and should be accompanied by an appropriate legend. It is strongly suggested that all contributors follow the guidelines of Allen's (1977) "Steps Toward Better Scientific Illustrations" published by the Allen Press, Inc., Lawrence, Kansas 66044.

The author is responsible for correcting galley proofs. He is also responsible for checking all literature cited to make certain that each article or book is cited correctly. Extensive alterations on the galley proofs are expensive and costs will be borne by the author. Reprints are to be ordered when the galley proofs are returned by the Editor.

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***Daphnia lumholtzi*: Appearance and Likely Impacts of an
Exotic Cladoceran in the Ohio River**

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ABSTRACT

Exotic species can have a profound impact on the ecosystems they invade. Over the period 1992-1994, the exotic cladoceran *Daphnia lumholtzi* has become increasingly common in the Ohio River. This cladoceran has prominent helmet and caudal spines which may protect it against invertebrate and some vertebrate predators. This well-defended cladoceran may eventually dominate the Ohio River plankton during the warm months of the year, potentially altering the composition of the potamoplankton assemblage and the trophic processes within the river.

INTRODUCTION

The Ohio River and its tributaries have been invaded by several exotic species in recent history. Species with obvious direct economic impacts such as the zebra mussel (*Dreissena polymorpha*) have been the object of intense research attention, but there are new arrivals in the plankton community of the Ohio that may have less direct but equally serious effects. Among these is the exotic water flea, *Daphnia lumholtzi* Sars [Crustacea: Branchiopoda] which may have been accidentally introduced from its native habitat in Africa, Asia, or Australia to the southern United States in association with either fish-stocking efforts or the release of exotic aquarium fish into the environment (1). It is distinguished from other cladocerans in the Ohio River by its relatively large size (adults average about 1.1 mm long, [2]) and the prominent spines on the head (helmet) and caudal regions. The plankton community in the McAlpine pool of the Ohio has been dominated for the past 4 years by small cladocera, such as *Bosmina longirostris*, which have no obvious spines (3).

The presence of spines in cladocera is often considered an anti-predator mechanism to re-

duce success by vertebrate and invertebrate predators in capturing and ingesting these organisms. The recent invasion of Lake Michigan by the predatory cladoceran *Bythotrephes cederstroemii* shows the impact that such well-defended zooplankton can have. This northern cladoceran also has a prominent caudal spine, which makes capture and ingestion by small fish such as juvenile yellow perch (*Perca flavescens*) difficult. Apparently these fish learn to avoid attacking *B. cederstroemii* (4). Such aversive behavior by the fish may prevent them from wasting pursuit and handling energy attempting to capture *B. cederstroemii*, but it also allows this cladoceran to compete with these small fish for other, less well-defended zooplankton prey.

To determine the seasonal occurrence, densities and distribution of *D. lumholtzi* at sites in the lower McAlpine and upper Cannelton pools of the Ohio River, we collected numerous plankton samples from spring through fall of 1992-1994. If the densities of *D. lumholtzi* are increasing in the Ohio, it is likely this cladoceran will have a negative impact on the native zooplankton and on the fish that rely on these zooplankton for food.

METHODS

We took plankton samples every 10 days from April to November at 2 sites in the Ohio River in 1992–1994. Two shore (within about 10 m of the shoreline) and one midchannel sample were taken at Cox's Park (Ohio River Mile [ORM] 600) near Louisville, KY and the Gallagher Power Station in New Albany, IN (ORM 610). Fifty liter samples were retrieved using a battery-operated water pump (rate ≈ 26 liters min^{-1}) from 1.0 m depths through a 63 μm -mesh plankton net. The samples were concentrated through a dolphin bucket (also with 63 μm mesh) at the cod-end, placed in 75 ml Nalgene screw-top containers transported back to the laboratory on ice. The samples were counted and identified within 4 hours of their collection. The zooplankton samples were enumerated at 45 \times using a Nikon SMZ-10 or a Nikon SMZU stereomicroscope. *Daphnia lumholtzi* was identified using the drawings of Havel and Herbert (1).

In 1994, various physical data were also taken in the river at the Cox's Park site. Temperature and conductivity data at the river surface were collected using a YSI Model 57 oxygen meter. Water samples (75 ml) were taken from the river surface, stored in acid-washed plastic containers and later analyzed for turbidity in nephelometric turbidity units (NTUs) using a Hach model 2100P turbidity meter.

RESULTS AND DISCUSSION

We collected no physical data from the Ohio with our plankton samples during 1992–1993, but in 1994 the conditions in the river varied seasonally. Turbidity was highest in April (mean ≈ 8.2 NTUs) and dropped through the year until the final sample in November (≈ 3.9 NTUs). Temperature varied from 17°C (April) to a high of 28°C in late August. Temperatures had dropped to about 15°C in November when the sampling ended. Conductivity did not vary much through the sampling period, remaining between 320 and 490 μmohs .

Daphnia lumholtzi was present in low densities in the summers (June through August) of 1992 and 1993 (5), but the occurrence and densities were too sporadic and low to distinguish any meaningful trends. In 1994, *D. lumholtzi* appeared suddenly in our samples in

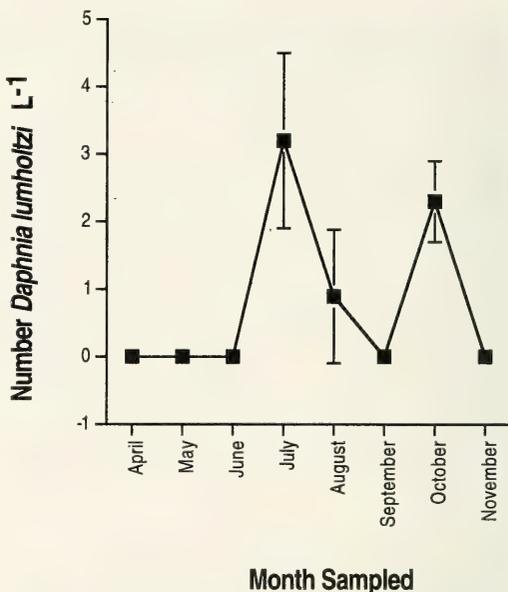


FIG. 1. Abundance of *Daphnia lumholtzi* in the Ohio River in 1994. Means are the result of at least three samples within the month; error bars indicate standard deviations. See text for comparison to densities in 1992–1993.

late July (see Fig. 1) at significant densities in the river. Through most of August, *D. lumholtzi* was a significant component (about >50% numerically) in our samples of the crustacean zooplankton in the Ohio River. Its population levels dropped soon afterward to undetectable levels in late August, although it reappeared briefly at lower densities in October (see Fig. 1).

The appearance of this exotic zooplankter is cause for concern both in terms of the trophic relationships in the Ohio River plankton community and its resultant impact on fisheries. Since its first confirmed report in 1991, *D. lumholtzi* has been found in reservoirs throughout much of the Southeast and Midwest regions of the United States (1); its rapid dispersal would indicate it could potentially colonize much of the Ohio River within the next few years.

While *D. lumholtzi* may not prey directly on other cladocerans as *B. cederstroemii* does, it is still likely to have a negative impact on lotic and lentic systems in this country. The presence of the spines on its helmet and caudal regions may provide some protection against invertebrate and small vertebrate predators.

Such structures have been shown to be effective against invertebrate predators in rotifers (6) and daphniids (7), as well as against fish (4). As *D. lumholtzi* continues its colonization of the Ohio River and tributaries, its defenses may result in it becoming a dominant member of the zooplankton during the warmer portions of the year. It seems likely that invertebrate predators such as the phantom midge larva, *Chaoborus* (especially early instars), may not be able to feed efficiently on this well-protected cladoceran, which may result in more predation pressure on co-occurring but less well-defended cladocera such as *Bosmina*. Increased feeding on *Bosmina* would further drive the plankton community toward dominance by *D. lumholtzi*, although *Bosmina* may be able to compensate for its losses through increased reproduction.

We will be performing both in situ and large tank experiments this spring and summer, investigating the impact of this new cladoceran on the pelagic communities in the river. Our experiments will enable us to understand better the probable impact of this new member of the Ohio River plankton community on trophic processes in the Ohio River.

SUMMARY

In systems dominated by *D. lumholtzi*, a new population bottleneck for large invertebrates and small vertebrates may emerge. Densities of small zooplanktivorous fish and

the macroinvertebrate predators such as *Chaoborus*, which can be important food for the larger fish in a community, may drop due to poor success with the new dominant cladoceran. Decreases in their densities would probably have an impact on higher trophic levels, including the larger fish popular with sport fishermen.

ACKNOWLEDGMENTS

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Comparing Epizootic Systems Using Spectral Analysis and Autoregression: A Case Study on *Tetranychus-Neozygites* Mycosis

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ABSTRACT

Host-pathogen interactions at the population level were studied using the twospotted spider mite, *Tetranychus urticae* Koch, and the pathogenic fungus, *Neozygites floridana* Weiser and Muma, as a model system. The generation time (egg to adult) of the twospotted spider mite is approximately 10 days while the generation time of *N. floridana* (host penetration to release of spores) is approximately 4 days when both are reared at 21°C. Three different systems were studied in relation to epizootic events. In System 1, leaf disks were infested with 250 eggs, 125 immatures, 5 male adults, and 20 female adults. In System 2, 25% of the immatures were added every other day and in System 3, 25% of the immatures were removed every other day. Our objective was to change the rate of increase of the host population. These systems were observed for 200 days. Spectral analyses showed that regulation of the host existed under 3 conditions. First, the pathogen could regulate the host in an epizootic system in which no hosts were removed but in which pathogens were periodically added. Regulation also occurred in systems in which hosts were added or removed, as long as a critical number of pathogen units (mummies) was maintained. Finally, a single introduction of pathogens could regulate the host in systems in which a portion of hosts was periodically removed. Autoregression and cross-correlation analyses of this system indicated that the number of mites that became infected can almost always be predicted by the number of eggs, but it cannot be predicted by the number of immatures. Moreover, densities of infected hosts could be forecast from a range of time lags of 2-16 days except for a time lag of 8 or 10 days.

INTRODUCTION

An epizootic system consists of 3 basic components: host, pathogen, and environment (1, 2, 3). Under a given set of environmental conditions, host-pathogen interactions are affected by host density, susceptibility, behavior, interspecific characteristics (4), pathogen density, infectivity, latency, survival, spatial distribution (5) and transmission characteristics (6). Simple models have long been used to study the elementary dynamics of such systems (7, 8, 9). These authors differentiate the host population into 2 distinct subpopulations of susceptible and infected hosts which were treated separately. Similar approaches have also been proposed by Anderson and May (10, 11, 12) who treated the host component as a composite of the 2 subpopulations. These studies have resulted in establishment of the threshold density concept with various epizootiological implications (13, 14) and unification of ecological (predator-prey) and epizootiological (pathogen-host) models (15).

Epizootic systems are often periodic which means that they can be analyzed by identifying the dominant cycles (16). One way to identify those cycles is to use spectral analysis. This

analysis has been successfully used in identifying periodicities in human epidemics (17). When the spectral density (variance of evenly spaced data points) is plotted against its frequency, power spectra peak in the dominant cycle(s) of the system. The biological importance of the cycle(s) is identified by correlating it with the biological characteristics of the system of interest.

In this study, we evaluated 2 methods, spectral analysis and autoregression, to analyze epizootic systems using the twospotted spider mite, *Tetranychus urticae* Koch as infected by the pathogenic fungus, *Neozygites floridana* Weiser and Muma, as an experimental model system.

MATERIALS AND METHODS

Experiments were carried out in the Insect Pathology Laboratory, Department of Entomology, University of Kentucky, Lexington, KY, during January-April 1991. The epizootic model for this study consisted of the twospotted spider mite, *Tetranychus urticae* Koch, reared on bush bean plants (Taylor Strain) and the pathogenic fungus, *Neozygites floridana* Weiser and Muma, maintained in mummified mite cadavers. The original source of *N. flor-*

idana was obtained as a gift from Dr. George G. Kennedy of North Carolina State University, Raleigh, NC. Only adult mite cadavers were used as inoculum in this study. On those days when inoculation was to be performed, a separate population of cadavers was inspected and all cadavers removed. Four hours later, newly-killed cadavers were selected for use as inoculum as described by Brown and Hasibuan (18). Therefore, age of inoculum (newly-formed cadavers) never exceeded 4 hours. Cadavers always sporulated if humidity was at 100% RH.

Experimental units were plastic trays (14 × 17 cm) with wet cotton batting supporting 4 bush bean leaf disks (dia. 3 cm) connected to each other by 1 × 5 cm hardware cloth bridge spans. From an initial pool of 36 units, immatures and adults were placed on them and were randomly assigned to 3 groups of epizootic systems, i.e., System 1 without additions or removals of host mites. In System 1, each leaf disk was infested with 250 eggs, 125 immatures, 5 male adults, and 20 female adults. System 2 was similar except 25% of mite immatures were added every other day whereas in System 3, 25% of mite immatures were removed every other day. Each system was further subdivided into three subsystems or treatments, i.e., control with mites only, treatment A with a single pathogen introduction of 5 mummies, and treatment B with repeated pathogen introductions of 1 mummy every other day. Observations on prevalence of mycosis (number of infected hosts or new mummies) and the surviving mites (number of susceptible hosts) were done every other day. Between any 2 observations, all trays were kept in a growth chamber (Percival, Model I-35 L) set at $21 \pm 1^\circ\text{C}$ and 100% RH. These systems were run until all mites died of mycosis or 200 days which ever came first.

The data series of susceptible hosts were documented as the number of eggs, immatures, adult males, and adult females. The series of susceptibles and infecteds (cadavers) were smoothed using a five point polynomial method (19). Spectral analyses and autoregression were then conducted on these smoothed data.

1. Spectral Analysis

In this study, the SAS SPECTRA procedure (20) was used to generate spectral density

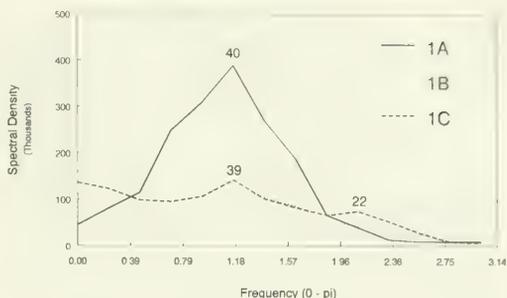


FIG. 1. Spectral density plots for total mites in System 1. System 1A (solid line) has a single obvious peak while system 1B (dotted line) has no peak. System 1C (dashed line) has two peaks that satisfy the criterion that the peak must be at least 10% higher than the preceding and succeeding spectral densities. The numbers on the graph correspond to the entries for total mites, System 1 A, B, and C in Table 1.

plots in each epizootic system from the time series of susceptibles and infecteds. Some predictions about the epizootic systems were then inferred.

2. Autoregression and Cross-Correlation Analysis

This analysis sought to correlate a data point with its future value with the expectation that the value at some future time (Δt) could be predicted by the original data point. To do this, pairs of regressor-predicted variables to be analyzed were $S(t)$ versus $S(t + \Delta t)$, $S(t)$ versus $I(t + \Delta t)$, $I(t)$ versus $S(t + \Delta t)$, and $I(t)$ versus $I(t + \Delta t)$, where S is the number of susceptibles and I is the number of infecteds. The values of Δt used were 2, 4, 6, 8, 10, 12, 14, or 16 days. The upper limit of 16 days lag was chosen in this study because the total developmental period of the mites is about 16 days (21).

RESULTS AND DISCUSSION

The criterion for identifying peaks in spectral density (which imply periodic fluctuations in these population systems) was that an increase of at least 10% in the spectral density was followed by a decrease by at least 10%. An example of the spectral density curves obtained from the spectral analyses is shown in Figure 1. This example, chosen because it displays the array of observed responses and demonstrates the use of this criterion, corre-

sponds to the total mite curves for System 1, populations with no additions or removals of immatures. System 1A, with a single introduction of the pathogen, has a single obvious spectral peak at 40 d. System 1B, continuous pathogen introduction, has no peaks; declining throughout the frequency interval. System 1C, mites without the pathogen, has 2 peaks (using the 10% criterion), 1 of which is at nearly the same frequency as System 1A and another of which is at 22 days.

The periodicities of all 9 systems are shown in Table 1. Over half (52%) of the variables investigated had one periodicity while 29% had none and the remaining 19% had more than one. Populations in System 2 (host additions) had more periodicities than those in the other systems. System 1 had the largest overall mean (32.8 ± 2.1 d), System 3 had the smallest (26.2 ± 3.1 d) and System 2 was intermediate with a mean (\pm S.E.) periodicity of 30.3 ± 2.9 d. None of these differences were significant.

The mean periodicity for each population is also shown in Table 1. These means were compared using all possible pairings in a two-tailed *t*-test ($P = 0.05$). In Systems 1 and 3, the mite population with no pathogen had significantly faster periods than the system with a single pathogen introduction. In contrast, these 2 population means were almost identical in System 2. Examining these means in order of increasing population growth rate (3—removal of immatures, 1—no manipulation of immatures, 2—adding immatures), reveals a clear trend of increasing periodicity with increasing growth rate on the populations with no pathogen. However, populations with the pathogen did not show an obvious trend.

This trend is somewhat clearer if one examines the individual variables in order of increasing growth rate. This trend is observable for immatures (System C), total mites (Systems A and C), proportion of eggs (System C).

Susilo had predicted that the infected hosts would show periodicities of 15–16 d with longer periods possible. This prediction was based, in part, on the spider mite's longevity which, under the conditions used here, is 15–17 d (21). The results in Table 1 confirm this in Systems 1 and 2 but not in 3 and, even then, only in the cases where the pathogen was repeatedly introduced. No regular peri-

TABLE 1. Periodicities (days) of the infecteds and classes of susceptibles in three epizootic systems tested. System 1 = no addition or removal of hosts; System 2 = addition of 25% of hosts every other day; System 3 = removal of 25% hosts every other day; C = no pathogens introduced; A = a single introduction of pathogens (five mite mummies initially); B = repeated introductions of pathogens (five mummies initially and one mummy every other day); p = proportion of corresponding host class to total susceptibles; x = no data; — = no periodicities; single values indicate single cycles; double or triple values indicate multiple periodicities.

Host classes	System 1			System 2			System 3		
	C	A	B	C	A	B	C	A	B
Infecteds	x	—	28; 17	x	—	71; 16	x	—	—
Suscept.									
Eggs	38; 22	40	42	32; 17	—	23	21	40	17
Immat.	37; 24	39	34	56; 17	—	21	21	23	—
Males	—	40	—	50; 17	54; 19	21	34	—	63
Femal.	24	—	—	—	53; 25; 16	54; 17	—	76; 18	20
Total	39; 22	40	—	46	53	23	25	24	21
pEggs	24	38	48	27; 17	17	17	21; 17	18	17
pImn.	24	37	60	55; 17	53; 18	18	—	51; 18	20; 17
pMales	23	—	23	48; 20; 18	20	—	22	—	17
pFem.	25	—	—	—	16	—	—	16	18
Mean \pm S.E.	27.46 \pm 2.06	39.0 \pm 0.51	36.0 \pm 5.67	31.21 \pm 4.30	31.27 \pm 5.31	28.10 \pm 5.93	23.00 \pm 2.04	31.56 \pm 6.82	23.33 \pm 4.99
Sign. Diff.	c	a	ab	bd	bed	bde	f	bcde	ef

TABLE 2. Significance test for time lags for predicting susceptibles or infecteds.

Variables		Lags (days)							
Predicted	Predictor	2	4	6	8	10	12	14	16
$S(t + \Delta t)_{Eggs}$	$S(t)_{Eggs}$	**	**	—	—	—	*	**	*
$I(t + \Delta t)$	$S(t)_{Eggs}$	—	**	**	**	**	**	*	*
$S(t + \Delta t)_{Eggs}$	$I(t)$	—	—	—	—	—	—	*	*
$S(t + \Delta t)_{Imm}$	$S(t)_{Imm}$	**	**	**	—	—	*	**	**
$I(t + \Delta t)$	$S(t)_{Imm}$	—	—	—	—	—	—	—	—
$S(t + \Delta t)_{Imm}$	$I(t)$	—	—	—	—	—	—	—	—
$I(t + \Delta t)$	$I(t)$	**	**	*	—	—	*	**	**

— Non-significant

* Significant at $P < 0.05$.** Significant at $P < 0.01$.

odicity was observed in the populations subjected to a single introduction of the pathogen. The pathogen did persist in these populations, sometimes at high levels of incidence, there just wasn't a regular periodic fluctuation. Brown (in press) has presented evidence that the interaction between the host and pathogen is chaotic and aperiodic.

Brown (22) and Hasibuan (23) have both suggested that mite populations on the leaf disks used here tend to overdamp. This is why periodicities would tend to increase with increasing growth rates when growth rates are manipulated by increasing the population by a predefined *percentage* of those already present. However, when the pathogen is present, it serves to reduce the mite growth rate thereby lessening the overdamping effect. Consequently, in these populations, the pathogen would tend to cause the periodicities to decrease as more immatures are added. It is this apparent conflict between the mite's intrinsic dynamics and those of the mite-pathogen system that cause some of the periodicities to decrease in System 2.

For the autoregression analysis, numbers of mite eggs, immatures, and infected hosts in System 2B were used to represent a continuum of susceptible and infected host classes tested because the prediction about host-pathogen regulation in that system was the resistant to perturbation. Results of autoregression and cross-correlation analyses on the above three host-pathogen classes revealed that the number of infected hosts can almost always be predicted by the number of eggs (Table 2). Results indicate that the number of eggs can be predicted by the number of infected hosts using the time lags of 14 or 16

days. However, the number of infecteds cannot be used to predict the number of immatures, and vice versa. Moreover, a future value of infecteds can almost always be predicted by a previous value of infecteds, except when time lags of 8 or 10 days were used. The same exception was true for self-predicting the number of immatures. Self-prediction of the number of eggs was possible using time lags of 2, 4, 12, 14, and 16 days.

CONCLUSION

Spectral density analysis on 3 epizootic systems of *Tetranychus-Neozygites* mycosis showed that pathogen cycles were maintained in systems where pathogens were repeatedly introduced. Host-pathogen regulation may occur in epizootic systems when a portion of that host was routinely added or removed or in systems with host additions or removals as long as the pathogen base-level was maintained.

Autoregression analysis on susceptibles (mite eggs or immatures) and infecteds (mite mummies) demonstrated that the number of infecteds can almost always be predicted by the number of eggs. Future values of infecteds, eggs, or immatures can be predicted by their corresponding previous values for a range of time lags of no more than the developmental time of the host (16 days), except for the time lags of 8 or 10 days.

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Foraging of Red-cockaded Woodpeckers (*Picoides borealis*) in Kentucky

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ABSTRACT

The 5 groups of red-cockaded woodpeckers (*Picoides borealis*) remaining on the Daniel Boone National Forest in 1990-1991 were observed during the winter and nesting seasons to determine the importance of different tree species as foraging substrates. On the average, red-cockaded woodpeckers foraged on hardwoods 14% of the time during the winter and 44% of the time during the nesting season. During the winter season, birds in 3 groups exhibited statistically significant preferences for foraging on pines compared to hardwoods ($P < 0.001$), and birds in 2 groups exhibited no preferences. During the nesting season, birds in 2 groups preferred hardwoods ($P < 0.004$), birds in 1 group preferred pine ($P < 0.003$), and birds in 2 groups exhibited no preferences. Southern red oak (*Quercus falcata*), black oak (*Q. velutina*), and white oak (*Q. alba*) were the hardwood species used most frequently by foraging red-cockaded woodpeckers. Our results suggest that management based primarily on the removal of hardwoods to improve red-cockaded woodpecker habitat contributes to loss of biodiversity and may ultimately lead to the extirpation of the local population of the species.

INTRODUCTION

The northern-most population of the endangered red-cockaded woodpecker (*Picoides borealis*) occurs on the Daniel Boone National Forest in southeastern Kentucky. As of November, 1994, only 4 or 5 birds in 2 groups survived. The range of this population is disjunct from others to the south, with little possibility of gene flow into Kentucky. The habitat is mixed pine-oak forests with well-developed lower canopy strata (1), vegetation typical for dry sites in the Appalachians but atypical for red-cockaded woodpecker habitat.

Management of this declining red-cockaded population by the U.S. Forest Service is based on the assumption, developed in the pine forests of the southeastern Coastal Plain, that habitat quality for red-cockaded woodpeckers is reduced in proportion to the occurrence of hardwoods in a stand (e.g., 2). This implies that red-cockadeds prefer pines and discriminate against hardwoods as a foraging substrate. To date, this assumption has not been tested in hardwood-dominated regions such as Kentucky. The objective of this study was to determine if red-cockaded woodpeckers in Kentucky exhibit a preference for pines as a foraging substrate. This is an important question

because recent management by the Forest Service has been largely based on this belief, and has relied on cutting and prescribed burning to remove hardwoods from active and inactive red-cockaded colony sites and other areas judged potentially suitable as colony sites for the species (3).

MATERIALS AND METHODS

Study Area.—The study area was located in Laurel and Whitley Counties on the Daniel Boone National Forest in southeastern Kentucky (36°48'N, 84°18'W). This area lies along the escarpment defining the western edge of the Cumberland Plateau physiographic region and is included in the Mixed Mesophytic Forest region (1).

The topographic heterogeneity of the landscape explains the presence of numerous forest types. Ridge tops are predominantly covered with pine and mixed pine-hardwood stands. These stands are often small and occur as narrow bands along elevational contours. Common species include shortleaf pine (*Pinus echinata*), white oak (*Quercus alba*), various red oak species, and hickory (*Carya* spp.). The mid- and understories present throughout the study area are composed of red maple (*Acer rubrum*), sourwood (*Oxydendrum aboum*), blackgum (*Nyssa sylvatica*), and dogwood (*Cornus florida*) (1). The lower slopes are

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dominated by hardwood stands and contain a low percentage of pines. Typical species are chestnut oak (*Quercus prinus*), red and white oaks, yellow poplar (*Liriodendron tulipifera*) and beech (*Fagus grandifolia*). On northerly slopes and in ravines, characteristic species include hemlock (*Tsuga canadensis*) and rhododendron (*Rhododendron maximum*). Hardwood stands occupy approximately 65% of the area, and pine and pine-oak stands, which are considered suitable habitat for red-cockaded woodpeckers, account for the remaining 35% of the forested area (1).

Field Methods.—Birds were observed at the 5 active colony sites from October to June, 1990–1991, and from December to March, 1991–1992. Four of the sites were occupied by pairs of birds, and the other site was occupied by a single bird. Data were systematically collected only during the 1991–1992 observation period to statistically describe foraging during the winter (December to March) and nesting seasons (April to June). During the winter period, foraging data were collected during 31 field sessions, 18 during the morning (2-hour period following sunrise) and 13 during evening (2-hour period preceding sunset). A total of 311 minutes of observation was collected. During the nesting period foraging data were collected during 19 field sessions, 14 during midday, 3 during the morning and 2 during the evening. A total of 426 minutes of observation was collected.

All foraging data were collected when the birds were near their cavity trees since cliffs, cut trees and brush prevented us from following the birds. During the nesting season, red-cockaded woodpeckers could be observed for relatively long periods because they spent much of their time close to the nest tree when feeding young. Birds were observed in areas that were approximately circular around cavity trees and ranged from 2.4 to 4.1 ha.

When recording data, we assumed that a red-cockaded woodpecker was foraging when it probed, picked or flaked bark as described by Hooper and Lennartz (4). Birds were not considered to be foraging when active on the cavity tree around the cavity entrance or resin wells. Observations were made at 1-minute intervals to record the species of tree on which the bird was foraging. Observations collected in this way may be considered independent for

TABLE 1. Importance (expressed as percentage of basal area and density) of tree species in red-cockaded woodpecker colonies.

Species	Percentage			
	Basal area (stems ≥ 10 cm DBH)		Density (stems ≥ 2 & <10 cm DBH)	
	Mean	Median	Mean	Median
<i>Pinus echinata</i>	55	57	0	0
<i>Quercus coccinea</i>	10	6	1	2
<i>Carya</i> spp.	7	6	7	8
<i>Quercus alba</i>	6	4	0	1
<i>Quercus velutina</i>	4	2	2	4
<i>Pinus virginiana</i>	4	8	0	0
<i>Pinus rigida</i>	0	1	0	0
<i>Quercus stellata</i>	3	3	1	1
<i>Quercus falcata</i>	2	1	5	6
<i>Quercus prinus</i>	2	2	1	1
<i>Acer rubrum</i>	2	2	24	21
<i>Liriodendron tulipifera</i>	1	2	8	17
<i>Cornus florida</i>	1	2	21	26
<i>Nyssa sylvatica</i>	1	2	2	2
<i>Tsuga canadensis</i>	1	2	6	11
<i>Oxydendrum arboreum</i>	1	1	12	13
<i>Magnolia</i> sp.	0	0	5	11
<i>Amelanchier arborea</i>	0	0	0	9
<i>Carpinus caroliniana</i>	0	0	2	4
<i>Sassafras albidum</i>	0	0	2	4
<i>Fagus grandifolia</i>	0	0	1	2

the purpose of testing the foraging preferences of individual birds (M. Lacki, pers. comm.). Since the birds were not banded it was impossible to distinguish between individuals in groups composed of 2 birds, and, therefore, for statistical analyses, data recorded for groups were treated as if they were recorded for an individual bird.

At each colony site, the vegetation within the area used for foraging was described. At each site, circular 0.05-ha plots were randomly located at a density of 5 per ha, and the diameter at a height of 1.4 m (DBH) and species of every tree ≥ 10 cm DBH was recorded. Within a 4 \times 25 m strip across each circle, the number and species of stems <10 cm DBH and ≥ 2 cm DBH were recorded (Table 1).

Statistical Analysis.—Chi-square tests were used to determine whether or not woodpeckers preferred pines or hardwoods as foraging substrate. The availability of pines and hardwoods was expressed as the percentage of the total basal area; for each site this percentage was used to calculate the expected number of foraging observations from the total number of observations. Expected values calculated in

this way represented the case where red-cockaded did not discriminate between pines and hardwoods, but foraged in the two types of trees in proportion to their occurrence.

Since the total population consisted of only 9 birds, and these were divided into 5 groups with members that were indistinguishable, it was impossible to collect enough independent observations to make statistical statements regarding the foraging preferences of the entire red-cockaded population on the Daniel Boone National Forest. Statistical analyses were therefore only performed for the individual groups of birds at each site, and observations pertaining to the entire population were summarized, but not analyzed, statistically. The numbers of observed and expected foraging observations on both pines and hardwoods were used to calculate chi-square values for each colony site to test for preference for either tree type. Winter and nesting season data were analyzed separately.

RESULTS

Basal areas of stems ≥ 10 cm DBH ranged from 5 m²/ha to 13.3 m²/ha, with an average of 12 m²/ha. Pine accounted for 48 to 74% of the basal area. Shortleaf pine was the most important species in red-cockaded woodpecker colonies accounting for 55% of the total basal area with a range of 40 to 70% (Table 1). In contrast, pitch pine (*Pinus rigida*) and Virginia pine (*P. virginiana*), respectively, accounted for <1 and 4% of the basal area. Six species of oaks accounted for 27% of the total basal area and 68% of the non-pine basal area. Other individual hardwood species accounted for <2% of the basal area (Table 1). For stems <10 cm and ≥ 2 cm DBH, total stem density ranged from 90 to 650 stems/ha over all 5 colony sites. In contrast with conditions on the study area (Table 1), Forest Service recommendations for optimum red-cockaded habitat specify that hardwood density per acre should be <10/ac in the upper canopy level and <3/ac in the mid-story (3).

During both winter and nesting seasons, red-cockaded woodpeckers spent more time on shortleaf pine (74% in winter; 50% in nesting season) than on any other tree species. The hardwoods used by red-cockaded woodpeckers were scarlet oak (*Quercus coccinea*), white oak, southern red oak (*Q. falcata*), post

TABLE 2. Levels of significance (*P*) for individual colonies of tests of red-cockaded woodpeckers' preference for pines or hardwoods (Hdwd) as foraging substrate during the winter and nesting seasons.

Colony	Nesting Season		Winter Season	
	<i>P</i>	Preference	<i>P</i>	Preference
I	0.19	None	<0.001	Pine
II	0.003	Pine	0.096	None
III	0.004	Hdwd	>0.250	None
IV	<0.001	Hdwd	<0.001	Pine
V	>0.250	None	<0.001	Pine

oak (*Q. stellata*), black oak (*Q. velutina*) and hickory. Red-cockaded woodpeckers were sometimes seen foraging on red maple during the winter, and on sourwood, chestnut oak and, rarely, dogwood during the nesting season. Red-cockaded woodpeckers were never observed on yellow poplar, black gum, or eastern hemlock.

During the winter, 311 (86%) foraging observations were on pines and 52 (14%) on hardwoods. Statistical analyses of the individual colonies showed that red-cockaded preferred pine over hardwoods at 3 colonies but did not exhibit a preference at the other two colonies (Table 2).

During the nesting season, 239 (56%) foraging observations were on pines and 187 (44%) were on hardwoods. Among the 5 colonies, foraging ranged from 34 to 89% on pines, and from 11 to 66% on hardwoods. During the nesting season the birds statistically preferred pine at one colony, hardwoods at 2 colonies, and showed no preference at 2 colonies (Table 2).

During the winter red-cockaded woodpeckers showed no apparent preference for any individual hardwood species as foraging substrate. During the nesting season, however, red-cockaded woodpeckers, on average, utilized the southern red oak/black oak and the white oak/post oak (*Q. stellata*) groups far above their proportional occurrence in the stands. Conversely, during the nesting season, red-cockaded woodpeckers seldom foraged on scarlet oak and hickory, although these taxa were among the most important hardwoods on all sites.

DISCUSSION

Red-cockaded woodpeckers in our study area spent most of their time foraging on pine

trees, and statistically preferred pines on 3 sites during the winter season (Table 2). This result was consistent with past research (4, 5, 6, 7, 8). From previous reports of red-cockaded foraging on hardwoods (4, 6, 9, 10), the highest reported use was in Mississippi where birds spent 22% of their time on hardwoods (6). Our finding that during the nesting season, on average, red-cockaded woodpeckers foraged on hardwoods 44% of the time, and statistically preferred hardwoods on 2 sites, contradicts the results of research in more typical habitat where red-cockaded woodpeckers seem to always prefer pines over hardwoods (4).

Our results support previous reports (4, 9) that red-cockaded woodpeckers exhibit a seasonal change in foraging preference for pines and hardwoods (Table 2). The seasonal change in preference that we documented may have been due to a shortage of arthropods on bole surfaces during the winter (11). This would force red-cockaded woodpeckers to concentrate their winter foraging on the thick, flaky bark of pines which offers protective habitat for a large number of over-wintering arthropods (12). In contrast to other woodpeckers, red-cockaded woodpeckers forage chiefly by scaling and flaking bark rather than by excavating holes (4, 8). Flaking is especially critical during winter when most prey are hidden beneath bark. During the nesting season, arthropods are probably more abundant on bole surfaces, allowing red-cockaded woodpeckers to forage on both pine and hardwood species.

The foraging behavior of red-cockaded woodpeckers in the pine-hardwood forests of the Daniel Boone National Forest is clearly different from that of red-cockaded living in pure pine forests further south. The birds in Kentucky seem to readily use hardwoods as a foraging substrate, especially during the warmer parts of the year. This result supports prior assertions (13, 14) that red-cockaded woodpeckers are opportunistic foragers that take advantage of a wide array of food sources, and demonstrates adaptation of the local population to the foraging habitat typical of the Appalachians.

Our results have important implications for management of red-cockaded woodpeckers on the Daniel Boone National Forest, especially since the results of this one-year study were

in agreement with our previous observations of foraging behavior on the study area. Present management is based on regional guidelines that in turn are based primarily on research in other, ecologically very different, parts of the red-cockaded woodpecker's range. Specific recommendations are to remove hardwoods from both the upper and lower canopy strata within areas ≥ 4 ha surrounding all active and abandoned cavity trees, and in areas designated as recruitment and replacement stands; prescribed fire at 2-5 year intervals is recommended to control sprouts from the stumps of the cut hardwoods (3). Implementation of these recommendations will make the species-rich and structurally-diverse forests of the area more uniform and less complex. Such management contrasts with the national trend towards preserving and restoring natural patterns of biodiversity, and with the regional tendency towards hardwood dominance. In particular, the drastic habitat alteration caused by the removal of hardwoods may cause the local population to become maladapted to its habitat, and may contribute to a loss of genetic diversity and ultimately to the extirpation of red-cockaded woodpeckers in Kentucky.

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Extended Monitoring of Mussels (*Bivalvia: Unionidae*) in the Rockcastle River at Billows, Kentucky, an Historical Site

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ABSTRACT

Surveys of unionid mussels in 1985, 1991, 1993, and 1994 in the Rockcastle River at Billows, Kentucky, yielded 18 live species. *Elliptio dilatata* was the most numerous, followed by *Amblema plicata* and *Actinonaias ligamentina*. Combined, those species represented 62% of the individuals collected in all surveys. The 1991 survey showed declines (25% to 40%) in 12 of 16 species observed when compared to the 1985 survey. Those declines were sequential to increased mining activities in the Billows quadrant. During that time, *Actinonaias ligamentina*, *Quadrula pustulosa*, and *Villosa taeniata* remained stable with slight increases above 1985 numbers and appear to be more tolerant of intermittent sedimentation. *Villosa trabalis* was not collected live at this location. Compared with historical data, these surveys indicate that changes in species composition and shifts in abundances will continue in response to changes in habitat conditions at the Billows site.

INTRODUCTION

One of the objectives of the Biological Monitoring Program for the Division of Water (DOW) is to gather biological information through time. This resulted in a database that began in 1976 and continues presently, for selected stream sites across the state. In 1982, the Rockcastle River at Billows, Kentucky, was added to the program. This particular site had prior historical mussel data that began with the Neel and Allen survey of 1947-1949 (1). Most of the mussel collections in the drainage afterwards excluded the Billows site until the Thompson study in 1985 (2). Her study was the most thorough assessment of the Rockcastle River mussels; however, her collections were above and below the DOW site. More recently, collections from this site by Layzer and Anderson (3), yielded 8 live species. Also, Cicerello (4), observed 10 live species at Billows, as part of an effort to document rare and threatened species for the United States Forest Service (USFS) in the Daniel Boone National Forest (DBNF), and for the Kentucky State Nature Preserves Commission (KSNPC) state inventory.

The objective of the DOW surveys at Billows was to document the resident mussel community on a continuing basis, so that changes in the community can be used (along with other components) in the evaluation of present water-quality conditions and to help

characterize future trends in water quality of the Rockcastle River.

STUDY SITE

The DOW study site at Billows is located downstream of the SR 1956 bridge. The study site extends from the bridge to a few meters downstream of the canoe launch. This area is the upper boundary of the Rockcastle Wild River segment. A long straight reach allowed bed load materials to be deposited across the width of the river, which created a long shallow run and a partially exposed shingle bar that extends the length of the study site (approximately 75 m). The substrate is dominated by rubble and cobble-sized flat rocks mixed with larger boulders, gravels, pebbles and sands that create ample mussel habitats.

METHODS

All surveys were conducted between mid-June and mid-July. Instream observations and identifications were made by both authors, while one person served as the data recorder for both observers. Mussel searches were made easier by visually striking transects from bank to opposite bank across the stream. Starting from opposite banks, each observer waded toward the other on the same transect. At a meeting point about mid-stream, one observer stepped around the other on the downstream side, allowing each to continue along the transect. In effect, each transect was vi-

TABLE 1. Species abundances and survey years for Rockcastle River at Billows.

	DOW				*L&A	RRC	N&A
	1985	1991	1993	1994	1992	1993	1964
<i>Actinonaias ligamentina</i>	61	66	46	72	—	2	—
<i>Actinonaias pectorosa</i>	2	5	—	5	—	6	C
<i>Amblema plicata</i>	104	66	47	53	L	8	A
<i>Cyclonaias tuberculata</i>	27	17	21	24	L	—	A
<i>Elliptio dilatata</i>	150	102	139	142	L	30	C
<i>Lampsilis cardium</i>	12	20	26	5	L	10	C
<i>Lampsilis fasciola</i>	9	17	7	6	—	5	C
<i>Lampsilis ovata</i>	5	2	—	—	L	—	—
<i>Lasmigona costata</i>	1	2	4	—	—	—	C
<i>Ligumia recta</i>	23	14	16	12	L	—	C
<i>Obovaria subrotunda</i>	—	1	—	—	—	—	—
<i>Pleurobema coccineum</i>	7	4	5	8	—	—	C
<i>Potamilus alatus</i>	1	1	—	1	—	3	C
<i>Ptychobranchus fasciolaris</i>	74	44	39	32	—	6	C
<i>Quadrula pustulosa</i>	12	9	11	19	—	3	—
<i>Strophitus undulatus</i>	1	—	—	2	—	—	—
<i>Tritogonia verrucosa</i>	—	—	—	1	L	—	—
<i>Villosa taeniata</i>	19	15	31	31	L	5	C
<i>Villosa trabalis</i>	—	—	—	—	—	—	C
Totals	507	371	393	412			

* Layzer & Anderson (L&A), R.R. Ciccerello (RRC), Neel & Allen (N&A). Abundant (A), Common (C), Live (L), Absent (—)

sually searched for mussels twice, once by each observer moving from opposite directions. That particular feature was useful because it helped discover mussels that were missed in one direction by the other observer, either by being overlooked or because of sun glare and/or wind action on the water surface. When undisturbed mussels were located, the mussels were removed from the substrate, identified, and cleaned before replacement, so as to prevent recounting by the observer searching from the opposite direction. Starting at the downstream edge of the study site, a total of 75 transects were visually searched in an upstream direction. The transects were approximately one meter wide and varied from 34 to 12 meters in width across the stream. A few mussel shells were retained in the DOW Mollusc Collection.

RESULTS AND DISCUSSION

Eighteen species have been collected alive at this site since 1985 (Table 1). In the 1991 survey, 12 of 16 species reflected 25–40% declines in numbers observed (see Table 1), which correlated with the bulk (24 of 30) of permitted mining sites that began operations between 1985–1991 (5). *Elliptio dilatata*, the most abundant species at the site, showed a decline in 1991. *Actinonaias ligamentina* has

steadily increased, replacing *Amblema plicata* as the next most numerous species. Combined, those species represented 62% of the total numbers of individuals collected in all surveys. *Cyclonaias tuberculata*, *Pleurobema coccineum*, *Quadrula pustulosa*, and *Villosa taeniata* populations have remained stable, with slight increases since the declines of 1991. Examples of *Lampsilis ovata* have been infrequent and were not observed in the last 2 DOW surveys; however, Layzer and Anderson (3) noted its occurrence at Billows, as well as *Tritogonia verrucosa*, which appeared only in the 1994 DOW survey. *Villosa trabalis* was not observed during the DOW surveys. *Actinonaias pectorosa*, *Quadrula pustulosa*, *Ligumia recta*, and *V. taeniata*, although low in numbers, were observed in each survey and have shown signs of recruitment in this area.

When comparing DOW data with the original Neel and Allen (1) survey, it can be concluded that changes in species composition and shifts in abundances have occurred. For instance, *Actinonaias ligamentina* and *Quadrula pustulosa* have become well established at Billows, and other species, such as *Lampsilis ovata*, *Obovaria subrotunda*, *Strophitus undulatus*, and *T. verrucosa*, have been infrequently observed. *Actinonaias pectorosa*, *P. coccineum*, *Potamilus alatus*, and *Lasmigona*

costata, originally considered common to the site, were observed in very low numbers. Explanations for those declines include natural cycles and the effects of habitat alterations from sedimentation. The most obvious effects to the mussel community at Billows are severe shell erosion (sand blasting) in some species, and alterations in substrate compositions (excessive sands). Surface mining in the headwaters (south and middle forks) were noted by Thompson (2) Layzer and Anderson (3), and Cicerello (4), but, much farther downstream and adjacent to the Billows site, (Hawk Creek drainage) there have been 30 permits issued for surface mining since 1985 (5). The permits range from 1.99 to 646 acres, totaling nearly 2,500 acres. Presently, only 5 permits are active, with disturbed lands about 1,600 acres and on the increase. If mining continues at the same rate, it could continue in the Billows quadrant (USGS) for another decade with existing permits.

Vannote and Minshall (7) reported that an influx of sediments appeared to be responsible for a shift in species dominance in the Salmon River Canyon, Idaho. Neel and Allen noted that *E. dilatata* became dominant in the Cumberland River system sometime after Wilson and Clark's survey (6) in 1911. Table 1 shows that *E. dilatata* was the most abundant species in all DOW surveys, while *Actinonaias ligamentina* made slight increases in numbers observed each survey. Houpp (8) previously documented that *E. dilatata* replaced *Alasmidonta marginata* as the most abundant mussel in the Wild River segment of the Red River, while *Actinonaias ligamentina*, and *Pleurobema coccineum* increased in abundances after the onset of coal mining in the headwaters of that eastern Kentucky stream. The above species, common to both stream systems, as well as *Quadrula pustulosa* and *Villosa taeniata*, of the Rockcastle River surveys, appear to be more tolerant to the effects of intermittent sedimentation than other mussel species in those drainages. Certainly, the timing of innate behavioral mechanisms (burrowing, and reproductive cycles) are central to individual species tolerances or intolerances of sedimentation.

Nearly all the mussel species previously recorded from this historical site are still pres-

ent. Although several species were few in number and infrequently observed, our work does validate their existence at this site and in the drainage.

ACKNOWLEDGMENTS

We wish to show our appreciation to fellow workers and others who have helped with field work over the years. Allen Robison, USFWS, Parrish Roush (DAQ), Gary Beck, Cliff Schneider, Giles Miller and John Brumley, DOW workers, and George Wesley Houpp. Also, Dru Hawkins, for her knowledge and help with word processors, Terry Anderson, Water Quality Branch, Manager, DOW and Dr. G. A. Schuster, EKU, for helpful manuscript reviews and Mike Mills, Ecological Support Section, Supervisor, DOW, and Dr. B. A. Branson, EKU, for their positive support. We thank them all.

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Mössbauer Study of Single Crystal Biotite and Phlogopite

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ABSTRACT

At room temperature, Mössbauer spectra of single crystal biotite and phlogopite were found asymmetric quadrupole doublets. This asymmetry is largest when the angle between the gamma-ray and the normal to the surface of the absorber is 0° , and it vanishes at about 55° .

Based on previous work in the literature, the biotite spectra have been fitted with 2 lorentzian doublets, presumably due to Fe^{2+} on M1 and M2 sites, while the phlogopite spectra have been resolved into 3 sets of lorentzian doublets, 2 due to Fe^{2+} on M1 and M2 sites and the third possibly due to Fe^{3+} on M2 sites.

This work is intended to answer the question of how many doublets can be used in fitting a certain spectrum. In some of the previous work, assignments of doublets seem somewhat arbitrary due to their overlap. One possible way of determining the number of doublets of Fe^{2+} and Fe^{3+} to be used in fitting a spectrum is by changing the oxidation state of iron in some samples, using heat treatment without changing their crystal structures.

INTRODUCTION

Some of the questions about minerals containing iron are: in a given mineral, how many iron sites are there, in what oxidation state, and what is the relative population of these states? Such questions are important, for example, in learning about the conditions of rock-formation.

Methods such as wet chemistry are not completely reliable in answering the above questions. For one thing, in wet-chemistry experiments, the sample is usually destroyed as a result of the method, unlike Mössbauer spectroscopy where the sample is unaltered after the study is done. If one ignores the possibility of different sites having different recoil-free fractions, then Mössbauer spectroscopy lends itself reasonably well to identifying the states of Fe^{2+} and Fe^{3+} ions and the crystal structure of their surroundings. In micas, these states are represented through doublets in a Mössbauer spectrum.

The number of doublets that could be fitted in a complicated Mössbauer spectrum is still uncertain. A complicated spectrum here means that there exists an overlap of doublets. Some of these doublets are for Fe^{2+} and some are for Fe^{3+} , both of which might be in the same or different sites.

Mössbauer spectra of biotite and phlogopite single crystal micas were taken at different orientations and different thicknesses, all at room temperature. We fitted the biotite data with 2 sets of doublets. The phlogopite spectra were

fitted with 2 and with 3 sets of doublets. Our biotite results are consistent with what is found in the literature. But in the phlogopite spectra, the Fe^{3+} doublet is small and buried under the Fe^{2+} doublet. It is not clear at all that the Fe^{3+} exists.

The sign of the electric field gradient (efg) for Fe^{2+} is known to be opposite of that of the Fe^{3+} ion. So, we annealed the mica at low enough temperature that the Fe^{2+} loses one electron and becomes an Fe^{3+} ion without changing the crystal structure of the sample. Then, by taking different spectra at different orientations it is possible to determine the number of sites and the oxidation states of iron occupying those sites, as shown below. X-ray analysis was done on the sample before and after the heat treatment as a way of insuring the crystal structure did not change during the heat treatment.

METHOD

The intensity ratio of the two quadrupole components is given by

$$R = \frac{I\left[\begin{matrix} \pm\frac{3}{2} \\ \rightarrow \\ \pm\frac{1}{2} \end{matrix}\right]}{I\left[\begin{matrix} \pm\frac{1}{2} \\ \rightarrow \\ \pm\frac{1}{2} \end{matrix}\right]} = \frac{3(1 + \cos^2\theta)}{(2 + 3 \sin^2\theta)} \quad (1)$$

where θ is the angle between the efg principal axis (z-axis) and the gamma-ray propagation direction (Fig. 1), and where the intensity ra-

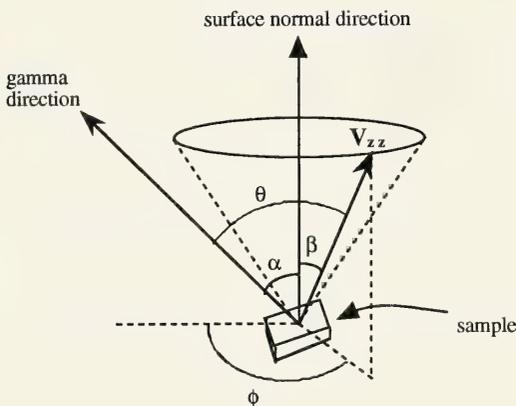


FIG. 1. Geometry of the experiment.

tion is obtained using the areas under the absorption peaks.

For polycrystals (powders), the 2 lines have equal intensities (i.e., $R = 1$) because of the averages of:

$$\langle \sin^2\theta \rangle = \frac{1}{4\pi} \int \sin^2\theta \sin\theta \, d\theta \, d\phi = \frac{2}{3},$$

and

$$\langle \cos^2\theta \rangle = \frac{1}{4\pi} \int \cos^2\theta \sin\theta \, d\theta \, d\phi = \frac{1}{3}.$$

For single crystals, $R = 5/3$ when the gamma-ray is perpendicular ($\theta = 90^\circ$) to the principal axis of the electric field gradient, $R = 1/3$ when the gamma-ray is parallel ($\theta = 0^\circ$) to the efg axis, and $R = 1$ for the magic angle $\theta = 54.7^\circ$. However, if the recoilless radiation is anisotropic and the recoil-free fraction f is itself a function of θ , then the ratio becomes

$$R = \frac{\int_0^\pi (1 + \cos^2\theta) f(\theta) \sin\theta \, d\theta}{\int_0^\pi (2/3 + \sin^2\theta) f(\theta) \sin\theta \, d\theta} = F[f(\theta)] \neq 1. \quad (2)$$

In other words, an anisotropic recoilless fraction should produce an asymmetry in the $\pm(3/2) \rightarrow \pm(1/2)$ quadrupole doublet. The above effect was discovered by Goldanskii (1).

By measuring R (ratio of areas under the peaks) as a function of the angle, θ , in single crystals, it is possible to determine which of

the spectral lines corresponds to the $\pm(3/2) \rightarrow \pm(1/2)$ transition and which to the $\pm(1/2) \rightarrow \pm(1/2)$ transition. In this way, the sign of the quadrupole interaction constant (e^2Qq) is determined. The sign of eQ of the Mössbauer nuclide is usually known from other nuclear techniques (i.e., $eQ = +0.18 \times 10^{-24} \text{ cm}^2$ for the 14.4 keV of ^{57}Fe nuclide). Then the sign of eQ ($=V_{zz}$) can be extracted. For example, the meaning of a positive sign is that the $\pm(3/2)$ energy level is higher in energy than that of the $\pm(1/2)$ level of the ^{57}Fe nuclide as shown in Figure 2.

The above method is under the assumption that the experimenter knows the number of doublets and the oxidation states of the iron in advance before meaningful analysis can take place.

PREVIOUS MICA STUDIES

Many types of mica have been studied extensively by many authors. A review of Mössbauer data on trioctahedral micas was done by Darby (2). The review was done in order to determine the existence of tetrahedral Fe^{3+} and cation ordering in micas and to find reasonable ranges of the hyperfine parameters of different Fe sites.

Hargraves and Rancourt (3) studied single crystal phlogopite mica at room temperature and at different orientation angles. They claimed that thickness effects are not negligible, the Fe^{2+} and Fe^{3+} area ratios can not be obtained due to the overlap, and the resolution of the cis and trans components of the Fe^{3+} is at best questionable.

Darby (4) did a Mössbauer and wet-chemistry study on 52 biotite samples. In one set of data, which she called O-L-10, she fitted the data with 4 doublets, 2 for Fe^{2+} and 2 for Fe^{3+} , all in the octahedral sheet, and she fitted the same data with 2 doublets for the Fe^{2+} and 1 doublet for octahedral Fe^{3+} and the other for the tetrahedral Fe^{3+} . She claimed that "even by visual inspection" the latter was a better fit. After examining her spectra carefully, it was not clear to us how such conclusions can be made.

Rancourt (5) studied 6 trioctahedral micas to determine the spectral features of $^{57}\text{Fe}^{3+}$. Similar studies were also done by Darby (6). But that aside, after examining their spectra, it is still hard to assign such specific oxidation

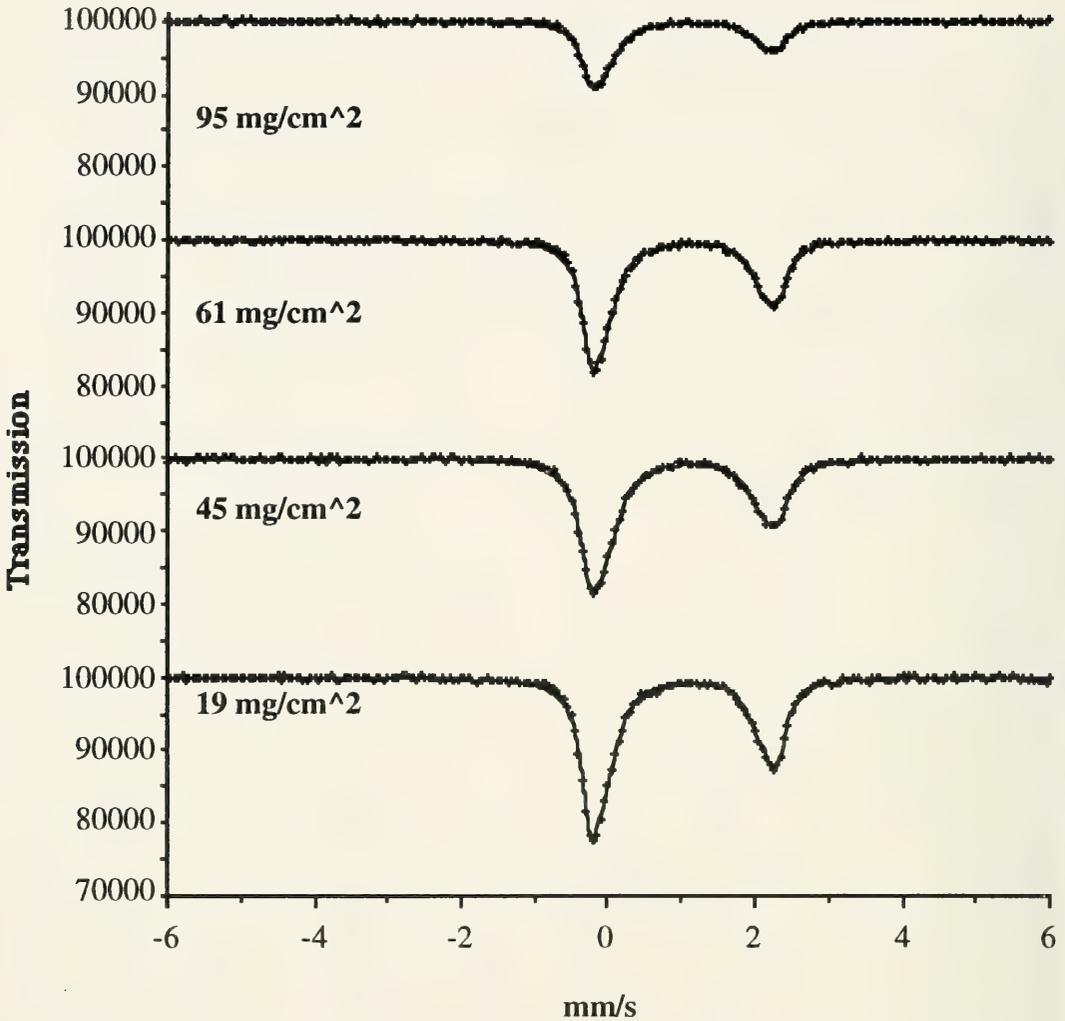


FIG. 3. Mössbauer spectra of biotite of different thicknesses all at $\alpha = 0^\circ$.

Rancourt responded by reiterating that single crystals are better suited and offer more insight (8).

In this study, we tried to address all of the above issues and to examine some of the claimed results. We have performed Mössbauer spectroscopy of single crystals as well as powder biotite and phlogopite for different orientation and different thicknesses. We only heat-treated the phlogopite since it is the controversial mica. We have done powder X-ray diffraction studies on the phlogopite before and after the heat treatment. The results of this study are outlined in the next section.

DATA ACQUISITION AND ANALYSIS

Biotite

A standard Mössbauer spectrometer was utilized in collecting the data. The source used was ^{57}Co in a rhodium matrix. The absorber used in the present study was single crystal biotite and phlogopite mica [$\text{K}(\text{Mg},\text{Fe})_3(\text{OH},\text{F})_2\text{AlSi}_3\text{O}_{10}$].

The biotite comes in dark sheets (flakes). About 1 centimeter by 1 centimeter square of biotite was cut for an absorber which was weighed and then mounted on a polarimeter, and data were accumulated for 20 hours at

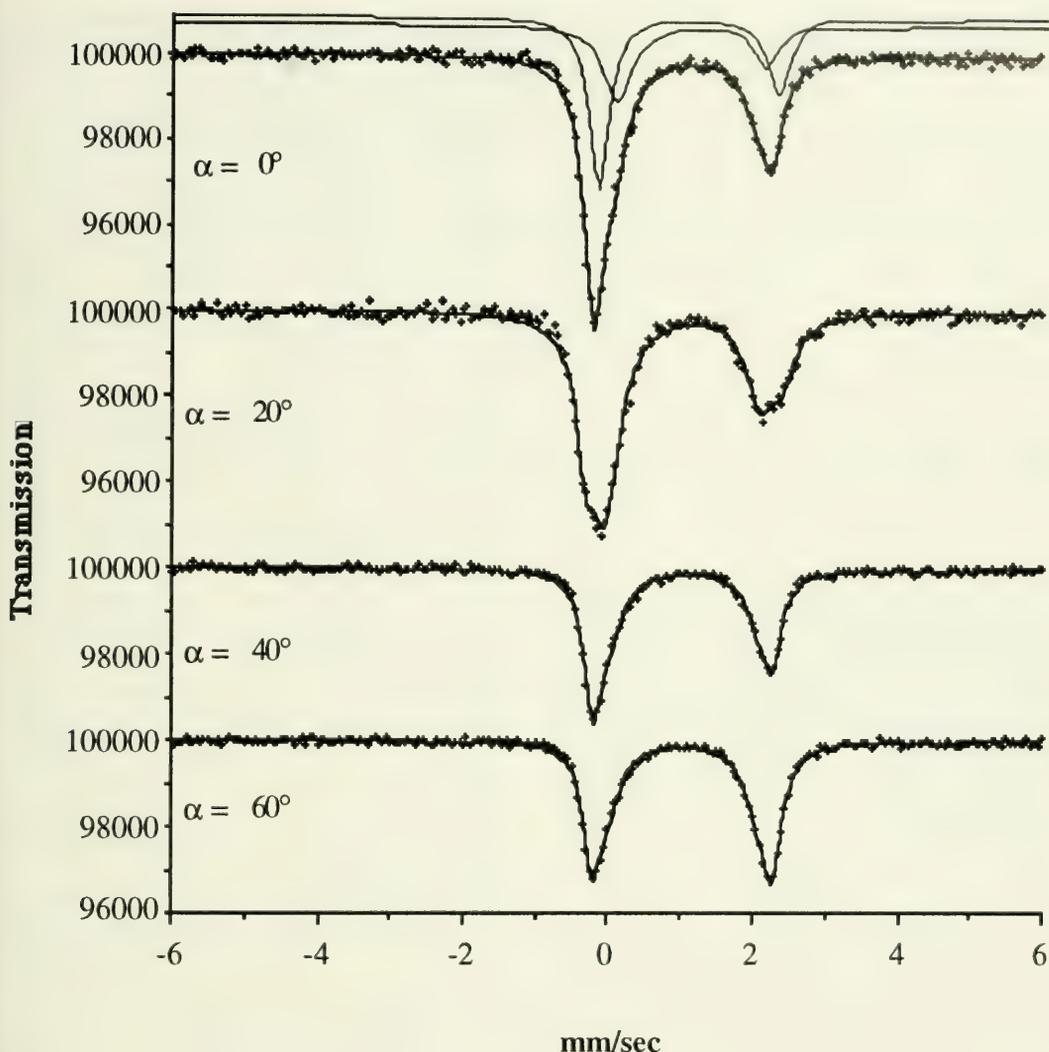


FIG. 4. Mössbauer spectra of biotite at different orientation angle α .

each orientation angle. Six sheets were peeled off and stacked consecutively for different thickness measurements.

Since we did not know the exact direction of the efg in advance nor, did we know the appropriate thickness, we measured the transmission rate as a function of the angle between the direction of the gamma-ray and the perpendicular to the surface of the absorber which we call α (Fig. 1). A typical spectrum of the results of thickness experiments for biotite in this case is shown in Figure 3.

After the appropriate thickness was selected, different spectra at different orientation

angles were taken (Fig. 4). The biotite data were fitted using a least squares fitting routine with 4 single lorentzian lines. By graphing the widths of the lines as a function of the orientation angle, it seems that line 1 and line 4 are coupled while line 2 is coupled to line 3 (Fig. 5). Such an approach was first introduced by Hargraves and Rancourt (3). The results of the fitting routine using the above doublets are summarized in Table 1.

From Table 1 we see that $R_{\text{exp}}(\alpha)$ is not quite equal to the theoretical ratio $R_{\text{th}}(\theta)$, which may mean that the principal axis of the efg is not perpendicular to the surface of the

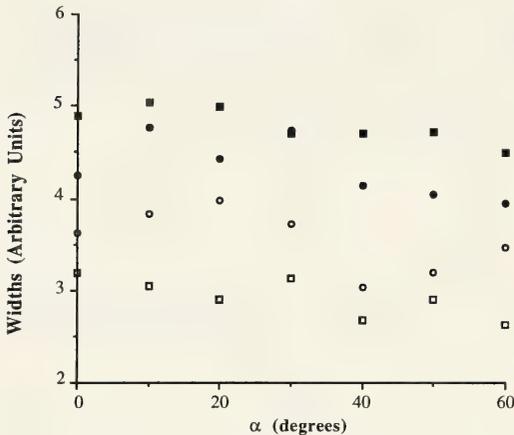


FIG. 5. Widths versus angle. □ for line 4, ○ for line 1, ● for line 3, ■ for line 2.

sample. It is clear though, from the systematics of Figure 4 and Table 1, that the left peak corresponds to the $\pm(3/2) \rightarrow \pm(1/2)$ transitions, which means that the sign of the interaction e^2qQ is negative. The sign of eQ for ^{57}Fe is known to be positive, implying that the sign of the efg (eq) is negative. Here, negative efg means that the $\pm(1/2)$ energy level is higher than the $\pm(3/2)$ energy level in the quadrupole splitting.

Phlogopite

The phlogopite we studied came in 2 colors: one is light in color (iron poor) and the other is dark (iron rich). The present results are for the dark phlogopite only. Again, a 1 centimeter by 1 centimeter square of phlogopite was cut for an absorber which was mounted on the polarimeter after being weighed, and data were accumulated for 20 hours at each orientation angle. For the thickness experiments,

a stack of these squares was used. The intensity of the quadrupole lines has been measured as a function of the angle, α , between the normal to the surface and the direction of the gamma-ray (Fig. 1). Typical spectra for thickness and angular orientation measurements for the phlogopite are shown in Figures 6 and 7.

The summary of the fitting routine for the phlogopite is shown in Table 2. Here, the data were fitted with 3 sets of doublets. Two doublets were attributed to Fe^{2+} on M1 and M2 sites and the third to Fe^{3+} on the M2 site.

Again, from Table 2 we see that $R_{\text{exp}}(\alpha)$ is not quite equal to the theoretical ratio $R_{\text{th}}(\theta)$ for Fe^{2+} . It is clear, though, from the systematics of Figure 7 and Table 2 that the left peak corresponds to the $\pm(3/2) \rightarrow \pm(1/2)$ transitions, which means that the sign of the interaction e^2qQ is negative. The sign of eQ for ^{57}Fe is known to be positive from other nuclear techniques, implying that the sign of the efg (eq) is negative for Fe^{2+} . Notice that the Fe^{3+} doublet is buried under the Fe^{2+} doublet. The suggested doublet for the Fe^{3+} had the right peak larger than the left peak which implied that the sign of the efg of Fe^{3+} is positive. As can be seen from the last column of the table, the fit was good. Other hidden doublets may be assumed but are difficult to justify.

The phlogopite can also be fitted with only 2 doublets yielding the same interpretations as that of biotite. The obtained Mössbauer fit parameters for Fe^{2+} on M2 site are: $\Delta = 2.54$ mm/sec and $\delta = 1.07$ mm/sec, while $\Delta = 2.08$ mm/sec, and $\delta = 1.08$ mm/sec for Fe^{2+} on the M1 site. An illustrative spectrum is shown in Figure 8. The misfit is the same as that of the biotite and is of the order of 0.2% which is within the statistical error.

TABLE 1. Results of the fitting routine to biotite. Δ is the quadruple splitting and δ is the isomer shift with respect to iron metal. RHHWM stands for the ratio of the half widths at half maximum. M1 and M2 refer to two sites.

α	R_{th}	R_{exp} (M1)	R_{exp} (M2)	RHHWM		δ (mm/sec)		Δ (mm/sec)		Misfit (%)
				M1	M2	M1	M2	M1	M2	
0°	3.00	2.60	2.24	1.25	0.88	1.04	1.03	1.98	2.47	0.098
10°	2.83	2.39	2.13	1.06	0.64	1.04	1.06	2.15	2.69	0.451
20°	2.40	2.18	2.08	1.03	0.75	1.03	1.06	2.15	2.73	0.423
30°	1.91	2.00	1.92	1.00	0.84	1.06	1.04	2.06	2.50	0.186
40°	1.47	1.40	1.55	1.13	0.88	1.05	1.03	2.10	2.50	0.334
50°	1.13	1.11	1.25	1.17	0.91	1.05	1.04	2.08	2.50	0.295
60°	0.88	0.60	0.81	1.14	0.76	1.00	1.02	2.06	2.49	0.262

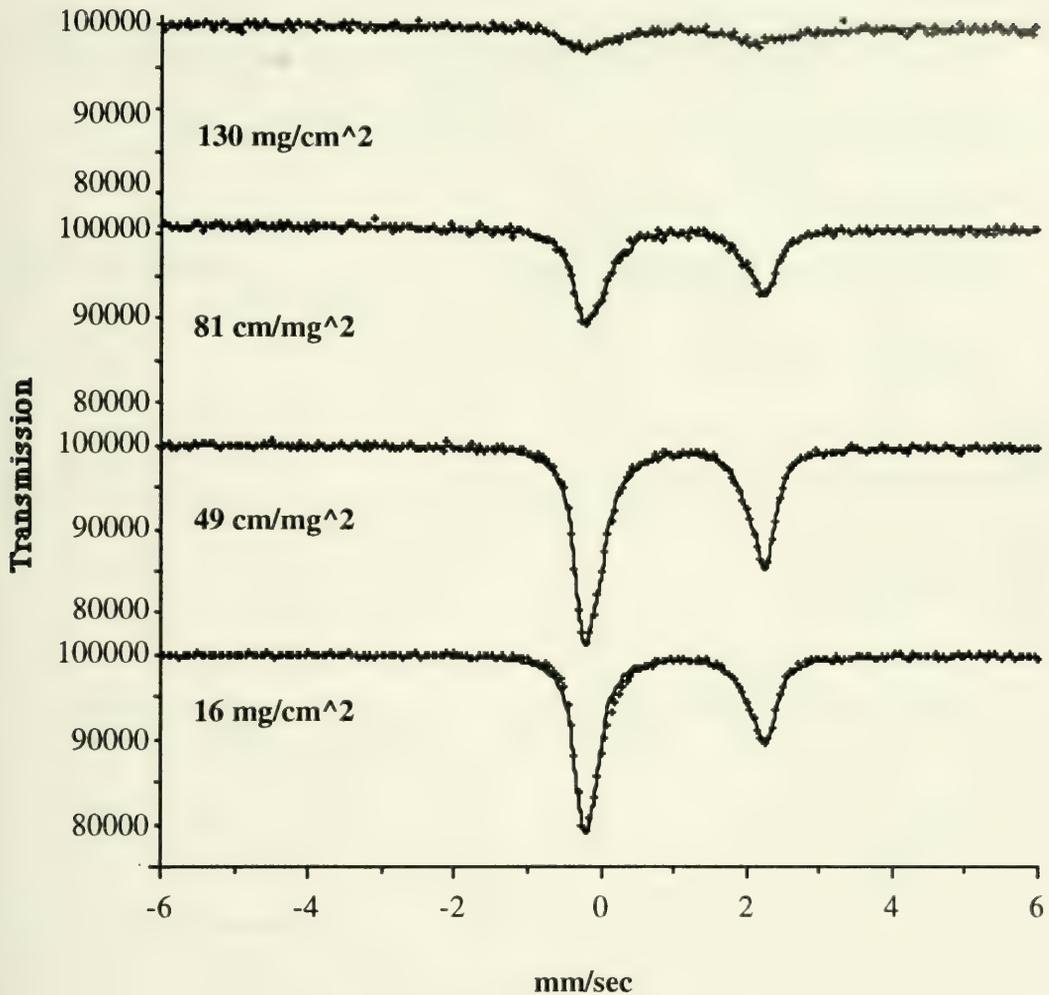


FIG. 6. Mössbauer spectra of phlogopite at different thicknesses all at $\alpha = 0^\circ$.

We annealed chosen sheets of the phlogopite at different temperatures in air and slow-cooled or quenched in room temperature, water, or liquid nitrogen. We found that the method of quenching had no effect on the spectra. The temperature range that produced clean spectra is between 600°C and 800°C . Typical spectra of annealed phlogopite are shown in Figure 9.

The Mössbauer spectra of Figure 9 are fitted with two doublets. It is clear from the quadrupole splitting values in Table 3 that all of the Fe^{2+} is converted to Fe^{3+} . Notice the quadrupole splitting of the Fe^{3+} in the untreated phlogopite is much lower than for the annealed one. Based on this it is safe to as-

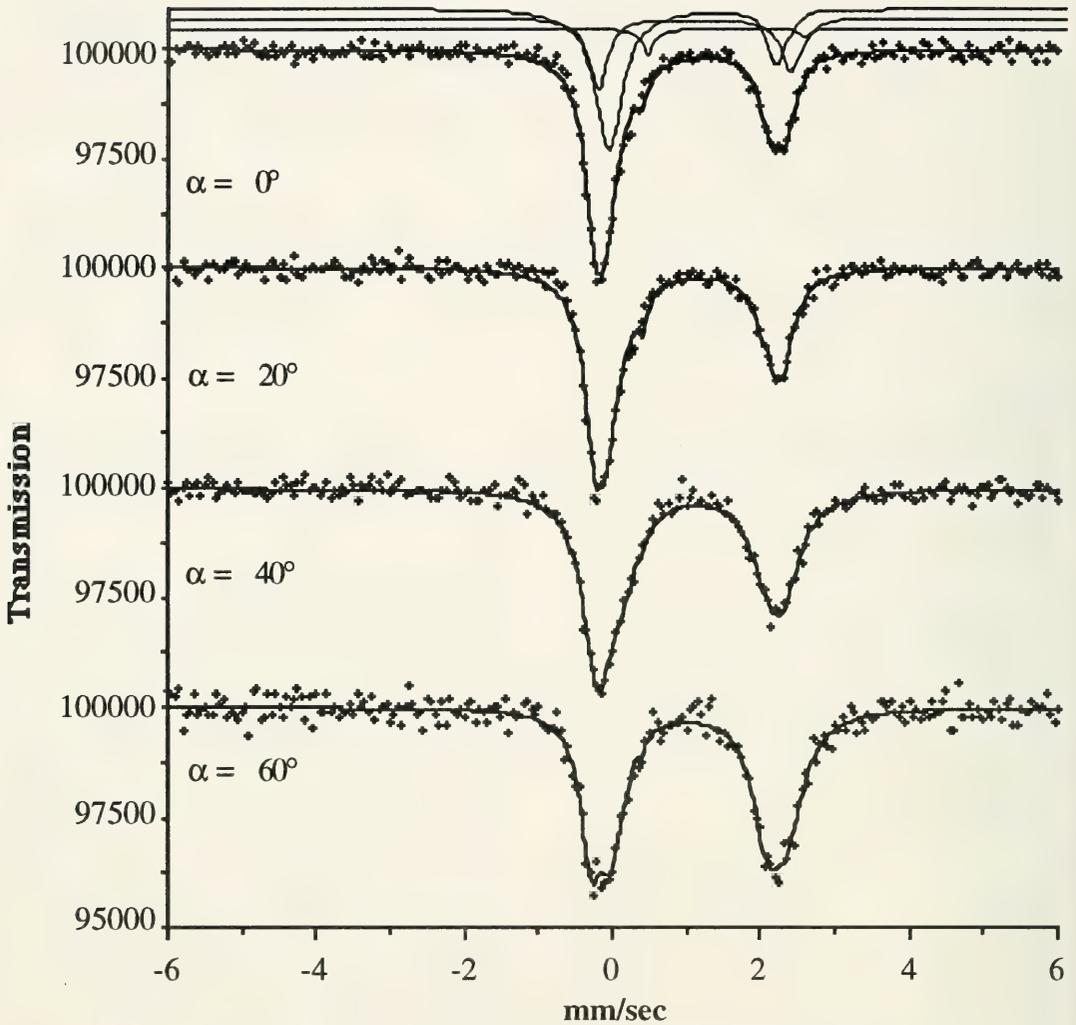
sume that the Fe^{3+} in the untreated phlogopite is too small to be statistically significant.

From Figure 9, it is clear that the sign of efg of the Fe^{3+} ion is opposite of that of Fe^{2+} ion.

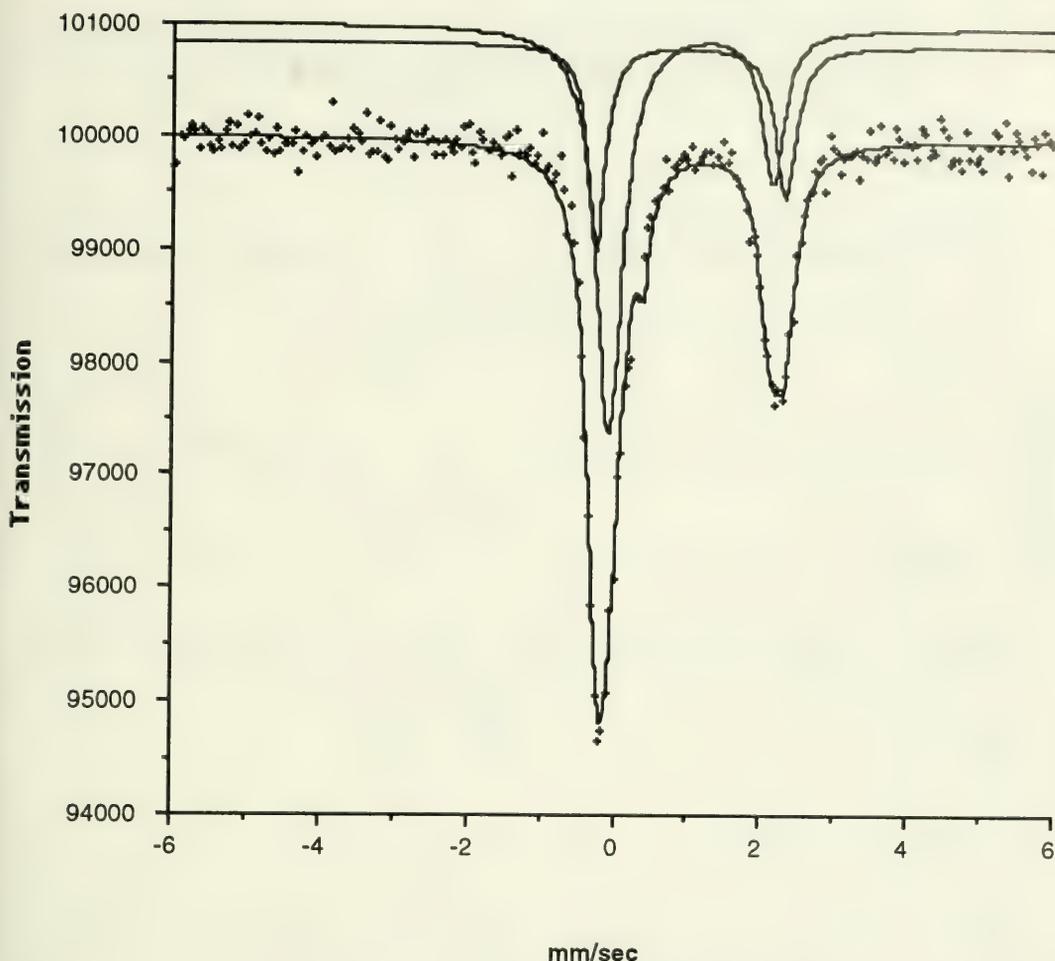
We did powder X-ray diffraction studies on the phlogopite before and after the heat treatment where the results were shown in Figure 10. By looking to the X-ray spectrum, it seems the heat treatment did not change the crystal structure significantly on the long range scale.

CONCLUSIONS

The sign of the electric field eq (V_{zz}) was found to be negative for Fe^{2+} in biotite and phlogopite and to be positive for Fe^{3+} in

FIG. 7. Mössbauer spectra of phlogopite at different orientation angle α .TABLE 2. Summary of the results of the fitting routine to phlogopite. Δ is the quadrupole splitting and δ is the isomer shift with respect to iron. M1 and M2 refer to two sites.

α	Rth	Fe^{2+}						Fe^{3+}			3+/2+ Ratio M2	Misfit (%)
		Rexp (M1)	Rexp (M2)	δ (mm/sec)		Δ (mm/sec)		Rexp (M2)	δ M2	Δ M2		
				M1	M2	M1	M2					
0°	3.00	2.71	2.69	1.01	1.04	2.24	2.59	0.20	0.26	0.30	0.07	0.339
20°	2.40	2.09	2.39	1.04	1.03	2.13	2.54	0.21	0.26	0.30	-0.09	0.456
40°	1.47	1.33	1.42	1.07	1.04	1.93	2.49	0.62	0.26	0.30	0.04	0.388
60°	0.88	1.02	1.03	1.02	1.02	2.63	2.10	0.70	0.26	0.30	0.06	0.159

FIG. 8. Phlogopite spectra at $\alpha = 0^\circ$ fitted with only two doublets.

phlogopite. Negative quadrupole interaction results in the $\pm(1/2)$ energy level being higher than the $\pm(3/2)$ level which is consistent with the present understanding in the literature.

We were able to get a good fit of the data of the biotite with only two doublets which we attribute to Fe^{2+} on the M1 and M2 sites re-

spectively. We saw no evidence of Fe^{3+} in our biotite sample. This is also consistent with most results in the literature.

We measured the gamma-ray absorption as a function of thickness for both biotite and phlogopite in order to determine the appropriate size (Fig. 3 and 6). We found the app-

TABLE 3. Summary of the results of the fitting routine to the annealed phlogopite. Δ is the quadrupole splitting and δ is the isomer shift with respect to iron metal. This is interpreted as Fe^{3+} at both sites.

α	Rth	Rexp (M1)	Rexp (M2)	RHWHM		δ (mm/sec)		Δ (mm/sec)		Misfit (%)
				M1	M2	M1	M2	M1	M2	
0°	0.33	0.65	0.67	1.00	1.00	0.285	0.292	0.859	0.559	0.386
20°	0.42	0.69	0.70	1.00	1.00	0.271	0.302	0.877	0.577	0.677
40°	0.68	0.89	0.90	1.00	1.00	0.280	0.292	0.863	0.551	0.551
60°	1.14	1.12	1.16	1.00	1.00	0.295	0.307	0.882	0.552	0.439

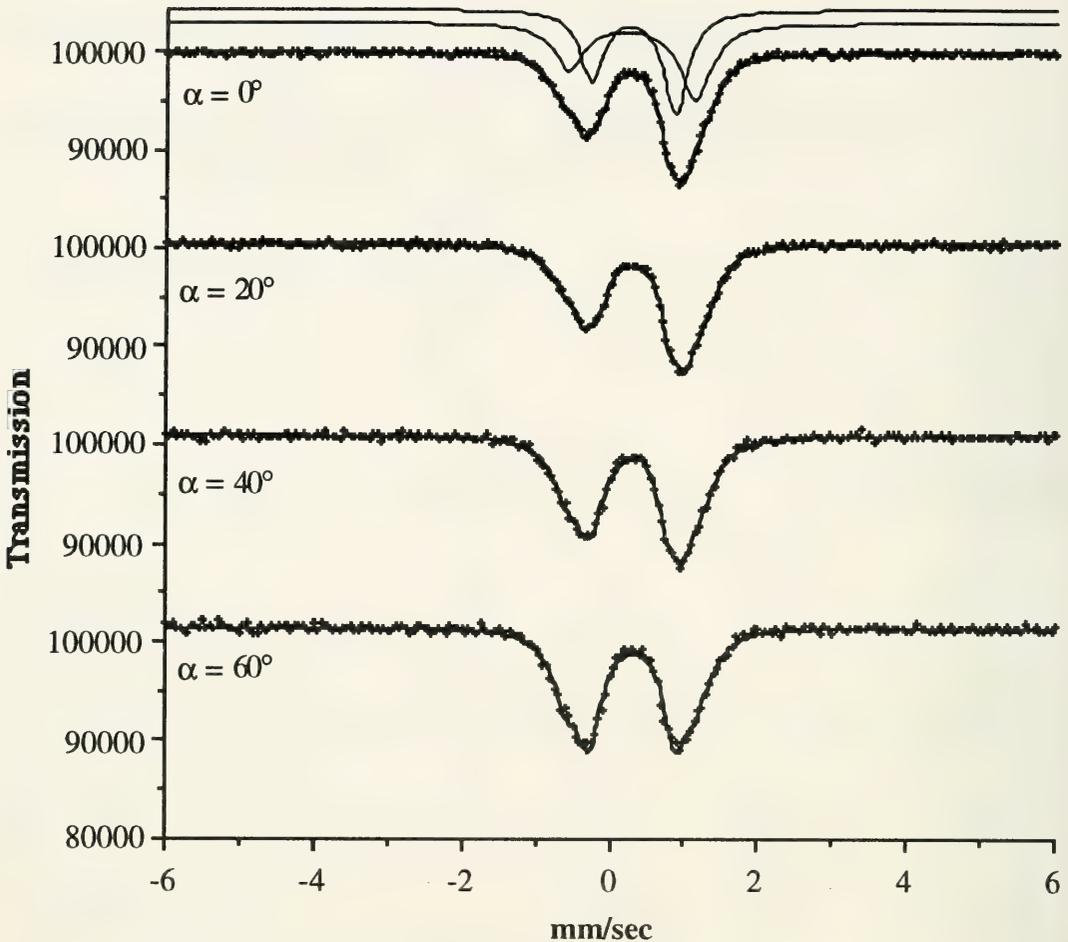


FIG. 9. Mössbauer spectra of annealed phlogopite at different orientation angle α .

appropriate range is of the order of 10-to-30 mg/cm².

The isomer shift and the quadrupole splitting values of Fe²⁺ for both samples are consistent with those found in the literature (Tables 1 and 2). The corresponding values for Fe³⁺ are not consistent.

We believe that the amount of Fe³⁺ in our original (unannealed) phlogopite was not enough to be detected. If there had been enough Fe³⁺ present, the quadrupole splitting values for Fe³⁺ would have been enhanced while the positions of its doublets would have stayed in the same channel numbers. Instead, the quadrupole splitting values and their positions of the doublets were different from those of the Fe³⁺ in the unannealed phlogopite.

The question about the existence of Fe³⁺ with different coordination, namely [4] and [6], probably can be resolved if the experiment could be done at liquid helium temperatures which might separate the Fe²⁺ and Fe³⁺ doublets further through the magnetic phase transitions.

We believe this technique can be applied to a variety of iron-containing samples to find the true oxidation states of the iron.

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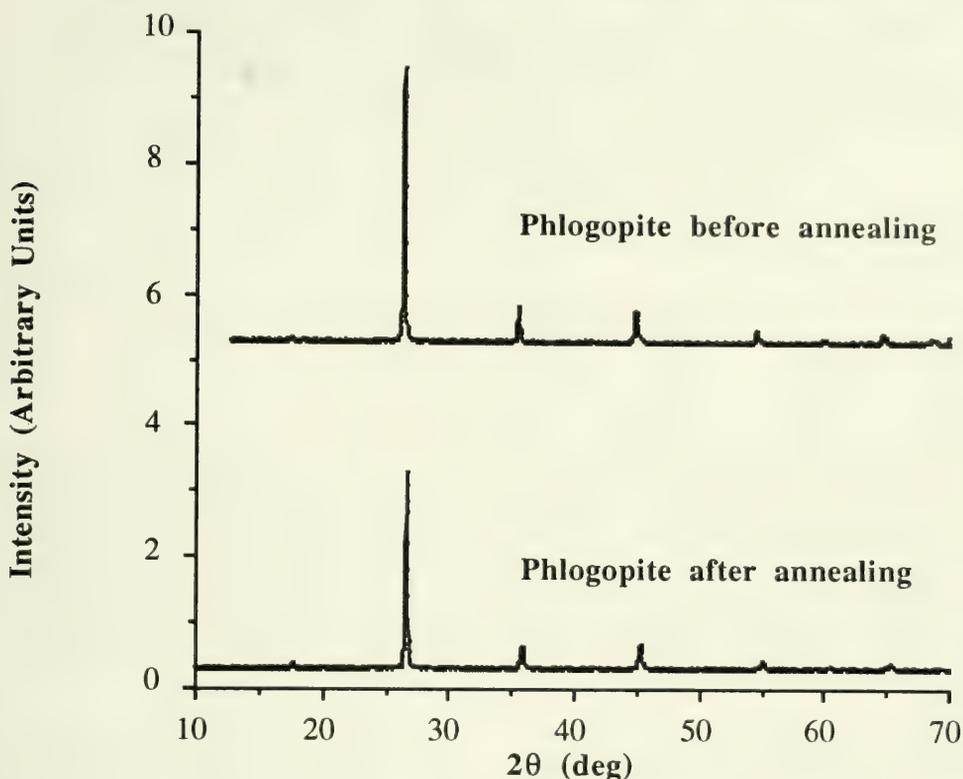


FIG. 10. Powder X-ray diffraction pattern observed for the phlogopite samples.

with the mica. We thank Dr. Glenn Julian from the Physics Department of Miami University in Oxford, Ohio; Dr. S. Jha from the Physics Department at the University of Cincinnati, Cincinnati Ohio; Dr. R. A. Dunlap, the Department of Physics at Dalhousie University, Halifax, Nova Scotia, Canada; and Dr. Smith T. Powell III, Berea College, Berea, Kentucky for their useful discussions.

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Nematodes Found in the Opossum (*Didelphis virginiana*) and Four Other Species of Mammals in Central Kentucky in 1991

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ABSTRACT

Opossums (n = 50), striped skunks (n = 3), eastern cottontail rabbits (n = 2), muskrats (n = 2), and a coyote, collected on horse farms (n = 14) in central Kentucky in the fall of 1991, were examined for internal helminths. All parasites (nematodes, cestodes, and trematodes) recovered were counted. Only nematodes were identified. Species of nematodes recovered from opossums were *Capillaria* spp., *Physaloptera turgida*, *Strongyloides* spp., *Longistriata didelphis*, *Viannia viannai*, and *Cruzia americana*; striped skunks were *Gongylonema* spp., *Physaloptera maxillaris*, *Baylisascaris columbaris*, and *Strongyloides* spp.; eastern cottontail rabbits were *Obeliscoides cuniculi*, *Trichostrongylus calcaratus*, and *Trichuris leporis*; muskrats were *Physaloptera* spp. and *Trichostrongylus* spp.; the coyote (only small intestine examined) were *Molinueus barbatus*, and *Uncinaria stenocephala*.

INTRODUCTION

Several species of wild mammals were collected in central Kentucky in 1991 on farms where leptospirosis had been found in horses. The purpose was to determine if they were carriers for this disease in horses. Collecting the mammals provided an opportunity to examine some of them for internal parasites. Mostly opossums, and lesser numbers of skunks, eastern cottontail rabbits, muskrats, and a coyote, were examined for helminths. Total counts were made for all parasites recovered, but only nematodes were identified.

MATERIALS AND METHODS

A total of 50 opossums (*Didelphis virginiana*), 3 striped skunks (*Mephitis mephitis*), 2 eastern cottontail rabbits (*Sylvilagus floridanus*), 2 muskrats (*Ondatra zibethica*), and 1 coyote (*Canis latrans*) were collected from 14 horse farms in central Kentucky between November 8 and December 12, 1991 for parasitologic examination. They were trapped by personnel from the Southeastern Cooperative Wildlife Disease Study, University of Georgia. The age/sex categories were: opossums—38 adults and 12 juveniles/26 males and 24 females; skunks—2 adults and 1 juvenile/1 male,

1 female, and 1 sex not recorded; eastern cottontail rabbits—2 adults/1 male and 1 female; muskrats—1 adult and 1 juvenile/2 females; coyote—1 adult male.

Organs examined for parasites were: lungs (including the trachea), liver, esophagus, stomach, small intestine, and large intestine. Snips of the lungs, liver, and esophagus were removed for other researchers before a parasitologic examination was done. Therefore, some parasite specimens from these tissues, which were examined in fresh condition for parasites with a stereoscopic microscope (at about 30 \times), may have been "lost." The gastrointestinal tract was separated into stomach, small intestine, and large intestine, including cecum. Contents were emptied into separate containers and then each segment was washed several times with water. Rinses were added to the appropriate container of contents and this material was preserved with 5% formalin. The gastrointestinal tract was then opened with scissors and any parasites observed by gross examination were removed. Next, each portion of the gastrointestinal tract was placed in containers of artificial digestive juice (1% pepsin and 1% HCl in an incubator at 37 $^{\circ}$ C) for about 3 hours; then, the mucosal side was

TABLE 1. Data on internal parasites recovered from opossums in a survey in central Kentucky.

Parasites	No. of parasites			Opossums (n = 50)	
	Range	Mean*	Total	No. infected	(%) infected
Nematodes					
<i>Capillaria</i> spp.**					
Lungs					
M	—	3.0	3	1	(2)
F	0-7***	2.1	46	22	(44)
M&F	0-7	2.2	49	22	(44)
G.i. tract					
M	0-8	2.1	30	14	(28)
F	0-11	3.1	68	22	(44)
M&F	0-11	3.9	98	25	(50)
<i>Physaloptera turgida</i> ****					
Imm	2-4,300	474.8	23,265	49	(100)
M	3-53	18.4	899	49	(100)
F	2-79	20.0	978	49	(100)
M&F	6-131	38.3	1,877	49	(100)
Total	10-4,314	518.4	25,918	50	(100)
<i>Strongyloides</i> spp.					
	0-42	15.0	45	3	(6)
<i>Longistriata didelphis</i>					
M	0-23	4.7	85	18	(36)
<i>Longistriata/Viannaia</i>					
F	0-21	7.3	198	27	(54)
<i>Viannaia viannai</i>					
M	0-2	1.7	5	3	(6)
<i>Cruzia americana</i>					
Imm	0-1,800	63.1	2,271	36	(72)
M	0-242	62.7	2,821	45	(90)
F	0-305	75.9	3,568	47	(94)
M&F	0-547	135.9	6,389	48	(96)
Total	0-1,804	180.4	8,660	48	(96)
Imm-Unidentified	0-39	6.8	129	19	(38)
Total Nematodes	12-6,119	703.7	35,187	50	(100)
Cestodes					
	0-510	114.4	1,487	13	(26)
Trematodes					
	0-421	69.1	2,419	35	(70)

* Mean = value for infected opossums; ** *Capillaria* spp.—from lungs, probably *C. didelphis* and from gastrointestinal tract, probably *C. longicauda*; *** Sex of one specimen not determined, but included data with females; **** Includes 776 from one opossum for which only total number counted; M = male; F = female; Imm = Immature.

washed under running water while being rubbed by hand to remove loosened material. This material and the digest were preserved with 5% formalin; the remainder of the walls was discarded. Digestion was done to free parasites associated with the walls and mucus. For the single coyote collected, only the small intestine was examined for parasites.

The fixed contents, water rinses, and digests from the gastrointestinal tract were washed into a series of sieves (10, 20, 40, 60, and 100 mesh). Residue was examined for helminths with the aid of a stereoscopic microscope at about 30 \times . Total counts of all helminths were done. Nematodes were identified by usage of

various reference publications (1-29). Cestodes and trematodes were not identified.

RESULTS AND DISCUSSION

For the opossums (Table 1), the 2 most prevalent species of nematodes were *Physaloptera turgida* (100%) and *Cruzia americana* (96%). The high prevalence of both of these species is similar to that reported by several other investigators (4, 11, 18, 30-32). Numbers of male versus female specimens per infected animal ranged (mean number/infected opossum) from 3 to 53 (18.4) and 2 to 79 (20), respectively, for *P. turgida* and from 0 to 242 (62.7) and 0 to 305 (75.9), respectively, for *C.*

TABLE 2. Data on number of opossums infected with internal parasites relative to farm where collected.

Farm no.	No. exam.	Internal parasites									
		Nematodes									
		<i>Capillaria</i> spp.		<i>Physaloptera</i>	<i>Sides.</i> spp.	Im. Un-ID	<i>Longistriata</i> **	<i>Viannaia</i>	<i>Cruzia</i>	Cestodes	Trematodes
		Lungs	GI tract								
No. of opossums infected											
1	3	1	2	3	0	1	3	0	3	0	2
2	3	2	2	3	0	0	2	1	2	1	2
3	2	0	0	2	0	0	1	0	2	0	0
4	5	2	2	5	0	3	0	0	5	1	2
5	1	0	0	1	0	1	0	0	1	0	1
6	6	2	5	6	1	4	6	0	6	5	6
7	1	1	0	1	0	1	0	0	1	0	1
8	3	1	0	3	0	3	1	0	2	1	2
9	4	3	1	4	0	3	1	0	4	0	4
10	4	2	1	4	0	3	1	0	4	0	0
11	1	0	0	1	0	0	1	0	1	0	1
12	3	2	0	3	0	0	0	0	3	0	3
13	8	2	7	8	1	0	6	2	8	1	6
14	6	4	5	6	1	0	6	0	6	4	5
Total	50	22	25	50	3	19	28	3	48	13	35
(T) inf.		(44)	(50)	(100)	(6)	(38)	(56)	(6)	(96)	(26)	(70)

* Complete names—*Physaloptera turgida*; *Strongyloides* spp. Im. = Immature (Unidentified); *Longistriata didelphis*, *Viannaia viannai*, *Cruzia americana*.

***Longistriata didelphis* category includes *L. didelphis* males and *longistriata/Viannaia* females.

americana. Immature specimens (determined only by much smaller size than adults) varied in number (mean) from 2 to 4,300 (474.8) for *P. turgida* and 0 to 1,800 (63.1) for *C. americana*. The 2 next highest prevalent nematode species were *Longistriata/Viannaia* females (54%) and *Capillaria* spp. (probably *C. longicauda*) (50%) from the gastrointestinal tract. *Longistriata didelphis* males were found in 36% of the opossums. Two other reports indicated higher prevalence (18, 30) for *L. didelphis*. For *C. longicauda*, lower infection rates were found in 2 studies (4, 18) than for *Capillaria* spp. from the gastrointestinal tract in the present study. The number of male *L. didelphis* varied (mean) from 0 to 23 (4.7) and of female *Longistriata/Viannaia* from 0 to 21 (7.3). *Capillaria* spp. males vs. females were 0 to 8 (mean = 2.1) and 0 to 11 (3.1), respectively. Most of the *Longistriata/Viannaia* female specimens are probably *L. didelphis* because so few male *V. viannai* were found. The females were grouped together because of similarities in features. Other nematode species recovered (prevalence) were: from the lungs—*Capillaria* spp. (probably *C. didelphis*) (44%), and from the intestines—unidentified immatures (38%), *Strongyloides* spp. (6%), and *Viannaia viannai* (6%). Prevalency for

Capillaria didelphis was less in 1 study (32) and more in another (18) compared to that of *Capillaria* spp. in the lungs of opossums currently investigated. *Strongyloides* spp. (21) and *Viannaia viannai* (14) are apparently uncommon in opossums. The highest total number of nematodes in an individual opossum in the present study was 6,119; it had the greatest number of immature *P. turgida* and *C. americana*.

Cestodes (0 to 510; mean = 114.4) were recovered from 26% of the opossums; trematodes were highly prevalent (70%). The number of specimens of trematodes varied from 0 to 421 (mean = 69.1) per opossum. Data on the number of opossums infected with internal parasites on each farm are recorded (Table 2). Prevalences of the various species or types of parasites were highest on Farm No. 6.

The 3 skunks examined (Table 3) were all infected with the nematodes *Physaloptera maxillaris*, *Baylisascaris columnaris*, and *Strongyloides* spp.; *Gongylonema* spp. were found in 1 skunk. Cestodes were present in all 3 individuals, but trematodes in none. Prevalence of *P. maxillaris* in several other studies varied greatly (16, 33-35). *Baylisascaris columnaris* were much more prevalent in 2 other studies (34, 35). *Strongyloides* spp. were

TABLE 3. Internal parasites recovered from skunks, eastern cottontail rabbits, muskrats, and a coyote.

Parasites	No. of parasites			Animals infected	
	Range	Mean*	Total	No.	(%)
Skunks (n = 3)					
Nematodes					
<i>Gongylonema</i> spp.					
M	—	1	1	1	(33)
F	—	1	1	1	(33)
M&F	1-1	2	2	1	(33)
<i>Physaloptera maxillaris</i>					
Imm	295-1,828	921.67	2,765	3	(100)
M	30-53	39.00	117	3	(100)
F	71-97	80.00	240	3	(100)
M&F	102-150	119.00	357	3	(100)
Total	400-1,978	1,040.67	3,122	3	(100)
<i>Balisascaris columnaris</i>					
M	1-18	7.00	21	3	(100)
F	3-9	6.67	20	3	(100)
M&F	4-27	13.67	41	3	(100)
<i>Strongyloides</i> spp.					
	1-93	38.33	115	3	(100)
Cestodes					
	10-92	39.67	119	3	(100)
Eastern cottontail rabbits (n = 2)					
<i>Obeliscoides cuniculi</i>					
M	3-4	3.50	7	2	(100)
F	1-1	1.00	2	2	(100)
M&F	4-5	4.50	9	2	(100)
<i>Trichostrongylus calcaratus</i>					
M	2-2	2.00	4	2	(100)
F	4-6	5.00	10	2	(100)
M&F	6-8	7.00	14	2	(100)
<i>Trichuris leporis</i>					
M	—	1.00	1	1	(50)
Cestodes					
	—	3.00	3	1	(50)
Trematodes					
	—	2,570	2,570	1	(50)
Muskrats (n = 2)					
Nematodes					
<i>Physaloptera</i> spp.					
(Imm)	1-1	1.00	2	2	(100)
<i>Trichostrongylus</i> spp.					
M	—	3.00	3	1	(50)
F	—	4.00	4	1	(50)
M&F	3-4	3.50	7	1	(50)
Cestodes					
	—	2.00	2	1	(50)
Trematodes					
	37-3,758	189.75	3,795	2	(100)
Coyote (n = 1)					
<i>Molineus barbatus</i>					
M	—	3.00	3	1	(100)
F	—	5.00	5	1	(100)
M&F	3-5	8.00	8	1	(100)
<i>Uncinaria stenocephala</i>					
M	—	6.00	6	1	(100)
F	—	2.00	2	1	(100)
M&F	2-6	8.00	8	1	(100)
Cestodes					
	—	39.00	39	1	(100)

* Mean = value for infected animals.

Imm = Immature; M = male; F = female.

found to be less prevalent by 2 researchers (34, 36). *Gongylonema longispiculum* were of low prevalence in 1 study (36).

For both cottontail rabbits examined (Table 3), 2 nematode species, *Trichostrongylus calcaratus* and *Obeliscoides cuniculi*, were recovered. One rabbit was also infected with *Trichuris leporis*. Cestodes and trematodes were present in 1 rabbit. Four other reports (2, 17, 31, 37) indicated greatly variable prevalence of *O. cuniculi* and *T. calcaratus* in this species of rabbit. For *T. leporis*, 3 investigators reported (2, 17, 37) lower infection rates than in the current survey.

The 2 muskrats examined (Table 3) were infected with the nematodes, *Physaloptera* spp. (immature) and *Trichostrongylus* spp. In addition, cestodes were recovered from 1 muskrat and trematodes from both muskrats. *Physaloptera* spp. have been previously found (23). *Trichostrongylus calcaratus* were found in a few animals in 2 studies (5, 20).

In the small intestine of the coyote, the nematodes, *Molineus barbatus* and *Uncinaria stenocephala*, were present. Also, cestodes (not *Echinococcus* spp.), but no trematodes, were found. Both of the species of nematodes were evident previously in low numbers of coyotes (10, 24).

This survey of internal parasites gave an indication of species, prevalence, and numbers in some of the wild mammals in the central Kentucky area. Especially meaningful are data from opossums for which a much greater number were examined than for the other hosts. Also, further information was obtained for Kentucky, supplementing previous surveys for helminths in muskrats in Madison County (20) and in eastern cottontail rabbits in western Kentucky (37).

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Index Herbariorum Kentuckiensis III

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ABSTRACT

A survey conducted during the academic year 1994-1995 provides information on herbarium collections at 13 Kentucky institutions. Totals of approximately 243,000 vascular plant specimens and 2,700 nonvascular plant specimens are reported for the state.

INTRODUCTION

The objective of this study was to determine the current status of collections of vascular and nonvascular plants in the state. This is an update of previous surveys published by Lassetter (1) and Jones (2). In the earlier report, 119,000 specimens were reported from 12 herbaria, and in the latter study 187,000 specimens were reported from 15 institutional herbaria. For this update, a survey was conducted by mail and by phone. All known Kentucky herbaria at public and private institutions were included. Private collections were excluded. The following data were requested: name and address of collection, phone/fax/e-mail numbers, emphasis and special collections, loan and exchange policies, and names of curators. In addition, information was gathered on the status of data-basing efforts. We appreciate the cooperation of all who supplied information.

RESULTS

This survey presents information on 13 institutional herbaria. Totals of approximately 243,000 mounted vascular plant collections and 2,700 nonvascular plant collections are reported. Vascular plant collections at the University of Kentucky (two herbaria) and the University of Louisville number 101,000; at the 5 regional institutions there are about 122,000 specimens; collections at the 5 private colleges total approximately 20,000. The great majority of private college collections are at Berea College. More than half of nonvascular plant collections are at the University of Kentucky.

Data gathered in this survey are listed below. Acronyms (given in parenthesis after the institution) and general format are based on

Index Herbariorum (3). Proposed but unpublished acronyms are indicated by an asterisk.

BEREA: Herbarium of Berea College (BEREA), Biology Department, Charles Martin Hall Science Building, Rm 212B, Berea, KY 40404. 606-986-9341, ext. 6320. Fax: 606-986-4506, e-mail: Ralph-F.Thompson@Berea.Edu. Established about 1961. 15,000 vascular plant specimens. Special interest in Fabaceae and Campanulaceae of Kentucky, surface-mine flora, and general floristics of Kentucky. Collections of J. D. Pittillo, D. D. Taylor, and J. R. Abbott. On-going projects: floras of Madison and Laurel Counties. No loans, visitation open to qualified researchers. Ralph L. Thompson, Curator.

BOWLING GREEN: Herbarium of Western Kentucky University (WKU), Biology Department, Rm 218 Thompson Complex NW, Bowling Green, KY 42101. 502-745-6008. Fax: 502-745-6856. e-mail: murreze@wkuvl.wku.edu. Established in 1967. 15,000 vascular plant specimens, teaching collection of 600 fungi. Special interests in *Calycanthus*, *Cornus*, molecular and morphological data in understanding the evolutionary history of plants, and local and pathogenic fungi. Collections of E. O. Beal, R. Athey, and G. Johnson. On-going projects: Systematics of *Cornus*, variability in the internal transcribed spacer region (ITS) of nuclear ribosomal DNA, demographics of *Spiraea virginiana*. Standard loan and exchange policies. Kenneth A. Nicely, Zack E. Murrell, Jeff Jenkins, Curators.

CAMPBELLSVILLE: Biological Collection, Science Building Rm 101, Campbellsville College, Campbellsville, KY 42718. 502-465-8158. Established in 1965. About 1,000 vascular plant specimens. Flora of Taylor County and

surrounding counties. Exchanges and loans available. G. Weddle, M. Rogers, Curators.

GEORGETOWN: Herbarium of Georgetown College, Department of Biological Sciences, Georgetown, KY 40324. 502-863-8085. e-mail: brafai1@gtc.georgetown.ky.us.edu. Established in 1945. 1,000 vascular plant specimens. General floristics. Standard loan and exchange policies. Barbara Raffail, Curator.

HIGHLAND HEIGHTS, Herbarium of Northern Kentucky University (KNK), Department of Biological Sciences, Highland Heights, KY 41099. 606-572-6390. Fax: 606-572-5639. Established in 1973. 26,500 vascular plant specimens. Special interests and current research in *Carex*, Poaceae, cultivated plants, general flora. Standard loan and exchange policies. Robert F. C. Naczi, John W. Thieret, Curators.

LEXINGTON: Herbarium, College of Agriculture, Department of Agronomy, University of Kentucky, Agricultural Science Building North Rm A-4, Lexington, KY 40546. 606-257-4898. Fax: 606-257-2185. e-mail: jgreen@ca.uky.edu. Established about 1887. About 21,000 vascular plant specimens. Emphasis on agricultural weeds of Kentucky. Many specimens of Harrison Garman and Mary Didlake from 1896–1930. A public service collection, providing identifications of specimens sent to the College. No exchange or loans; visitors welcome. J. D. Green, Curator.

LEXINGTON: Herbarium of the University of Kentucky (KY), Thomas Poe Cooper Building, Department of Forestry, Lexington, KY 40546. 606-257-7596. Fax: 606-323-1031. e-mail: for121@ukcc.uky.edu. Reestablished in 1948 after fire destroyed the previous collection. Estimated 50,000 vascular plant specimens, 1,500 bryophytes. Flora of the Bluegrass region and general floristics of Kentucky. Collections of Mary Wharton and various county floras. Standard loan and exchange policies. Robert Paratley, Curator.

LOUISVILLE: Davies Herbarium, Department of Biology, University of Louisville (DHL), Louisville, KY 40292. 502-852-5940. Fax: 502-852-0725. e-mail: wsdavi01@ulkyvm.louisville.edu. Established in 1953. 30,000 vas-

cular plant specimens. Floristics of nearby counties and natural areas. Research collections of *Malacothrix* (Asteraceae). Standard loan and exchange policies. W. S. Davis, Curator.

MOREHEAD, Herbarium of Morehead State University (MOKY*), Lappin Hall, Room 306, Morehead, KY 40351. 606-783-2947. Fax: 606-783-5002. e-mail: h.setter@msuacad.morehead-st.edu. Established in 1930s. 12,000 vascular specimens, 455 bryophytes. Data-base project on-going. Flora of Eastern Kentucky, Rowan County and adjacent counties, Neotropical Tiliaceae. Fern collections of T. McCoy. Current research on local wetlands and flora of Rowan County. Standard loan and exchange policies. Howard L. Setser, Curator.

MURRAY, Herbarium of Murray State University (MUR), Department of Biological Sciences, Blackburn Science Building, 5th floor, Murray, KY 42071. 502-762-2786. Established in 1967. 35,000 vascular plant specimens; 600 nonvascular plants. Data-base project on-going. Special interest and current research in flora of the Jackson Purchase. Fern collections of T. McCoy, and recently acquired the Athey Herbarium, formerly located in Paducah, and many Athey specimens from Memphis State University. Standard loan and exchange policies. Marian J. Fuller, Curator.

RICHMOND: Herbarium of Eastern Kentucky University (EKY), Department of Biological Sciences, Memorial Science Rm 170, Richmond, KY 40475. 606-622-6257. Fax: 606-622-1020. e-mail: bioclark@acs.eku.edu. Established in 1974. 33,000 vascular plant specimens; 500 bryophytes. Special interests and current research in woody plants, wetland flora, Asteraceae, Aquifoliaceae, regional county floras, rare species studies, statewide generic treatments, and bryophytes of Kentucky. Recent acquisitions include a set of Mary Wharton collections and specimens from the Kentucky State Nature Preserves Commission. Sets of specimens from several natural areas and local county floras. Interested in exchanges of vascular plants and bryophytes of southeastern United States. Standard loan policies. Ronald L. Jones, Ross C. Clark, and David A. Eakin, Curators.

WILLIAMSBURG: Herbarium of Cumberland College, Biology Department, Science Building 116, Williamsburg, KY 40769. 606-539-4399. Fax: 606-539-4490. e-mail: tyetter@cc.cumber.edu. Established in 1984. 2,000 vascular plant specimens. Special interest in Lamiaceae. Standard loans and exchange policies. Todd Yetter, Curator.

WILMORE: Herbarium, Department of Biology, Asbury College, Wilmore, KY 40390. 606-858-3511, ext. 2233. Fax: 606-858-3921. Established in 1967. 350 vascular plant specimens. Herbaceous flora of Jessamine County. No exchange or loans. John Brushaber, Curator.

DISCUSSION

The current estimated total of 243,000 vascular plant specimens held in 13 Kentucky institutions represents an increase of 56,000 specimens in the past 8 years. About 70% of this increase occurred at the regional public universities. It should be noted that some of this increase was due to the incorporation of old collections, i.e., Wharton and Athey specimens, and therefore not all was the result of new collecting. The number of institutions with herbaria has decreased by two since the previous survey. In addition, with collections now at West Virginia University (S. Studlar, pers. comm.), there is no longer a bryophyte herbarium at Centre College in Danville, and the number of nonvascular plant collections in the state has decreased by 50%.

Other changes since the 1987 survey (2) are the presence of new curators at Western Kentucky University, Northern Kentucky University, Georgetown College, University of Kentucky (both herbaria), Richmond (2 additional curators), and Williamsburg. The KY Herbarium is being transferred from the School of Biological Sciences to the Department of Forestry. The EKY Herbarium recently has been moved into a larger remodeled facility; storage capacity has approximately doubled. Fax numbers and e-mail addresses are now generally available. The only active data-basing projects are at Morehead and Murray Universities, but several other curators plan to begin such projects in the future. The Kentucky State Nature Preserves Commission (502-573-2886) maintains a database for their rare plant records, as

well as a small reference collection. Discussions have already started among curators on the possibilities of sharing databases generated at different Kentucky herbaria over the Internet.

The total number of collections in Kentucky herbaria is still far below that of nearby states. For example the 1990 edition of *Index Herbariorum* (3) lists over 800,000 vascular plant specimens in 8 herbaria in Tennessee, over 259,000 in 8 herbaria in West Virginia, over 700,000 in 11 herbaria in Indiana, over 400,000 in 22 herbaria in Virginia, and over 1 million vascular plant specimens in 14 Ohio herbaria. The discrepancy is even greater for nonvascular collections. For example there are 22,500 mosses and liverworts at the University of Cincinnati (CINC) (J. Snider, pers. comm.), and 165,000 nonvascular cryptograms at the University of Tennessee (TENN) (D. Smith, pers. comm.), compared to the 2,700 in Kentucky collections. There has been considerable bryophyte collecting in the state over the last two decades, but the majority of these collections have been deposited outside the state, particularly in CINC and TENN. There are also many Kentucky vascular plant specimens housed in other states—a list of herbaria with considerable Kentucky holdings was given by Jones (2). Obviously much collecting remains to be done in Kentucky, in particular, many counties in east-central and south-central Kentucky which have received little attention from collectors. There is a need to investigate these and many other areas in Kentucky in order to gain a better understanding of the presence and distribution of vascular and nonvascular plant species. An atlas of the state vascular flora is now a possibility in the foreseeable future and, perhaps soon thereafter, a manual. Relatively little is known about Kentucky's nonvascular plants, but with new studies of these plants now occurring at several institutions, available information is likely to grow rapidly.

In this survey, some curators expressed concerns about the future of their institutional collections. Others thought there should always be a place for even small research and teaching herbaria, especially when the value of these collections for varied data-gathering activities (from molecular to floristic to ecological to geographic) is recognized. Another com-

ment was that, if departments and institutions appreciate the critical role of organismal biology in broad biological training, then systematics and biological collections will continue to be valued.

In summary, this survey indicates to us that (1) active improvement of the documentation of Kentucky's flora is occurring, primarily by regional public institutions and private institutions which retain active systematists on their faculties; (2) Kentucky's flora remains much more poorly documented than that of most southeastern states; (3) Kentucky's collections of nonvascular plants are particularly depauperate; (4) there is an increasingly urgent need for additional, active documentation of Kentucky plants; and (5) factors and a cooperative spirit are emerging that could lead to significant improvement in coverage and in-

formation sharing in the near future. Of major significance is the fact that in the last 3 years 3 new systematic botanists have been added to the faculties of the public regional universities. All curators participating in this survey expressed a willingness to cooperate, contribute, and support our common goal of increasing our knowledge of the flora and vegetation of Kentucky.

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FORUM

Biodiversity and Kentucky's Heritage

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It is crucial to the future of our mental and physical health, and that of our species, that we decide what we think about biodiversity and what we will try to do about preserving or destroying it. Human progress has never been shaped by those who are comfortable with quick, simple answers, and it should not be otherwise today in Kentucky. Those who are willing to address the issue thoughtfully are those who should call the tune for the future.

Before European settlement, Kentucky was a place where resources were shared by all Indian tribes close enough to benefit—not the domain of any single tribe. The current status of Kentucky as a commonwealth implies the same thing: Kentucky's natural resources should benefit all its citizens. Even though our heritage of English common law permits us to hold title to land and its resources and to do largely what we please with them, it is ecologically impossible to own anything in this world. From a functional viewpoint, we simply use what is in this world to survive and reproduce, and then pass out of the picture. We do not own the world. It owns us.

Money is an artificial and very incomplete currency. Leaving aside societal values, the only real currency in the world—the only things of fundamental value—are the energy and nutrients that support the metabolism of organisms, including ourselves. Modern civilization uses money to value the extraction and circulation of resources, but does not use money to value the end products of resources. If we were consistent about how we use money, we would use it to value all aspects of resource extraction and use, including the integrity of the atmosphere, water resources and habitat alteration and loss.

But we do not live in a world where the use of natural resources is valued completely. Our species is raping the world, and every one of us is participating. All of us use more than we

need. None of us recycles our waste products so that all of them can be reused by someone or something else.

What does this have to do with biodiversity? Biodiversity is an issue because the activities of our species threaten it. What does it matter? Why should we care? There are two fundamental reasons why we should care. One reason is practical and one is ethical.

The practical reason why we should care about preserving biodiversity is that we are the product of diverse ecosystems. A couple of years ago, I asked students in one of my classes to write down where they would live and what their surroundings would be like, if they could live anywhere in the world they desired. Every person wrote that he or she wanted to live simply in the country, with trees, wild animals, clean air and clean water.

Those students didn't choose their preferred surroundings by accident. For more than 99% of humans' existence on earth, we lived in general equilibrium with the ecosystems we inhabited. We have practiced agriculture and lived in large settlements for only 15,000 years out of the 2+ million years our genus has existed. The makeup of our brains was not shaped in cities. It was shaped in nature. Biologically, our real home is nature, not towns and cities. It is not coincidental that we need diversity of landscape, diversity of habitat, diversity of plants and animals around us to be mentally healthy individuals and cultures. The main values to us of tropical rainforest or mixed mesophytic forest are not the economic products or medicines they may contain. *The most fundamental value of intact ecosystems and their organisms is that without them, we do not know who we are.* Without a connection to nature we cannot actually feel, we are strangers in a strange land. We should not have to lose our connection with nature to discover the obvious truth.

The second main reason we should care

about biodiversity is ethical. Ethics is the property of being positively influenced by the needs of others, of keeping the welfare of others in mind. Whether one is ethical or not is not as fundamental as where our natural home is. We cannot choose, and no one can choose for us, whether or not we are children of nature; we just are, because it happened that way. One *can* choose whether or not to be ethical; or, whether we feel responsibility toward others may be determined by how we are raised, or by what happens to us during our lives. Ethical people and societies will feel some responsibility toward the needs of other species that share the earth. If we are conscientious, we will try to live lightly on the earth, so that other species also will experience a decent quality of life. They did not choose to be here with us. They are at our mercy. If we are ethical, we will save them a share of a decent living. If we are not, we will not care whether they exist or not, or will always place what we want above their well-being. If we are ethical, we will actively preserve diversity in ecosystems which all organisms require to get their food and raise their young.

What does all this mean to people who say "no one has the right to tell me what I can do with my land?" What does it mean to the corporation claiming a right to pollute with impunity because it gives people jobs, or the corporation claiming the right to destroy thousands of acres of diverse forest to make chipboard or run a biomass electrical generating station?

For Kentucky, the answer should be clear. Has Kentucky forgotten how the timber barons from the northeast gave its citizens poorly paid temporary jobs and shipped its magnificent forests (and most of the money) somewhere else? Has Kentucky forgotten how the coal barons did the same thing with its coal, leaving the State with a legacy of black lung, dead streams, ruined property and poor people? How many times must we see the process repeated to know that people "doing whatever they want to with their land" is an idea that doesn't benefit the Commonwealth in the long run?

This Commonwealth is responsible for the welfare of its people. Public officials are elected and appointed to serve that welfare, not to serve their own interests. We don't live as is-

lands; we live together. When the common good must be served, private property "rights" must give way. Those "rights" are not inalienable. They were invented by humans. For the common good, traditional rights are often limited by humans for the benefit and future of all, including other species. If individuals are ecologically unethical, then state regulations can help to impose ethics. Bad habits are hard to break, but for the good of all, Kentucky must change.

Independence, an admirable quality, is the only thing some Kentuckians have left to be proud of. What would help persuade people that it is in their own best interest to give up some of that independence? Perhaps more people would be willing to give up some independence if they could feel worthwhile some other way, if they could see the hope of a better future, of assured, sustainable jobs that did not require the destruction of the landscape which they value. Kentuckians say they are proud of their state. They love the mountains, lakes and rivers. What if they could sell that pride to outsiders and make a decent living doing it? What if Kentucky once again got serious about attracting tourists by repairing and upgrading its state park facilities? What if people could work in secondary timber industries, exporting finished products instead of sawlogs and chipboard? What if they could retrofit houses for energy conservation and solar assisted heating instead of burning whole forests to generate more electricity? What if state government could work very hard to attract nonpolluting industries, instead of plowing the same old eroded resource extraction-ecosystem destruction furrow?

But what about unskilled people who cannot handle sophisticated jobs? Consider South Carolina. About 30 years ago, South Carolina built a system of technical schools to give its citizens access to the real jobs of the future. When a major corporation considers locating to South Carolina, the tech schools sit down with company management and work carefully with the industry to design a special curriculum to train workers to work in that industry. The state and the people pay the training costs. By the time the industry has built its facility, there are plenty of well-trained workers available for the specific jobs the industry offers. That's how Spartanburg came to have

the highest concentration of foreign companies doing business of any location in the Southeast. It's also how South Carolina landed several Michelin facilities and Mercedes Benz. It's also how my stepson, without a college education, learned how to be a technical draftsman and worked his way up to being project manager in a major construction firm, making more money than I do. He loves his work. It's rewarding. He's proud of himself. He should be. South Carolina's vision made his success possible. The same thing could happen in Kentucky.

There were only two prerequisites for the South Carolina success story: people who are willing to work, and a government dedicated to their future. It took vision and serious investment in education on all levels.

With the constructive insistence of its enlightened citizens, more Kentuckians could someday be proud of something more than just being Kentuckians. Proud of a better, more livable environment; proud of better

jobs; proud of healthy ecosystems that remind visitors where all of us came from. Proud of a progressive government which anticipates the future. All these things are intertwined. You can't separate them from each other. To produce a success story, all these factors must be addressed at the same time for a sustained period. The reason we should work to create better jobs and lives in the midst of resolutely preserved biodiversity is because it is the right thing to do for ourselves and our children.

Does Kentucky have the vision and the guts to do what is right for the future of the people of this Commonwealth, or must we remain an increasingly degraded resource extraction colony for the rest of the nation? Will timber tide us over so coal can tide us over so Maxie Flats can tide us over so oil can tide us over so biomass and chipboard plants can tide us over so chicken processors can tide us over, or is there a better way?

Think, Kentucky. Think Kentucky. Long-term independence and pride come from having it all together and keeping it that way.

NEWS AND COMMENTS

ANNUAL MEETINGS

The annual meeting of the Kentucky Academy of Science for 1995 will be a joint affair with the Tennessee Academy of Science, 16, 17, 18 November, in Bowling Green, Kentucky. All members should make an effort to attend this forum.

The 1996 meeting will be at Kentucky State University in Frankfort, and the 1997 meeting will be at Morehead State University.

EDITOR'S ADIOS

It has been my pleasure to serve the Academy as Editor of the *Transactions* for the last 15 years or so, mostly to good purpose, I trust. But all good things, as the old saw goes, must come to an end. I would be remiss if I failed to acknowledge all the great help I have had from many people, including John Thieret, Vince DiNoto, Tom Green, Bob Naezi, and many others. And, of course, Varley E. Weideman who, through all those years, served faithfully as Index Editor. I wish our new Editor, John Thieret of Northern Kentucky University, good luck and peace in his duties.

And finally, since I have you as a captive audience, I wish to make a few parting com-

ments. During the last 30 years technological advancement has been nothing if not astounding. Yet, our attention to natural history has failed to keep pace. Those among us who have scant interest in organisms above the cellular and sub-cellular level tend to view ecologists, naturalists, macro-evolutionists, and population geneticists as relicts from some long-past era. Not true. It has become patently clear, at least to me and other field biologists, that if there is going to be any salvation of the natural world it will have to come from the camp of the naturalist, not the technological one. We cannot let our love affairs with machines cause an erosion of scientific insight into ecological matters. Machines like computers are fantastic in their abilities to urge along the thought processes that are involved in deducing from evidence, but they are little else. Computers can never replace contemplative consideration of the nature of things. We need well-educated naturalists today more than we have ever needed them in the history of science, but we are not educating enough of them. Not training—educating.

And all this from a life-long naturalist who is not apt to change his thought patterns—Branley Allan Branson.

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**Intersexual Differences in Feeding Frequency and
Prey Size in the Robber Fly *Promachus albifacies*
(Diptera: Asilidae): Possible Influence of
Male Mating Behavior**

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ABSTRACT

The robber fly *Promachus albifacies* (Williston) is an ambush aerial predator that feeds on a wide variety of insects. Late in the flight season, males spend most of the daytime on or near shrubs in which females oviposit. Males use the oviposition shrubs as mate encounter sites. We studied a population of *P. albifacies* in a desert grassland habitat in central New Mexico, testing the hypothesis that male occupation of oviposition shrubs constrains foraging. Results did not support the hypothesis. Late in the flight season, females did feed more frequently and on larger prey than did males. However, we observed no difference in feeding frequency or in prey size for males between early and late season, suggesting that intersexual differences in feeding can be attributed to factors other than constraints imposed by male mating behavior.

INTRODUCTION

Many field studies of robber flies described foraging behavior and cataloged the taxon and size of their insect prey. Several of these studies compared feeding frequencies and prey size of male with female flies; the patterns vary among species. In certain species, females feed more frequently than males (Lavigne 1979; Weeks and Hespenheide 1985); in others no intersexual difference in feeding frequencies has been observed. In some species, females capture larger prey (Scarborough 1979); in others, males capture larger prey (Lavigne 1979); and in others no intersexual difference in prey size is reported (Weeks and Hespenheide 1985). While most of these studies provided excellent documentation of asilid foraging, few offered explanations for observed intersexual differences (exceptions: Dennis 1979; Scarborough 1979), and none proposed and tested hypotheses attempting to account for the differences.

In many species of insects sexual selection has led to evolution of behaviors in males that enhance mating success relative to other males (Thornhill and Alcock 1983). Behavior evolved in the context of competition for mates may be in conflict with behavior that enhances survival (Gwynne 1987), including, conceivably, foraging behavior.

Late in the flight season male *Promachus albifacies* spend most of the daytime perched on or near shrubs in which females oviposit (Hastings et al. 1994). Males use these shrubs as mate encounter sites. However, these sites appear to be inferior foraging sites (Hastings et al. 1994). We therefore hypothesized that male occupation of these sites constrains their foraging. During the late season, males should feed less often and on lower quality (i.e., smaller) prey than do females. During the early season, males should feed more often and on larger prey than during the late season, as

males are less constrained by mating behavior during the early season.

MATERIALS AND METHODS

We studied a population of *P. albifacies* in central New Mexico between 20 May and 19 June 1993. The 18.4-ha study site is located on an alluvial fan at the north end of the Magdalena Mountains ca. 10 km east of Magdalena, Socorro County. The habitat is a desert grassland containing sparse staghorn cholla (*Opuntia arborescens*) and narrow leaf yucca (*Yucca elata*) within a matrix of short grasses, a variety of short annual plants, and bare soil.

Censusing Procedures

Female *P. albifacies* oviposit in cracks and small openings in woody tissues of dead yucca flowering stalks and dead cholla branches that are at least 1 m above the ground (Hastings et al. 1994). We term cholla and yucca plants containing such sites as "oviposition shrubs." We subdivided the study site into two areas: OS—oviposition shrubs and all land area within 1 m of an oviposition shrub, and OTHER—all area not within 1 m of an oviposition shrub. We counted the number of oviposition shrubs within the study site, estimated the average diameter of shrubs to be 1 m, and then calculated the percentage of the study site covered by the two area subdivisions to be 1.2% OS, 98.8% OTHER.

In 1993 the flight season of *P. albifacies* extended from 20 May to at least 25 June at the study site; few adults were alive on or later than 25 June. To determine daily activity and spatial distribution of the robber flies we conducted censuses each day from 26 to 31 May and from 4 to 19 June. Censuses conducted from 26 to 31 May are defined as early-season censuses; those conducted from 4 to 19 June are defined as late-season censuses. Censusing effort each day was constant between 0900 and 1600 MDT. During censusing we walked along a grid of lines parallel to a border of the study site and used visual and auditory cues to locate flies. Grid lines were spaced 20 m apart to minimize repeated observations of the same individuals. We recorded the location (OS vs OTHER) and the activity (perch, feed, mate, oviposit) of each fly. We defined feeding as a fly having a prey item in its possession. A fly

was considered to be perching if it was not engaged in any of the other activities.

Prey Size and Prey Taxon

We captured samples of feeding male and female *P. albifacies* during the early and late season. Dial calipers were used to measure body length of their insect prey to the nearest 0.05 mm. We grouped prey into size classes of 5 mm increments, and used Kolmogorov-Smirnov tests to compare the size-class distributions of prey captured by male vs female robber flies, and by males in the early vs the late season. We identified the order of each insect prey item retrieved from the robber flies.

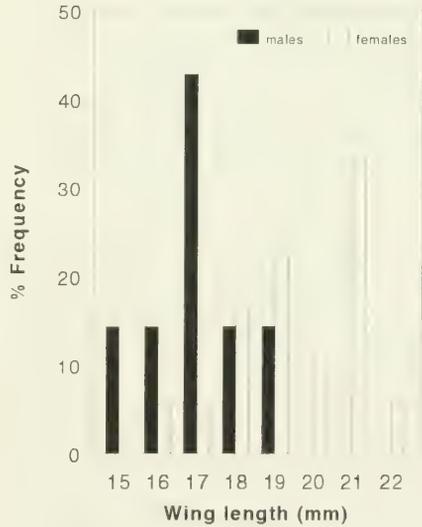
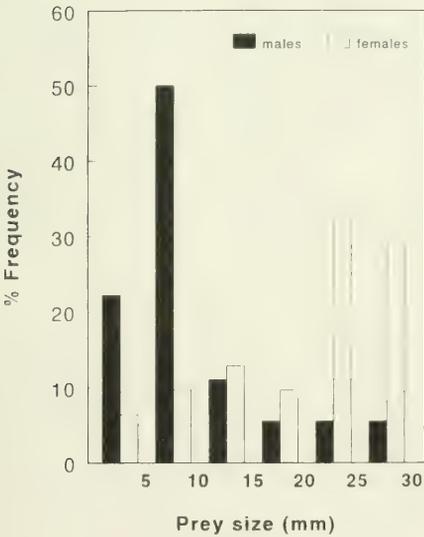
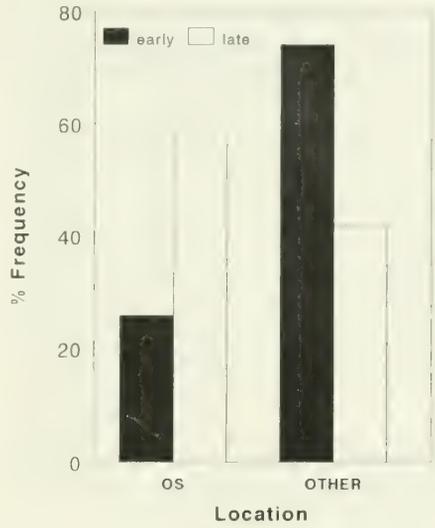
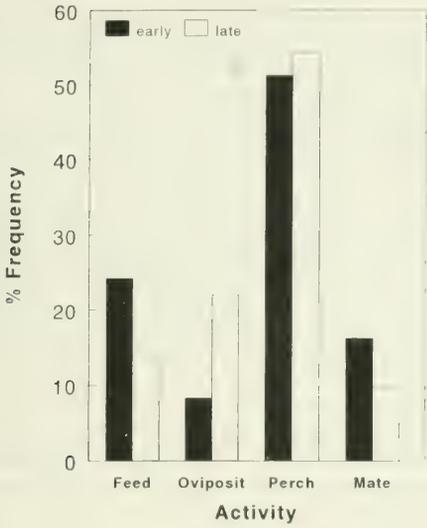
Intersexual Body Size Comparison

To estimate body size of flies in the field, we used dial calipers to measure the length of the right wing to the nearest 0.05 mm. Wing length and dry body mass are highly correlated in both sexes (Hastings et al. 1994). We measured the wing length of a random sample of male and female *P. albifacies* and used a student's t-test to compare the sample means.

RESULTS

As Hastings et al. (1994) previously reported, the activity of female *P. albifacies* varied between seasons (Chi-square = 28.9, $P < 0.0001$, $N = 536$) (Fig. 1). Ovipositioning frequency was higher during the late season (57 of 259 sightings of females) than it was during the early season (23 of 277 sightings). Also as Hastings et al. (1994) reported, we found that late season male spatial distribution was non-random (Chi-square = 569.7, $P < 0.0001$, $N = 735$). Despite the fact that OS area covered only 1.2% of the study site, 427 of the 735 sightings of males were in OS locations. Males were also nonrandomly distributed within the study site during the early season (Chi-square = 157.5, $P < 0.0001$, $N = 602$), but males were found less frequently in OS locations than they were in the late season (Chi-square = 139.4, $P < 0.0001$, $N = 1337$) (Fig. 2).

Though *P. albifacies* males and females feed on a wide variety of insects (Table 1), they feed largely on insects that forage on flowers; of the 134 Hymenoptera prey captured by males and females combined, 121 were bees (Apidae); of the 119 Diptera prey, 80 were bee



Figures 1–4. The robber fly *Promachus albifacies*. Fig. 1 (upper left). Percent frequency of females engaged in different activities during the early (N = 277) and during the late flight season (N = 259). Fig. 2 (upper right). Percent frequency of males sighted in OS (oviposition shrubs) vs OTHER locations within the habitat during early season (N = 602) and late season (N = 735) censuses. Fig. 3 (lower left). Percent frequency of size (total body length) classes of prey of males (N = 54) vs females (N = 31) captured during the late season. Fig. 4 (lower right). Percent frequency of size (wing length) classes of males (N = 78) vs females (N = 76).

flies (Bombyliidae). During the late season, females fed at a higher frequency than did males (Chi-square = 5.3, $P = 0.02$, N = 994). Of the 735 males sighted, 63 had prey in their possession; 36 of 259 females were observed

with prey. Prey size also varied between sexes during the late season (Kolmogorov-Smirnov = 0.56, $P < 0.0001$, male N = 54, female N = 31) (Fig. 3); females generally captured larger prey.

TABLE 1. Number and percent composition of prey by order taken by the robber fly *Promachus albifacies* for 1992 and 1993 in central New Mexico.

Order	Males		Females		Total	
	Number	Percent	Number	Percent	Number	Percent
Coleoptera	84	32.1	25	13.0	109	24.0
Diptera	55	21.0	64	33.3	119	26.2
Hemiptera	27	10.3	21	10.9	48	10.6
Homoptera	4	1.5	10	5.2	14	3.1
Hymenoptera	78	29.8	56	29.2	134	29.5
Lepidoptera	9	3.4	3	1.6	12	2.6
Neuroptera	2	0.8	3	1.6	5	1.1
Orthoptera	3	1.1	10	5.2	13	2.9
Totals	262		192		454	

Male feeding frequency did not vary significantly between seasons (Chi-square = 0.99, $P = 0.32$, $N = 1337$); of 602 late-season sightings of males, 63 had prey; 65 of 735 males censused during the early season had prey. Size of prey captured by males also did not vary significantly between seasons (Kolmogorov-Smirnov = 0.08, $P = 0.09$, late $N = 54$, early $N = 60$).

The mean (\pm SEM) wing length of a random sample of 76 female *P. albifacies* (18.05 ± 0.30 mm) was larger than the mean wing length of a random sample of 78 males (16.30 ± 0.20 mm; $t = 6.9473$, $P < 0.0001$) (Fig. 4).

DISCUSSION

We observed the same basic seasonal changes in activity of male and female *P. albifacies* that Hastings et al. (1994) reported. Ovipositioning frequency within the female population was higher in the late than in the early flight season. In the late season males spent more of the daytime within the relatively sparse OS locations than they spent at these sites early in the season when visits to them by females were relatively infrequent. Male perch selection, at least during the late season, appears to be largely driven by competition for mates.

Hastings and others (1994) reported that the OS locations were inferior foraging sites. This is consistent with our finding that *P. albifacies* of both sexes feed to a large extent on pollinating insects. During the flight season many plants within the habitat are in bloom, but the oviposition shrubs, which consist of dead yucca flower stalks or partly to completely dead cholla, are generally devoid of flowers.

Consequently, fewer preferred prey should be available in oviposition shrubs than in other locations. Therefore, we expected that male occupation of the shrubs would reduce the number and/or quality of prey available to them. However, our prediction that the increased male occupation of ovipositioning shrubs during the late season would constrain foraging was not supported by the results. The observation that male feeding frequency and prey quality did not differ between seasons suggests that male mating behavior does not constrain foraging.

If male mating behavior does not impose constraints on foraging, then what accounts for the observed intersexual differences in feeding frequency and prey size? In general, the eggs of female robbers flies are not mature when they eclose, and ovipositioning is delayed 10 to 11 days following emergence (Lavigne et al. 1978). Vitellogenesis, which continues throughout the adult life of females (Scarborough 1978) increases the nutritional demands of females over those of males (Scarborough 1979); females simply must feed more often and/or on larger prey. Shelly (1985) reported that, in general, larger robber flies capture and feed on larger prey. The fact that female *P. albifacies* are generally larger than males probably enables them to capture and feed on larger prey.

SUMMARY

During the late flight season, male *P. albifacies* spend most of the daytime on shrubs in which females oviposit. The shrubs, which males use as mate encounter sites, have previously been reported to be inferior foraging locations. We tested the hypothesis that male occupation of these shrubs would constrain their foraging. Results did not support the hypothesis. Though during the late season, females fed more often and on larger prey than did males, male foraging frequency and prey size did not vary between the early and the late season. This suggests that intersexual differences in foraging during the late season result from factors other than constraints imposed by male mating behavior. We suggest that the nutritional demands of vitellogenesis and the fact that females are generally larger than males may account for the observed in-

tersexual differences in foraging frequency and prey size.

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Effects of Wastewater Discharge from Houseboats on Water Quality in a Large Oligotrophic Reservoir, Lake Cumberland, Kentucky

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ABSTRACT

Effective water quality management depends on availability of site-specific data on nutrient inputs, outputs, and transport. We examined the potential importance of wastewater discharge from houseboats as a source of nutrient and organic matter loading to a large, oligotrophic reservoir (Lake Cumberland, Kentucky). Spatial variation in chlorophyll, nutrient, and dissolved oxygen concentrations were compared between areas differing in levels of houseboat activity. Upstream-downstream gradients in chlorophyll, transparency, and nutrient availability generally followed expected patterns based on longitudinal variations in flow velocity and water residence time. Consistently elevated total phosphorus (TP) levels were observed immediately downstream from a large houseboat marina complex. Although a corresponding increase in chlorophyll was not observed, higher rates of nitrate and silica depletion at this site suggested greater phytoplankton production. Overall, the data provided no compelling evidence of water quality deterioration in areas frequented by houseboats. The magnitude of nutrient and organic matter loading from direct wastewater inputs appeared to be small in relation to lake volume.

INTRODUCTION

Nutrient loading to surface waters is a widespread problem contributing to water quality deterioration in lakes, rivers, and estuaries. Nutrient inputs arise from agricultural runoff, industrial discharge, and municipal and domestic wastewater. Symptoms of excess nutrient loading include proliferation of certain kinds of algae and associated reductions in water clarity. Algal blooms and subsequent microbial decomposition promote high biological oxygen demand, which can lead to periodic fish kills. Although effects of eutrophication have been well documented through monitoring and experimental approaches (e.g., Edmondson 1972; Schindler 1974), site-specific information on nutrient sources and transport are often lacking. This limits the effectiveness of lake and watershed management plans for reducing nutrient inputs. We examined one potential source of nutrient inputs (wastewater discharge from houseboats) to a large reservoir where perceived changes in water quality have been of recent concern.

Lake Cumberland, a large (20,336 ha), oligotrophic reservoir in south-central Kentucky (Figure 1), was formed upon completion of Wolf Creek Dam on the Cumberland River in 1952. Its purpose was to provide hydroelectric power to parts of southern Kentucky and Ten-

nessee. In the last 20 years a thriving recreation industry has developed with tourists from nearby metropolitan centers (Louisville, Cincinnati, and Indianapolis) visiting the lake and providing income for resorts and marinas around the reservoir. The growing tourism industry is one factor that may pose a threat to water quality conditions in the lake. Particularly controversial has been the increase in numbers of houseboats (more than 500 in peak season) using the lake. Federal guidelines require that boats be outfitted with Marine Sanitation Devices to provide primary treatment of wastewater. Boaters are not required to hold chemically treated wastewater for subsequent processing at an on-shore secondary treatment facility. This has led to the common practice of flushing chemically treated wastewater directly into the lake.

In our study we examined spatial variations in water quality in relation to levels of houseboat activity. Wolf Creek embayment is an area frequently used by houseboats; Indian Hills Resort Marina and Alligator Dock are located along this embayment. Caney Creek, the other studied embayment, has no houseboat rental facilities and consequently has lower levels of houseboat activity. Movement of houseboats between the embayments is limited due to lack of adequate mooring sites in the Caney Creek embayment and to the long travel time resulting in high operational costs.

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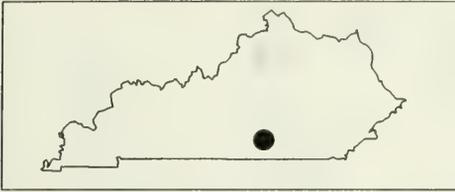


Figure 1. Outline map of Kentucky, showing location of Lake Cumberland.

We compared water quality conditions in Wolf Creek and Caney Creek embayments as an index of effects of houseboat activity. Measured parameters included algal abundance (as chlorophyll *a*), nutrient concentrations (total phosphorous, inorganic nitrogen, and dissolved silica), and bacterial activity (rates of hypolimnetic oxygen depletion). We hypothesized that nutrient and organic matter loading associated with houseboat discharge would result in elevated nutrient concentrations, increased algal growth, and increased bacterial activity. We predicted that chlorophyll concentrations, nutrient availability, and rates of oxygen depletion would be greater at the site experiencing more intense houseboat activity (Wolf Creek embayment).

SITE DESCRIPTION AND SAMPLING DESIGN

Lake Cumberland has a surface area of 20,336 ha, a mean depth of 24.2 m, and a maximum depth of 57 m; it exhibits strong thermal stratification from April through October. The lake's watershed (1,479,170 ha) is a mosaic of land-use types including 21% agricultural, 55% forestlands, and 3% urban areas (DEP 1984). Topography of the region is mountainous in the east, leveling to gentle plateau in the west; deep narrow valleys etched into the landscape form the reservoir's basin. Major inflows include the Cumberland River and its Big South Fork; minor contributors are numerous creeks throughout the length of the reservoir. The sole major outflow is the remaining Cumberland River below Wolf Creek Dam.

To characterize spatial variation in each of the embayments, we sampled three stations along a transect from the main channel of the lake toward the headwaters (Figure 2). The three Caney Creek stations and the furthest

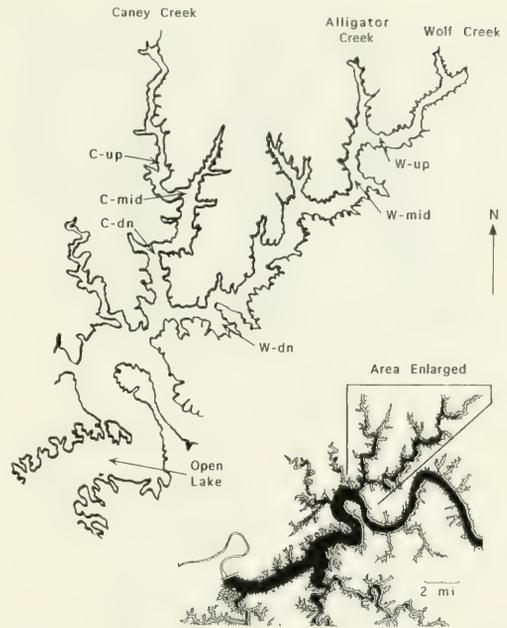


Figure 2. Map of Lake Cumberland, Kentucky, showing location of sampling sites in Wolf Creek embayment (W-up, W-mid, and W-dn), Caney Creek embayment (C-up, C-mid, and C-dn), and open lake (OL).

downstream Wolf Creek site (W-dn) are in undeveloped areas. The upstream and mid-embayment Wolf Creek stations (W-up and W-mid, respectively) are close to marinas with associated parking and recreation areas. Station W-mid is 100 m downstream from Indian Hill Resort Marina and directly across from a small shale beach on the west bank heavily used for swimming and recreation. Station W-up is 200 m upstream from Alligator Dock. Lake depth varies among sampling sites: from 10 m at the upstream stations to >25 m at sites further downstream.

Samples were collected also at an open-lake (main channel) site to assess differences in water quality relative to the embayments. The sampling site in the main body of the lake (at Cumberland River mile 469.7) was chosen because it is a U.S. Army Corps of Engineers water quality monitoring site (samples collected twice yearly, in mid-summer and early fall). The site is exposed to prevailing winds and, as a result, displays much deeper mixing patterns than the embayments. Water level in the reservoir varies considerably, decreasing from maximum pool in spring to minimum pool in

late summer. Depth at the open-lake site varies from 45 m in March to 38 m in October. Samples were collected at monthly intervals between 1 May and 1 Oct 1994. To characterize water quality conditions while the lake was at maximum pool, one set of samples was collected in mid-March (Wolf Creek sites only).

METHODS

Temperature and irradiance profiles were obtained for each of the seven sites from measurements taken at 1 m intervals (surface to 25 m or to bottom at shallower sites). Temperature was measured with a YSI Model S-C-T thermistor. Irradiance profiles (PAR) were measured with a Protomatic photometer equipped with upward and downward spherical sensors. Depth profiles of downwelling and upwelling irradiance were analyzed using the methods of Kirk (1983) to estimate coefficients of light attenuation, absorption, and scattering. The attenuation coefficient for downwelling irradiance (K_d) was determined from a linear regression of the natural log of downwelling irradiance against depth. Correlation coefficients derived by fitting linear regressions to irradiance data were uniformly high, and estimates for the standard error of the slope were consistently less than 10%. A detailed description of analytical procedures used for irradiance data is presented in Bukaveckas and Driscoll (1991).

Water samples for chlorophyll analyses were taken at three depths spaced equally between the surface and the 1% light level. Samples were stored in coolers and processed, within 1 to 3 hours after collection, by filtration through Gelman A/E glass fiber filters. Filters were stored frozen and in the dark. Within 3 to 10 days the filters were macerated and pigments extracted overnight in 90% acetone. Extracts were analyzed using a Varian DMS 70 spectrophotometer equipped with long pathlength (4 cm) cells and narrow (1 nm) bandwidth. Concentrations of degraded chlorophyll *a* were corrected for pheophytin *a* using the Lorenzen equations as modified by Speziale et al. (1984).

Water samples for dissolved oxygen determinations were collected at 5 m intervals to 20 m (or to the bottom). Samples were analyzed using the azide modification of the

Winkler titration method (APHA 1985). Water samples for nutrient analyses were taken at depths of 3 m, 10 m, and 17 m (where possible). All nitrate and dissolved silica analyses were performed on an autoanalyzer (Skalar San Plus) using unfiltered water samples. Nitrate concentrations were determined using the automated cadmium reduction method; dissolved silica was measured by the automated molybdosilicate method (APHA 1985). Total phosphorous was analyzed on unfiltered samples using the manual two reagent ascorbic acid method following persulfate digestion (APHA 1985).

RESULTS

Spatial variation in water column transparency followed a consistent trend of increasing light penetration with increasing distance downstream (Figure 3). Attenuation coefficients (K_d) ranged from 0.40 to 0.75 (summer averages) with highest light attenuation measured at upstream sites (W-up and C-up). Variation in light attenuation corresponded to differences in depth of the photic zone (1% light level), which ranged from 7 to 8 m at upstream sites to 10 to 12 m at downstream and open-lake sites. At all stations, vertical gradients of chlorophyll showed a distinct peak at depths ranging from 5 to 10 m. These depths corresponded to light levels approximating 2% to 10% of subsurface irradiance.

Differences in light attenuation among sites located in the Caney Creek embayment were related to variation in light scattering. The importance of scattering in regulating overall light attenuation was inferred from the higher values measured upstream (coefficient = 2.0) and the lower values occurring downstream (coefficient = 0.5). By comparison, coefficients of light absorption were smaller (0.4–0.5) and exhibited little variation along the upstream-downstream gradient. In the Wolf Creek embayment, variation in both scattering and absorption contributed to between-site differences in light attenuation. Light attenuation, highest at the furthest upstream site, decreased downstream despite higher scattering at W-mid.

Depth profiles of dissolved oxygen differed markedly between the embayments. Data collected on 6 July typify the differences that were apparent throughout the summer (Fig-

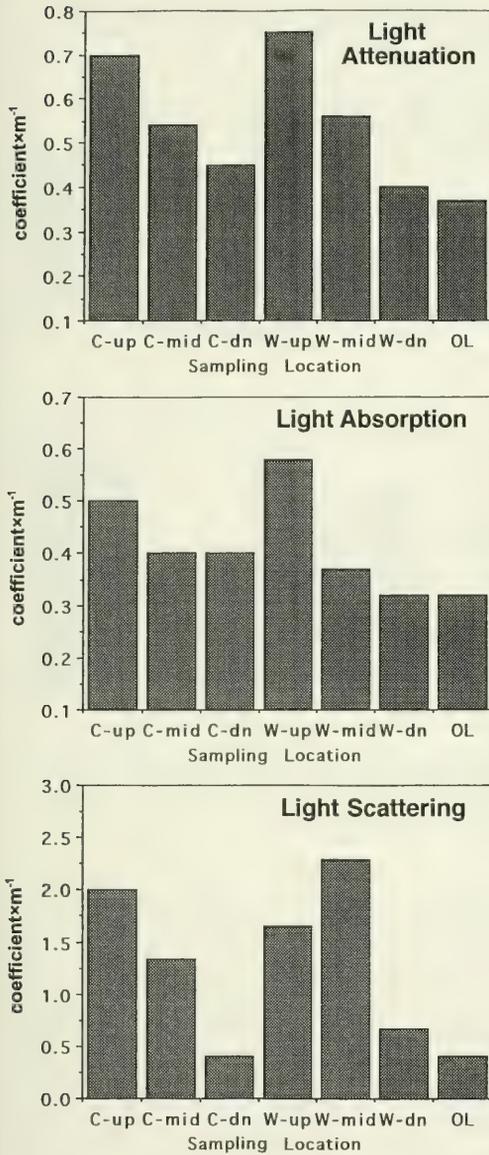


Figure 3. Spatial variation in coefficients of light attenuation, light absorption, and light scattering along an upstream-downstream gradient in two embayments and the main channel of Lake Cumberland, Kentucky (mean values for four sampling dates in July–October 1994).

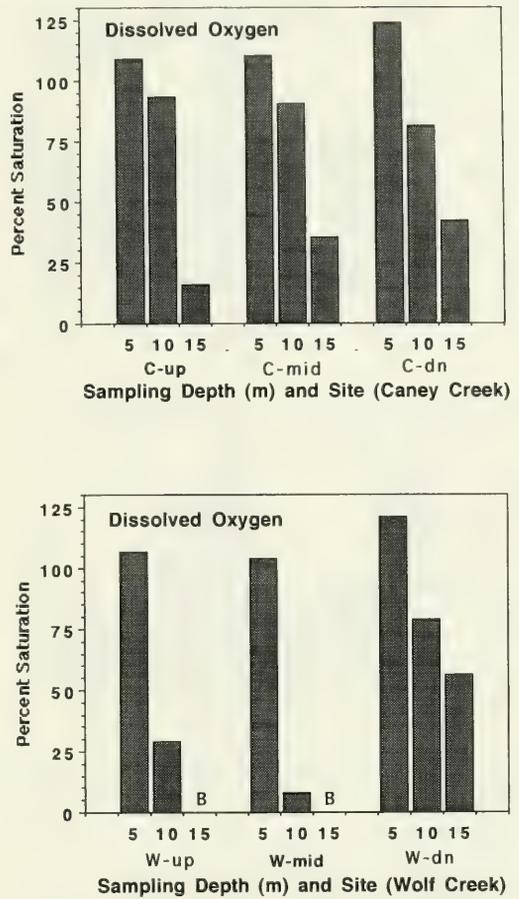


Figure 4. Oxygen saturation at 5, 10, and 15 m along an upstream-downstream gradient in the Wolf Creek and Caney Creek embayments Lake Cumberland, Kentucky, for samples collected on 6 July 1994. B denotes missing samples where lake depth < 15 m.

ure 4). At all stations, upper water column samples (0–5 m) were at or above saturation (90% to 110%). Differences between the embayments were evident in samples collected at 10 m. At Caney Creek sites, 10 m samples were consistently within 10% to 20% of atmospheric equilibrium (saturation >80%),

whereas 10-m samples from Wolf Creek sites were substantially depleted in dissolved oxygen (10% to 50% saturation). Differences in oxygen resources of the lower water column were most pronounced at upstream and mid-stream sites, whereas oxygen profiles obtained at downstream sites were generally similar.

Patterns of spatial variation in chlorophyll and nutrient concentrations were similar between embayments (Figure 5) with highest chlorophyll concentrations observed at sites furthest upstream. Chlorophyll concentrations upstream (C-up and W-up) typically exceeded 5 $\mu\text{g/liter}$ (photic zone average), whereas values for open-lake and midstream and down-

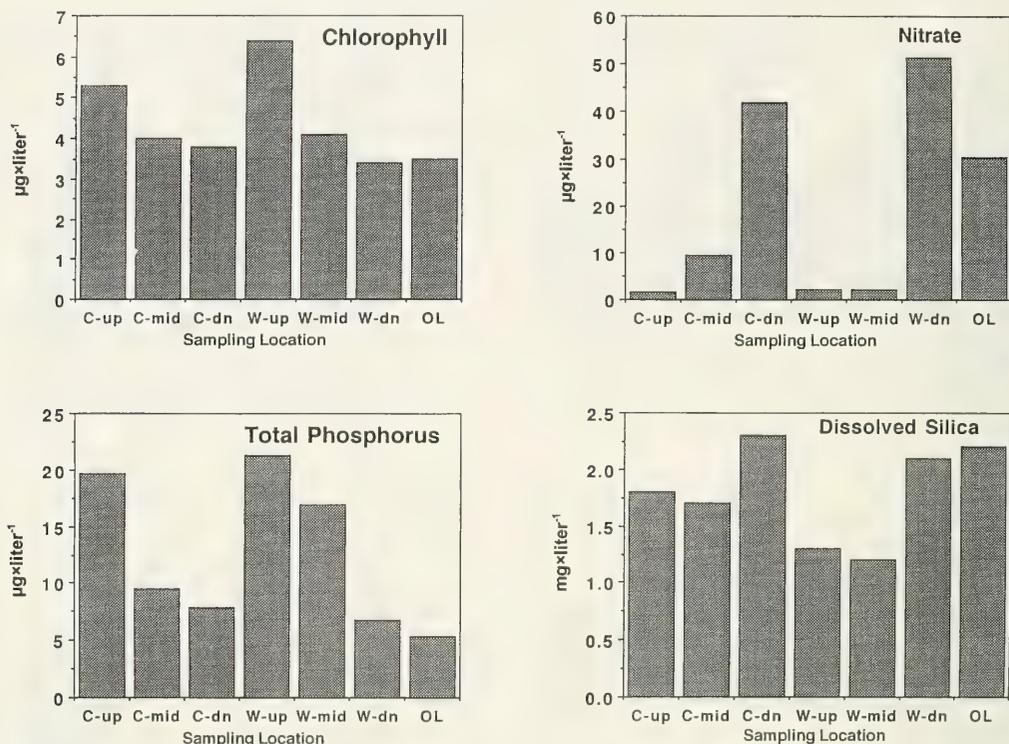


Figure 5. Spatial gradients in chlorophyll, total phosphorus, nitrate, and dissolved silica concentrations along an upstream-downstream gradient in two embayments and the main channel of Lake Cumberland, Kentucky. Data shown are mean values for samples collected between 0 and 10 m on four sampling dates in July–October 1994 ($n = 30$).

stream sites were generally lower. Spatial variation in TP followed patterns similar to those for chlorophyll. In both embayments, higher phosphorus concentrations were measured at upstream sites (15–20 $\mu\text{g}/\text{liter}$) with lower values (<10 $\mu\text{g}/\text{liter}$) at downstream sites. The embayments differed in that phosphorus concentrations were consistently higher at the midstream site in Wolf Creek (nearest to the marina) in comparison to the midstream site at Caney Creek.

Patterns of spatial variation for nitrate differed from those observed for chlorophyll and total phosphorus. Nitrate concentrations at upstream and midstream sites were extremely low (<10 $\mu\text{g}/\text{liter}$) and in a few cases below analytical detection limits. Somewhat higher values were observed at downstream and open-lake sites (30–50 $\mu\text{g}/\text{liter}$). Spatial patterns in dissolved silica were similar between embayments, with highest concentrations measured at downstream sites (>2 mg/liter)

and lower concentrations upstream (1–2 mg/liter).

Seasonal variation in chlorophyll and nutrient concentrations were similar between embayments (Figure 6) although interpretation of these trends is somewhat compromised by the incomplete record for Caney Creek sites (no March data). Chlorophyll concentrations were notably higher at W-up and W-mid sites during the March sampling. Concentrations were generally low at all sites during summer (photic zone averages <10 $\mu\text{g}/\text{liter}$). Nutrient concentrations in surface waters were also highest in March and declined throughout the summer. In the Wolf Creek embayment, total phosphorus decreased from a range of 50–75 $\mu\text{g}/\text{liter}$ in March to 10–20 $\mu\text{g}/\text{liter}$ by early July. Nitrate was depleted more rapidly with concentrations decreasing from 400–600 $\mu\text{g}/\text{liter}$ to <5 $\mu\text{g}/\text{liter}$ by July. The relative availability of nitrogen to phosphorus was consistently low at all stations and throughout the

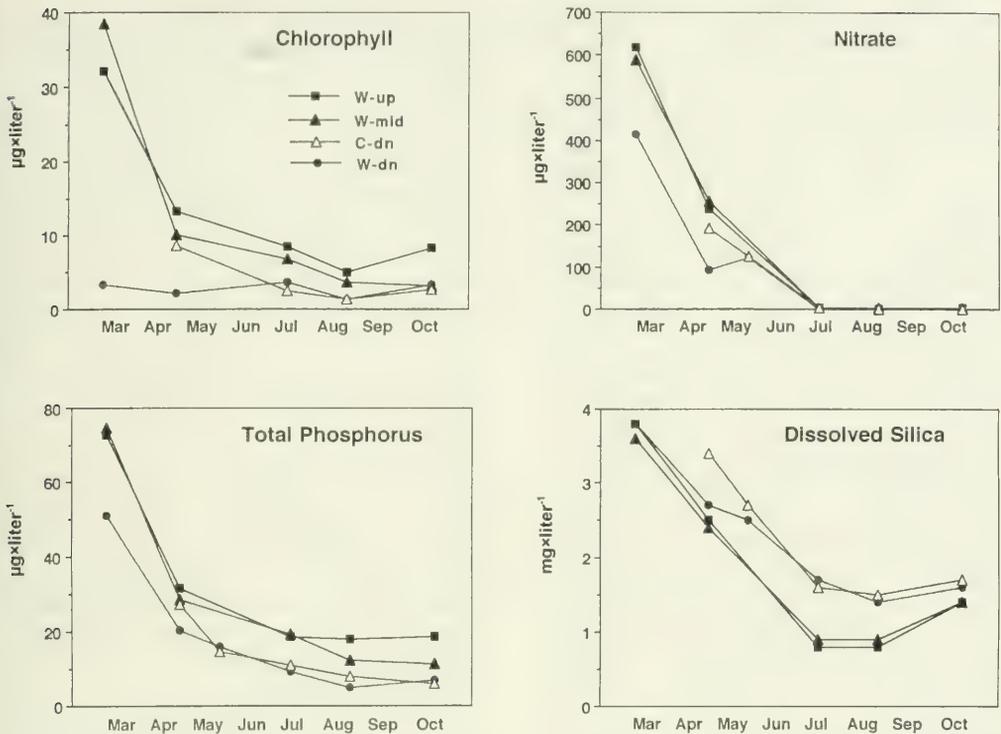


Figure 6. Temporal trends in chlorophyll, total phosphorus, nitrate, and dissolved silica concentrations (mean values for samples collected in the photic zone of Lake Cumberland, Kentucky, in March–October 1994; 0–10 m).

study. Ratios of N:P at Wolf Creek decreased from 8:1 in March to <1:1 during July–October (atomic ratios based on NO_3 and TP determinations). Dissolved silica was also substantially depleted in the upper water column as indicated by decreases in concentration from 3–4 $\text{mg}\times\text{liter}^{-1}$ in March to 1–1.5 $\text{mg}\times\text{liter}^{-1}$ by early July. Silica depletion was apparently greater at W-mid and W-up sites relative to downstream sites (W-dn and C-dn) as evidenced by lower mid-summer silica concentrations.

Chlorophyll and nutrient data were analyzed using a Generalized Linear Models Procedure (SAS Version 6.03) to partition variance arising from four sources: embayment (Caney and Wolf), sampling date (July, August, September), station (upstream, midstream, downstream), and depth (epilimnion, metalimnion, and hypolimnion). In addition to the four main effects, the ANOVA model included three interaction terms: embayment with date (to determine if seasonal patterns differed between embayments), embayment with station

(to determine if longitudinal patterns differed between embayments), and embayment with depth (to determine if vertical profiles differed between embayments). The empirical models were generally found to be good predictors ($p < .05$) of variation in chlorophyll ($R^2 = 0.52$), pheophytin ($R^2 = 0.55$), nitrate ($R^2 = 0.75$), and total phosphorus ($R^2 = 0.48$). The model describing variation in dissolved silica concentrations was not significant. Among the main effects, date and station were found to be significant predictors of chlorophyll and pheophytin, date and depth were significant predictors of nitrate, and station was a significant predictor of total phosphorus (Table I). Embayment did not explain a significant proportion of the variance in measured parameters; in only one model were any of the three interaction terms significant (embayment with depth in the chlorophyll model).

DISCUSSION

We interpreted our data for Lake Cumberland in the context of the generalized reservoir

Table 1. Results of statistical analyses partitioning variation in chlorophyll and nutrient concentrations associated with the effects of embayment, date, station, and depth (SAS Generalized Linear Model Procedure) in Lake Cumberland, Kentucky.

Source	df	Mean square	F	p	Dependent variable
Embayment	1	0.01	0.01	NS	Chlorophyll R ² = 0.52
Date	2	15.21	3.82	0.033	
Station	2	14.16	3.55	0.041	
Depth	2	11.00	2.76	NS	
Emb × Date	2	0.95	0.24	NS	
Emb × Station	2	1.10	0.28	NS	
Emb × Depth	2	18.25	4.58	0.018	
Model	13	10.24	2.57	0.015	
Error	31	3.99			
Embayment	1	1,210	0.23	NS	Nitrate R ² = 0.75
Date	2	26,532	5.07	0.013	
Station	2	9,428	1.80	NS	
Depth	2	128,221	24.51	0.001	
Emb × Date	2	2,693	0.51	NS	
Emb × Station	2	388	0.07	NS	
Emb × Depth	2	977	0.19	NS	
Model	13	35,564	6.80	0.001	
Error	29	5,231			
Embayment	1	10.12	0.13	NS	Total phosphorus R ² = 0.48
Date	2	6.79	0.09	NS	
Station	2	670.51	8.68	0.001	
Depth	2	53.52	0.69	NS	
Emb × Date	2	72.76	0.94	NS	
Emb × Station	2	26.91	0.35	NS	
Emb × Depth	2	60.01	0.78	NS	
Model	13	159.77	2.07	0.050	
Error	29	77.25			

model described by Kennedy and Walker (1990) and Kimmel, Lind, and Paulson (1990). Their model considers spatial heterogeneity in nutrient availability and phytoplankton production in relation to longitudinal gradients in water flow and basin morphology. A typical reservoir is considered to comprise three zones: uplake riverine zone, transition zone, and lacustrine (near dam) zone. The riverine zone is characterized by higher flow velocities, shorter water residence time, high concentrations of suspended solids, and low light penetration. The transition and lacustrine zones are characterized by increasing basin breadth and depth, decreasing flow, longer water residence time, and deeper light penetration (due to sedimentation of suspended solids). According to the model, nutrient concentrations are predicted to be highest in the riverine zone due to elevated nutrient concentrations in watershed run-off and low rates of phytoplankton uptake under light-limiting conditions. Nutrient concentrations should de-

crease downstream (toward the dam) because greater light penetration allows for increased phytoplankton production.

Spatial gradients in chlorophyll, total phosphorus, and light penetration for Lake Cumberland were consistent with predictions of the generalized model described above. Light penetration was lowest at upstream stations and increased with distance downstream. Overall, light availability did not appear to impose severe constraints on phytoplankton growth because the photic zone included the upper 8–12 m of the water column at all sites (maximum depth 15–25 m). Chlorophyll concentrations were higher at upstream stations compared to samples collected downstream in the embayments and at the open-lake site. Light conditions and trends in chlorophyll suggest that our furthest upstream stations would be considered transition zone sites (area of high phytoplankton productivity) in the context of the generalized model. The Caney Creek and Wolf Creek tributaries, rela-

tively small, were likely near base flow during most of the study period. It is therefore not surprising that we did not observe conditions typical of the riverine zone even at our furthest upstream sites.

Spatial gradients in nutrient availability were more difficult to interpret because total phosphorus decreased downstream whereas nitrate and dissolved silica increased downstream. The close correspondence between chlorophyll and total phosphorus concentrations suggests that phosphorus inputs via tributary streams may have stimulated phytoplankton production in upstream areas. This would likely result in greater upstream depletion of nitrogen and dissolved silica and may account for the inverse gradients (increasing downstream) observed for NO_3 and SiO_2 . This argument assumes that phosphorus limits phytoplankton production; however, our data suggest that the availability of nitrogen relative to phosphorus is very low in Lake Cumberland ($\text{N:P} < 10:1$ at all sites during our study). Our measurements of phosphorus concentrations (based on TP) may overestimate phosphorus availability if the nonlabile (particle-bound) P fraction is large. Concentrations of suspended matter were low from May to September as indicated by low chlorophyll concentrations and low light attenuation coefficients. We feel, therefore, that our TP data are indicative of labile (dissolved) P in summer. Nitrogen availability was inferred from measurements of nitrate only; to the extent that NH_4 may contribute to the inorganic nitrogen pool in epilimnetic waters, we may be underestimating N:P . For reservoirs where phytoplankton growth has been shown to be N-limited on the basis of physiological indicators (Groeger and Kimmel 1988), nitrogen availability was reported to decrease downstream (opposite of the NO_3 gradients we observed in Lake Cumberland). On the basis of data presented here, it is unclear which of these nutrients limits phytoplankton production in Lake Cumberland. Bioassay experiments and additional measurements of P fractions (TP and SRP) would serve to clarify the relative importance of P and N in limiting phytoplankton production.

We hypothesized that effects of recreational activities on water quality would be superimposed on upstream-downstream gradients de-

scribed above. Specifically, we predicted that nutrient and organic matter loading from houseboat discharge would result in elevated nitrogen, phosphorus, and chlorophyll concentrations and greater hypolimnetic oxygen depletion in the Wolf Creek embayment. Consistently elevated TP levels measured at the station located immediately downstream (100 m) from a large marina complex (W-mid) support this hypothesis. Although a corresponding increase in chlorophyll was not observed, higher rates of nitrate and silica depletion (in comparison to the downstream site) suggest greater phytoplankton production. Localized waste inputs containing inorganic or organic phosphorus could account for the observed trends in TP, NO_3 , and SiO_2 . An alternative hypothesis is that differences in TP between embayments are a result of differences in nutrient inputs from associated tributaries. If tributaries were an important source of phosphorus, we would expect that upstream sites would show a similar pattern of differences (Wolf Creek TP > Caney Creek TP). This was not the case as there were no consistent differences in TP concentrations at upstream sites. The midstream station in the Wolf Creek embayment occurs below the confluence of two smaller bays formed by the Wolf Creek and Alligator Creek tributaries. Water quality at this site may be influenced by inputs from Alligator Creek, which enter the reservoir 4.4 km (2.75 mi) upstream. As tributary sampling was not included in our study, we cannot exclude the possibility that elevated TP at this site was in part due to tributary influences.

We compared dissolved oxygen profiles to test the hypothesis that organic matter loading resulted in greater hypolimnetic oxygen depletion in the Wolf Creek embayment. Oxygen saturation of 10 m samples collected at W-up and W-mid were consistently lower relative to corresponding sites in the Caney Creek embayment. Differences in oxygen resources between the embayments are consistent with the hypothesis of higher organic matter loading in upper reaches of the Wolf Creek embayment. It is important to note, however, that stations W-up and W-mid were shallower (15 m) in comparison to corresponding sites at Caney Creek (25 m). Because the position of the thermocline was similar among sites (8–12 m), the volume of the hypolimnion in upstream

reaches of Wolf Creek was smaller in comparison to that of the Caney Creek sites. Thus, comparable rates of organic matter loading would result in greater apparent oxygen depletion per unit volume at shallower sites.

In summary, our analyses of spatial variation in water quality revealed that the site near a major houseboat marina complex showed consistently elevated TP concentrations and higher suspended particulate matter (as indicated by light scattering). Sites upstream and downstream in the same embayment did not exhibit pronounced differences in water quality compared to sites in the other embayment, where houseboat activity was substantially lower. Overall, longitudinal gradients in both embayments followed expected patterns based on changes in flow velocity and basin morphology; our study does not provide compelling evidence of water quality deterioration resulting from houseboat discharge. This conclusion is supported by statistical models in which between-embayment differences were not found to be significant predictors of variation in chlorophyll and nutrient concentrations. Our findings may be due in part to the low resolution of our sampling (monthly intervals), which is not suitable for detecting transient changes in water quality. Periods of intense houseboat activity, such as occur during the 4th of July holiday, may result in short-term reductions in water quality. These effects would not be detected by our design and would require a more intensive field effort.

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Survey of Cereal Aphids in Kentucky Wheat Fields: Common Species and Distribution

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ABSTRACT

Species determination and relative abundance of cereal aphids in samples from winter wheat were investigated in major production areas of Kentucky in 1992-1993 and 1993-1994. Cereal aphids were more abundant in 1992-1993 than in 1993-1994. The bird cherry-oat aphid (*Rhopalosiphum padi*) was the most common aphid followed by English grain aphid (*Sitobion avenae*), corn leaf aphid (*R. maidis*), and greenbug aphid (*Schizaphis graminum*), respectively. All species were widely distributed in the Kentucky wheat production area.

INTRODUCTION

Barley yellow dwarf (BYD), a disease resulting from infection by a viral pathogen (BYDV), is the most important viral disease of cereals worldwide (Plumb 1983). The pathogen is resident in a large number of crop and non-crop species of the grass family (Poaceae), e.g., corn (*Zea mays*) and tall fescue (*Festuca arundinacea*) and is vectored within and among these hosts/crops by a complex of aphid species (Homoptera: Aphididae). Fields of small grains infected with this disease often produce significantly reduced yields (Irwin and Thresh 1990). In Kentucky, BYD in wheat may be found at low levels each year; it occasionally reaches epidemic status.

Much literature on BYD and associated hosts and vectors has been published by researchers around the world (Burnett 1989; Irwin and Thresh 1990). In Kentucky, however, little information about BYD in production fields is available. We wish to establish which species of cereal aphids are most common in Kentucky soft red winter wheat (*Triticum aestivum*) fields and if the species vary from fall to spring and across the wheat-growing region. Specifically we wish to see if the situation in Kentucky is substantially similar to or different from that in surrounding areas.

MATERIALS AND METHODS

Aphid collections were made in fall and spring in each of the 1992-1993 and 1993-

1994 growing seasons. Collection sites were selected to represent the distribution of wheat production in Kentucky (KAS 1991). Ten (1992-1993) and five (1993-1994) fields were sampled in each of 15 counties (Ballard, Bourbon, Calloway, Christian, Daviess, Graves, Hardin, Henderson, Hickman, Logan, Shelby, Simpson, Todd, Trigg, and Warren), producing a possible 150 and 75 samples, respectively (Figure 1). During both seasons fall samples were taken in the last 2 weeks of November and the first 2 weeks of December; spring samples were taken in April.

The sample for each field was a composite of cereal aphids collected from foliage of five plants at five locations, randomly spaced along a diagonal line across the field, and were no closer than ca. 15 m from the field edge or one another. Aphid populations were not overly abundant in either year, and no effort was made to differentiate the cereal aphids by location in a field. Aphids were obtained by collecting leaf sections on which they rested into appropriately labeled vials of 70% ETOH and held in the laboratory until examination. Identification was accomplished by microscopic examination and by reference to Pike, Boydston, and Allison (1991) and Stoetzel (1987). Several samples were discarded as unusable due to physical damage to the aphids from improper handling.

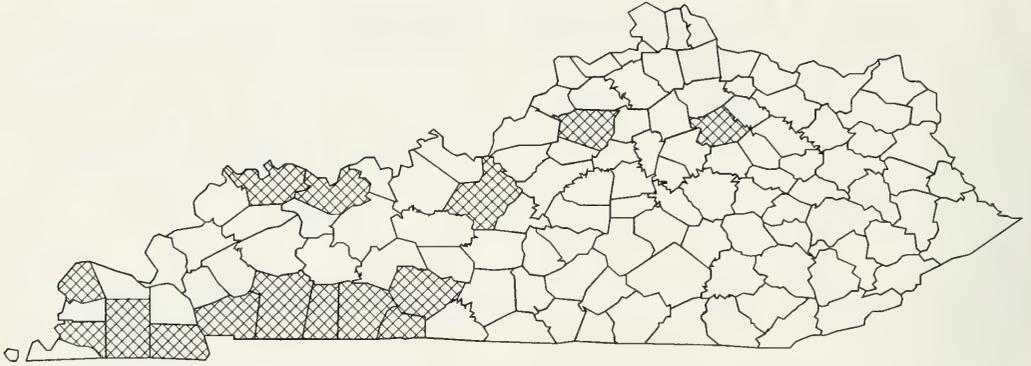


Figure 1. Kentucky counties containing fields of soft red winter wheat sampled for cereal aphids in 1992–1993 and 1993–1994.

RESULTS

Cereal aphids were more abundant in 1992–1993 than in 1993–1994. Of the 150 fields visited in 1992–1993, the fall collection yielded 109 samples of which 104 were usable; of these, three contained mixed species. In spring, 109 samples were collected, and 100 were usable; of these, four contained mixed species. In 1993–1994, of the 75 fields visited, the fall collection produced only 17 samples yielding 16 usable, with one containing mixed species. The spring yielded 50 samples of which 49 were usable, with three containing mixed species.

Four species of cereal aphids common in wheat production were found: *Rhopalosiphum padi* (L.), bird cherry–oat aphid; *R. maidis* (Fitch), corn leaf aphid; *Schizaphis graminum* (Rondani), greenbug aphid; and *Sitobion avenae* (Fab.), English grain aphid. All these species are able to serve as vectors for BYDV. Additionally, BYDV isolates associated with these aphids have been confirmed from Ken-

tucky soft red winter wheat fields (unpublished data).

The bird cherry–oat aphid (BCOA) was the most commonly encountered species. It was collected in all four survey periods and was present in the greatest numbers of samples in three of the four collections. The English grain aphid (EGA) was the second most common aphid. It was collected in all four collection periods and dominated the samples in spring 1994. The corn leaf aphid (CLA) and greenbug aphid (GB) were found in much fewer numbers (Table 1).

These cereal aphids are ubiquitous in Kentucky soft red winter wheat. When all samples are considered, BCOA was collected from every county surveyed; EGA was collected in all but four (Graves, Hardin, Hickman, and Simpson) of the 15 surveyed counties. The three GB samples were collected from Daviess and Graves counties; the two CLA samples, from Ballard and Logan counties.

DISCUSSION

The species collected and their wide distribution in Kentucky soft red winter wheat were expected. Gildow (1987), in his summary of regional BYD research, pointed out that this same series of aphid species is commonly encountered in Illinois, Indiana, Missouri, and many other states in the United States. However, when working with a pathosystem as complex as BYD, unsubstantiated assumptions can quickly lead to erroneous results. For this reason and to allow for the use of research in

Table 1. Numbers of samples containing various species of common cereal aphids, collected during a survey of soft red winter wheat fields in Kentucky in fall and spring 1992–1993 and 1993–1994.

	1992–1993		1993–1994	
	Fall	Spring	Fall	Spring
Total samples	145	141	74	74
Samples with aphids	104	100	16	49
Bird cherry–oat aphid	99	85	16	3
Corn leaf aphid	1	0	0	0
English grain aphid	4	17	1	49
Greenbug aphid	3	0	0	0

nearby states it is important to know that these species are present.

The commonness of BCOA is also to be expected. However, the differences among BCOA, EGA, and CLA may be due, at least in part, to the timing of sample collection. CLA might be expected to occur in wheat early in fall and to decrease with the onset of cold weather; EGA is generally encountered late in spring after wheat begins to produce inflorescences (personal observation). Our samples were generally collected later in fall and earlier in spring than one would expect to encounter large numbers of CLA and EGA, respectively. A similar shift in species composition was reported in Virginia (McPherson and Brann 1983). The large increase in EGA samples in spring 1994 is probably due to the early occurrence of spring weather that year. Additional research concerning the timing of shifts of aphid species in Kentucky wheat is certainly warranted.

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***Cryptosporidium parvum* in the Domestic Dog Population of Central Kentucky**

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ABSTRACT

Cryptosporidium parvum oocysts were recovered from fecal specimens of 17 of 100 healthy or diseased adult and puppy dogs tested in central Kentucky. The specimens were collected and placed in 10% formalin, concentrated by the formalin-ethyl acetate method, and stained by a modified hot acid-fast technique. The presence of oocysts in domestic dogs may represent a possible reservoir that could infect immunocompromised and/or immunocompetent humans.

INTRODUCTION

Cryptosporidium parvum is an enteric coccidian protozoan assigned to phylum Apicomplexa, class Sporozoa, and order Eucoccidiorida (Current 1985). In the suborder Emeriorina, *C. parvum* is found with three other important human pathogens, *Toxoplasma gondii*, *Isospora* sp., and *Sarcocystis* spp. (Current 1985; Dubey, Speer, and Fayer 1990). The life cycle of *C. parvum* exhibits asexual and sexual endogenous stages; oocysts are discharged in the feces of the host (Dubey, Speer, and Fayer 1990; Fayer and Ungar 1986; Loose, Sedergran, and Cooper 1989; Tzipori 1988).

Oocysts are found in the general environment including water and food (Hayes et al. 1989; Tzipori 1988). Widespread in nature, *C. parvum* infects many animals (Fayer and Ungar 1986). Spread by the oral-fecal route, it has been implicated as a zoonosis (D'Antonio, Winn, and Zajac 1985; Hayes et al. 1989).

Cryptosporidiosis, reported to cause disease in both immunocompetent (CDC 1982; Current et al. 1983; Fayer and Ungar 1986; Soave 1988; Tzipori 1987, 1988) and immunocompromised humans (CDC 1982; Current et al. 1983; Soave and Johnson 1988; Tzipori 1987), is a significant source of morbidity in both groups. In the immunocompromised patient it

is a source of severe prolonged diarrhea. No specific effective treatment is available at the present time (Cotton 1991; Soave and Armstrong 1986), although paromycin has been used with some success (Armitage et al. 1992; Bissuel et al. 1994; Cotton 1991; Goodgame et al. 1993). Therapy studies are difficult to design because patients are severely immunosuppressed and may have multiple intestinal infections. Rat models indicate successful treatment with paromycin at higher dosages than the 1-2 g/day human patients usually receive (Verdon et al. 1995).

Associations between calves and human disease are firmly established (Rahaman et al. 1984). Evidence suggests a link between domestic cats and cryptosporidiosis in humans (Fayer and Ungar 1986). Puppies have been suspected as a source of *Cryptosporidium* oocysts (Current et al. 1983). Serological studies suggest that cryptosporidiosis in dogs is a frequent occurrence (Tzipori and Campbell 1981). Several surveys have been conducted on domestic dogs to detect *C. parvum* oocysts (Chermette and Blondell 1989; Fayer and Ungar 1986; Pohjola 1984). Dogs excreting the oocysts may be asymptomatic carriers or may exhibit disease symptoms such as diarrhea. Because of the lack of treatment for cryptosporidiosis, and because of the severe discom-

fort seen in immunocompromised patients, guidelines for these patients in regard to their association with pet dogs may need to be established. Similar guidelines concerning food counseling for persons infected with HIV have been issued by the U.S. Public Health Service (Archer 1989). These state that the risk of contracting cryptosporidiosis for HIV patients can be reduced by avoiding raw foods of animal origin and by washing all foods to avoid contamination from soil.

MATERIALS AND METHODS

Specimens

Fecal specimens were collected from 100 domestic, diseased or healthy adult and puppy dogs in central Kentucky in September, October, and early November 1991 and 1992. After the specimens were collected or had arrived at the veterinary clinic, they were placed in a preservative (10% formalin) in screw-capped containers. A questionnaire, designed to gather pertinent information, asked the dog's name, breed, age, sex, illnesses at the time, and whether the dog had an active case of diarrhea. Of the 100 dogs, 40 were males, 56 were females, and 4 were of unknown sex; 49 were 1 year of age or less, 33 were 1–10 years, 11 were over 10 years, and 8 were of unknown age; and 81 were well dogs and 19 had reported illnesses.

Processing Methods

The stool specimens were concentrated using a modified formalin-ethyl acetate procedure (Baron, Peterson, and Finegold 1994) based on Ritchie's methodology (Ritchie 1948). The concentrate was spread thinly on a pre-cleaned slide and heat fixed at 70°C for 10 minutes before staining. The smears were stained with a modified hot Ziehl-Neelsen carbol fuchsin (ZN) for 5 minutes while heating the smear to steaming. The decolorizer was 5% H₂SO₄ for 30 seconds; the counterstain, methylene blue for 1 minute. Stained smears were evaluated by bright field microscopy at 1000× for *Cryptosporidium* oocysts.

Oocysts stain bright red. Yeasts have been reported to stain blue by some researchers, but many of the yeasts encountered in our specimens stained red. Therefore, the size of the object was important in distinguishing oo-

cysts from yeasts. *Cryptosporidium parvum* is 4–6 μm; many yeasts are smaller.

To distinguish the yeast that was in the size range for *C. parvum*, an iodine stain was used on all specimens where the ZN smears appeared red. When exposed to iodine for less than 15 minutes, yeasts stain but *C. parvum* does not (Baron, Peterson, and Finegold 1994; Ma and Soave 1983). Therefore, the criteria to call a specimen positive for *C. parvum* oocysts included (1) bright red color on modified hot ZN with the object viewed falling into the 4–6 μm range and (2) concentrated sediment showing structures comparable in size that did not stain with iodine. Each batch of acid-fast stains included a positive control for *C. parvum* purchased from Trend Scientific, Inc., St. Paul, Minnesota 55112. Each control was read with the specimens; satisfactory results were demonstrated for each control.

RESULTS

Cryptosporidium parvum oocysts were present in 17 fecal specimens. Of the seven dogs with oocysts nine were female, seven were male, and one was in the unknown category (fecal specimen delivered to clinic without information). These dogs represent 16% of the female population and 18% of the male population.

The infected dogs ranged from a few weeks to 12 years old. Fifteen were 1 year or under, one was 3 years, and one was 12 years. Of the 100 dogs examined, 31% of age 1 year and under were positive for oocysts, 3% of age 2 to 10 years were positive, and 9% of age 10 years and over were positive.

Oocysts were found in two dogs with diarrhea, but nine dogs with diarrheal symptoms were negative. Fecal specimens from 81 apparently healthy dogs revealed 15 cases of cryptosporidiosis. It was difficult to tabulate information concerning the breeds of dogs represented. In the entire population, 41 breeds were represented by at least one animal. Of these, only English setters and German shepherds showed more than one positive case. Two out of three English setters (66%) and four of four (100%) German shepherds demonstrated oocysts.

DISCUSSION

We think that ours is the first report of *C. parvum* oocysts from dogs in central Kentucky

(where the incidence was 17%) and the first comprehensive analysis of a cross section of domestic dogs in the United States. Dogs develop natural infections with *C. parvum* (Current et al. 1983), but the prevalence rate for dogs is largely unknown (Dubey, Speer, and Fayer 1990).

The group of dogs demonstrating the highest incidence was 1 year old and under. In the study by Current et al. (1983), puppies were suspected as a source of infection for cryptosporidiosis; the high incidence rate in young dogs from central Kentucky appears to support this speculation.

The relatively high rate of *C. parvum* cases in the domestic dog population in our study suggests a possible reservoir for oocysts that might infect immunocompromised and immunocompetent humans. Because most dogs exhibiting *C. parvum* oocysts were asymptomatic, their owners would probably consider them healthy and not a potential source of infection. With only two dogs (12%) testing positive and showing diarrheal symptoms, such symptoms cannot be used to implicate a dog as a reservoir.

Geographical factors may have an effect on positive rates. We had insufficient data to determine the proximity of positive cases to each other. Clustering effects may have occurred with dogs from the same household. In future studies, this factor would need to be delineated more clearly.

Since this is thought to be the first comprehensive study on a cross section of domestic dogs in central Kentucky, it remains to be determined if our data are representative of dog populations in general. More research on populations in Kentucky and other states is needed before definitive prevalence rates can be determined conclusively.

Cryptosporidiosis is a significant threat to immunocompromised individuals such as AIDS patients. Public health statistics released 30 Jun 1995 in the Kentucky AIDS Surveillance Report (HSD 1995) listed 189 cases (11.5% of total AIDS cases) of HIV Wasting Syndrome from 1982 with 11.5% of all Kentucky AIDS patients exhibiting this as primary disease. Cryptosporidiosis remains largely untreatable except for limited success with paromycin. Therefore, prevention may be the best path to follow for immunocompromised

individuals. Public health guidelines for preventing listeriosis and salmonellosis in such patients (Archer 1989) include avoiding raw milk and eggs and washing fruits and vegetables before eating. If prevalence rates were better delineated for domestic dogs and the results of our study are supported in the future, guidelines may include avoidance of dogs for these patients as one method for preventing cryptosporidiosis.

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Age, Growth, and Food of Freshwater Drum, *Aplodinotus grunniens* (Sciaenidae), in Kentucky Lake, Kentucky/Tennessee

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ABSTRACT

Zebra mussels (*Dreissena polymorpha*) have recently been discovered in the lower Tennessee River. Information on age and growth rates of freshwater drum in Kentucky Lake, Kentucky/Tennessee, before colonization of zebra mussels is important, especially if freshwater drum were to be managed to control expansion of this rapidly spreading exotic. Age and growth of freshwater drum in Kentucky Lake were determined from fish supplied by local commercial fishermen in 1985 and 1986. Growth in the reservoir was generally slower than in other bodies of water. The diet of freshwater drum was also examined to determine feeding habits. The fish were feeding on the exotic mussel *Corbicula fluminea*. Zebra mussels in the diet of freshwater drum have already been documented for Lake Erie. These facts suggest that once zebra mussels colonize Kentucky Lake, freshwater drum will be a substantial predator on them.

INTRODUCTION

The freshwater drum (*Aplodinotus grunniens*, Sciaenidae) is a common fish of streams, rivers, and reservoirs in much of midwestern North America. Because of its strong molariform pharyngeal teeth, the fish is capable of feeding on mollusks (Moen 1955; Price 1963; Wrenn 1968). With the recent invasion of the zebra mussel (*Dreissena polymorpha*) into North America, the freshwater drum may play an important role as a biological control of this rapidly spreading exotic. French and Bur (1993) showed that freshwater drum greater than 250 mm in length fed on zebra mussels in Lake Erie. They suggested that anglers should release all freshwater drum to increase biological control of the mussels. French (1993) recommended that fishery managers in eastern North America should manage for native molluscivores to suppress zebra mussel populations. He recommended research on prey-preference and mollusk-eating efficiency by freshwater drum because their pharyngeal teeth make them the most efficient zebra mussel predator.

Zebra mussels were first reported in the lower Ohio and Tennessee rivers in autumn 1991 (Sickel and Leek 1994). Since that time they have been found on native mussels in Kentucky Lake, Kentucky/Tennessee, in the area of Camden, Tennessee. In July 1992 a cluster of seven adult zebra mussels was found near the west shore of Kentucky Lake at Ten-

nessee River Mile (TRM) 23.8. In autumn 1992 the locks at Kentucky Dam on the Tennessee River were dewatered for maintenance. The locks contained many attached zebra mussels, which probably came from barges and other vessels traveling from areas where the mussel populations are denser (Sickel and Leek 1994). By autumn 1993 the lower Ohio River had been completely colonized by zebra mussels; the lower Tennessee River remained relatively uncolonized.

As freshwater drum may become important to Kentucky Lake fishery managers, information on age and growth rates of this species before colonization by zebra mussels would be valuable if freshwater drum were to be managed to control expansion of zebra mussel populations. Also, Kentucky Lake presently supports a substantial commercial fishery for buffalo (*Ictiobus* spp.), common carp (*Cyprinus carpio*), and catfish (*Ictalurus* spp.). Freshwater drum is the most common non-target species in the commercial catch in Kentucky Lake, but currently no market exists (Timmons et al. 1989). If a commercial fishery for this species was ever developed, growth data would be important for determining gear and harvest regulations.

Several authors studied freshwater drum in Tennessee River impoundments (Dendy 1946; Wrenn 1968), but we are not aware of any study of Kentucky Lake populations. The objectives of our research were to describe the

diet of freshwater drum and to examine the age and growth of this fish in Kentucky Lake from samples collected prior to colonization by zebra mussels. These data may be valuable in assessing the effect of zebra mussel colonization on the freshwater drum population in Kentucky Lake.

MATERIALS AND METHODS

Kentucky Lake is in western Kentucky and western Tennessee on the Tennessee River. This reservoir was formed by the impoundment of the Tennessee River at Tennessee River mile (TRM) 22 in 1944. Surface area of the reservoir is ca. 64,800 ha with 21,000 ha within Kentucky and 43,800 ha within Tennessee. Freshwater drum were collected from throughout the reservoir, stretching from Kentucky Dam (TRM 22) to Pickwick Dam (TRM 207).

Freshwater drum used for age determination were collected in 1985 and 1986 by commercial fishermen using gill nets, baitlines, snaglines, and hoopnets in Kentucky Lake. Small individuals not present in the commercial harvest were collected concurrently by using small mesh gill nets (<76 mm). Total length (TL) and weight were recorded for each fish. Scales were removed from the left side of each fish for age determination, placed on a heated acetate slide, and pressed with a Wildeo laboratory press. Scale impressions were viewed using a Bell and Howell Model ABR-IV microfiche reader and measured from the focus to each annulus and the scale margin along the anterior-median primary radius. Annuli were determined by using the standard criteria of crowding and crossing over of circuli (Lagler 1956). Lengths at previous annuli were calculated using the Fraser-Lee method (Bagenal and Tesch 1978). The correction factor "a" was extrapolated with a length-margin linear regression model and was equal to the Y-intercept.

The fish used for diet analysis were collected in a 12-month period beginning in November 1987 and ending in November 1988. The stomachs of 122 freshwater drum were removed and examined. Food items were identified and the percent frequency of occurrence for several freshwater drum size classes was calculated by dividing the number of stomachs

containing the item by the number of non-empty stomachs.

RESULTS

Scales from 322 freshwater drum were aged and measured for backcalculation. Age classes represented in the sample ranged from 0 to 13 with one fish at 16. Fish lengths at capture ranged from 86 to 775 mm. Linear regression of TL on margin resulted in the equation $\text{length} = 64.49 + 2.27 \text{ margin}$, which was significant at $p < 0.0001$, $r^2 = 0.914$. The Y-intercept (64.49) was used as the correction factor in the Fraser-Lee formula for backcalculation of length at annulus formation.

The first year of growth of freshwater drum in Kentucky Lake was rapid, with a mean length at age 1 of 130 mm (Table 1). Growth rate was consistent between ages 1 and 10, averaging 41 mm/year. Freshwater drum averaged 503 mm at age 10 and grew at an average rate of 31 mm/year from age 10 through 13. The length-weight equation $\log_{10} \text{ weight} = -6.10 + 3.46 \log_{10} \text{ length}$ was significant at $p < 0.0001$, $r^2 = 0.951$.

Kentucky Lake freshwater drum fed primarily on benthic organisms (Table 2). Small fish from 100 to 300 mm fed about equally on larvae of Ephemeroptera (*Hexagenia*) and Diptera. The Asiatic clam *Corbicula fluminea* appeared in stomachs of only 2.0% of the small fish. However, after the fish attain 300 mm, the diet shifted from insect larvae to *Corbicula*, with the shift becoming more pronounced with increasing fish length. The frequency of occurrence of Asiatic clams was 71% for individuals greater than 300 mm and 84% for those greater than 400 mm. *Corbicula* was often the only food of fish longer than 500 mm. Crayfish were occasionally consumed by larger freshwater drum; fishes as prey were present in low numbers in all freshwater drum size classes.

DISCUSSION

The growth of freshwater drum in Kentucky Lake was generally slower than that reported in many other waters (Table 3). The differences in growth rates do not appear to be related to climate as some of the populations exhibiting more rapid growth than in Kentucky Lake are more southern in latitude (Benton, Jackson, and Davies 1988; Houser

Table 1. Mean backcalculated lengths (mm) and weighted mean increment of growth (mm) for 322 freshwater drum collected from Kentucky Lake in 1985-1986.

Age	n	Mean length at annulus formation															
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
0	10																
1	28	117															
2	52	125	161														
3	46	125	169	201													
4	60	128	172	206	241												
5	41	131	176	212	244	279											
6	16	131	178	216	248	284	322										
7	28	139	186	219	257	293	328	364									
8	18	131	176	214	251	291	331	365	411								
9	7	141	187	221	259	288	333	367	402	443							
10	6	142	180	221	279	323	363	405	451	510	561						
11	6	138	189	231	272	307	355	395	431	488	539	578					
12	2	138	191	250	284	327	352	376	412	452	498	566	595				
13	1	115	151	171	210	261	285	349	391	431	468	499	510	535			
14	0	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
15	0	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
16	1	121	155	192	228	253	289	337	396	416	448	477	577	657	677	697	722
Weighted mean		130	175	213	252	291	329	370	413	457	503	530	561	596	677	697	722
Weighted mean increment			45	38	39	39	38	41	43	44	46	27	32	35	81	20	25

1960) and one is more northern (Butler and Smith 1950). Freshwater drum in several natural northern lakes have faster growth rates early in life and slower growth rates later in life compared to those in Kentucky Lake (Bur 1984; Priegel 1969). The growth of freshwater drum in Kentucky Lake most closely resembled that of this species in two other reservoirs. Wheeler Reservoir (Wrenn 1968), another Tennessee River impoundment is more southern, located in Alabama. Kentucky Lake fish grew faster initially, but by 5 years of age, fish from Wheeler Reservoir had surpassed the growth of freshwater drum in Kentucky

Lake and continued to widen the differential with increasing age. Lewis and Clark Lake (Swedberg 1965) is a more northern reservoir, located on the Nebraska-South Dakota border. Growth rates there were similar to the present study, although fish in Kentucky Lake lived slightly longer.

Freshwater drum feed on clams and mussels after reaching some size threshold between 250 and 300 mm TL. In Kentucky Lake, fish greater than 300 mm TL fed extensively on the Asiatic clam, often the only food of fish longer than 500 mm TL. Native mussels are also occasionally consumed by freshwater drum in the lake (Paul Rister, pers. comm.). Individuals between 100 and 300 mm TL fed on *Hexagenia* and dipteran larvae. Food habits reported here (Table 2) are similar to those reported from Wheeler Reservoir (Wrenn 1968). In both reservoirs, freshwater drum less than 254 mm TL fed predominantly on dipteran larvae, with copepods and the larvae of Ephemeroptera and Plecoptera of lesser importance. Freshwater drum greater than 254 mm TL fed predominantly on *Corbicula* and fishes in Wheeler Reservoir (Wrenn 1968). The same pattern was evident more recently in Lake Erie with freshwater drum

Table 2. Percent frequency of occurrence of food items from freshwater drum collected from Kentucky Lake in 1987-1988. n = 122. (sample size in parentheses).

Food	Total length (mm)				
	100-199 (23)	200-299 (52)	300-399 (16)	400-499 (12)	≥500 (19)
<i>Corbicula</i> sp.	—	2.0	62.5	83.3	84.2
Crayfish	—	—	12.5	—	10.5
Diptera (larvae)	52.2	50.0	12.5	—	—
Ephemeroptera (larvae)	47.8	49.0	37.5	16.7	—
Odonata (larvae)	—	3.9	—	8.3	—
Miscellaneous insects	—	—	—	8.3	—
Miscellaneous fishes	4.3	7.8	12.5	8.3	10.5

Table 3. Mean backcalculated lengths (mm) at each annulus for freshwater drum in Kentucky Lake, Kentucky and Tennessee 1985–1986, and other studies.

	Length at each annulus											
	1	2	3	4	5	6	7	8	9	10	11	12
Kentucky Lake (present study)	130	175	213	252	291	329	370	413	457	503	530	561
Jordan Dam Tailrace, AL												
Benton, Jackson, and Davies (1988) ¹	148	214	269	312	362	406	444	456	479			
U. Mississippi River Butler & Smith (1950)	124	229	297	340	376	419	460	485				
Lake Erie Bur (1984)												
males	127	186	223	251	278	307	334	359	407	451		
females	134	190	227	259	287	315	351	382	425	488	567	
Oklahoma Reservoirs Houser (1960) ²	107	216	300	353	389	467	518	572	592	635	678	754
Wheeler Reservoir, AL Wrenn (1968)	81	145	198	241	295	348	406	475	551	607	647	676
Lake Winnebago, WI Priegel (1969)	130	218	277	312	338	356	368	381	391	427		
Lewis and Clark Lake, NE–SD Swedberg (1965)	94	166	209	241	271	311	355	410	451	502	573	

¹ Unweighted mean.² Unweighted mean from four reservoirs with the sample size from each greater than 100 fish.

feeding on zebra mussels. French and Bur (1993) found that predation on zebra mussels occurs in fish greater than 250 mm and increases with total length. Zebra mussels were the most abundant food item by volume and dry weight in both medium (250–374 mm TL) and large (375–574 mm TL) fish, with some larger specimens eating almost exclusively zebra mussels. They believe freshwater drum will likely feed on zebra mussels wherever their ranges coincide.

If zebra mussels reach densities in Kentucky Lake similar to those reported for Lake Erie, the freshwater drum population in Kentucky Lake may be profoundly affected. We predict that freshwater drum will be a significant predator of zebra mussels when the mussels colonize Kentucky Lake. This may cause an increase in the numbers, growth rate, and production of freshwater drum, already the most common incidental fish in the Kentucky Lake commercial catch (Timmons et al. 1989). Whether they will select thin shelled zebra mussels over thicker shelled *Corbicula* is not known. We are not aware of any studies documenting the effects of freshwater drum predation on zebra mussel populations, an area certainly in need of further study.

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Understanding the Mass Distribution of the Dwarf Galaxy DDO 170

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ABSTRACT

Using a two-component mass model consisting of (1) a luminous disk with constant mass to light ratio and (2) an HI gas disk, we determined the rotation curve for the dwarf galaxy DDO 170 in the conformal model of gravity. The rotation curve we derived using the two-component, “maximal disk” mass model is in excellent agreement with the measured rotation curve.

INTRODUCTION

Compared to studies of mass distribution in bright spiral galaxies, much less is known about the distribution of mass in dwarf spiral and irregular galaxies. Recent studies of dwarf galaxies have added to the evidence that dark matter is a significant contributor to the mass of these galaxies (Broeils 1992; Carignan and Beaulieu 1989; Lake 1989; Lake, Schommer, and van Gorkom 1990). Mass models for most dwarf galaxies studied to date include three components (1) a luminous disk with a constant mass to light ratio, (2) a gas disk, and (3) a dark matter halo.

As is the case for bright spiral galaxies, dark matter distributions for dwarf spiral and irregular galaxies can be determined by comparing the “measured mass” of stars and gas with the dynamical mass via a comparison of the rotation curve predicted using the measured mass, and of the rotation curve determined by making measurements of the Doppler shifts of light from the galaxy at several radii. The well-documented result of this mass comparison indicates the existence of dark matter (Binney and Tremaine 1987). The specific distribution of dark matter in every galaxy is different, but in general the dark matter makes up 50% to 90% of bright galaxies and is distributed in an isothermal sphere or halo. The basic halo parameters (halo core radius, phase density, and asymptotic velocity) are very sensitive to the mass model used to describe the galaxy. Even though observation of a galaxy and measurements of its luminosity and total flux indicate the general distribution of stars and gas, respectively, the overall mass of the galaxy cannot be determined with great precision, and

the ratios $M_{\text{gas}}/M_{\text{star}}$ and M_{total}/L_B are variable parameters in most rotation curve fits. These factors lead to the somewhat unsettling conclusion that the percentage and distribution of dark matter and the ratio $M_{\text{gas}}/M_{\text{star}}$ vary widely from galaxy to galaxy for no fundamental physical reason.

Based upon the many unanswered questions concerning the nature of dark matter and the fact that there seems to be a great deal of arbitrariness when determining the amount and distribution of dark matter in galaxies, it is reasonable to ask whether or not two-components mass models can be used to understand the mass distributions in galaxies and, if so, how. These are the questions in which we are interested.

We chose to study DDO 170 for several reasons. The most important of these was the high quality of the HI images and photometry that has led to a well-determined rotation curve for this particular dwarf galaxy. In addition, to study the mass distribution of a galaxy using galactic rotation as the comparator, we note that the galaxy must be relatively isolated so that the perturbative effects of neighbors are minimal. The gas distribution in the galaxy must be smooth and symmetric, otherwise the assumption of circular motion may not be appropriate. Also, to assure that our model is one describing a galaxy that exists in nature, the measured rotational velocities derived separately from opposite sides of the galaxy should agree to within 10%. It is also desirable to study a galaxy with an observed inclination between 50° and 80° so it can be verified that a disk describes the plane of the galaxy accurately all the way to the

edges and that the azimuthal distribution of the gas can be accurately determined. For DDO 170, all of these properties were observationally verified (Lake, Schommer, and van Gorkom 1990) and this galaxy is, therefore, exceptionally useful for our purposes.

METHODS

The Potential-Velocity Relationship

If the galaxy is assumed to be an axisymmetric collection of gravitationally bound masses rotating about some axis, we can derive an expression for the relationship between the gravitational potential and orbital velocity of matter in the galaxy. The result of such an exercise gives us the rotation curve. This procedure is described well in the literature (e.g., Binney and Tremaine 1987).

Because it is known that the Newtonian potential cannot be used to accurately derive the rotation curve for the galaxy DDO 170 using only a two-component mass model (Lake, Schommer, and van Gorkom 1990), we must look to modifications of the gravitational potential to derive an acceptable potential-velocity relationship. The non-Newtonian theory of gravity known as MODified Newtonian Dynamics (MOND) (Milgrom 1983) could possibly provide us with such a modification. However, MOND has failed in its attempts to provide a solution to the galactic rotation curve problem without the use of dark matter for this and several other galaxies (Lake 1989; Lake, Schommer, and van Gorkom 1990).

Another possibility lies in the framework of a modification of classical general relativity known as conformal gravity. Kazanas and Mannheim (1989) derived an exact expression for the gravitational potential on all distance scales in the presence of a static, spherically symmetric source. This solution is the analog of the Schwarzschild solution in general relativity. In conformal gravity the Newtonian potential is modified to include a linear term and a quadratic term in radius. Mannheim and Kazanas (1992) argued that,

although the Schwarzschild-like solution of the conformal gravity field equations produces the expected short-distance (Newtonian) potential, on larger distance scales the modifications become important. These authors have used this solution up to the linear modification of the potential to successfully describe the rotation curves for galaxies NGC 3198, NGC 2903, NGC 5907, and DDO 154 without the use of dark matter (Mannheim 1993). These four galaxies are representative of four luminosity classes: intermediate, compact bright, large bright, and dwarf. Because we are particularly interested in dwarf galaxies we note that the rotation curve for DDO 154 was successfully derived using only the visible mass. Mannheim and Kazanas (1992) noted that their treatment of DDO 154 led to some anomalous results in light of the rotation curve behavior of the other three galaxies. We also note that the fits to the rotation curve in conformal gravity using only the linear modification for DDO 154 indicate that the galaxy appears to be gas-rich where $M_{\text{gas}}/M_{\text{stars}} = 5.2$ and $M_{\text{total}}/L_B = 1.4$.

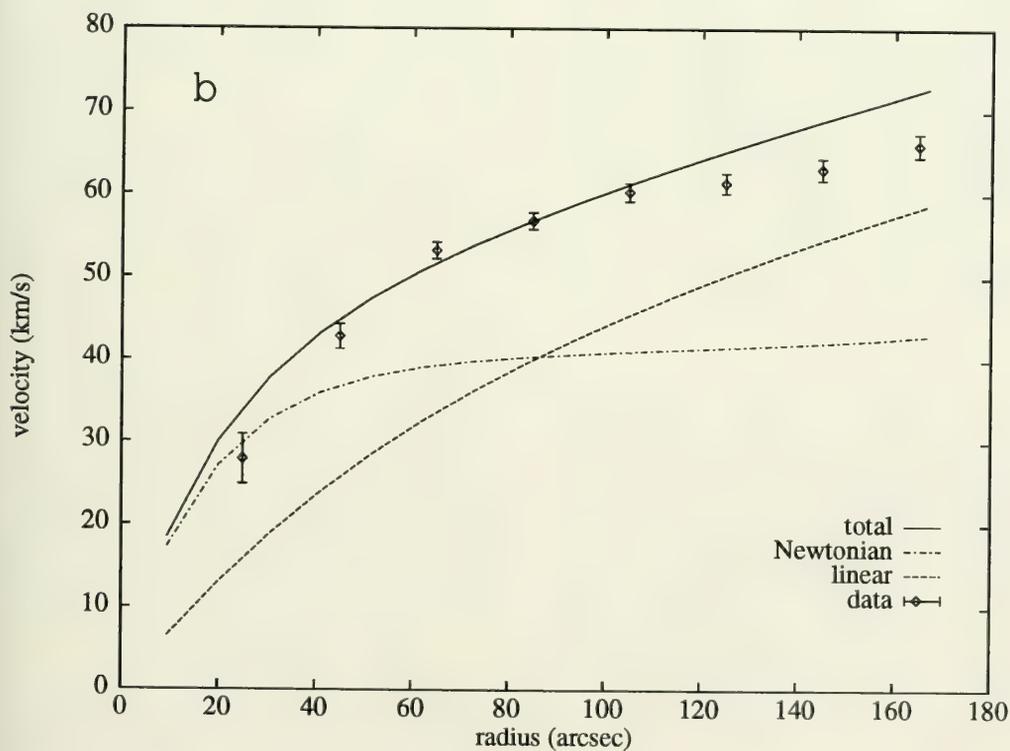
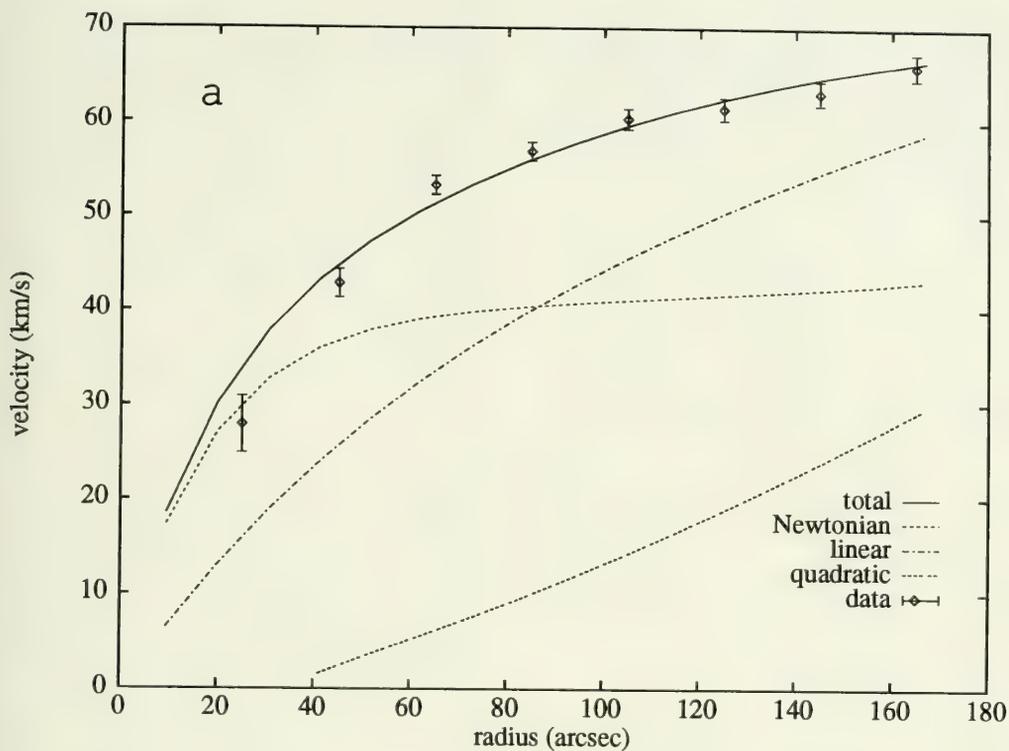
Although it is interesting that conformal gravity seems to have had some success predicting rotation curves by using two-component mass models, the reasons for using only the linear modification to make a prediction are unclear. Therefore, we use two methods to derive a two-component mass model for DDO 170. The first employs a modified Newtonian potential including only the linear modification, which we refer to as the "truncated model"; the second incorporates both linear and quadratic modifications given in Mannheim (1993) and Mannheim and Kazanas (1992).

In conformal gravity the gravitational potential for a static, spherically symmetric gravitating object V is given in Mannheim (1993)

$$V(r) = -2\beta/r + \gamma r - kr^2 \quad (1)$$

where the constants β , γ , and k are integration constants depending on the mass of the gravitating system under study. Because a galaxy is

Figure 1. Rotation curve fits for the galaxy DDO 170. The solid line is the fit to the data points when (a) the full conformal gravity model is used and (b) the linear potential model is used. The data are from Lake, Schommer, and van Gorkom (1990).



a collection of stars with potential given in equation (1) we can derive the potential of the galaxy if we know the stellar and gas distribution functions so that the gravitational potential for an extended, axially symmetric disk with matter distribution given by $\rho(R, z')$ is

$$\begin{aligned}
 V(r, z) = & -2\beta \int dR d\phi' dz' \\
 & \cdot [R\rho(R, z')] [r^2 + R^2 \\
 & \quad - 2rR \cos \phi' \\
 & \quad + (z - z')^2]^{1/2} \\
 & + \gamma \int dR d\phi' dz' \\
 & \cdot R\rho(R, z') [r^2 + R^2 \\
 & \quad - 2rR \cos \phi' \\
 & \quad + (z - z')^2]^{1/2} \\
 & - k \int dR d\phi' dz' \\
 & \cdot R\rho(R, z') [r^2 + R^2 \\
 & \quad - 2rR \cos \phi' \\
 & \quad + (z - z')^2] \quad (2)
 \end{aligned}$$

where the primed coordinates represent source coordinates and the unprimed coordinates refer to observational coordinates. The complete expression (2) was used in the full conformal gravity model while we set $k = 0$ in the truncated model. Given the correctness of the assumptions about DDO 170 listed above, the potential given in (2) can then be used in the well-known expression relating circular velocity and potential:

$$v(r) = [rV'(r)]^{1/2}. \quad (3)$$

Once the matter distribution $\rho(R, z')$ for the galaxy is determined via observation, the right hand side of equation (3) can be evaluated, and a rotation curve can be derived. Several authors have been able to analytically reduce equation (2) to a more compact expression for several special cases (Binney and Tremaine 1987; Cuddeford 1993; Mannheim 1993); however for the sake of generality we chose to leave it in this form and to perform the necessary numerical integration to evaluate equation (3).

The gravitational potential for every gravita-

tionally bound system depends on the specific distribution of matter in the system via the function $\rho(R, z')$, which is determined by observation. We used only the visible mass of stars and gas in DDO 170 to determine the exact form for the gravitational potential and used this potential to determine the rotation curve. The variable parameters in the rotation curve fits are the integration constants in the conformal gravity model and the ratio $M_{\text{gas}}/M_{\text{stars}}$ so that equation (3) becomes

$$v(r) = [r_{\text{stars}} V'_{\text{stars}}(r) + \frac{M_{\text{gas}}}{M_{\text{galaxy}}} r_{\text{gas}} V'_{\text{gas}}(r)]^{1/2}. \quad (4)$$

Observed Star and Gas Mass

DDO 170 is a dwarf galaxy of absolute magnitude $M_B = -15.15$ with the approximate location of $13^{\text{h}}13^{\text{m}}$ RA $25^{\text{d}}42'$ DEC. The galaxy is inclined at an angle of 84° and has major and minor axis lengths of 4.7 and 0.9 arcsec, respectively. The radius of the HI gas disk is about five times larger than the optical disk radius. Details of the optical and radio observations as well as the details of the rotation curve derivation can be found in Lake, Schommer, and van Gorkom (1990). Here we quote only the details necessary to describe the two-component mass model and resulting rotation curve.

The luminosity profile can be described as an exponential while the HI profile is described well by a Gaussian. We use these expressions in equation (2) so that $\rho(R, z') = (M/L)e^{-R/22}f(z')$ and $\rho(R, z') = e^{-(R/95)^2}f(z')$ for the HII and HI distributions, respectively. In accordance with the mass model used by Lake, Schommer, and van Gorkom (1990), the function $f(z')$ is taken as 1 in the case of the gas distribution and an exponential in the case of the stellar distribution. Since $f(z')$ has not been precisely determined by observation, we numerically studied the dependence of the rotation curve on $f(z')$ (unpublished results) and concluded that the dependence is small when $f(z')$ assumes a form common to that observed for other axisymmetric galaxies (Cuddeford 1993; van der Kruit and Searle 1981).

RESULTS AND DISCUSSION

Galactic Rotation Curve for DDO 170

Using only the visible mass and gas along with the procedure described above, we have

derived a rotation curve for DDO 170 using two methods (Figure 1). According to our best fit, we have determined a constant mass-to-light ratio of about four for the stellar disk and derived our rotation curve using observations of R in units of arcsec and not kpc to minimize the effects of error due to measurements of distance to the galaxy. In Figure 1 we show the results of fitting our model to the data using the full conformal gravity potential and truncated potential model, respectively, when the ratio $M/L_{\text{disk}} = 4.0$ (as determined by observation) and $M_{\text{gas}}/M_{\text{star}} = 3$. This last ratio is over an order of magnitude lower than that used in three-component dark matter models; however, this is to be expected given the modification of the potential. We have maximized the contribution of the stellar disk to the rotation curve (hence the term “maximum disk” model) in order to compare more directly the fits for this particular dwarf galaxy with rotation curve fits for bright spirals.

It is obvious from comparison of Figure 1a and Figure 1b that the full conformal gravity fit models the observed rotation curve more accurately. Note that the quadratic term becomes important far from the edge of the stellar disk and serves only to keep the rotation curve from rising too fast. Mannheim (1993) found the quadratic term to be negligible in the rotation curve fits and suggested that the quadratic term becomes important only on cosmological distance scales. Here, however, we find that $k_{\text{galaxy}} = 3.6 \times 10^{-36} \text{cm}^{-2}$ and has a noticeable affect on the rotation curve beginning at about three times the radius of the stellar disk. It is also true that the linear term ($\gamma = 8.4 \times 10^{-28} \text{cm}^{-1}$) works well to describe the rotation curve up to that point.

These results lead us to question the physical interpretation of k and the quadratic term as cosmological. To further address this issue, we plan to analyze the rotation curve of another dwarf galaxy, DDO 154, using the full conformal gravity potential to see if the “anomalous” behavior referred to be Mannheim (1993) might be accounted for in this way, or if this behavior is truly an indicator of some basic physical property of low luminosity (dwarf) galaxies.

SUMMARY

We have shown that it is not necessary to employ dark matter as part of a three-com-

ponent model to derive the rotation curve for the dwarf galaxy DDO 170. By use of the full conformal gravity potential, the measured rotation curve for the dwarf galaxy DDO 170 can be derived using a two-component mass model that consists of (1) a luminous disk with constant mass-to-light ratio and (2) an HI gas disk. A good fit to the measured rotation curve can be obtained when a “maximal disk” model is employed.

To understand whether or not two-component models can in general be used to understand the mass distribution in other dwarf galaxies as well as spiral galaxies, the procedure employed here to determine the rotation curve will continue to be used. Future studies will help us to better understand (1) how the integration constants in conformal gravity scale with mass, (2) overall trends regarding the distribution of visible stars and gas in bright spiral galaxies as opposed to dwarf spiral and dwarf irregular galaxies, and (3) the dependence of the ratio $M_{\text{gas}}/M_{\text{star}}$ on morphology.

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Unionid Fauna of the Lower Cumberland River from Barkley Dam to the Ohio River, Kentucky (Mollusca: Bivalvia: Unionidae)

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ABSTRACT

Unionids of the lower Cumberland River, Kentucky, were surveyed by brail and SCUBA from Barkley Dam to the Ohio River. The only previous survey was conducted in 1910-1911 by Wilson and Clark. The two surveys are compared and show a significant decrease in the number of surviving species. Live individuals of 24 species were found in our surveys compared to 45 species found by Wilson and Clark in the region now inundated by Lake Barkley. Three species were found that had not been reported previously from the Cumberland River. Alteration of habitat resulting from impoundment, pollution, and rapid fluctuation of discharge from hydroelectric dams apparently are the major causes for the decline in native species, while impoundment probably has enhanced invasion by species from the Ohio River.

INTRODUCTION

In recent years the number of species of unionids on the federal endangered species list (USFWS 1993) has increased, and there is growing interest in the scientific community in conserving biodiversity and understanding its importance to a healthy environment (Wilson 1994). In contrast, there is an apparent waning interest in protecting rare, threatened, and endangered species and the environment by the current U.S. Congress. With the rapid spread of zebra mussels into the Ohio and Mississippi river systems, an already impoverished native unionid fauna is faced with greater threats, making it imperative that unique, rare, and endangered species be studied and documented. Documentation of species and recent changes in their abundance and distribution is critical for understanding the importance of complex ecosystems if biodiversity is to be preserved. With these issues in mind, the results of several surveys of the lower Cumberland River conducted by us in 1981 and 1988 are reported here and compared to a 1910-1911 survey by Wilson and Clark (1914).

STUDY AREA

The Cumberland River arises in the Cumberland Mountains in southeastern Kentucky.

It flows westerly, arching southward into central Tennessee. From there it flows westerly, gradually arching northward into western Kentucky and northwesterly to its junction with the Ohio River at Smithland, Kentucky. The "official" U.S. Army Corps of Engineers junction, Cumberland River mile (CRM) 0.0, occurs at Ohio River mile (ORM) 922.7 (Anonymous 1994). The actual confluence of the two bodies of water where mixing begins is at the official Cumberland River mile 2.2 (Figure 1). The Cumberland River and the larger and nearly parallel Tennessee River are two of the most ancient rivers of North America. This in part is the reason for the great diversity of unionids that once populated the rivers (Wilson and Clark 1914). Documentation of the unionids of the middle and upper Cumberland River by numerous authors in recent years indicates major declines of the unionid fauna of those areas (Anderson, Layzer, and Gordon 1991; Blankenship and Crockett 1972; Call and Parmalee 1982; Clark 1981, 1985; Harker et al. 1980; Isom, Gooch, and Dennis 1979; Miller, Rhodes, and Tippit 1984; Neel and Allen 1964; Parmalee, Klippel and Bogan 1980; Schmidt 1982; Schuster, Butler, and Stansbery 1989; Starnes and Bogan 1982). Two important reviews providing gen-

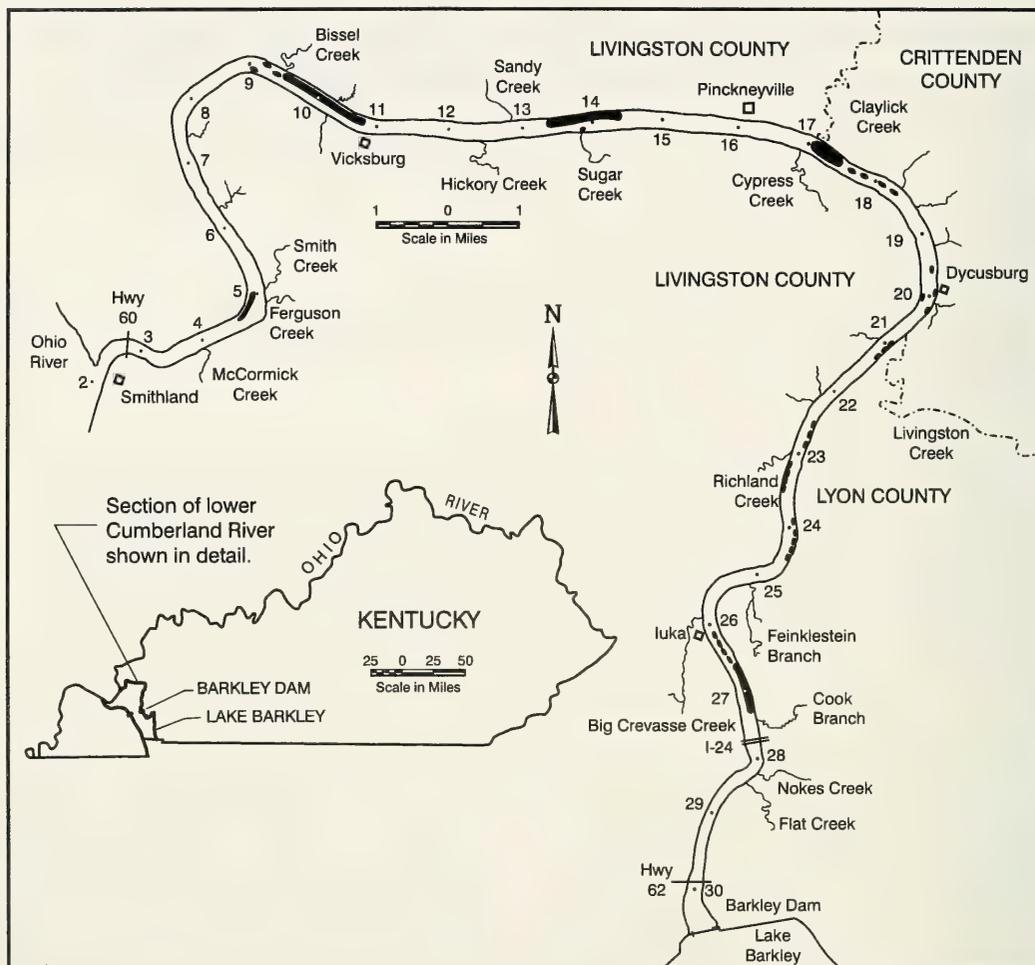


Figure 1. Map of lower Cumberland River, Kentucky, from Barkley Dam at Cumberland River mile 30.6 to the Ohio River showing major unionid beds as elongated, solid areas and river mile points as solid dots. Numbers 2 through 30 along the river represent miles from its confluence with the Ohio River. (Width of river is shown as twice actual scale.)

eral information about unionid distribution in the Cumberland River are those by Cicerello, Warren, and Schuster (1991) for Kentucky and Starnes and Bogan (1988) for Tennessee.

WILSON AND CLARK 1910–1911 SURVEY

Charles B. Wilson and H. Walton Clark (1914) published the results of a freshwater unionid survey of the Cumberland River conducted by them, John F. Boepple, and Ernest Danglede and supervised by Robert E. Coker. Their project involved several expeditions in summers of 1910 and 1911 to evaluate the shell resources then being utilized in the pearl button industry. By sampling with crowfoot

brails, tongs, and rakes in deep water, wading in shallow water, and examining the catch of commercial shellers, they described the species composition and relative abundance of unionids from the headwaters above Cumberland Falls in eastern Kentucky to near the mouth where the Cumberland River enters the Ohio River. Their sample location farthest downstream was at Horse Ford near Kuttawa, Lyon County, Kentucky, at Cumberland River mile (CRM) 36.5 (58.7 km from the Ohio River at Smithland, Livingston County, Kentucky). They reported extensive beds from Cumberland Falls to Kuttawa. That study constitutes the only published report prior to our

surveys of the unionids inhabiting the lower Cumberland River, including the region presently inundated by Lake Barkley. In their report, Wilson and Clark (1914) briefly discussed ecological requirements of some unionids and the influence of dams on this fauna.

The effect of dams on unionids living in once free-flowing rivers has been extensive (Bates 1962; Fuller 1974). When Wilson and Clark (1914) were conducting their survey, only one dam had been constructed on the lower Cumberland River below Nashville, Tennessee; eight had been completed upstream from Nashville. Lock and Dam A at CRM 150.6 were completed by the Army Corps of Engineers in 1904 near the present site of Cheatham Dam (CRM 148.7), Cheatham County, Tennessee, which was completed in 1957. Cheatham Dam forms the upstream boundary of Lake Barkley. Dam A formed a pool that backed up the Harpeth River entering at CRM 153. Wilson and Clark (1914) reported that siltation in the lower Harpeth River resulting from Dam A had eliminated a large unionid bed. Similar results were observed immediately upstream from other dams. However, Wilson and Clark reported rapid growth of unionids in the backwaters above the silt, and they believed that the dams would be more beneficial than harmful. They were referring to low dams, however, that did not raise the water above the original river banks. They had no experience with the high dams such as Barkley and Cheatham dams presently on the lower Cumberland River.

From near the present location of Cheatham Dam (CRM 148.7) to Barkley Dam (CRM 30.6), Lyon County, Kentucky, Wilson and Clark (1914) collected at 28 stations. From their descriptions many of their stations can be located on current maps or navigation charts. In the following discussion, locations given as approximate river miles could not be located precisely. Below mile 148, the upstream boundary of the present Lake Barkley, Wilson and Clark reported 47 species (Table 1). Eleven of the 47 species are listed on the federal endangered species list (USFWS 1993): *Cyprogenia stegaria*, *Dromus dromas*, *Epioblasma florentina*, *E. haysiana*, *E. obliquata*, *Hemistena lata*, *Lampsilis abrupta*,

Obovaria retusa, *Plethobasus cooperianus*, *Pleurobema plenum*, and *Quadrula fragosa*. *Cyprogenia stegaria* was reported at Seven-mile Ferry (CRM 132.5), Owl Hollow Bar (ca. CRM 130), and Guisers [Geisers] bar (CRM 128.5). *Dromus dromas* occurred at Seven-mile Ferry and Red Rock Bar (ca. CRM 125) below Clarksville, Montgomery County, Tennessee. Only a shell of *Epioblasma florentina* was found at Half Pone Bar (CRM 145). *Epioblasma haysiana* was found at Clarksville (CRM 126) and above Ball [Bald] Island (CRM 97.6). *Epioblasma obliquata* was reported alive only from Half Pone Bar; a dead shell was found at Elk Creek Shoals (CRM 98.6). *Hemistena lata* was reported from Half Pone Bar. *Lampsilis abrupta* was reported from Seven-mile Ferry and Kuttawa, Kentucky (CRM 41). *Obovaria retusa* was reported from above Ball Island, foot of Dover Island (CRM 90.5), Canton, Kentucky (CRM 62), and Horse Ford (CRM 36.6). *Plethobasus cooperianus* was reported from Owl Hollow Bar, Guisers Bar, Clarksville, Red Rock Bar, Meeks Spring Bar (ca. CRM 118), Walters' Camp (ca. CRM 98), above and below Ball Island (CRM 97.4), Linton (CRM 73), Donelson [Donaldson] Creek (CRM 67), Canton (CRM 63), and Horse Ford below Kuttawa. At Walters' shell camp in 1911 Wilson and Clark noted a pile of ca. 150 tons of shells with *Plethobasus cooperianus* ranking second in abundance of the commercial species. *Pleurobema plenum* was found at Half Pone Bar, Seven-mile Ferry, and Jones Landing (ca. CRM 80) somewhere between Dover, Stewart County, Tennessee, and Linton, Trigg County, Kentucky. *Quadrula fragosa* was reported from Half Pone Bar, Seven-mile Ferry, Owl Hollow Bar, Clarksville, Red Rock Bar, Trices Landing (ca. CRM 124), above Meeks Spring Bar (ca. CRM 119), Meeks Spring Bar, foot of Dover Island, Jones Landing, Linton, below Canton, Eddyville Bar (CRM 44), above Kuttawa, below Money Cliff (CRM 39), and at Horse Ford. Wilson and Clark did not collect downstream from Horse Ford at CRM 36.5. Therefore, no records exist before our 1981 and 1988 surveys of the unionid fauna in the region now the tailwater of Barkley Dam from CRM 30.6 to the Ohio River.

Historically, the Cumberland River contained at least 85 species of unionid bivalves

Table 1. Unionid clams of the lower Cumberland River, Kentucky, from the region now inundated by Barkley Lake to the Ohio River as reported by Wilson and Clark (1914) from CRM 36 - 148 and surveys in 1981 and 1988 from CRM 2.5 - 47.

Taxa ¹	Common Name	Nomenclature of Wilson and Clark (1914)	Reported in 1914	Present Surveys
<i>Actinonaias ligamentina</i> (Lamarck, 1819)	Mucket	<i>Lampsilis ligamentina gibba</i> (Simpson, 1900)	+	-
<i>Amblema plicata perplicata</i> (Conrad, 1841)	Roundlake	<i>Quadrula perplicata</i> (Conrad, 1841)	+	-
<i>Amblema plicata plicata</i> (Say, 1817)	Threeridge	<i>Quadrula undulata</i> (Barnes, 1823)	-	+
<i>Anodonta suborbiculata</i> Say, 1831	Flat floater		-	+
<i>Arcidens confragosus</i> (Say, 1829)	Rock pocketbook		-	+
<i>Cumberlandia monodonta</i> (Say, 1829)	Spectaclecase	<i>Margaritana monodonta</i> (Say, 1829)	+	-
<i>Cyclonaias tuberculata</i> (Rafinesque, 1820)	Purple wartyback	<i>Quadrula tuberculata</i> (Rafinesque, 1820)	+	shell
<i>Cyclonaias tuberculata</i> (Rafinesque, 1820)	Purple wartyback	<i>Quadrula granifera</i> (Lea, 1838)	+	-
<i>Cyprogenia stegaria</i> (Rafinesque, 1820)	Fanshell	<i>Cyprogenia irrorata</i> (Lea, 1828)	+	-
<i>Dromus dromas</i> (Lea, 1834)	Dromedary mussel	<i>Dromus dromas</i> (Lea, 1834)	+	-
<i>Ellipsaria lineolata</i> (Rafinesque, 1820)	Butterfly	<i>Plagiola securis</i> (Lea, 1829)	+	+
<i>Elliptio crassidens</i> (Lamarck, 1819)	Elephant-ear	<i>Unio crassidens crassidens</i> Lamarck, 1819	+	+
<i>Elliptio dilatata</i> (Rafinesque, 1820)	Spike	<i>Unio gibbosus</i> Barnes, 1823	+	+
<i>Epioblasma flexuosa</i> (Raf., 1820)	Leafshell		-	shell
<i>Epioblasma florentina florentina</i> (Lea, 1857)	Yellow blossom	<i>Truncilla florentina</i> (Lea, 1857)	shell	-
<i>Epioblasma haysiana</i> (Lea, 1833)	Acornshell	<i>Truncilla haysiana</i> (Lea, 1834)	+	-
<i>Epioblasma obliquata</i> (Rafinesque, 1820)	Catspaw	<i>Truncilla sulcata</i> (Lea, 1829)	+	-
<i>Fusconaia ebena</i> (Lea, 1831)	Ebonyshell	<i>Quadrula ebena</i> (Lea, 1831)	+	+
<i>Fusconaia flava</i> (Rafinesque, 1820)	Wabash pigtoe	<i>Quadrula undata</i> (Barnes, 1823)	+	+
<i>Fusconaia subrotunda</i> (Lea, 1831)	Long solid	<i>Quadrula subrotunda</i> (Lea, 1831)	+	-
<i>Hemistena lata</i> (Rafinesque, 1820)	Cracking pearly	<i>Lastena lata</i> (Rafinesque, 1820)	+	-
<i>Lampsilis abrupta</i> (Say, 1831)	Pink mucket	<i>Lampsilis orbiculata</i> (Hildreth, 1828)	+	shell
<i>Lampsilis ovata</i> (Say, 1817)	Pocketbook	<i>Lampsilis ovata</i> (Say, 1817)	+	-
<i>Lampsilis ventricosa</i> (Barnes, 1823) ²	Pocketbook	<i>Lampsilis ventricosa</i> (Barnes, 1823)	+	-
<i>Lampsilis anodontoides</i> (Lea, 1831) ²	Yellow sandshell	<i>Lampsilis anodontoides</i> (Lea, 1831)	+	-
<i>Lampsilis teres</i> (Rafinesque, 1820)	Yellow sandshell	<i>Lampsilis fallaciosa</i> (Smith, 1899)	+	-
<i>Lasmigona complanata</i> (Barnes, 1823)	White heelsplitter	<i>Symphnota complanata</i> (Barnes, 1823)	+	+
<i>Leptodea fragilis</i> (Rafinesque, 1820)	Fragile papershell	<i>Lampsilis gracilis</i> (Barnes, 1823)	+	+
<i>Ligumia recta</i> (Lamarck, 1819)	Black sandshell	<i>Lampsilis recta</i> (Lamarck, 1819)	+	+
<i>Megalonaias nervosa</i> (Rafinesque, 1820)	Washboard	<i>Quadrula heros</i> (Say, 1829)	+	+
<i>Obliquaria reflexa</i> Rafinesque, 1820	Threehorn wartyback	<i>Obliquaria reflexa</i> Rafinesque, 1820	+	+
<i>Obovaria olivaria</i> (Rafinesque, 1820)	Hickorynut	<i>Obovaria ellipsis</i> (Lea, 1828)	+	-
<i>Obovaria retusa</i> (Lamarck, 1819)	Ring pink	<i>Obovaria retusa</i> (Lamarck, 1819)	+	shell
<i>Obovaria subrotunda</i> (Rafinesque, 1820)	Round hickorynut	<i>Obovaria circulus</i> (Lea, 1829)	+	-
<i>Plethobasus cicatricosus</i> (Say, 1829)	White wartyback		-	shell
<i>Plethobasus cooperianus</i> (Lea, 1834)	Orangefoot pimpleback	<i>Quadrula cooperiana</i> (Lea, 1834)	+	shell
<i>Plethobasus cyphus</i> (Rafinesque, 1820)	Sheepnose	<i>Pleurobema aesopus</i> (Green, 1827)	+	-
<i>Pleurobema catillus</i> (Conrad, 1836) ²	Solid pigtoe	<i>Quadrula solida</i> (Lea, 1838)	+	-

Table 1. (continued)

Taxa ¹	Common Name	Nomenclature of Wilson and Clark	Reported in 1914	Present Surveys
<i>Pleurobema cordatum</i> (Rafinesque, 1820)	Ohio pigtoe	<i>Quadrula obliqua</i> (Lamarck, 1819)	+	+
<i>Pleurobema plenum</i> (Lea, 1840)	Rough pigtoe	<i>Quadrula plena</i> (Lea, 1840)	+	-
<i>Pleurobema rubrum</i> (Rafinesque, 1820)	Pink pigtoe	<i>Quadrula pyramidatum</i> (Lea, 1831)	-	shell
<i>Pleurobema sintoxia</i> (Rafinesque, 1820)	Round pigtoe	<i>Quadrula coccinea</i> (Conrad, 1834)	-	shell
<i>Potamilus alatus</i> (Say, 1817)	Pink heelsplitter	<i>Lampsilis alata</i> (Say, 1817)	+	+
<i>Potamilus ohioensis</i> (Rafinesque, 1820)	Pink papershell	<i>Lampsilis laevisissima</i> (Lea, 1829)	+	shell
<i>Ptychobranthus fasciolaris</i> (Rafinesque, 1820)	Kidneyshell	<i>Ptychobranthus phaseolus</i> (Hildreth, 1828)	+	-
<i>Pyganodon grandis</i> (Say, 1829) ³	Giant floater	<i>Anodonta grandis</i> Say, 1829	-	+
<i>Quadrula cylindrica cylindrica</i> (Say, 1817)	Rabbitsfoot	<i>Quadrula cylindrica</i> (Say, 1817)	+	-
<i>Quadrula fragosa</i> (Conrad, 1835)	Winged mapleleaf	<i>Quadrula fragosa</i> (Rafinesque, 1820)	+	-
<i>Quadrula metanevra</i> (Rafinesque, 1820)	Monkeyface	<i>Quadrula metanevra</i> (Rafinesque, 1820)	+	+
<i>Quadrula nodulata</i> (Rafinesque, 1820)	Wartyback		-	+
<i>Quadrula quadrula</i> (Rafinesque, 1820)	Mapleleaf	<i>Quadrula lachrymosa</i> (Lea, 1828)	-	+
<i>Quadrula pustulosa pustulosa</i> (Lea, 1831)	Pimpleback	<i>Quadrula pustulosa</i> (Lea, 1831)	+	+
<i>Strophitus undulatus undulatus</i> (Say, 1817)	Squawfoot	<i>Strophitus edentulus shaefferiana</i> (Lea, 1852)	+	-
<i>Tritogonia verrucosa</i> (Rafinesque, 1820)	Pistolgrip	<i>Quadrula tritogonia</i> Ortmann, 1909	+	+
<i>Truncilla donaciformis</i> (Lea, 1828)	Fawnsfoot	<i>Plagiola donaciformis</i> (Lea, 1828)	+	+
<i>Truncilla truncata</i> Rafinesque, 1820	Deertoe	<i>Plagiola elegans</i> (Lea, 1831)	+	+
<i>Utterbackia imbecillis</i> (Say, 1829) ³	Paper pondshell	<i>Anodonta imbecillis</i> Say, 1829	-	+
<i>Villosa lienosa</i> (Conrad, 1834)	Little spectaclecase	<i>Lampsilis lienosa</i> (Conrad, 1834)	+	-

(Hyphen indicates not found, plus indicates found alive, and "shell" indicates found only as a dead shell)

¹ Nomenclature agrees with that of Turgeon et al. (1988) except where noted otherwise

² Nomenclature of Ortmann and Walker (1922)

³ Hoeh (1990)

(Starnes and Bogan 1988). This number is second only to the Tennessee River, which once had ca. 95 species (Starnes and Bogan 1988), the largest assemblage found anywhere (Johnson 1978). Many species once found in the middle and upper reaches of the Cumberland River belong to the Cumberlandian fauna, those unionids that apparently had their origin in the Cumberland and Tennessee river systems. Ortmann (1924) defined the Cumberlandian Region as extending from the headwaters of the Cumberland River down to near Clarksville, Tennessee, and the headwaters of the Tennessee River down to some point beyond Muscle Shoals, Alabama, including part of the Duck River, Tennessee.

Of the species reported by Wilson and Clark (1914) in the lower Cumberland River, only three were considered to be Cumberlandian

forms (origin in Cumberlandian Region) by Ortmann (1925): *Dromus dromas*, *Epioblasma haysiana*, and *E. florentina*. Johnson (1980), however, added three more to the list, claiming that they had extended their range from the Cumberlandian Region into the Ohio drainage: *Epioblasma flexuosa*, *Lampsilis abrupta*, and *Plethobasus cicatricosus*. In the case of *Lampsilis abrupta*, Ortmann (1925) stated that it is a large river form and its "center of origin is in the Interior Basin."

With the demise of the pearl button industry, the lower Cumberland River saw few shellers. When the demand for shells for the cultured pearl industry sent shellers back to the rivers, the discovery that Cumberland River shells from below Nashville were too chalky or badly eroded and had a low value for the production of pearl nuclei kept shellers away.

Therefore, little was known about the recent unionid fauna until our surveys were conducted.

The purpose of our study was to determine the distribution and relative abundance of the unionid fauna of the lower Cumberland River from Barkley Dam to the Ohio River. The Nashville District Corps of Engineers had proposed channel maintenance operations that potentially could have an adverse impact on the mollusks. The Corps was required to ascertain whether any species on the federal endangered species list survived in the area of the river being considered for channel improvement. We present results of that survey and a comparison with the Wilson and Clark (1914) study along with a report on the range extension of three species into the Cumberland River and a comparison with archeological studies.

1981 AND 1988 SURVEYS

Methods

The 1981 survey was conducted using three commercial boats equipped with 4.9 m crow-foot brails and operated by commercial shellers. Brails have been used in the shell industry since the end of the 19th century (Coker 1919). Our brails consisted of a metal or wooden bar with 1 m lengths of line or light chain attached every 7.6 cm. Attached to the lines were bundles of four-pronged hooks made of heavy (10 to 14 gauge) wire. The ends of the wire were bent upward at various angles depending on the type of sediment in which the brail was used and on the method of hauling. The tips of the hooks were melted to form a bead to help hold the unionid, a modification invented by J. F. Boepple (Wilson and Clark 1914), one of the founders of the pearl button industry in the United States. One or two brails were towed slowly by a boat such that the bar was suspended just above the sediment surface and the hooks raked the bottom. A hook tip that enters the opening between the two valves of a unionid will cause the individual to clamp shut on the hook. If the hook is secure it will pull the individual from the sediment; the individual will remain attached until the brail is hauled to the surface. A jonboat equipped with a 2.4 m brail and operated by Murray State University person-

nel was also used. Three SCUBA divers sampled selected sites where brails were not effective, such as among large rocks and snags. Shorelines were searched for empty shells that may have washed ashore or piles of shells left by muskrats. Both conditions usually indicate that a bed is nearby. Beds discovered by brailing and areas in the vicinity of shell piles observed along the shoreline were also examined by divers. The commercial shellers brailed the middle, right, and left margins of the channel from river mile 30 to the Ohio River at CRM 2.2. Where unionids were encountered in numbers suggesting a bed, several brail hauls were made to more fully determine the location and extent of the bed and the species composition. SCUBA diving on beds was used to search for species not caught by the brails. Representative samples of brail catches and all SCUBA samples were counted to determine the relative abundance of species. Other brail samples were examined only for species composition. Empty shells found along shore were examined for species not encountered by other collecting methods. The 1988 survey was conducted by two SCUBA divers. One additional site was sampled by divers in Lake Barkley at Clay Bay, CRM 47.0. Names of species correspond to those given in Turgeon et al. (1988) except where otherwise noted.

Results and Discussion

The distribution of all species found during our 1981 and 1988 surveys is presented in Table 2. The sample locations are reported by river mile and position within the river as either left, middle, or right of the channel as viewed facing downstream.

Unionid beds. Unionid beds are considered to be locations of stable substrate, usually of gravel and sand stabilized by compact silt and clay, in which individuals of various age classes and species occur in significant densities, generally more than $1/m^2$. The establishment of a bed requires many years since recruitment is generally a slow process. It is not uncommon to find beds composed of individuals ranging from 5 to 25 years in age with very few juveniles.

Figure 1 shows the Cumberland River from Barkley Dam to the Ohio River. Elongated solid areas indicate the major beds found in the surveys. The small, oval, solid areas indi-

cate smaller concentrations. The only bed that several retired commercial shellers could recall as ever having produced commercial harvests was in the vicinity of mile 14 between Pinckneyville and Sandy Creek. Diving and brailing at the site revealed a bed with limits from mid-channel to the right bank from CRM 13.1–14.7. Nineteen species were found in the bed, which was the largest and most species rich in the Barkley Dam tailwater section of the river. The age composition of the bed indicated that some of the species in this section of the river may be on the verge of demise, most individuals being over 15 years old with no evidence of recruitment since the construction of Barkley Dam in 1965. The rapid rise and fall of water level caused by the intermittent discharge of water for power generation at Barkley Dam may be one cause for the lack of recruitment.

The surveys located other beds at CRM 4.5–5.0, middle; CRM 9.4–11.0, middle; CRM 17.0–17.3, right-middle-left; and CRM 26.5–27.2, middle-right. The bed at CRM 4.5–5.0, confined to a narrow mid-channel region, consisted mainly of *Megaloniaias nervosa* with minor representatives of 13 other species. The bed at CRM 9.4–11.0 was rather spread out down the mid-channel. Fourteen species were represented with *Fusconaia ebena* being the most abundant and *Megaloniaias nervosa*, *Elliptio crassidens*, and *Pleurobema cordatum* following in that order.

At CRM 17.0–17.3 a small bed was located in the vicinity of another old shell pile on the right bank at CRM 17.3. The bed was small but extended from bank to bank and contained 12 species.

The bed at CRM 26.5–27.1 was dense and extended from the bottom of the right bank out to mid-channel. Fifteen species were recovered alive along with empty shells of *Obovaria retusa* and *Pleurobema rubrum*. This bed is in danger of being destroyed by channel maintenance dredging.

Old shell piles along shore. Because of the steep river banks and bank slumping caused by the fluctuating discharge at Barkley Dam and wave action from passing vessels, there were few shoreline sites where empty shells were likely to wash ashore and few locations where old shell piles remained exposed. Only a few shells were found on gravel bars or clay

banks; most of these were *Corbicula fluminea* (Müller), one of the Asiatic clams.

Three large shell piles were found mostly buried in the bank, which had apparently slumped over them. These were located on the right bank at miles 14.3, 17.3, and 19.5. Digging in the bank uncovered many shells, mostly “pinks,” *Cyclonaias tuberculata*, *Elliptio crassidens*, and *Elliptio dilatata*, and “washboards,” *Megaloniaias nervosa*. “Pinks” are shells with a pink or purple nacre that were not used in the pearl button industry; they were usually culled by shellers along shore to remove them from the harvest and to prevent them from returning to the beds. Washboards in the Cumberland River were generally stained and of low value, so they too were frequently culled (Wilson and Clark 1914). Because of the species composition of the piles, it is assumed that they are old cull piles from pearl button days 40 to 90 years ago. Commercial shellers probably culled the unwanted shells along shore near the beds where they were working. Two of the three shell piles occurred adjacent to existing beds, which supports the idea that the beds are very old. Some of the living individuals in these beds could well be older than 30 years. It is difficult to determine accurately the age of a clam beyond about 15 years without thin sectioning the shell because the shell growth slows and the external rest lines tend to be poorly separated. The third pile was near a landing at Dycusburg, probably a location where commercial shellers processed their harvest.

Comparison with Wilson and Clark survey. All of the unionids found in our study and those reported by Wilson and Clark (1914) for the lower Cumberland River are listed in Table 1. Wilson and Clark reported 45 species from between CRM 148–125 and 33 species from CRM 124–36. Our survey lists 33 species (24 found alive) from CRM 47–2.2. Of the 14 species Wilson and Clark reported from above CRM 124 that did not occur below that point, two are Cumberlandian forms (Ortmann 1925): *Dromus dromas* and *Epioblasma florentina*. One Cumberlandian species, *Epioblasma haysiana*, was reported by Wilson and Clark below CRM 124 from above Ball Island at CRM 98. It is unlikely that these species still exist in the lower Cumberland River. Ac-

Table 2. Abundance of live unionid clams collected by brailing or SCUBA diving left, middle, or right of the river channel in surveys conducted in 1981 (a) and 1988 (b) in the lower Cumberland River, Kentucky, from CRM 47 to the Ohio River. (The letter S indicates that only one or more dead shells were found.)

Taxa	CRM 2.5 Right, Dive (b)	CRM 4.3 Left, Dive (b)	CRM 4.7-5.0 Middle, Brail (a)	CRM 4.9 Left, Dive (b)	CRM 9.0-9.3 Middle, Brail (a)	CRM 9.4-11.0 Middle, Brail (a)	CRM 10.6-11.0 Middle, Brail (a)	CRM 11.0-12.4 Middle, Brail (a)	CRM 13.5-14.0 Right, Brail (a)	CRM 13.9 Right, Dive (a)	CRM 13.9 Left, Dive (a)	CRM 14.2 Right, Dive (a)
<i>Amblema plicata</i>	20	2	2	5	1	3		3	3	2		1
<i>Anodonta suborbiculata</i>												
<i>Arcidens confragosus</i>			3		1					1		
<i>Cyclonaias tuberculata</i>												
<i>Ellipsaria lineolata</i>			4			4			6			
<i>Elliptio crassidens</i>			5			14	1		8			
<i>Elliptio dilatata</i>										1		
<i>Epioblasma flexuosa</i>												
<i>Fusconaia ebena</i>	1		6			25		1	10	2		2
<i>Fusconaia flava</i>	4		1			1				1		
<i>Lampsilis abrupta</i>												
<i>Lasmigona complanata</i>	1	1							1			
<i>Leptodea fragilis</i>	6	3		2		1						
<i>Ligumia recta</i>										1		
<i>Megalonaias nervosa</i>	51	4	41	8	3	19		2	31	10	1	18
<i>Obliquaria reflexa</i>	5		3		4			5	5			2
<i>Obovaria retusa</i>												
<i>Plethobasus cicatricosus</i>												
<i>Plethobasus cooperianus</i>												
<i>Pleurobema sintoxia</i>												
<i>Pleurobema cordatum</i>			2			13			6	4		
<i>Pleurobema rubrum</i>												
<i>Potamilus alatus</i>	4	7	3	7	3	2		1			1	
<i>Potamilus ohioensis</i>												
<i>Pyganodon grandis</i>	1											
<i>Quadrula metanevra</i>	1		1			3		1	6			
<i>Quadrula nodulata</i>			1		1	2			4			
<i>Quadrula p. pustulosa</i>			1			1		1		1		
<i>Quadrula quadrula</i>	10		3		3	3		1	4		1	1
<i>Tritogonia verrucosa</i>	2					1			5			
<i>Truncilla donaciformis</i>									1			
<i>Truncilla truncata</i>												
<i>Utterbackia imbecillis</i>												
TOTAL LIVE UNIONIDS	106	17	76	22	16	92	1	15	90	23	3	24
TOTAL SPECIES LIVE	12	5	14	4	7	14	1	8	13	9	3	5

Table 2. (continued)

Taxa	CRM 17.0-17.5 Left, Brail (a)	CRM 17.3 Right, Dive (a)	CRM 17.5-19.6 Middle, Brail (a)	CRM 19.6 Left, Dive (a)	CRM 19.8 Right, Dive (b)	CRM 20.0 Left, Dive (a)	CRM 20.0 Right, Dive (b)	CRM 20.0-20.2 Right, Brail (a)	CRM 23.3 Right, Dive (a)	CRM 23.8-24.4 Middle, Brail (a)	CRM 24.2 Left, Dive (b)	CRM 24.5 Right, Dive (b)
<i>Amblema plicata</i>	3	2			11			1		1	17	8
<i>Anodonta suborbiculata</i>												
<i>Arcidens confragosus</i>												
<i>Cyclonaias tuberculata</i>												
<i>Ellipsaria lineolata</i>	3											
<i>Elliptio crassidens</i>	11		9		1							
<i>Elliptio dilatata</i>												
<i>Epioblasma flexuosa</i>												
<i>Fusconaia ebena</i>	10		2		1			1	1	1		
<i>Fusconaia flava</i>			1		1			1		1		
<i>Lampsilis abrupta</i>												
<i>Lasmigona complanata</i>												
<i>Leptodea fragilis</i>	1		1		2		3				1	4
<i>Ligumia recta</i>												
<i>Megalonaias nervosa</i>	13		3		21		1		3	2		4
<i>Obliquaria reflexa</i>	3		1		2				1		33	3
<i>Obovaria retusa</i>												
<i>Plethobasus cicatricosus</i>												
<i>Plethobasus cooperianus</i>		S										
<i>Pleurobema sintoxia</i>												
<i>Pleurobema cordatum</i>	8		1									
<i>Pleurobema rubrum</i>												
<i>Potamilus alatus</i>	3		1		3	3						2
<i>Potamilus ohioensis</i>												
<i>Pyganodon grandis</i>						1						
<i>Quadrula metanevra</i>			1								1	
<i>Quadrula nodulata</i>		1	1					1			3	1
<i>Quadrula p. pustulosa</i>												
<i>Quadrula quadrula</i>	1	4	1	1	2			2			3	2
<i>Tritogonia verrucosa</i>		1	1		1							
<i>Truncilla donaciformis</i>												
<i>Truncilla truncata</i>											1	
<i>Utterbackia imbecillis</i>											1	
TOTAL LVE UNIONIDS	56	8	23	1	45	4	4	6	5	5	59	24
TOTAL SPECIES LIVE	10	4	12	1	10	2	2	5	3	4	8	7

Table 2. (continued)

Taxa	CRM 25.5 Right, Dive (a)	CRM 26.3-27.0 Middle, Brail (a)	CRM 26.9 Right, Dive (a)	CRM 27.1 Right, Dive (a)	CRM 27.6 Left, Dive (a)	CRM 28.1 Right, Dive (b)	CRM 28.3 Right, Dive (b)	CRM 28.5 Right, Dive (a)	CRM 47.0 Left, Dive (b)	TOTAL LIVE	% TOTAL
<i>Amblesma plicata</i>		5	6	6	1		13	1	6	123	13.23
<i>Anodonta suborbiculata</i>									2	2	0.22
<i>Arcidens confragosus</i>										5	0.54
<i>Cyclonaias tuberculata</i>			S							0	0.00
<i>Ellipsaria lineolata</i>			1							18	1.94
<i>Elliptio crassidens</i>		3	1	3						56	6.02
<i>Elliptio dilatata</i>										1	0.11
<i>Epioblasma flexuosa</i>					S					0	0.00
<i>Fusconaia ebena</i>		2	7	3						75	8.06
<i>Fusconaia flava</i>		1	1	2						15	1.61
<i>Lampsilis abrupta</i>					S					0	0.00
<i>Lasmigona complanata</i>			3	2						8	0.86
<i>Leptodea fragilis</i>	2		1				25			52	5.59
<i>Ligumia recta</i>		2								3	0.32
<i>Megalonaias nervosa</i>		3	10	7			1			256	27.53
<i>Obliquaria reflexa</i>		1					5			73	7.85
<i>Obovaria retusa</i>			S							0	0.00
<i>Plethobasus cicatricosus</i>					S					0	0.00
<i>Plethobasus cooperianus</i>										0	0.00
<i>Pleurobema sintoxia</i>					S					0	0.00
<i>Pleurobema cordatum</i>		3	3							40	4.30
<i>Pleurobema rubrum</i>			S							0	0.00
<i>Potamilus alatus</i>		1		1	1		2	1		46	4.95
<i>Potamilus ohioensis</i>								S		0	0.00
<i>Pyganodon grandis</i>							2			4	0.43
<i>Quadrula metanevra</i>		2								16	1.72
<i>Quadrula nodulata</i>	1									16	1.72
<i>Quadrula p. pustulosa</i>										4	0.43
<i>Quadrula quadrula</i>		7	4	4			7		31	95	10.22
<i>Tritogonia verrucosa</i>		3	2	2						18	1.94
<i>Truncilla donaciformis</i>										1	0.11
<i>Truncilla truncata</i>							1			2	0.22
<i>Utterbackia imbecillis</i>				S						1	0.11
TOTAL LIVE UNIONIDS	3	33	39	30	2	0	56	2	39	929	99.89
TOTAL SPECIES LIVE	2	12	11	9	1	0	8	2	3	24	

cording to Stansbery (1970) *Epioblasma florentina* is restricted to the South Fork Holston River, Virginia, while *Epioblasma florentina walkeri*, perhaps the same as *Epioblasma florentina* of Wilson and Clark, is "reduced to the lower Stones and Red Rivers of the Cumberland River system," and *Epioblasma haysiana* is restricted to a 16 km region of the Clinch River. *Dromus dromas* was recently found living in the Cumberland River 160 km upstream from Nashville at CRM 296.8 by Parmalee et al. (1980), and there is a possibility that it still exists in the upstream section of Lake Barkley above Clarksville within the original Cumberlandian Region where Lake Barkley still retains some of the characteristics of a free-flowing river.

Within the region now in Lake Barkley, the data from Wilson and Clark (1914) indicate a gradual change in species composition with the number of species declining downstream. This is to be expected because, as the river increases in size and decreases in gradient, the variability of habitats is reduced and fewer shoals occur, the shoals being a favored habitat for small river forms.

The 14 species Wilson and Clark (1914) reported only from above CRM 124 (CRM 124–148) were *Cyclonaias tuberculata*, *Cyprogenia stegaria*, *Dromus dromas*, *Epioblasma florentina*, *E. obliquata*, *Fusconaia subrotunda*, *Hemistena lata*, *Lasmigona complanata*, *Obovaria subrotunda*, *Pleurobema sintoxia*, *Ptychobranchus fasciolaris*, *Truncilla donaciformis*, *T. truncata*, and *Villosa lienosa*. With the exception of the two Cumberlandian species already mentioned (*Dromus dromas*, *Epioblasma florentina*) that were not reported below CRM 125, the other 12 species are widely distributed in other river systems, and three (*Lasmigona complanata*, *Truncilla donaciformis*, *T. truncata*) are present in the lower Cumberland today.

Several species were found in our study that were not reported by Wilson and Clark (1914) in the lower Cumberland River and several that have never been reported from the Cumberland River. Those not reported by Wilson and Clark are *Anodonta suborbiculata*, *Arcidens confragosus*, *Epioblasma flexuosa*, *Plethobasus cicatricosus*, *Pleurobema rubrum*, *Pyganodon grandis*, *Quadrula nodulata*, and *Utterbackia imbecillis*.

Epioblasma flexuosa was found in our survey at CRM 27.5 only as a single valve with part of the periostracum attached. Casey (1987) reported shells from archaeological sites at CRM 26L, 26.6R, and 27.3L that date between 1000 and 1300 A.D. Neel and Allen (1964) reported *Epioblasma lewisi* (Walker) from the upper Cumberland River where Wolf Creek Dam is now located. Both are assumed to be extinct (Stansbery 1970).

Plethobasus cicatricosus was recovered as a single, badly eroded valve at CRM 27.6. It has not been reported live from the Cumberland River but was previously reported from prehistoric rock shelters along the middle Cumberland River by Parmalee, Klippel, and Bogan (1980) and by Casey (1987) at CRM 26.6R and CRM 27.3L. Stansbery (1970) considered it restricted to a single population in the Tennessee River in northern Alabama below Wilson Dam.

Pleurobema rubrum was recovered only as a relic shell, both valves with most of the periostracum attached, at CRM 26.9. It was not reported by Wilson and Clark (1914) from the lower Cumberland, but they found a few in the upper Cumberland. It was found by Parmalee, Klippel, and Bogan (1980) in the prehistoric rock shelter deposits along the Cumberland River in Smith County, Tennessee.

Pyganodon grandis and *Utterbackia imbecillis* were not reported by Wilson and Clark from the main river, but they were found in a floodplain lake, Haynes Lake, a short distance downstream from Clarksville, Tennessee.

Three species found in our surveys were not reported from the Cumberland River by Wilson and Clark (1914), Neel and Allen (1964), or Parmalee, Klippel, and Bogan (1980): *Anodonta suborbiculata*, *Arcidens confragosus*, and *Quadrula nodulata*. All three were listed by Johnson (1980) as having originated in the Mississippian Region. He suggested that *Arcidens confragosus* had a refugium in the Meramec River, Missouri, during the Pleistocene and that all three found a refuge in the Ozarkian Region below the Ozark Crest. Johnson (1980) presumed that their extension into the Ohioan Region occurred after the Pleistocene; however he did suggest that the Green River, Kentucky, may have been a refugium during the Pleistocene. These three species apparently have entered the lower Cumberland Riv-

er since the Wilson and Clark survey, and the altered habitat caused by dams may have influenced the invasion. In 1929 the Army Corps of Engineers completed Lock and Dam 52 at Paducah, Kentucky. This dam maintained an upstream elevation of 92 m above mean sea level. This reduced the flow in the lower Cumberland River and may have encouraged the invasion by the three Mississippian species. The completion of Barkley Dam in 1965 may have contributed to the invasion as well. The impoundment conditions apparently have been favorable for their range extension. A similar event occurred in the Tennessee River following impoundment of Kentucky Lake in 1944. None of the three Mississippian species was reported from the Tennessee River by Ortmann (1918, 1924, 1925) or van der Schalie (1939) except for *Quadrula nodulata*, which van der Schalie (1939) listed from Paducah, Kentucky, at the junction of the Tennessee and Ohio rivers. Stansbery (1964) found *Anodonta suborbiculata* in the Tennessee River at Muscle Shoals, Alabama, in 1963; Isom (1969) found *Arcidens confragosus* in 1965; and Yokley (1972) reported *Quadrula nodulata*, *Arcidens confragosus*, and *A. suborbiculata* in Kentucky Lake in 1972.

To explain the previous absence of these three species—*Anodonta suborbiculata*, *Arcidens confragosus*, and *Quadrula nodulata*—in the Cumberland River, one must examine the geological history of the region. Ortmann (1925) pointed out that the Tennessee and Cumberland rivers were at one time joined and separated from the Ohio River, at least for some distance beyond the present confluence. Thornbury (1965) suggested that the two rivers had a pre-Pleistocene connection below Paducah, Kentucky, and continued westward through the Metropolis Lowland now occupied by the Ohio River, which at that time flowed through the Cache Lowland to the north. The junction with the Ohio River may have occurred near Cairo, Illinois, or perhaps as far south as Memphis, Tennessee. The isolation of the Tennessee River from the Cumberland River by the present Ohio River at the end of the Pleistocene in conjunction with reduced gradient in the lower sections of the rivers essentially isolated the Cumberlandian fauna in the upper reaches and allowed the invasion of the Ohioan and Mississippian fau-

nas to proceed. But, apparently, the direct connection of the Cumberland River to the Ohio River during the Pleistocene was not sufficient to allow all of the Ohioan and Mississippian species to invade. If length of time alone were the factor limiting the invasion, then all of the common Interior Basin (Mississippian and Ohioan) fauna might have extended into the Cumberland River. Obviously barriers, both ecological and geological, prevented wholesale invasion just as they prevented most of the Cumberlandian fauna from extending beyond the Tennessee and Cumberland rivers. Before successful invasion can occur, the appropriate host fish must be present and a sufficient number of unionids (as larvae, juveniles, or adults) or a gravid female must be transported to a suitable habitat where a reproducing population can become established. Then habitat suitability and lack of competition are required.

In fact, the lower Cumberland River may have experienced fluctuations between Ohioan and Cumberlandian species composition since the Pleistocene. During the dry, hypsithermal (Franklin 1994) from 8700 to 5000 years ago, Cumberlandian species may have been more common in the lower Cumberland River, and species such as *Epioblasma flexuosa*, *E. obliquata*, and *Pleurobema clava*, which may have originated in the Cumberlandian Region, moved into the Ohioan Region. Between 700 and 1000 years ago at least two species, *Dromus dromas* and *Epioblasma arcaeformis*, were common in the region now the Barkley Dam tailwater (Casey 1987). The changing climate with increasing moisture may have restricted the Cumberlandian fauna upstream as Ohioan species invaded the lower reach of the river, which was becoming larger and deeper and more like their natural habitat in the Ohio River.

Another notable change from 1911 to the present is the replacement of *Quadrula fragosa* with *Q. quadrula*. It could be argued that Wilson and Clark (1914) were actually looking at *Quadrula quadrula* but identified it as *Q. fragosa*. However, the figures by Coker (1919) clearly distinguish the two and we assume they recognized the difference. We are presently observing a changing species composition from *Quadrula quadrula* to *Q. apiculata* (Say, 1829) in Kentucky Lake within the last 15

years, suggesting that these changes can occur rapidly as better-adapted species invade. This, of course, has major implications for attempts at preservation of endangered species.

Impoundment and pollution have resulted in a large reduction in the number of native species in the Cumberland River. However, altered habitat and reduction in native species have allowed more Interior Basin species, well adapted to impoundment conditions, to invade the lower Cumberland River.

There were 30 species reported by Wilson and Clark (1914) in the Cumberland River downstream from CRM 148 that we did not find alive (Table 1). Remnants of these species may survive in the upper regions of Lake Barkley where riverine conditions occur, but it is probably safe to say that they all have disappeared from lower sections of the Cumberland River from approximately Dover, Tennessee, CRM 89, to the Ohio River.

CONCLUSIONS

Twenty-four species of unionids in 18 genera still survive in the lower Cumberland River from mile 47 to mile 2.2. Nine additional species in seven genera were found only as relic shells. No live specimens listed on the federal endangered species list were encountered, although relic or subfossil shells of six endangered species were found: *Epioblasma flexuosa*, *Lampsilis abrupta*, *Obovaria retusa*, *Plethobasus cicatricosus*, *P. cooperianus*, and *Pleurobema rubrum*. Thirty species reported in 1914 were not found alive in our study. It is unlikely that very many of these survive in the Barkley Dam tailwater section of the Cumberland River. Impoundment, both on the Ohio and Cumberland rivers, appears to be the major cause for change in species composition. However, the changing climate since the Pleistocene, changes in availability of host fish, pollution, and successful invasions by species from other regions have played various roles in determining the present species composition. Unionid communities are in constant flux. Better knowledge regarding what controls the makeup of unionid communities is needed if their biodiversity is to be preserved.

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NOTES

Nesting Association of the Cyprinid Fishes *Phoxinus cumberlandensis* and *Semotilus atromaculatus* (Cyprinidae).—*Phoxinus cumberlandensis*, a cyprinid endemic to small, upland streams in the upper Cumberland River basin of eastern Kentucky and Tennessee (1), is known to spawn only over fine, silt-free gravel in *Campostoma anomalum* nests (2). In silt-free streams, *P. cumberlandensis* presumably spawns over gravel in riffles or runs. However, siltation and habitat loss from coal mining, silviculture, agriculture, and road construction have reduced and fragmented *P. cumberlandensis* habitat and resulted in the fish being a threatened species by the U.S. Fish and Wildlife Service (3). Herein we report observations of an additional host species for *P. cumberlandensis*.

On 12 May 1993 we observed and photographed ca. 30 brilliantly colored *P. cumberlandensis* in an occupied *Semotilus atromaculatus* nest in Rock Creek, McCreary County, Kentucky. Rock Creek is a second-order tributary to Jellico Creek; it supports one of the best remaining populations of *P. cumberlandensis* in Kentucky (Cicerello and Laudermilk, unpubl. data). Upstream from the site of our observations, the watershed is densely forested and contains only one small, reclaimed strip mine along the Tennessee border. As a result, the sand, gravel, and scattered cobbles underlying the stream's alternating pools and short riffles are relatively silt-free. At the time of our observations, Rock Creek was clear, low, and ca. 3-4 m wide; flow was slow to negligible. The gravel *Semotilus* nest was at a depth of ca. 7-10 cm in the lower end of a pool immediately upstream from a small riffle.

When we approached the stream, the nest's occupants fled to the deeper, upper end of the pool, but the *P. cumberlandensis* aggregation slowly meandered back toward the nest after we took cover. Members of the aggregation included brilliantly colored males and females and smaller, apparently juvenile individuals lacking bright spawning colors. While moving toward the nest, males chased females individually and in groups in the manner described by Starnes and Starnes (2). After an estimated 10-15 minutes, the aggregation returned to and hovered over the ca. 25 cm diameter nest depression. About 5-10 minutes later, a nuptial male *Semotilus* (ca. 18 cm TL) returned to the nest where his presence was obscured by the *P. cumberlandensis* hovering above. Starnes and Starnes (2) witnessed aggressive behavior toward *Semotilus* by nesting *C. anomalum*, which they believed provided associated *P. cumberlandensis* with some protection from egg predation. We did not observe spawning by *P. cumberlandensis*, but their behavior and previously unreported association with a nesting *S. atromaculatus* (4) are consistent with spawning by *P. cumberlandensis* observed in a *C. anomalum* nest (2) and by *P. oreas* in *Nocomis* nests (5).

Phoxinus cumberlandensis occurs syntopically with *Semotilus* and *C. anomalum* above Cumberland Falls, and with *Semotilus* and *C. oligolepis*, a probable *P. cumberlandensis* nesting host, below the falls. However, *Semotilus*

occurs more frequently and is more abundant than *Campostoma* spp. in streams inhabited by *P. cumberlandensis*. Of 95 collections of *P. cumberlandensis* made in 1993-1994, 89 (94%) included *Semotilus* but only 24 (25%) included *Campostoma* spp. During April through June, when *P. cumberlandensis* spawns (2), *Semotilus* also was present more often than *Campostoma* spp. (51 collections vs. 11).

We believe these observations strongly suggest that *P. cumberlandensis* spawns in *Semotilus atromaculatus* nests, even in relatively silt-free streams such as Rock Creek; that *Semotilus* is more important than *Campostoma* spp. in providing spawning habitat for *P. cumberlandensis*; and that nest-building cyprinids probably play an important role in conservation of *P. cumberlandensis* by providing spawning habitat in relatively clean streams as well as those degraded by silt. However, additional observations are needed to confirm these findings and to document spawning by *P. cumberlandensis* independently of nest-building cyprinids.

We thank B.M. Burr (Southern Illinois University at Carbondale) for reviewing the draft, and V. Bishop and L. Perry (U.S. Forest Service) for providing land-use information. This study was supported in part by the Kentucky Department for Surface Mining Reclamation and Enforcement, Frankfort, Kentucky.

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***Sclerochloa dura* (Poaceae) in Kentucky.**—The European grass *Sclerochloa dura* (L.) Beauv. (Figure 1), hard grass, was first collected in North America in New York in 1895, but this introduction apparently did not lead to naturalization of the species. Thirty-three years later, in 1928 in Utah, the grass was collected again. Since then,

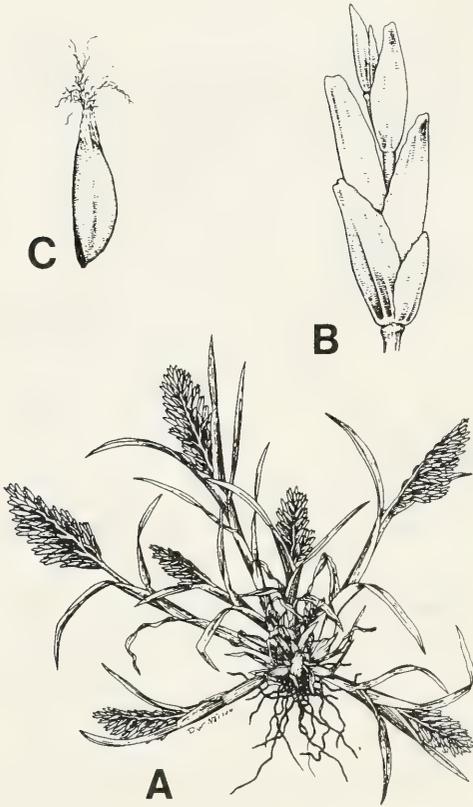


Figure 1. *Sclerochloa dura*. A, plant, $\times 0.45$. B, spikelet, $\times 5.0$. C, caryopsis, $\times 7.2$. Drawing by Paul W. Nelson.

the plant has become fully naturalized in the United States, having been found in 16 additional states from coast to coast by 1991 (1). The species has since been recorded from Illinois and Indiana (2) and from Iowa (3). It can now be added to the flora of Kentucky, where it is known from six counties (Bracken, Campbell, Grant, Jefferson, Kenton, Robertson), all in the northern part of the state. The specimens were collected from mid-April to mid-May.

All the Kentucky specimens were growing in athletic fields and associated disturbed areas. Of the 12 such fields

searched in 1995, the grass was in nine of them. In some places there were hundreds of individuals; in others, individuals were few and widely scattered. The recent—and first—record from Ohio (Hamilton County) (1) was also from an athletic field. Swink and Wilhelm (2) mentioned that *S. dura* is common in fairgrounds in parts of its range. The first Iowa collection was from a gravel parking lot (3).

We suggest that *S. dura* may be carried from field to field in soil on the shoes of ball players as the players peregrinate in search of victories. The grass turns brown and dies early in the season, providing abundant propagules in places frequented by players and onlookers: the fields, the bleacher areas, and the nearby parking lots.

We also suggest that the species might also be spread in "athletic field" turfgrass mixtures. Many of these mixtures are said to originate in Washington and Oregon, where *S. dura* has been known for about 60 years. We are unable to confirm or deny this idea.

Associates of the plant in Kentucky include other vernal annuals: *Capsella bursa-pastoris*, *Cerastium* spp., *Draba brachycarpa*, *D. verna*, *Holosteum umbellatum*, *Matricaria matricarioides*, *Poa annua*, *P. chapmaniana*, *Stellaria media*, *Veronica peregrina*, and *V. arvensis*.

The grass often grows with *Poa annua*, which bears a resemblance to it, especially in vegetative condition. Both grasses come into flower when they are quite young; they are then easily distinguished by their inflorescences, which in hard grass are distinctly one-sided, quite unlike the more or less symmetric ones of *P. annua*. The branches of *S. dura* are ascending to prostrate, forming clumps to 12 cm wide.

We cite here a voucher specimens for the presence of *S. dura* in Kentucky: KENTUCKY. Jefferson Co., Louisville, in "turf" of *Poa annua* in mowed ballfield along River Road near Ohio Street, 26 Apr 1994, Thieret 57202 (KNK).

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Abstracts of Some Papers Presented at the 1995 Annual Meeting of the Kentucky Academy of Science

AGRICULTURAL SCIENCES

Contents of field-grown turnips in relation to colored mulches. G. S. ANTONIOUS* and M. E. BYERS, Atwood Research Facility, Kentucky State University, Frankfort, KY 40601; M. J. KASPERBAUER, Coastal Plains Research Center, USDA-ARS, Florence, SC 29501.

Growth, development, and chemical composition of field-grown turnips (*Brassica campestris* cv. Purple Top) can be influenced by the spectrum of light reflected from the soil surface. The shoot/root weight ratio and the chemical composition of roots were affected by the quantity of blue light and the ratio of far-red relative to red (FR/R) in upwardly reflected light. A FR/R ratio higher than the ratio in incoming light generally resulted in a greater shoot/root biomass ratio. In a taste test of uncooked roots, 24 of 25 panelists indicated that turnips that received more reflected blue light during development had roots with a sharper flavor. The majority of the tasters also indicated that roots from plants grown with green mulch had the mildest (almost sweet) flavor. In a 2-year laboratory study, roots from turnips grown in field plots over blue, green, silver, and white surfaced plastic mulches were analyzed for concentrations of total glucosinolates (GSLs), ascorbic acid, and sugar. The greatest concentrations of GSLs and ascorbic acid were found in roots grown with blue mulches. Reducing sugars were higher in roots grown with green than in those grown with blue mulches. The comparison of chemical composition of roots from plants grown with blue versus green mulches is important because both blue and green surfaces reflected about the same amount of photosynthetic light, very little red light, different amounts of blue, and about the same FR/R ratio. The quantity of blue in reflected light received by leaves contributed to chemical differences related to flavor of roots from turnip plants grown in sunlight over blue- versus green-surfaced mulches. Differences in chemical composition of roots grown with white or silver mulches (both of which reflected more photosynthetic light and lower FR/R ratios than the blue or green mulches) were less pronounced, and concentrations were generally lower than those in roots grown with blue mulch.

Dermal and inhalation exposure of several mixers/applifiers to ag-chemicals during tobacco production. J. A. LONSWAY,* M. E. BYERS, H. A. DOWLA, and M. PANEMANGALORE, Community Research Service, Kentucky State University, Frankfort, KY 40601.

Exposure of limited-resource tobacco-farmers to ag-chemicals and natural products has been identified as an area for concern. This study was designed to determine routes and extent of exposure to insecticides and growth regulators that mixers/applifiers would encounter using

a tractor-mounted boom-sprayer or a highboy. Dermal and inhalation exposures were determined using a gauze pad monitoring technique and personnel type air samplers with charcoal adsorbents, respectively. Endosulfan, acephate, and its metabolite methamidophos were analyzed by gas liquid chromatography (GLC) and GLC/mass spectrometry (MS). Results from 1994 field studies indicated that dermal exposure to acephate was greater for mixing than for spraying. For spraying, dermal exposure to methamidophos well exceeded exposure to acephate. This difference was likely due to acephate chemically transforming into methamidophos during spraying. Inhalation values ranged from 0 to 3.9 mg/kg/hr for acephate and methamidophos. Some exposure to nicotine was also assessed. Field experiments were repeated in 1995.

Effects of waste composts on tomato production in containers. BRIAN D. LACEFIELD* and ELMER GRAY, Department of Agriculture, Western Kentucky University, Bowling Green, KY 42101.

The increasing waste production by municipalities and the growing interest in gardening by urbanites amply justify research on compost utilization in container gardening. Tomatoes are well suited for container production. Objectives of the present study were to compare two tomato cultivars—Patio and Celebrity—for production in containers and to compare different waste composts—leaf, brush, and N-Viro-Soil—for their effects on tomato yield. Ten growth media consisted of soil and the three composts alone plus 50:50 (by volume) mixtures of all combinations of the four media. Containers were plastic barrel sections (58 cm diameter, 38 cm depth) with capacity of 64 liters. Forty containers permitted four replications of the 10 growth media. Media fertilization consisted of one pre-transplant application, at kg/ha rates, of 56.0 N, 24.5 P, 46.5 K. Vine-ripe tomatoes were harvested twice weekly during the production season. Celebrity produced significantly fewer and significantly heavier fruits than Patio, resulting in no significant difference between cultivars for total season production per plant. There were no significant differences in tomato yields associated with growth media. There also were no significant cultivar × growth media interactions. These preliminary results indicated that composts of waste products can be effectively substituted for soil in container production of tomatoes.

Pawpaw research at Kentucky State University: an update. DESMOND R. LAYNE, Atwood Research Facility, Kentucky State University, Frankfort, KY 40601.

Kentucky State University (KSU) currently has a full-time research program directed at developing pawpaw (*Asimina triloba*) as a new commercial fruit crop for Ken-

tucky and the United States. Three concurrent research projects are underway at KSU: (1) overcoming the horticultural limitations to development as a new crop; (2) characterizing the morphological and molecular variation in a diverse germplasm collection; and (3) developing a centralized research support system. For project 1, we have conducted greenhouse experiments to establish optimal conditions (light regimen, fertilization, soil temperature, etc.) for production of robust seedlings. A new pawpaw regional variety trial orchard (300 trees) comparing the most promising 28 pawpaw clones or named varieties was also planted. This replicated research trial involves cooperators in 15 states who will plant identical orchards and evaluate clone suitability, etc. for their region. For project 2, we have planted a new orchard (1200 trees) of over 400 pawpaw accessions from 16 states. In addition, several inter- and intraspecific hybrids were planted in this same orchard for long-term evaluation. Molecular evaluation of isozyme polymorphisms began fall 1995. Project 3 began fall 1995 and is twofold in nature. First, we will develop an on-line computer database for information on any subject related to pawpaws. Second, we will develop improved techniques for seed propagation and clonal (vegetative) propagation. As a result of federal funding of the latter project, KSU has been designated the National Clonal Germplasm Repository for pawpaws.

Phenotypic variation in Indian mock strawberry (*Duchesnea indica*). ELMER GRAY* and BRIAN D. LACEFIELD, Department of Agriculture, Western Kentucky University, Bowling Green, KY 42101.

Indian mock strawberry, *Duchesnea indica*, is common on uncultivated open land in central and eastern U.S. Under conditions resulting from relaxed lawn management, the plant may dominate areas of lawns. It reproduces prolifically through vegetative propagules and seeds. The relative proportions of asexual and sexual reproduction impact variation in the resulting population. The objective of the study was to characterize phenotypic variability of local and more distant populations of the plant. Measurable plant traits included petiole length, leaflet length and width, and leaflet serrations. Linear correlations (r) among these traits were highly significant in each of the nine populations. However, relationships among the traits were not the same in the different populations as evidenced by significant heterogeneity among the correlation coefficients and variability among the coefficients of variation (standard deviation \times 100/mean). Overall, the results indicated a high level of phenotypic plasticity in Indian mock strawberry resulting from both genetic and environmental effects.

Testing alfalfa varieties for yield. LINDA G. BROWN,* Department of Agriculture, Western Kentucky University, Bowling Green, KY 42101; J. C. HENNING and L. M. LAURIAULT, Department of Agronomy, University of Kentucky, Lexington, KY 40506.

The Kentucky Alfalfa Variety Trials are a continuing co-

operative project between Western Kentucky University and the University of Kentucky. Alfalfa is an important crop in cash hay production enterprises of this state. Every year dozens of new varieties are released to the public for sale, making the task of selecting an appropriate variety for planting very challenging for producers. Each variety is distinctly different from others in one of the following ways: maturity date, dormancy rating, growth habit, number of leaflets per leaf, pest resistance, and/or yield potential. The objective of this research is to evaluate the yield performance of new alfalfa varieties at several locations in Kentucky. Plots are established at four locations in Kentucky on a rotating basis: Bowling Green, Lexington, Princeton, and Mayslick. Cumulative yield data will be presented as well as the recommended procedure for using the data to select a variety.

Tobacco field worker re-entry exposure to acephate, nicotine, and their metabolites. N. C. BAKER,* J. A. LONSWAY, M. E. BYERS, H. A. DOWLA, and M. PANEMANGALORE, Community Research Service, Kentucky State University, Frankfort, KY 40601.

Field workers were monitored for dermal and respiratory exposure to acephate and its metabolite methamidophos at re-entry intervals of 2, 4, 8, 24, and 48 hours after application. The task of field workers was to "top" bloom stage tobacco. Exposure to nicotine was also assessed. Dermal exposure was measured by analyzing 10 gauze pads attached to the clothing of workers to represent human body regions. Hand exposure was determined using cotton gloves. Respiratory exposure was determined using personnel type air samplers equipped with charcoal adsorbent traps. Gas liquid chromatography was used to quantify residues of acephate, methamidophos, and nicotine in pads, gloves, and charcoal air sampler adsorbents. Dermal exposure was greatest for the hands at all re-entry intervals. There was not a consistent trend of decreasing pesticide residue over time. This can probably be attributed to heavy dew or rainfall on the tobacco plants for 24 and 48 hour re-entries. Nicotine exposure was random by body region, but residues increased consistently with moisture levels. Our preliminary results suggest that a 48-hour post-spraying waiting period may not be sufficient.

BIOCHEMISTRY & PHARMACOLOGY

Effect of manipulation of central histaminergic activity on locomotor activity in rats. DANITA SAXON KELLEY,* Department of Consumer and Family Sciences, Western Kentucky University, Bowling Green, KY 42101; L. PRESTON MERCER, Department of Nutrition and Food Science, University of Kentucky, Lexington, KY 40506.

Physical activity is reported to have a circadian rhythm (period of about 24 hours) in rats and humans. This study examined the relationships between locomotor activity, diet composition, and histaminergic activity in the central nervous system (CNS). Dietary treatments altering the histaminergic parameters of central histamine and/or H₁-

receptor concentrations were performed. Male rats were ad libitum fed a 25% casein diet (NP), ad libitum fed a 1% casein diet (LP), or pair-fed a 25% casein diet (PF). Significant ultradian rhythms (period < 20 hours), in addition to diurnal changes, in locomotor activity were identified for all treatment groups. Pair-fed rats had increased, though not significant, maximum activity compared to NP rats. Administration of the H₁ antagonist doxepin significantly decreased mean activities of LP and PF rats and significantly decreased maximum activity of PF rats. This study supports the involvement of CNS histaminergic activity in locomotor activity at the H₁-receptor.

Effects of chloropropanes on glycolysis. YI ZHOU and ROBERT F. VOLP* Department of Chemistry, Murray State University, Murray, KY 42071.

Chloropropanes are a group of organic chemicals produced in large quantities for a variety of uses. Human exposure occurs during the production and use of these compounds and via contaminated groundwater. The toxicity of chloropropanes has received little attention although analogies to chloroethanes suggest that chloropropane toxicity is determined by their biotransformation. Structural similarity between putative chloropropane biotransformation products and intermediates of glycolysis suggests that toxicity occurs when chloropropane biotransformation products inhibit glycolytic enzymes. To investigate the interactions of chloropropanes, their biotransformation products, and glycolytic enzymes, rat liver cytosol was incubated at pH 7.5 and 37°C with 3-phosphoglycerate (3PG) and necessary cofactors. Glycolytic reactions initiated by 3PG were monitored by measuring NADH absorbance at 340 nm. The effects of 1,2,3-trichloropropane (TCP; 0.5, 5.0 mM), glutathione (GSH; 1.0 mM), and rat liver microsomes (0.1, 0.5 mg/ml) were tested by adding each individually and in all possible combinations. In the absence of microsomal protein, TCP did not affect glycolysis rate, suggesting that biotransformation is necessary for an effect. With 0.1 mg/ml microsomal protein, TCP decreased glycolysis rate; inclusion of GSH abolished this effect. These results suggest that a microsomal biotransformation product of TCP inhibits glycolysis and that GSH conjugation detoxifies either this product or TCP itself. With 0.5 mg/ml microsomal protein, TCP did not affect glycolysis rate in the absence of GSH; it increased the rate in the presence of GSH. An explanation for these effects is likely to require identification of multiple effects mediated by the microsomes.

BOTANY & MICROBIOLOGY

Analysis of morphological and genetic variation in *Hexastylis contracta*. PATRICK E. CARROLL* and ZACK E. MURRELL, Department of Biology, Western Kentucky University, Bowling Green, KY 42101.

Hexastylis contracta, an herbaceous evergreen plant in the Aristolochiaceae, is generally restricted to remnant old-growth hemlock forests in the Cumberland Plateau of Kentucky and Tennessee, with three disjunct populations

in western North Carolina. Extensive field work identified 18 new populations of *H. contracta* in Tennessee. The apparent hybrid found appears to be the result of crosses between *H. contracta* and *H. arifolia*. Genetic variability within *H. contracta* and the putative hybridization between *H. contracta* and *H. arifolia* are the subjects of ongoing molecular analyses of the internal transcribed spacer (ITS) regions of nuclear ribosomal DNA. The putative hybrid exhibits floral and leaf similarities with *H. arifolia*, but the fleshy perianth has a flare at or below the middle that is very similar to the *H. contracta* perianth structure. The hypothesis of hybridization is explored through morphometric analyses of leaf and perianth structure of the putative hybrid and the parent species.

Blue- and white-fruited dogwoods (*Cornus*): internal transcribed spacer (ITS) region sequence data and species relationships. JEFFREY J. BAKER* and ZACK E. MURRELL, Department of Biology, Western Kentucky University, Bowling Green, KY 42101.

The genus *Cornus* is currently recognized with eight segregate subgenera and ca. 50 species. The blue- and white-fruited dogwoods include about 30 species that have traditionally been segregated, on the basis of leaf position and branching patterns, into opposite-leaved (subg. *Kranioopsis*) and alternate-leaved (subg. *Mesomora*) groups. Recent studies have separated the Asian blue-fruited species *C. oblonga* and the South American blue-fruited species *C. peruviana* into segregate subgenera or sections. Our study involves analyses of relationships in *Cornus* using the internal transcribed spacer (ITS) region of nuclear ribosomal DNA. The sequences were generated using cycle sequencing on an automated sequencer, aligned using CLUSTAL, and analyzed using PAUP. The alignments, with the exception of length variation in *C. oblonga* and *C. peruviana*, showed a high degree of similarity. The trees produced by the PAUP analyses suggest that the blue-fruited dogwoods are monophyletic, with the exceptions of *C. oblonga* and *C. peruviana*, which appear to be basal lineages within *Cornus*. Within the monophyletic clade of blue- and white-fruited dogwoods, there is strong support for an alternate-leaved clade and an opposite-leaved clade. The opposite-leaved clade can be divided into two monophyletic groups, the "stolonifera group" and the "stricta group." The support for these groups based upon molecular data is in agreement with morphological data from plant architecture and leaf venation patterns.

Dogwood (*Cornus*) subgeneric relationships: evidence from the internal transcribed spacer (ITS) of nuclear ribosomal DNA. ZACK E. MURRELL* and JEFFREY J. BAKER, Department of Biology, Western Kentucky University, Bowling Green, KY 42101.

Molecular sequence data from the internal transcribed spacer (ITS) region of 18–26S nuclear ribosomal DNA have been derived for eight subgenera of the genus *Cornus* and from representatives of the putative outgroups *Nyssa*, *Alangium*, and *Davidia*. These sequences show ex-

tre variations in length, from 250 to 350 bases in both ITS I and ITS II. Attempts have been made to align these data using the computer program CLUSTAL, as well as manual alignment; results suggest that multiple repeats and length variation are difficult to align using CLUSTAL. The manually aligned complete data set was analyzed using PAUP, and subsets of this data set, omitting the INDELS, were also analyzed and compared to the total data set. The results of these analyses support the monophyly of the blue-fruited dogwoods and also the sister relationship of the big-bracted dogwoods and the cornelian cherries. Evolutionary positions of *C. oblonga*, *C. peruviana*, and *C. volkensii* in relationship to the remainder of *Cornus* are equivocal, largely due to the extreme length variation of their sequences and the effects of long branch attraction.

Molecular analysis of the internal transcribed spacer (ITS) region of nuclear ribosomal DNA in a dogwood (*Cornus*) hybrid complex. CHRISTOPHER P. REED* and ZACK E. MURRELL, Department of Biology, Western Kentucky University, Bowling Green, KY 42101.

The distributions of two species of blue-fruited dogwoods, *Cornus amomum* and *C. obliqua*, have a broad area of sympatry, from Maine to Tennessee. Within this area, putative hybrids have been identified on the basis of several morphological characters. Although the habit, periderm, and inflorescences are similar in the two, the leaf pubescence and abaxial epidermal protuberances can be used to separate them; these characters are intermediate in the putative hybrids. Molecular sequence data derived from the internal transcribed spacer region (ITS) of 18–26S nuclear ribosomal DNA has been generated for the putative hybrids using Polymerase Chain Reaction (PCR) and standard dideoxy sequencing. Initial sequence data exhibit double banding at several nucleotide sites, suggesting a lack of uniformity among a putative hybrid's multiple ITS copies. Ongoing cloning studies of amplified PCR products from the putative hybrids are being sequenced in exploration of this multiple copy region. This aspect of the research is an attempt to test the use of cloned PCR amplified products as a method to detect hybrids and hybridization. The generated data are being used to determine the extent of hybridization between the two species.

Preliminary bryoflora of an old growth forest, Pine Mountain, Kentucky. JUDITH E. ROZEMAN,* Office of Institutional Research and Effectiveness, Berea College, Berea, KY 40404; TIMOTHY J. WECKMAN, Department of Biological Sciences, Eastern Kentucky University, Richmond, KY 40475.

The bryophytes, including the mosses, liverworts, and hornworts, have been undercollected compared to vascular plants in Kentucky. Fewer than 2,500 vouchered specimens are known to be on deposit in Kentucky herbaria, representing about one percent of total plant collections. A recent checklist of Kentucky bryophytes in-

cludes nearly 300 taxa of mosses and 105 hepatics and hornworts. The primary purpose of the present study is to provide a preliminary bryoflora of the Blanton Forest, a 2,300-acre tract of old growth woods located on the south face of Pine Mountain near Harlan, Kentucky. The secondary purpose of the project is to augment the number of vouchered bryophyte specimens in the state. Collections have been made in six major habitats including bogs and seeps, high elevation xeric sites, mixed mesophytic communities, hemlock-beech communities, creeks, and rock houses and rock outcrops in both wet and dry conditions. Specific substrates include rock, soil, water systems, decaying trees and nursery logs, and living trees. Collections have been made over a 2-year period in all seasons. All phases of the work (collections and identifications) are still in progress. Preliminary results have yielded 58 genera of mosses (the liverwort collection is still to be examined). The degree of similarity of the genera list with that of Lilley Cornett Woods, a comparable tract of old growth forest in eastern Kentucky, is 96%. The degree of similarity at the species level is still to be determined. Notable finds include *Andreaea rothii*, *Campylopus tallulensis*, *Dicranodontium demudatum*, and *Fontinalis sullivantii*.

Seed dispersal in *Rhus aromatica*. XIAOJIE LI,* JERRY M. BASKIN, and CAROL C. BASKIN, School of Biological Sciences, University of Kentucky, Lexington, KY 40506.

Attempts were made to quantify dispersal of the 1-seeded drupaceous fruit in *Rhus aromatica*. In 1994, observations began on 12 June. By 3 July, $48.3 \pm 7.0\%$ ($\bar{x} \pm SE$) of the fruits had been dispersed; the percentage reached 99.9 ± 0.1 by 13 August. Compared to 1994, rate of dispersal began faster but finished slower in 1995. On 19 June, $51.5 \pm 6.1\%$ of the fruits had been dispersed; by 3 July, the percentage was 87.2 ± 2.8 . About 5.5% of the fruits were still on the mother plants on 4 September. During the dispersal period, fruits and/or seeds (i.e., seed plus endocarp) were collected in seed traps on the ground, mostly at weekly intervals. The fruit/seed rain was 66.3 m^{-2} in 1994 and 62.5 m^{-2} in 1995, with the seed component being $77.8 \pm 8.3\%$ and $79.7 \pm 8.6\%$, respectively. The percentages of fruits and seeds that disappeared from the seed traps due to predation/emigration were 84.2 ± 7.3 and 71.7 ± 8.0 , respectively, leaving a transient seed bank of $18.2 \text{ seeds m}^{-2}$ in 1994 and $19.1 \text{ seeds m}^{-2}$ in 1995. An additional loss of seeds from the transient seed bank was due to natural softening of the endocarp. After 1 year of burial in the field, $29.4 \pm 2.7\%$ of the seeds had softened. Thus, the input to the persistent seed bank was $12.8 \text{ seeds m}^{-2}$ in 1994 and $13.5 \text{ seeds m}^{-2}$ in 1995.

Species delineation and population structure of *Spiraea virginiana*. JAMES C. ESTILL* and ZACK E. MURRELL, Department of Biology, Western Kentucky University, Bowling Green, KY 42101.

Spiraea virginiana is a rare rhizomatous shrub found in

heavily scoured areas of high-gradient streams in the southern Blue Ridge and Appalachian Plateau provinces and the edge of the Blue Grass Province in Kentucky. Although the species is distributed in six states, the majority of known individuals occur in Kentucky, Tennessee, and West Virginia. An intensive field search for *S. virginiana* in Tennessee has yielded 22 new populations to add to the 13 previously known Tennessee populations. Seventeen new populations were located in four drainages that contained known populations, and five populations were found in three "new" drainages. Three known populations, not relocated, are presumed extirpated. All other known populations were relocated and appeared relatively unchanged in number and density since they were last surveyed. Examination of molecular and morphological character variation suggests that the three species of section *Calospira* found in southeastern North America—*S. corymbosa*, *S. japonica*, and *S. virginiana*—can be recognized as segregate species. Previous germination studies, examination of total seed production, and field analyses of populations suggest that *S. virginiana* is limited to asexual reproduction. Ongoing studies utilizing molecular markers address the issues of population and drainage genetic identity of the species.

Survey of Ohio River bacteria: Gram-negative organisms. STEVE KRISTOFF, Department of Biology, Thomas More College, Crestview Hills, KY 41017.

The Ohio River is extensively used for transportation, recreation, and drinking and industrial water. Although coliform studies of the river are performed on a regular basis, a literature search reveals little information concerning the bacterial flora. As knowledge of the role of microorganisms in the environment grows, it becomes clear that knowledge of the bacterial flora of the river can contribute to our understanding of the river as an ecosystem. Also, since thousands of people use the river for recreational purposes (including physical contact with the water) and as a source of drinking water, it is clear that the river has a significant impact on public health. This survey was undertaken with these concerns in mind. Samples were taken from the Ohio River at the Thomas More College Biology Field Station in Campbell County, Kentucky (River Mile 451). Our preliminary data indicate that the majority of cultivable Gram-negative organisms are *Pseudomonas*, with smaller numbers of *Acinetobacter*, and occasional members of the Enterobacteria group. A test of susceptibility to antimicrobial agents reveals that a majority of the *Pseudomonas* are resistant to several classical agents including ampicillin, streptomycin, and chloramphenicol. Most, however, are susceptible to tetracycline.

Symbiotic nitrogen fixation by the turfgrass weed black medic (*Medicago lupulina*). DAVID LOWELL ROBINSON,* Biology Department, Bellarmine College, Louisville, KY 40205; CARROLL P. VANCE, Agronomy and Plant Genetics Department, and USDA-ARS, Plant Sci-

ences Research Unit, University of Minnesota, St. Paul, MN 55108.

Black medic (*Medicago lupulina*), a common weed of commercial and residential turfgrass in the Midwest, is a close relative of alfalfa (*Medicago sativa*), a forage crop known for its high rates of atmospheric nitrogen (N) fixation. This process is due to a symbiotic relationship between plant and soil bacteria (*Rhizobium meliloti*) that results in formation of N-fixing root nodules. Previous research has shown that, in alfalfa, N fixation rates decline when top growth is frequently harvested, when N fertilizers are applied to soil, or when the plant blooms. All three of these conditions occur in turf where black medic is a weed. Therefore, we evaluated nodulation and N fixation in black medic after frequent harvesting, N applications, and flowering to evaluate its N-fixing potential under these conditions. Although frequent harvesting of the shoot caused N fixation rates to decline, black medic nodules still fixed significant amounts. Nitrate applications to black medic and alfalfa caused a greater decline in nodulation of the latter than the former. Therefore, it appears that black medic maintains the potential to fix N under common turf management scenarios. This information is useful not only in describing the ecological role of this weed in turf but adds to our understanding of symbiotic N fixation.

CHEMISTRY

Evaluation of the Toledo Chemistry Placement Exam—Form 1981. L. C. BYRD and D. R. HARTMAN,* Department of Chemistry, Western Kentucky University, Bowling Green, KY 42101.

Attempts to identify under-prepared students encouraged investigations into correlation of success in college chemistry with high school grade-point average, class rank or chemistry grade, ACT, SAT-math, aptitude test scores and/or customized tests to measure chemistry/math skills. Hovey and Krohn were leaders in the investigations and developed the Toledo Chemistry Placement Examination (TCPE). Several universities require TCPE raw scores above 35–50 for entrance into college chemistry. The Department of Chemistry at Western Kentucky University (WKU) determined the correlation coefficients between success in college chemistry (grade of A, B, C) and the TCPE total raw scores (0.55). The next-best correlation (0.49) was with the TCPE, part I (math section). The correlation of ACT composite score and high school grade-point average with the TCPE scores was extremely poor (0.093–0.296). Average TCPE raw scores (part I, 13.5/20; part II, 11.8/20; part III, 9.4/20; total, 34.7/60); common TCPE questions missed were discussed.

GEOGRAPHY

Federal land policy and the Wise Use movement. MARY M. SNOW, Department of Geography and Geology, Western Kentucky University, Bowling Green, KY 42101.

Policy makers in the U.S. increasingly are pressured by

the Wise Use movement (WUM) to open all public lands to unconstrained mining, off-road vehicle use, logging, and grazing. While posing as a coalition of grassroots organizations working on behalf of labor and private property owners, WUM is funded by some of the nation's largest corporations. This paper examines the background of WUM; WUM's leaders, their perspectives and goals; and major funders of WUM. It analyzes legislation influenced by WUM.

Maximizing rural tourism: Horse Cave, Kentucky. RICHARD K. SNOW, Department of Geography and Geology, Western Kentucky University, Bowling Green, KY 42101.

The town of Horse Cave, Kentucky, long has been associated with tourism due to the numerous show caves in the area as well as its proximity to Mammoth Cave National Park. However, the pollution of Hidden River Cave on the town's main street devastated the tourism industry in 1944. A new sewage treatment plant has helped Horse Cave reclaim some of its former glory. Yet a survey of area businesses reveals that most merchants feel that city officials are not doing enough to attract tourists to Horse Cave. Among recommendations for Horse Cave to maximize its tourism potential are attracting a major motel and restaurant, converting historic homes into bed-and-breakfasts, creating a downtown handicraft market, and establishing a bus service from neighboring Cave City.

GEOLOGY

Glacial deposition and erosion of the Mile 605 area, Ohio River, Kentucky/Indiana. GRAHAM HUNT, Department of Geography and Geosciences, University of Louisville, Louisville, KY 40292.

A chronostratigraphic analysis of mainly unconsolidated sediments, soils, and processes of glacial deposition and erosion may be used to reconstruct environmental history of Quaternary age rocks overlying Paleozoic age bedrock in the study area. The exact extent of the Illinoian and Kansan glaciations is unknown in the Louisville area; however, the Kansan drift underlies the Illinoian drift within a few miles of each other. These data suggest that the two ice sheets had about the same southerly extent of advance. Numerous glacial erratics of possible lateral and/or terminal moraines are found near the banks of the Ohio River in northern Kentucky. Sandstone and conglomerate erratics are found near grooved, striated, polished, and exfoliated bedrock of mainly Devonian age carbonates. If reassembled, the erratics would be of several cubic meters; the total original weight may be up to 10 tons. Recent drilling in this area indicates a small, U-shaped, and bedrock-walled valley at depth of Silurian age rocks. The Pleistocene continental deposits at this locality may be divided into mappable sequences of lacustrine, outwash, and eolian units overlying the uranium-bearing New Albany Shale. Geologic mapping in population areas is important to evaluate urban mineral deposits of water supply rocks and to help identify and remedy geologic hazards (radon-

222). Radon-222 is commonly released from glacial deposits.

MATHEMATICS

A quasi-classical polynomial family. JAMES B. BARKSDALE JR., Department of Mathematics, Western Kentucky University, Bowling Green, KY 42101.

This investigation explored some of the properties of a family, $\mathbb{H} = \{h_n\}_{n=2}^{\infty}$, of polynomial functions (indexed by degree) created by integrating the Legendre polynomials, $\{P_n\}_{n=0}^{\infty}$. Specifically, the elements of \mathbb{H} are defined by

$$[1] \quad h_n(x) = \frac{n}{c_{n-1}} \int_{-1}^x P_{n-1}(t) dt,$$

where c_{n-1} denotes the leading coefficient of P_{n-1} . In view of Item [1], it may not be surprising that members of \mathbb{H} inherit some of the properties and recursive relations enjoyed by the Legendre polynomials. In addition to these "expected" properties, the elements of \mathbb{H} possess other curious and interesting attributes, which include the following: (a) \mathbb{H} is the unique family of polynomials such that each h_n is monic, has $(x^2 - 1)$ as a factor, and has only real, distinct zeros in $[-1, 1]$ coinciding with the inflection point abscissas; and (b) \mathbb{H} is a quasi-orthogonal family of vectors in the sense that $|n - m| \notin \{0, 2\}$ implies $0 = (h_n, h_m) = \int_{-1}^1 h_n(t)h_m(t) dt$.

PVM and parallel computation. KEITH ROE, Department of Mathematics, Morehead State University, Morehead, KY 40351.

PVM (Parallel Virtual Machine) is a message-passing computer language. In this project, PVM was used to parallelize a program, solving a problem of particle transport theory in two dimensions by the invariant imbedding method. This is a computationally intensive large-scale problem, which implies it may be a problem best solved by parallel methods. This program was run on a cluster of HP 9000 workstations and also on a Convex Exemplar machine. The matrix-multiply routine was found to be using 90% of CPU time. This, therefore, was the module that was parallelized. The method for parallelizing the matrix multiply was to set up child processes on the other nodes of the cluster and divide the dot product computations among them. Next the program was timed to determine if this was an efficient method of solving this problem. Although the parallel version was consistently slower than the serial version, due to message passing overhead, the parallel version consistently grew more efficient as the size of the problem grew. The results of this project demonstrated a basic trend that indicates a parallel version may be efficient for sufficiently large problems.

Some concerns in axiomatic set theory. ANDREW MARTIN, Department of Mathematical Sciences, Morehead State University, Morehead, KY 40351.

The publication of Paul Cohen's *A Minimal Model for Set Theory* (1963) and *The Independence of the Continuum Hypothesis* (1964) demonstrated two cases of set the-

oretic statements (the axiom of choice and the generalized continuum hypothesis) that he showed could not be proved or disproved from the Zermelo-Fraenkel axioms. His method of forcing has since been elegantly refined and developed to prove the undecidability of many other set theoretic statements. It was the view of Goedel that there are other basic principles yet undiscovered that will enable basic set theoretic to be decided. This expository paper reviewed some candidates, in particular Martin's axiom, proper forcing axiom, diamond, projective determinacy, and some large cardinal axioms.

When is a partially ordered set well ordered? ANDREW MARTIN,* Department of Mathematical Sciences, Morehead State University, Morehead, KY 40351; ALEXANDER ABIAN, Department of Mathematics, Iowa State University, Ames, IA 50011.

Let S be a subset of a partially ordered set P . We call an element i of P an immediate successor of S if and only if i is a strict upper bound of S and no element of P less than i is a strict upper bound for S . A subset PI of P is called pseudo-inductive provided that for every subset A of PI , if A has immediate successors in P , then at least one of these immediate successors must be an element of PI . We have proved that well-ordered sets can be characterized in terms of their pseudo-inductive subsets, in the following sense. THEOREM: A partially ordered set is well-ordered if and only if it has no pseudo-inductive proper subset.

MOLECULAR & CELL BIOLOGY

Analysis of alpha-fetoprotein enhancer III activity in transgenic mice. JERHONDA M. BRYANT and BRETT T. SPEAR, Department of Microbiology and Immunology, University of Kentucky College of Medicine, Lexington, KY 40536.

Alpha-fetoprotein (AFP), a 70,000 dalton glycoprotein, is the major serum protein in the developing mammalian fetus. AFP functions primarily as a transport protein; it may also protect the developing fetus from the maternal immune system. AFP is synthesized at high levels in the liver and the yolk sac and at low levels in the gut. AFP transcription decreases ca. 10,000 fold after birth; this repression is reversible as the AFP gene can be reactivated during liver regeneration and in certain cancers. Our lab studies aspects of AFP gene regulation. Three distinct enhancers, EI, EII, and EIII, are located upstream from the AFP gene. Previous studies in our lab have identified binding sites for HNF-3 and C/EBP within Enhancer III. HNF-3 and C/EBP sites are liver-specific transcription factors. To further investigate the role of the HNF-3 and C/EBP binding sites, we have altered these sites by PCR-mediated site-directed mutagenesis. Four constructs were produced, the wild type EIII, EIII lacking the HNF-3 site, EIII lacking the C/EBP site, and EIII lacking both sites. These four fragments are currently being ligated to a promoter fused to the lacZ reporter gene. The constructs will be analyzed in tissue culture cells and trans-

genic mice. The effects of these mutations on EIII activity will be determined by measuring lacZ activity. We predict that each single mutation will reduce EIII activity and that the double mutant will completely eliminate EIII activity.

Antibody response and histopathology of *Trypanosoma cruzi* infection in mice held at elevated temperature. AHMED A. ARIF and CHERYL D. DAVIS, Department of Biology, Western Kentucky University, Bowling Green, KY 42101.

Highly susceptible C3H mice survive an otherwise lethal infection with *Trypanosoma cruzi* when held at an elevated temperature of 36°C. The body temperature of the mice increases 3° to 4°C and the mice experience decreased parasitemia and enhanced parasite-specific and nonspecific immune responses. The present study was designed to determine whether heat shock proteins (hsp) of the parasite might be playing a role in this phenomenon. Antibody responses to parasite hsp were analyzed using metabolic labelling with ³⁵S methionine/cysteine followed by SDS PAGE and autoradiography. The results showed that by day 15, serum from RT-infected mice recognized proteins of approximately 93 kDa, 83 kDa, and 66 kDa, while in mice held at 36°C the major proteins recognized were 93 kDa and 83 kDa. By day 25, a high MW band of ca. 183 kDa was recognized by serum from mice held at RT and 36°C. A stronger response to the 93 kDa, 83 kDa, and 66 kDa proteins was seen in RT-infected mice as compared to 36°C infected mice. By day 35, another high MW protein of approximately 192 kDa was recognized by sera from both RT and 36°C infected mice. Strong reactivity with the 183 kDa, 93 kDa, 83 kDa, and 66 kDa proteins was seen in both groups of mice on day 35. By day 45 of infection, serum from 36°C infected mice recognized proteins of ca. 183 kDa, 93 kDa, 83 kDa, and 66 kDa more intensely than in the RT-infected mice group. In addition, a low MW protein of approximately 27 kDa was recognized only by serum from 36°C infected mice.

Comparative analysis of plant mitochondrial small subunit ribosomal RNA. CANDACE L. GLENDENING,* CRAIG A. TUERK, and GERALD DEMOSS, Department of Biological and Environmental Sciences, Morehead State University, Morehead, KY 40351.

The small subunit of the ribosomal RNA has long been used as a tool in determining phylogenetic relationships. This project explored a region of the mitochondrial small subunit ribosomal RNA (MSrRNA) found only in the plant mitochondrial genome referred to as Variable Region 7 (V7). The V7 region of cauliflower and radish, members of the Brassicaceae, was amplified with the polymerase chain reaction, cloned into the expression vector PBSSK+, and sequenced. There was only a 0.3% sequence difference between cauliflower and radish V7 region, thus suggesting that there will not be sequence variation in this region within a plant species. Cauliflower and radish V7 sequence was compared with the six other se-

quences of plant MSrRNA V7 region available: wheat, corn, oats, evening-primrose, soybean, and lupine. Based on percent difference between the V7 region sequences, a phylogenetic tree was constructed that supports placement of these species in Cronquist's morphologically based phylogenetic tree of flowering plants.

Determination of behavior of antigen-specific T-cells following immunization to an immune privileged site. DAVID PEYTON,* RITA EGAN, and JEROLD WOODWARD, Department of Microbiology and Immunology, University of Kentucky, Lexington, KY 40502.

The immune system cannot react the same way in all situations. Sites such as the eye and central nervous system would be harmed or destroyed if they received the same immune response to antigen that most other sites in the body receive. These special sites, known as immune privileged sites, can be studied using adoptively transferred mice and fluorescent antibody stained T-cells. Adoptively transferred mice are created by intravenous injection of a small population of ovalbumin-specific T-cells from a transgenic mouse into a non-transgenic mouse. The adoptively transferred mice receive an ovalbumin injection into the anterior chamber of the eye. To monitor the activity of the T-cells, the lymph nodes and spleen from the injected mice are removed and the T-cells from these organs are isolated. Next, the T-cells are stained with two separate fluorescent antibodies: one specific for the ovalbumin-specific T-cells and one that binds to all CD4+ T-cells. Using a flow cytometer to measure the amount of staining, the T-cells specific for ovalbumin can be distinguished, quantified, and compared to non-ovalbumin-specific T-cells. Data from this study indicated that there was a local draining lymph node response in the cervical and submandibular nodes following antigen administration in the eye. These data also suggest that there is not a response in the venous circulation, which would have been indicated by a population of ovalbumin-specific T-cells in the spleen.

Determinations of residues involved in interchain formation and stability of hamster aspartate transcarbamylase. PANNA PATEL* and JEFFREY N. DAVIDSON, Department of Immunology and Microbiology, University of Kentucky, Lexington, KY 40502.

Aspartate transcarbamylase (ATCase) is an independent monofunctional enzyme in *Escherichia coli*. In hamster, ATCase is one of the three domains of the multifunctional CAD protein. CAD, a multimer of identical subunits, is named after its three enzymes: carbamyl-phosphate synthetase, ATCase, and dihydroorotase. Together these three enzymes catalyze the first three steps of de novo pyrimidine biosynthesis in which ATCase specifically catalyzes the second step. In *E. coli* the enzyme is a trimer of catalytic chains; adjacent catalytic subunits form shared active sites. The objective here was to identify residues of the hamster ATCase not directly involved in the shared active site but involved in stabilizing the interactions be-

tween CAD monomers. To achieve this aim, an expression plasmid encoding the ATCase domain of hamster CAD was expressed in *E. coli*, and five changes were introduced by site-directed mutagenesis: Phe286 → Alanine, Phe286 → Tyrosine, Arg287 → Glutamine, Gln288 → Asparagine, and Gln288 → Glutamic acid. Reduced enzymatic activities were observed. Heat treatment of bacterial extracts at 50°C for 5 minutes showed the most drastic effects on enzymatic activities of Phe286 → Ala and Phe286 → Tyr. For these two mutants, the hamster CAD cDNA was cloned into the pMALc2 expression vector, a special vector that permits one step purification of the recombinant protein. After purifying pMALc2-Phe286 → Ala protein, its multimeric structure was determined by native gel electrophoresis. Because the protein migrated to a smaller pore size than the original wild-type ATCase, this indicated that the protein has an altered multimeric structure, suggesting that Phe286 plays a role in CAD structure and that substitution at this point can disrupt the multimer.

Dietary activation of the hypothalamic-pituitary-adrenal axis through the histaminergic system. AMY TIU,* HOLLY BUNDRANT, and L. PRESTON MERCER, Department of Nutrition and Food Science, University of Kentucky, Lexington, KY 40506.

During protein deficiency, increased histaminergic activity increases adrenocorticotropic hormone (ACTH) and corticotropin releasing factor (CRF), which may lead to increased levels of corticosterone in rats and cortisol in humans. Hypercortisolemia is a condition found in cases of anorexia nervosa (AN). Corticosterone levels were examined in 19 male and 19 female Sprague-Dawley rats fed diets containing either 25% casein or 1% casein. One group of seven male rats and six female rats was fed a 1% casein diet and received a subcutaneous injection of doxepin, a central histamine receptor antagonist (10 mg/kg; DOX). Plasma from the rats were then analyzed by a corticosterone solid phase ¹²⁵I radioimmunoassay. The mean levels of corticosterone in each treatment group (25% casein, 1% casein, and 1% casein + DOX) of males were 1618.54 nmol/liter, 1236.45 nmol/liter, and 1115.22 nmol/liter, respectively. The mean levels of corticosterone in each treatment group (25% casein, 1% casein, and 1% casein + DOX) of females were 1969.88 nmol/liter, 1491.53 nmol/liter, and 1512.89 nmol/liter, respectively. All groups were significantly different from each other ($P = 0.05$). Rats injected with doxepin showed improved food intake, weight gain, and efficiency. Even though no statistical differences were found in comparison of corticosterone levels between gender and between treatment groups within gender, the results suggest that corticosterone levels may be influenced by protein deficiency and the histaminergic system.

Effects of lead acetate on host susceptibility to *Trypanosoma cruzi*. TABITHA ELLIS and CHERYL DAVIS, Department of Biology, Western Kentucky University, Bowling Green, KY 42101.

Lead has been found to be an immunotoxicant that suppresses the resistance of a host organism to infection. C57Bl/6J mice, which are not highly susceptible to the Brazil strain of *Trypanosoma cruzi*, were chosen to determine if and how lead would affect their immune response upon infection. The mice were acclimated for 2 weeks on deionized water and rodent chow, prior to lead treatment. They were divided into four groups and were given oral dosages of lead acetate ranging from 0 to 1,000 p.p.m. in their drinking water for 3 weeks. On day 35 of the experiment, the mice were injected with 10^4 blood form trypomastigotes of *Trypanosoma cruzi*; the lead treatment continued. Beginning at 14 days post infection, parasitemias were conducted two times weekly. The mean peak parasitemias for each group were as follows: 0 p.p.m.— 6.49×10^6 , 10 p.p.m.— 9.61×10^6 , 100 p.p.m.— 1.31×10^7 , and 1000 p.p.m.— 3.62×10^7 . The concentrations of lead in blood in the first replicate of the experiment were: 0 p.p.m.—5.34 $\mu\text{g/liter}$, 10 p.p.m.—19.72 $\mu\text{g/liter}$, 100 p.p.m.—16.36 $\mu\text{g/liter}$, and 1000 p.p.m.—204.41 $\mu\text{g/liter}$. Although no increased mortality was observed in lead-treated mice, the results of this study suggest that lead does enhance the susceptibility of infected mice, resulting in higher parasitemias.

Effects of viral infection on insect immune cell function. AARON CAMERON* and BRUCE A. WEBB, Department of Entomology, University of Kentucky, Lexington, KY 40502.

The parasitoid wasp *Campoletis sonorensis* injects certain immunosuppressive factors when it preys upon its lepidopteran host. One of these factors is the polydnavirus, the only known DNA virus with a segmented genome. The polydnavirus has proven to have adverse effects upon the host; in particular, suppression of the cellular immune response. A procedure for collecting insect hemolymph and purifying hemocyte cell populations has been optimized for use in determination of viral gene expression in parasitized insect RNA. The viral gene of interest has been partially sequenced, creating a sequence-specific tag, allowing for its identification and characterization into the rep-gene family. A Northern has been performed on RNA extracted from parasitized insects to examine phases of viral gene expression post-injection. Research of this nature is pertinent to both agriculture and human immunology.

Electrophoretic analysis of carotid plaque lipoproteins. AMANDA R. TATRO* and ROBERT A. LODDER, College of Pharmacy, University of Kentucky Medical Center, Lexington, KY 40536.

Determining the number of proteins present in an atheroma cell and the roles played by these proteins will be essential in the future to diagnosis and treatment of atherosclerosis. In vitro plaque electrophoresis and in vivo near-IR spectrometry are used to provide qualitative and quantitative information on the proteins involved in carotid atherosclerotic lesions. The information garnered

from electrophoresis is used to test the hypothesis that particular proteins in combination show significant correlation to patient medical history and lesion pathology. Bruit, from medical history, and fibrous cap, from pathology reports, are shown to involve similar concentrations of the same proteins. Near-IR spectra of these proteins indicate that all but one of the protein factors characteristically found are probable lipoproteins. In addition, the concentration of the 18 kDa protein is related to lesion size, which in turn has been related to severity of disease. Duplex ultrasound measurements are used to determine lesion size and are compared to protein concentrations determined by electrophoresis to demonstrate a relationship between the concentration of the 18 kDa protein and the size of the lesion.

Expression of human NAD⁺-dependent 15-hydroxyprostaglandin dehydrogenase in yeast. GARY HALL,* MARK ENSOR, and HSIN-HSIUNG TAI, Department of Medicinal Chemistry and Pharmaceutics, College of Pharmacy, University of Kentucky, Lexington, KY 40536.

Prostaglandins are fatty-acid-derived compounds exhibiting a broad spectrum of biological activities. Once produced, they are rapidly metabolized and inactivated. The first step of their metabolism is catalyzed by NAD⁺-dependent 15-hydroxyprostaglandin dehydrogenase (PGDH). For this reason PGDH is considered to be the key enzyme controlling inactivation of the prostaglandins. However, the nature of the interaction of the prostaglandins with the active site of the enzyme is not known. A future goal is to determine the three-dimensional structure of PGDH by x-ray crystallography. Determining the structure will aid in determining the catalytic mechanism of the enzyme as well as how pharmacological agents interact to exert their effects. The levels of PGDH expression in recombinant mammalian and bacterial systems make it difficult to produce and purify PGDH in sufficient amounts for x-ray crystallographic studies. Recently, a yeast expression system (*Pichia*) was developed that should allow for production of recombinant proteins in much higher amounts. The cDNA for PGDH was inserted into the yeast expression plasmid (pHIL-D2), which was then used to transform the yeast *Pichia pastoris*. A recombination event occurred within the yeast between the yeast alcohol oxidase (AOX1) gene sequences contained in the plasmid and the AOX1 gene in the yeast chromosome. This recombination results in replacement of the yeast chromosomal AOX1 gene with the PGDH cDNA. The PGDH cDNA is then under the control of the AOX1 promoter. This promoter is regulated by the presence of methanol. When methanol is present, very high levels of expression from the AOX1 promoter occur. Maximum expression of PGDH was obtained after 2 days of induction with methanol. Levels of expression were 25 times higher than that obtained in *Escherichia coli*. Expression of PGDH using this yeast system will greatly

facilitate the purification of the enzyme for x-ray crystallography.

Identification of cytochrome P450 monooxygenase enzyme genes in corn. JOHN DAVID GREULICH*, MICHAEL BARRETT, NICHOLAS POLGE, and JOHN RAWLS, Department of Agronomy, University of Kentucky, Lexington, KY 40546.

Most organisms contain cytochrome P450 monooxygenase enzymes (P450), which are involved in various biological reactions such as the production of secondary metabolites and the detoxification of environmental pollutants and xenobiotics. Presently, two inbred lines of corn have been found to express and not express P450 genes, which are responsible for the detoxification of particular pesticides. Further, these genes are found to be inducible and non-inducible with treatment by certain chemicals. Polymerase chain reaction (PCR) has been used previously to isolate partial sequences of corn P450 genes. The construction of a cDNA library is necessary to obtain full-length sequences. A cDNA library was produced from one inbred line using a library construction kit to make cDNA from isolated mRNA. This library and another produced commercially will be screened by P450 probes centered around a conserved heme binding region characteristic of P450s. Clones showing homology to probes will be sequenced in an attempt to obtain full-length sequences of P450 genes of interest. Comparison between the two lines will give insight into the manner in which the p450 genes are regulated. Further study into the regulation of p450 genes would require construction of a genomic DNA library. Additionally, the inducible line could be studied in greater detail, as it shows multiple substrate activity. This could be due either to a single enzyme acting upon a variety of substrates or to many enzymes with genes closely associated on the same chromosome. These would not show segregation with classical genetic analysis.

Interactions of the tobacco vein mottling virus's mutant cylindrical inclusion and nuclear inclusion B protein. BETH SMITH* and ARTHUR HUNT, Department of Agronomy, University of Kentucky, Lexington, KY 40508.

The tobacco vein mottling virus (TVMV) is a single-stranded RNA plant potyvirus containing six classified coding regions: the Cylindrical Inclusion gene, the Nuclear Inclusion A gene, the Nuclear Inclusion B gene, the Helper Component gene, the Coat Protein gene, and the P1 gene. The coding regions of interest, the CI and NIB genes, both have putative roles in viral replication as a helicase and polymerase, respectively. Previous research identified moderate protein-protein interactions between the CI and NIB proteins. To determine if the active sites of these proteins were responsible for the identified interactions, CI and NIB fusin proteins were designed to contain mutations within regions of these proteins' putative active sites. The NIB gene expresses a 58 kDa protein that has been implicated in viral replication as an RNA-dependent RNA polymerase. The most conserved of the potyviral

proteins, the NIB protein's putative active site is a GDD domain. The CI gene codes for a protein 70 kDa known as the cylindrical inclusion protein. Cylindrical inclusion proteins aggregate within the cytoplasm of plant cells infected by the TVMV virus to form pinwheel-like shaped structures. The CI protein has been included in a proposed superfamily of proteins that have putative ATP-dependent helicase activity. There are six regions within the CI domain that contain significant sequence homology with the other proteins included in the proposed helicase superfamily. A CI mutant containing a point mutation in region VI was used to determine if one of the putative active sites for helicase activity were involved in the protein-protein interactions identified, while a NIB clone designed with a point mutation in the GDD domain was provided by Dr. Tom Pirione's laboratory. The data suggest that the mutations in the putative active sites of the CI and NIB proteins insignificantly affected the identified interaction.

Lipoprotein determination in single cells by near-infrared spectromicrography. JENNIFER L. MOSES* and ROBERT A. LODDER, College of Pharmacy, University of Kentucky Medical Center, Lexington, KY 40536.

A near-infrared indium-antimonide (InSb) focal plane array camera is being used to collect images of carotid plaques during carotid endarterectomies. The excised plaques are later analyzed for lipoprotein content by ultracentrifugation and gel electrophoresis. The results from the electrophoresis indicate a correlation between the size of atherosclerotic plaque and the concentration of a 93 kDa protein in the plaque, and plaque size is correlated to stroke. Further study of the 93 kDa protein may lead to a drug to block its receptor in plaque cells. This report describes tests of two hypotheses: (1) that a near-infrared platinum-silicide (PtSi) charge-coupled device camera provides better *in vivo* spatial resolution and more functional pixels on a plaque image than the InSb focal plane array camera, with sufficient signal-to-noise ratio for macroscopic lipoprotein determination in individual plaque cells, and (2) that the oxLDL 93 kDa protein is transported into plaque cells from serum by infiltrating macrophages. The InSb camera has more dead pixels and a lower spatial resolution than the PtSi camera. The standard error of estimate and standard error of prediction for analyses using the InSb camera and PtSi camera were compared in the laboratory using prepared samples to create a calibration curve. The increased spatial resolution of the PtSi camera makes it easier to select spectra from microscopic sections made from the excised plaque. The spectra collected by attaching the PtSi camera to a microscope are also compared to a visual picture of a stained slide from the same plaque for reference.

Nicotine metabolite contribution to the central nervous system effect of nicotine. SUSAN MOORE,* WENDY SHAW, SUSAN BUXTON, and LINDA DWOSKIN, De-

partment of Pharmacology and Experimental Therapeutics, University of Kentucky, Lexington, KY 40536.

Dopamine is believed to play a major role in the reinforcing properties of drugs of abuse, including nicotine. The purpose of this research was to determine if the CNS effects of nicotine exposure are due in part to the nicotine metabolite-induced activation of the dopaminergic receptors. The nicotine metabolites—normicotine, cotinine, and norcotinine—have been shown to be in the CNS following nicotine exposure. These nicotine metabolites were investigated to determine their ability to evoke [³H]dopamine release from rat striatal slices and to inhibit [³H]dopamine uptake in rat striatal synaptosomes. [³H]dopamine release evoked by normicotine was found to be equivalent to release evoked by nicotine, whereas cotinine was less effective, and norcotinine was ineffective. Further, the evoked release was not the result of inhibition of dopamine uptake. Thus, these nicotine metabolites activate dopaminergic receptors indirectly, and therefore may contribute to the abuse liability of tobacco smoking.

Role of EGR-1 in prostate cancer cell programmed cell death. REZA F. SAIDI* and VIVEK M. RANGNEKAR, Department of Surgery, University of Kentucky, Lexington, KY 40536.

Androgens stimulate both normal and malignant growth in prostate. Androgen-ablation is a standard therapy for metastatic prostate cancer. However, this therapy is rarely curative because prostate cancer is heterogeneous in cell content, including both androgen-dependent and androgen-independent cells. Our studies indicate that phorbol 12-myristate 13-acetate (PMA) causes death in both androgen-dependent (LNCaP) and androgen-independent (PC-3) cells. PMA-inducible death of LNCaP and PC-3 cells exhibited molecular features indicative of programmed cell death (apoptosis) particularly oligonucleosome-length DNA fragmentation. Our data showed that this process was linked to an increase in expression of *EGR-1*, a zinc finger transcription factor. Functional studies using dominant-negative mutant of *EGR-1* and antisense oligomers indicate that *EGR-1* plays an important role in prostate cancer cell apoptosis.

Role of NAD as a P53 modulator and chemopreventive agent. MANDALA V. WILSON*, ARNOLD C. HUANG, and ELAINE L. JACOBSON, Department of Clinical Sciences, University of Kentucky, Lexington, KY 40506.

The primary metabolite of the vitamin niacin is NAD. Nicotinamide, nicotinic acid, and tryptophan are reported precursors of NAD; however until now studies have not been conducted to determine whether they also serve as precursors in breast epithelial cells, and the quantitative requirements of precursor to metabolite are unknown. Recently a new function of NAD was shown to be as a substrate in synthesis of ADP-ribose polymers when cancer-causing chemicals induce DNA strand breaks. These polymers function in recovery of DNA damage. About 15% of the western world is predicted to be niacin defi-

cient. HME cells are principle precursor cells for breast cancer and are at particular risk for niacin deficiency due to periodic cycles of cell division. DNA damage is an important step in the process of developing cancer cells. Cellular responses to DNA damage such as DNA repair limit development of cancer cells. We have developed a method for extracting and measuring both oxidized and reduced NAD and NADP from any tissue in a single step; this allowed us to analyze cells for niacin status much faster than by previous methods. We have also been able to regulate NAD content in HME cells by growing them for several population doublings in nicotinamide free medium. Cellular NAD modulates the levels of p53, a tumor suppressor gene product. p53 assists in responses to DNA damage by stopping cell cycle progression in order for repair to take place. Below-normal p53 response is associated with an increased frequency of tumors in animals and humans; mutations in p53 are the most widely recognized mutation found in tumors. We have shown that the p53 levels in cells with depleted nicotinamide levels are lower than in cells with normal nicotinamide levels. Since p53 levels are known to be elevated following DNA strand breaks, the effect of niacin modulation are now being studied using oxidative stress as a DNA damaging agent. These final studies should establish the quantitative requirements for niacin and NAD in the p53 response to DNA damage.

Specific role of 92-kDa Type IV collagenase in metastatic tumor cells. DAVID DEREMER,* STEPHEN ZIMMER, and CONNIE ZIMMER, Department of Immunology and Microbiology, University of Kentucky, Lexington, KY 40502.

Studies have shown that metalloproteinases, specifically 92-kDa type IV collagenase, may mediate metastasis. Activity of 92-kDa type IV collagenase was evaluated in two distinct cell types: a non-metastatic C1300 neuroblastoma and a non-metastatic HKB1 fibroblast. The C1300 and HKB1 cell lines were evaluated by zymogram and western blot analyses for the presence of 92 kDa type IV collagenase. Those cells expressing high levels of 92 kDa type IV collagenase were subcutaneously injected in nude mice. In each case, 92 kDa type IV collagenase correlates with metastasis but is not the only molecule down regulated. In addition to 92 kDa type IV collagenase, development of metastatic potential is correlated with suppressed CD44V and stomelysin, as well as an increase in nm 23-H1 (a putative metastasis-suppressor gene). To date it is not known whether the 92 kDa type IV collagenase, by itself, is sufficient to establish a malignant phenotype.

Targeting the poly(ADP-ribose) glycohydrolase gene in cancer chemotherapy. CHRISTOPHER D. WATT* and ELAINE L. JACOBSON, Department of Clinical Sciences, University of Kentucky, Lexington, KY 40506.

One of the most rapid responses to DNA strand breaks is the formation of complex ADP-ribose polymers from

the substrate nicotinamide adenine dinucleotide (NAD). The modification of chromatin protein by these polymers is known to be a critical step in cellular recovery. Inhibition of this metabolism enhances the cytotoxicity of agents that generate DNA strand breaks. Our target for inhibition of this metabolism enhances the cytotoxicity of agents that generate DNA strand breaks. Our target for inhibition, in this multienzymatic process, is poly(ADP-ribose) glycohydrolase (PARG), which catalyses degradation of ADP-ribose polymers following DNA repair. Prior research has shown adenosine diphosphate (hydroxymethyl) pyrrolidinediol (ADP-HPD) to be a potent and selective inhibitor of PARG activity *in vitro*. We investigated the general cytotoxicity of the drug as well as its ability to amplify effects of N-methyl-N'-nitro-N-nitrosoguanidine (MNNG), and oxidative DNA damaging agent, in cultured animal cells. Based on colony forming and MTS cell proliferation assays, ADP-HPD is slightly toxic to undamaged cells. The cytotoxicity of MNNG is, however, amplified up to 10-fold when MNNG is applied in the presence of ADP-HPD. To verify the mechanism of this biological response, ADP-ribose polymer levels must be measured and compared in ADP-HPD treated and untreated cells following DNA damage. Initial experiments to quantify radiolabeled polymers in damaged cells showed that ADP-HPD decreased the amount of polymer formed. The effect of antisense mRNA targeting the PARG gene will provide confirmation of this observation. Our preliminary data suggest PARG is indeed a likely target for the modification of responses to chemotherapy.

T-cell responses of the channel catfish, *Ictalurus punctatus*, to outer membrane proteins of *Edwardsiella ictaluri*. BARRY R. HAMILTON* and GEOFFREY W. GEARNER, Department of Biological and Environmental Sciences, Morehead State University, Morehead, KY 40351.

Although the immune system of the channel catfish has been studied for many years now, the role of the channel catfish T-lymphocyte is still poorly understood. There are several efforts underway to develop a vaccine against *Edwardsiella ictaluri*, the causative agent of enteric septicemia of catfish. Current vaccines can induce a specific antibody response that confers some protection but is not 100% effective. An understanding of the cellular, as well as the humoral, immune response is needed to develop an effective vaccine. In this project, channel catfish T-lymphocyte mitogenic responses to outer membrane proteins of *E. ictaluri* was examined. Ten micrograms of protein was sufficient to induce a significant proliferative response. There was a significant proliferative response to *E. ictaluri* outer membrane proteins that occurred in the range of 55 to 66 hours in pre-exposed channel catfish and in the range of 66 to 78 hours in control catfish.

Tissue-specific regulation of renin angiotensin components by cold exposure. JERRI R. DYER,* ANN TRAN, and LISA A. CASSIS, Division of Pharmacology and Ex-

perimental Therapeutics, College of Pharmacy, University of Kentucky, Lexington, KY 40536.

Angiotensin II (AII) has been found to be a major peptide factor regulating blood pressure. Recent studies have shown that AII may be formed independently of the blood stream in a variety of tissues. One location for this formation is rat interscapular brown adipose tissue (ISBAT). ISBAT, a highly metabolic tissue, is regulated primarily by the sympathetic nervous system. Thus, an increase in ISBAT metabolism induced by cold-exposure is hypothesized to result in increased production of AII. This study will determine if increased metabolism regulates AII production in ISBAT. Molecular characterization of the synthetic components of the RAS will be determined in ISBAT following different periods of cold-exposure. An increase in mRNA expression for angiotensinogen and angiotensin converting enzyme in ISBAT and not in liver or kidney will provide evidence suggesting tissue-specific regulation of AII synthesis.

Use of a genetic technique to identify yeast components of the splicing apparatus. GUY EAKIN* and BRIAN RYMOND, T. H. Morgan School of Biological Sciences, University of Kentucky, Lexington, KY 40502.

The primary RNA transcripts (pre-mRNA) of most eukaryotic genes contain non-coding sequences (introns) within the protein coding segments. Removal of these introns is, in most instances, imperative for the RNA to direct protein synthesis. The splicing of pre-mRNA to yield mature mRNA is a multi-step process involving several intermediates. As DNA is transcribed within the nucleus, the introns are excised from the pre-mRNA transcript by the spliceosome, a complex enzyme consisting of 50-100 polypeptides and 5 small nuclear RNAs (snRNAs). Commitment of the pre-mRNA to the splicing pathway is dependent on stable association of the pre-mRNA molecule with the U1 small nuclear ribonucleoprotein particle (consisting of the U1 snRNA and associated polypeptides). In addition, our lab has previously shown that, in *Saccharomyces cerevisiae*, the PRP39 gene product (PRP39p) is essential for stable association of the U1 snRNP with the pre-mRNA transcript. The focus of this project was to identify other gene products that genetically interact with the PRP39p by use of a genetic technique, high copy number mediated suppression. A library of self-replicating plasmids containing random segments of the yeast genome was used to transform a temperature sensitive (ts) strain of yeast, ts307. Of ca. 30,000 transformants 20-40 colonies were obtained wherein the mutant ts prp39 conditional lethal defect was relieved. Preliminary characterization of 13 plasmids suggest that, in addition to isolation of wild-type PRP39, other genes that interact with PRP39p were represented. On-going studies will prove the possible role of novel genes in the pre-mRNA splicing process.

PHYSICS

Asteroid orbital determinations based on CCD observations. RICHARD HACKNEY,* KAREN HACKNEY,

ROGER SCOTT, and JIM PARVIN, Western Kentucky University Astrophysical Observatory, Department of Physics and Astronomy, Western Kentucky University, Bowling Green, KY 42101.

Students in observational astronomy courses, or who otherwise have an interest in participating in observational project experiences, can use CCD images of asteroids as a basis for determining the approximate distances of the asteroids from the sun. We describe a student activity using CCD observations of a selected asteroid made ca. 1 hour apart, along with the necessary derivations to use measurements of the change in position of an asteroid near opposition to evaluate the apparent angular velocity and to deduce the size of the orbit, assuming low orbital eccentricity. Students can make the determinations using either CCD images that they acquire themselves with available equipment, or using images recorded using the 24-inch reflector at the Western Kentucky University Astrophysical Observatory.

Heaviside operational exact analytical solutions of the time-dependent Hamilton-Jacobi equation initial-value-problem defined by model real/complex rheonomic Hamiltonians. VALENTINO A. SIMPAO, 108 Hopkinsville St., Greenville, KY 42345.

Exact analytical solutions of the initial-value-problem for the time-dependent Hamilton-Jacobi equation defined by broad classes of model real/complex rheonomic Hamiltonians, have recently been discovered via a formal Heaviside operational scheme.

Heaviside operational exact analytical solutions of the time-dependent Schrodinger equation initial-value-problem defined by model real/complex rheonomic Hamiltonians. VALENTINO A. SIMPAO, 108 Hopkinsville St., Greenville, KY 42345.

Exact analytical solutions of the initial-value-problem for the time-dependent Schrodinger equation defined by broad classes of model real/complex rheonomic Hamiltonians have recently been discovered via a formal Heaviside operational scheme.

NASA-Kentucky partnership opportunities for space-related science, technology, and economic development. KAREN HACKNEY,* RICHARD HACKNEY, and ROGER SCOTT, Kentucky Space Grant Consortium and NASA EPSCoR Program, Department of Physics and Astronomy, Western Kentucky University, Bowling Green, KY 42101.

NASA has established partnerships with Kentucky and its universities for the purpose of involving faculty and students in space-related research, as well as technological and economic development. We describe current opportunities in the Kentucky Space Grant Consortium for undergraduate scholarships and graduate fellowships for students in mentored projects. Funding opportunities for the development of space-related projects and activities to support the teaching of space science and related disciplines are outlined. Progress of existing pro-

jects and new opportunities in the Kentucky NASA EPSCoR Program are discussed.

Novel analytically soluble model multi-particle interacting time-dependent classical dynamical systems. VALENTINO A. SIMPAO, 108 Hopkinsville St., Greenville, KY 42345.

Developed herein is a theoretical model of multi-particle interacting time-dependent classical dynamics, which admits analytical solution. The program is based upon my recent studies in analytical solution schemes for the time-dependent Hamilton-Jacobi initial-value-problem, defined by broad classes of model real/complex time-dependent Hamiltonians and initial conditions. The objective is achieved by particularizing the cited solution schemes to generate and solve the proposed model system. Meeting the objective results in enhanced theoretical and computational capabilities (i.e., algorithms) for analysis of actual physical multi-particle interacting time-dependent classical dynamical systems.

Novel analytically soluble model multi-particle interacting time-dependent quantum dynamical systems. VALENTINO A. SIMPAO, 108 Hopkinsville St., Greenville, KY 42345.

Developed herein is a theoretical model of multi-particle interacting time-dependent quantum dynamics, which admits analytical solution. The program is based upon my recent studies in analytical solution schemes for the time-dependent Schrodinger initial-value-problem, defined by broad classes of model real/complex time-dependent Hamiltonians and initial conditions. The objective is achieved by particularizing the cited solution schemes to generate and solve the proposed model system. Meeting the objective results in enhanced theoretical and computational capabilities (i.e., algorithms) for analysis of actual physical multi-particle interacting time-dependent quantum dynamical systems.

Opportunities for minority students in astrophysics using robotic observing facilities in the Center for Automated Space Science. ROGER SCOTT,* CHARLES McGRUDER, KAREN HACKNEY, and RICHARD HACKNEY, Center for Automated Space Science, Department of Physics and Astronomy, Western Kentucky University, Bowling Green, KY 42101; MICHAEL BUSBY, Center for Automated Space Science, Center of Excellence in Information Systems Engineering and Management, Tennessee State University, Nashville, TN 37209.

NASA has awarded funding to support a University Research Center titled the Center of Automated Space Science (CASS). The program is centered at Tennessee State University (TSU) under the direction of Dr. Michael Busby, with major components at Western Kentucky University (WKU) and at South Carolina State University (SCSU). Objectives of the program include establishment of research programs using remotely operated ground-

based telescopes and NASA missions, and development of procedures for dynamic scheduling and conducting astrophysical observations using automated instrumentation. The multi-faceted program will provide opportunities for involving under-represented minority students in preparing for careers in space-related sciences. Research opportunities at TSU include developing and managing robotic observing facilities and using the instruments to study magnetic effects in cool stars, including solar-type stars with starspots and magnetic activity relevant to the solar cycle and its effects on the earth. At WKU, a related theme is the effect of celestial high-energy sources, including the sun, x-ray sources, and gamma-ray bursters, on the earth's ionosphere. At SCSU, the studies extend beyond the solar system to understand the physics of the interstellar medium and nebular concentrations within it. Probing still greater distances, far beyond our galaxy, a group at WKU is studying the behavior of active galactic nuclei—energetic objects such as quasars with rapidly varying energy output. Opportunities for minority students to participate in the development of automated remote observing programs, and in astrophysical research, will be described.

Spectral classification of B supergiants in the Small Magellanic Cloud with the International Ultraviolet Explorer. **RAYMOND C. McNEIL*** and **ANTHONY L. BORCHERS**, Department of Physics and Geology, Northern Kentucky University, Highland Heights, KY 41099.

The spectral classifications of 79 B stars known or suspected to be supergiants and members of the Small Magellanic Cloud (SMC) are being estimated by means of visual examination of their ultraviolet spectra. All program spectra are new or archival low-dispersion spectra obtained with the short wavelength prime camera ($1200 \text{ \AA} < \lambda < 2000 \text{ \AA}$) of the International Ultraviolet Explorer (IUE). The work is being carried out in collaboration with George Sonneborn and Richard P. Fahey of the Goddard Space Flight Center (GSFC) using the computing facilities of the IUE Data Analysis Center. The spectral classifications are based on criteria suggested by literature and/or illustrated by sequences of low-dispersion IUE spectra of galactic standards. Archival high-dispersion IUE spectra of additional galactic standards have also been obtained and resampled to approximate the resolution of the low-dispersion spectra. Spectral features selected for classification are limited to photospheric absorption lines within the stated range of wavelengths. Preliminary classification of 41 SMC spectra suggests that nearly half the program stars may fall outside the range defined by the low-dispersion sequence of B0 to B9 galactic supergiants. One-third of such spectra appear to be hotter than B0; the remaining two-thirds appear to be giants or bright giants. Spectral criteria are therefore being carefully re-examined before final program classifications are determined.

The galactic center's annihilation line (0.511 MeV).

TODD GRIFFITH,* JUNMEI PAN,* SHAWN BRADLEY,* and **ANDREW MARTIN**, Department of Mathematical Sciences, Morehead State University, Morehead, KY 40351.

Balloon-borne instruments in the 1970s first detected the galactic center's strong 0.511 MeV spectral line. As 0.511 MeV is the precise energy equivalent to one electron mass, it was generally accepted that this line was the signature of electron-positron annihilation. However, subsequent occasional monitoring has shown considerable variation in the flux of 0.511 MeV photons being received. In fact, in the early 1980s the flux dropped to nearly zero. But in the late 1980s the line "turned on" again and has remained at about the same level until now. It now seems generally accepted that there are two sources for the positrons whose annihilation causes this line. One is a compact time-variable source (probably 1E 1740.7-2942); the other is a galactic plane component, probably due to neutron stars.

PHYSIOLOGY & BIOCHEMISTRY

Antioxidant influence of platelet aggregation and oxidation of low-density lipoprotein in hypercholesterolemic rats. **R. W. THURMAN*** and **D. J. SAXON**, Department of Biological and Environmental Sciences, Morehead State University, Morehead, KY 40351.

The effects of dietary vitamin E supplementation, an antioxidant, on platelet aggregation and oxidation of low-density lipoprotein (LDL) were investigated. Male rats received either a normal diet (N), a N diet plus 5% cholesterol (C), or a C diet plus 300 mg vitamin E/kg diet (CE) for 10 weeks. There were no differences in platelet aggregation responses among the groups, as measured by impedance aggregometry of whole blood, to either 0.5 mM arachidonic acid or 5 μM adenosine diphosphate. Total cholesterol and LDL were elevated in plasma by the C and E diets in comparison to the N diet; the high-density lipoproteins were not different. Triglyceride levels in the E group were greater than in the N group. LDL was isolated by ultracentrifugation from C and CE diet animals, and thiobarbituric acid reactive substances (TBARS) were determined by fluorescence spectroscopy. TBARS were lower in the CE group. Dietary supplementation of vitamin E did not alter platelet aggregation responses but did reduce the oxidation of LDL in the hypercholesterolemic animals.

Effect of exercise and tamoxifen on induction of estrogen-dependent and estrogen-independent rat mammary tumors. **ROBERT DOEPKE*** and **DAVID MAGRANE**, Department of Biological and Environmental Sciences, Morehead State University, Morehead, KY 40351.

This research characterized the influence of moderate levels of exercise and the antiestrogen tamoxifen on induction and development of estrogen-dependent and estrogen-independent rat mammary tumors. Fifty-day-old female Sprague-Dawley rats were given a 10 mg dose of the carcinogen 7,12-dimethylbenz(a)anthracene (DMBA)

and then placed in one of four groups: I—Controls, II—Exercise, III—Tamoxifen, and IV—Tamoxifen + Exercise. Groups II and IV were given moderate treadmill exercise (15 minutes/day; 5 days/week/12 weeks) at a belt speed of 20 meters/minute with a 1° incline. Groups III and IV were given subcutaneous injections of 100 µg of tamoxifen citrate (TAM) in oil 5 days/week for the 12 weeks. Results show that rats receiving TAM had significantly lower body weights compared to controls. Rats in the exercise group had 54% fewer total tumors than controls after 12 weeks; rats receiving TAM alone or TAM + exercise each had 92% fewer tumors. Rats with tumors were ovariectomized, and tumors that regressed by 20% after a 2-week period were judged estrogen dependent. Control rats showed 47% estrogen-dependent tumors; the exercise group had 80% estrogen-dependent tumors; and the TAM group and TAM + exercise were all estrogen independent. This research determined that both moderate exercise and tamoxifen showed a reduction in tumorigenesis in the DMBA rat model.

Renal response to dehydration in the larval bullfrog (*Rana catesbeiana*). ENDANG L. WIDIASTUTI* and JOHN J. JUST, School of Biological Sciences, University of Kentucky, Lexington, KY 40506.

Bullfrog tadpoles can osmoregulate when placed in a wide range of medium osmolalities (7–200 mOsm/kg H₂O). Part of physiological mechanisms involved in this hyperosmotic regulation is an alteration of kidney function. About 125 tadpoles were cannulated; their urine was collected for ca. 24 hours. A decrease in urine formation rate (UFR) occurred during periods of dehydration. A group of 49 tadpoles maintained in artificial pond water (APW) at 7 mOsm/kg H₂O had a UFR of 12.0 ± 1.0 µl/g/hr; a group of 43 tadpoles maintained in 100 mOsm/kg H₂O of mannitol in APW had a UFR of 3.8 ± 0.4 µl/g/hr. Finally 39 animals maintained in 200 mOsm/kg H₂O of mannitol in APW had a UFR of 2.1 ± 0.3 µl/g/hr. The decrease in UFR was accompanied by an increase in urine concentration from 47 ± 3 mOsm/kg H₂O in normal APW, up to 62 ± 4 mOsm/kg H₂O in 100 mOsm/kg H₂O of mannitol in APW, and as high as 120 ± 6 mOsm/kg H₂O of mannitol in APW, to as high as 0.57 ± 0.03 in 200 mOsm/kg H₂O of mannitol in APW. Tadpoles were grouped into different Taylor-Kollros stages of development (<XV, XVI–XXI, and XXII–Froglets). Dehydration treatment caused a universal decrease in urine production, the pattern being not significantly different among the groups. Dehydration caused an increase in the U/P ratio in all three developmental groups of tadpoles. However, there was no difference among the tadpole groups in the same treatment.

PSYCHOLOGY

Relationship between learning style and self directed learning. FRANCIS H. OSBORNE, ALISON K. VELASQUEZ,* Department of Psychology, Morehead State University, Morehead, KY 40351; RONALD L. SKIDMORE, Department of Educational Psychology, University of Kentucky, Lexington, KY 40506.

Seventy subjects were administered Schmeck's Inventory of Learning Processes (ILS) and the Oddi Continuing Learning Inventory (OCLI). The ILS has four scales of cognitive processing: Deep, Elaborative, Fact Retention, and Methodical Study. Deep and Elaborative processing have been found to be predictive of college grade-point average. The OCLI measured self-directed learning behavior. The subjects were subdivided into three groups on the basis of their OCLI score: Low, Medium, and High self-directed learning. A two-factor mixed analysis of variance using OCLI group as a between factor and ILS categories as repeated measures was performed. This analysis indicated that Low self-directed learners generally had lower cognitive processing scores than did either of the other OCLI groups. Overall, all subjects were less likely to employ deep processing and more likely to employ fact retention as favored learning styles. It should be noted that these subjects were typically at the beginning of their first semester in college. Perhaps this factor resulted in unrealistic expectations of the tools necessary for adequate performance in college.

Relationship between personality and negative life events. FRANCIS H. OSBORNE,* Department of Psychology, Morehead State University, Morehead, KY 40351; RONALD L. SKIDMORE, Department of Educational Psychology, University of Kentucky, Lexington, KY 40506.

The purpose of this research was (a) to explore the interrelationship among four negative affective factors that might influence academic performance and (b) to determine the relationship between these negative affective factors and several additional experimental variables such as self-directed learning and perfectionism. The four negative affective factors examined were operationalized by the Brief College Student Hassles Scale, which measures negative life events; the Experience Log, which measures negative feelings; and Spielberger's two-part State-Trait Anxiety Inventory. Subjects were 70 college-age students enrolled in an introductory psychology course at a regional state university. Inter-correlations among the four negative affect variables ranged from 0.45 to 0.76. The Experience Log and the Hassles Scale were highly correlated ($r = 0.76$), suggesting that they measured similar constructs from somewhat different approaches—events versus feelings. Income was negatively correlated with three of the four negative affect variables. College grade-point average was positively associated with three of the four variables, suggesting that anxiety and life events may play a motivational role for college students. The Oddi Continuing Learning Inventory was significantly and negatively asso-

ciated with the four negative affect variables, suggesting negative affect is counterproductive for continuing learning. Perfectionism, representing negative coping skills, was significantly correlated with all four negative affect variables. It remains to be seen whether or not these negative affect variables play a significant role in academic performance in specific college classes.

SCIENCE EDUCATION

On homework and tests. JOHN G. SHIBER, Division of Biological Sciences, Prestonsburg Community College, Prestonsburg, KY 41653.

The majority of 616 parents, teachers, college, and pre-college students from eastern Kentucky who participated in a survey on homework and tests said both are essential to learning. About 1/3 of teachers disagreed. Virtually all teachers, however, and a majority of parents and college students, but only 50% of pre-college students, supported homework. Of all respondents, 52% said student grades should be based solely on homework assignments, 35% on test/quiz scores. Only teachers (45%) favored daily homework; 32% preferred every other day, along with 53% of parents and 37% of pre-college students. Of college students, 32% preferred every other day, too, but 54% said weekly assignments were best. Most adult respondents (68%) said students are not overloaded with homework at any level, but 53% of pre-college students disagreed. Of the total sample, 66% said that teachers make homework a useful involvement, but 80% said teachers should instruct students on *how* to do it. Of adults surveyed, 81% said elementary children should have adult supervision at homework, but 54% of pre-college students disagreed. Yet 79% of pre-college students, and 89% of adults, said parents should help children with homework. Pre-college student responses might appear conflicting. Perhaps they shun supervision and prefer/need *help* from parents/guardians, not just any adult. Homework should be done first, before all other activities, said 65% of all respondents, but a quarter of parents and teachers said it should come second, after play/sports. Of teachers, 10% and, of pre-college students, 20% said homework should be done last, before bedtime. Of teachers, parents, and college students with elementary-age children, 76%, 87%, and 91%, respectively, said homework is either done first, or right after play/sports, and 22%, 11%, and 3%, respectively, said it is not done until right before bedtime. Most of the adults with high-schoolers said their children do homework right after school or immediately after play/sports, too, but 34% of parents, 48% of teachers, and 6% of college students said their high-schoolers did it right before bedtime. Of adults, 65% preferred courses with tests, but 38% of teachers preferred not to have tests, and pre-college students were unsure. Most parents, college, and pre-college students said if they have to take tests, they preferred multiple choice questions first, then true/false, while most teachers said short essay, with multiple choice ques-

tions as a second choice. Matching came in third place for all.

Integrating water quality testing, school-to-work training, and international multiculturalism in a joint American-Russian "Eco-Bridge" project. CLOYD J. BUMGARDNER,* Calloway County Middle School; STEPHANIE L. WYATT, Calloway County High School, Murray, KY 42071.

In this model environmental project, five high school students and their principal from Ust-Donetsk School #2 in Rostov, Russia, traveled to Murray, Kentucky, where they and five Calloway County High School students and their science teacher were trained in environmental monitoring of drinking water sources. The American-Russian research team visited the Mammoth Cave and Horse Cave karst systems to learn about groundwater flow and potential for pollution. The research team also tested water from several streams in Kentucky and then traveled to Rostov, Russia, where they performed the same tests on springs supplying Ust-Donetsk with drinking water. As a result of the research, two springs supplying water to Ust-Donetsk were closed for drinking purposes due to unacceptably high levels of pollutants. The students presented their research at the first United States-Russian Environmental Teen Summit in Washington, DC and at Rostov University in Russia. The Water Watch Program through the Kentucky Division of Water provided scientific and school-to-work training. This model curriculum integrated environmental science, school-to-work training, governmental agencies, and multiculturalism with the project design.

Teachers' perception of student academic success. JOHN G. SHIBER and H. BOGALE, Divisions of Biological Sciences & Physical Sciences, Prestonsburg Community College, Prestonsburg, KY 41643.

Questionnaires on perception of what students believe is important for academic success were sent to 180 faculty members from 9 community colleges. The teachers' responses were compared with those of 975 students in a 1993 survey. One striking difference between the two groups was their perception of student confidence in their academic ability. Of the teachers, 40% said that most of their students were incapable of getting anything above a C in their courses and that most students agreed with this. Of the students, 94% said they were capable of getting a higher grade (i.e., 49% an A, 45% a B). Further, a much higher percentage of teachers (66%) than students (22%) believed students are under pressure to get As at the expense of learning the material. Another difference was seen in the attitude towards tests. Of teachers, 77% said test scores should not be the sole determinant of final grades, but 98% of students believed they should. Both groups did agree about the type of question students prefer: multiple choice. This was also favored by 25% of teachers, whereas 24% preferred problem-solving, 22% essay, 10% short answer, and 19% combinations of types.

For the best proof of comprehension, only 5% of teachers chose multiple choice. The majority chose essay, problem-solving, and a combination, in that order. While students showed a strong preference for lectures and note taking, teachers thought they prefer handling real things. With respect to teacher preference, a combination of instructional approaches was first choice, class participation second, then handling real things. Only 10% of teachers expressed preference for lectures and note-taking. Of teachers, 58% and, of students, 79% said that learning/retaining course material, irrespective of grades, best reflects student success, but 21% of students thought getting As and Bs was the primary indicator, irrespective of learning. Also, 38% did not seem to think that interest in a course is vital for success, while 84% of teachers did. Despite the differences, most students and teachers agreed that a serious but enthusiastic and optimistic student attitude, a good personal discipline, and a knowledgeable teacher who explains things well are essential to student success. It is suggested, however, that student success initiatives cannot be fully effective unless the students' opinions and perceptions are known and seriously considered.

ZOOLOGY & ENTOMOLOGY

Birds of Manchester 1, Manchester 2, and Brush Creek Islands, Lewis County, Kentucky. RUDY A. GELIS, Department of Biology, Berea College, Berea, KY 40404.

A bird survey was conducted on three Ohio River islands and associated water environs from 9 Mar 1995 through 30 Sep 1995. A total of 83 species was documented consisting of 54 summer residents and 29 transient species observed only in spring and/or fall. Unconsolidated shoreline, riverbank, old field, and forested bottomland were four island habitats important for feeding, roosting, and nesting. Cavity nesters of special interest were yellow-shafted flicker (*Colaptes auratus*), pileated woodpecker (*Dryocopus pileatus*), red-bellied woodpecker (*Melanerpes carolinus*), hairy woodpecker (*Picoides villosus*), downy woodpecker (*Picoides pubescens*), American kestrel (*Falco sparverius*), wood duck (*Aix sponsa*), eastern screech-owl (*Otus asio*), and great horned owl (*Bubo virginianus*). Black vultures (*Coragyps atratus*) and turkey vultures (*Cathartes aura*) were observed from egg through fledgling stages. Two species seen throughout the summer are listed by the Kentucky State Nature Preserves Commission (KSNPC) as Special Concern birds. A colony of bank swallows (*Riparia riparia*) was found on Manchester 2, and great blue herons (*Ardea herodias*) were observed at Manchester 1 and Brush Creek Island, although nesting was not confirmed. Spotted sandpipers (*Actitis macularia*), with KSNPC Endangered status, were also recorded during the breeding season with no evidence of nesting.

Changes in gut capacity of prairie voles (*Microtus ochrogaster*). TERRY L. DERTING, TIMOTHY P. BEGIN,* and MANDY L. CARTER, Department of Biological Sciences, Murray State University, Murray, KY 42071.

When energy demand increases in small herbivores, a sequence of changes in gut capacity may occur that accommodates increased food intake. To investigate the occurrence of sequential changes in gut capacity with increasing energy demand we established four groups of prairie voles (*Microtus ochrogaster*). Each group consisted of five adult male voles, implanted subcutaneously with a placebo or an established dose of triiodothyronine. The groups were labeled control, low-, medium-, or high-energy demand in correspondence with their level of daily metabolic rate (DMR) (0%, 12%, 23%, 42%, respectively). The pre- and post-implant basal and daily metabolic rates were measured for each vole. After a 21-day implantation period the animals were sacrificed and the gastrointestinal and vital organs removed. We compared the lengths, wet masses, and dry masses of organs among groups. There were no significant differences in any measurement of internal organs between the low-DMR and control groups. The medium-DMR group had significantly greater dry masses of the total gut, small intestine, and heart as compared to the controls. The group with high-DMR had significantly greater dry masses of the total gut, small intestine, caecum, and liver. There was no evidence for sequential changes in the length or mass of any individual gut organ with increasing DMR. There was a positive association, however, between the number of individual organs that exhibited a greater mass and increasing DMR.

Effects of *Acanthocephalus dirus* on pigments and proteins of *Lirceus lineatus*. DAVID F. OETINGER, Kentucky Wesleyan College, Owensboro, KY 42302.

From March 1985 to March 1995, 216 of 907 (23.8%) *Lirceus lineatus* collected from Rhodes Creek (Davies County, KY) were infected with larvae of *Acanthocephalus dirus*. Infected isopods had either decreased integumental pigmentation (and thus were "lighter"—9.4% of total) or increased integumental pigmentation ("darker"—13.8% of total). Only 0.7% of *L. lineatus* with normal integumental pigmentation were infected with *A. dirus*. While infected isopods harbored about equal numbers of male and female acanthocephalans, darker integumental pigmentation was more common, and female isopods exhibited altered integumental pigmentation more frequently than did male isopods. Spectrophotometric analyses of methanolic-HCl pigment extracts showed that lighter-pigmented isopods had decreased levels of ommochrome pigments while darker-pigmented isopods had increased levels. Native proteins from whole-body extracts and hemolymph of isopods were separated by sodium dodecyl sulfate polyacrylamide gel electrophoresis. Resulting electropherograms were photographed and compared qualitatively as well as densitometrically. Variations in protein constituents paralleled altered pigmentation patterns: extracts from comparable size, lighter-pigmented isopods were missing protein bands, and those from darker-pigmented isopods had larger, more darkly-staining bands than normal-pigmented isopods.

Increased gut capacity in prairie voles (*Microtus ochrogaster*): a response to metabolic demand or food intake? TERRY L. DERTING and MANDY L. CARTER,* Department of Biological Sciences, Murray State University, Murray, KY 42071.

We investigated two potential proximate causes for changes in gut capacity in small mammal species: energy demand and food ingestion. We established three experimental groups of juvenile male prairie voles (*Microtus ochrogaster*). The control group had ad libitum ingestion with normal energy demand. The first experimental group had ad libitum ingestion with elevated energy demand. The second experimental group had elevated ingestion and elevated energy demand. Energy demand was elevated through implants of triiodothyronine. Basal and daily metabolic rates (BMR and DMR) were estimated. After a 21-day experimental period the voles were sacrificed and the length, wet mass, and dry mass of the stomach, small intestine, caecum, and colon were measured. There were no significant differences in DMR among the groups. Therefore, differences in gut capacity due to elevated energy demand were due to elevated BMR alone. Elevated BMR was associated with mass increases of the total intestine, small intestine mucosa, kidney, and liver. Voles with elevated energy demand plus elevated ingestion showed additional differences, having greater stomach length, stomach mass, small intestine mass, duodenum mass, ileum mass, and spleen mass than the controls. We concluded that energy demand and food ingestion were associated with increases in gut capacity. Food ingestion, however, had greater and more diverse effects on gut capacity than did energy demand.

Stage dependent induced hatching in the fathead minnow (*Pimephales promelas*). SARAH A. MURPHY* and JOHN J. JUST, Department of Biological Sciences, University of Kentucky, Lexington, KY 40506.

All aquatic invertebrate and vertebrate embryos hatch from proteinaceous egg cases. Hatching involves two mechanisms: digestion of the egg case by a hatching enzyme secreted from unicellular glands located in epidermis of the head and breaking of the egg case by mechanical means. This study shows that, in the fathead minnow, hypoxia can induce premature hatching as early as Devlin's stage 28, four developmental stages, about 3 days,

earlier than normal hatching (stage 32). Embryos at developmental stages 22–31 ($n = 60$ for each stage) were subjected to various environmental O_2 pressures by bubbling oxygen ($P_{O_2} = 760$ mm Hg), air ($P_{O_2} = 160$ mm Hg), or nitrogen ($P_{O_2} = 0$ mm Hg) into vials containing 10 ml dechlorinated tap water and 20 embryos. Animals from developmental stages 22–27 failed to hatch after 2 hours in any of the above treatments. Stages 28–31 showed a progressive increase in percent hatch in the nitrogen treatment. Percent hatch of stages 28, 29, 30, and 31 were observed to be 22%, 51%, 68%, and 95%, respectively. Embryos at stage 30 ($n = 60$) were placed into various environmental O_2 pressures of 0, 38, 76, 160, or 760 mm Hg. Less than 21% of the embryos hatched at a P_{O_2} of 76 mm Hg or above, compared to a hatch rate of 60% or greater when the P_{O_2} was decreased to 38 mm Hg and below. In a closed system the average oxygen consumption of stage 30–31 embryos was $0.68 \pm 0.21 \mu\text{l/hr}$ /embryo, and the average oxygen pressure at hatching was 67 mm Hg. Thus the fathead minnow as several other aquatic species can be induced to hatch prematurely when exposed to hypoxic conditions. Work continues to isolate the hatching enzyme of the fathead minnow and to histologically identify the hatching glands. It is believed that premature hatching can occur only after the hatching glands and hatching enzymes make their developmental appearance.

Symmetry of gene expression in wing venation of *Drosophila melanogaster*. LYNN A. EBERSOLE, Department of Biological Sciences, Northern Kentucky University, Highland Heights, KY 41099.

Symmetry of wing venation of *Drosophila melanogaster* was measured in males and females in combinations of three major mutants, two modifier lines, and three developmental temperatures (18°C, 22°C, 26°C). The major mutant genes were *veinlet* (*ve*), *radius incompletus* (*ri*), and *cubitus interruptus-Dominant* (*ci^D*). High and low modifier lines had been selected for their effect on *cubitus interruptus* expression. Ratios comparing the interrupted lengths of the veins L2, L3, and L4 to the length of the wing were computed. Paired *t* tests of the left and right wing ratios were made. Of 64 comparisons, significant asymmetry was found in only 3: L3 length in high-line, *ve/ve*, *ci^D/+*, males at 22°C; L2 length in low-line, *ve/ve*, *ci^D/+*, females at 22°C; L2 length in low-line, *ve/ve*, *ci^D/+*, males at 22°C.

DISTINGUISHED SCIENTIST AND OUTSTANDING TEACHER AWARDS 1995

Distinguished Scientist Award

Dr. John T. Riley—professor of chemistry, Western Kentucky University, Bowling Green, Kentucky. B.S., chemistry/mathematics, Western Kentucky University; Ph.D., chemistry, University of Kentucky. The Distinguished College/University Scientist Award is given for significant contributions to science teaching but particularly for research achievements. The 1995 awardee is Professor John T. Riley, professor of chemistry, Western Kentucky University, Bowling Green, Kentucky.

Professor Riley's extensive academic career has been marked particularly by his development and encouragement of integrating research and education. His research has concentrated primarily on coal science, and atomic spectroscopy for identification of constituents of coal. In the 1980s he directed the work of some 20 students, undergraduate and graduate, in studies of causes of self-heating of coal and methods of minimizing this occurrence. Several resulting recommendations for loading and trimming coal in barges are being followed today. The students who worked on this project gained much field experience and saw a broader spectrum of research activities than students usually do. Later he worked on coal desulfurization, which led to a patented process for removing organic sulfur.

John has an equally extensive and impressive record of public service. Founder of the Coal and Fuel Laboratory at Western Kentucky University, he has helped build it into a nationally recognized facility. He has been involved in grants and contracts bringing more than two million dollars in research funds to Western. John's statewide activities include service on the state's EPSCoR committee and on the subcommittee for DOE/EPSCoR. On the national level, he is active in the ASTM and in ACS. John is a member of the fuel division of ACS, a member of its executive committee since 1993, and the 1996 program committee chairman for national meetings.

Although John is heavily occupied in research and research administration, he also is active in teaching at all undergraduate levels. He offers his "Chemical Magic Show" to public receptions for science and technology.

Clearly a scientist with strong national and international reputations, Professor Riley has enlarged the research strength and record of Western. He has done that while carefully incorporating his students into research so that they appreciate that research is education. Professor Riley, an outstanding example of the complete life of an active and eminently successful professor, is eminently qualified for the award of Distinguished College/University Scientist.

Outstanding College/University Science Teacher Award

Dr. Gordon K. Weddle—professor of biology, Campbellsville College, Campbellsville, Kentucky. B.S., biology,

Oakland City College; M.S., mammalogy, Fort Hays State University; Ph.D., zoology, Southern Illinois University. The Outstanding College/University Teacher Award is given for significant contributions primarily to science teaching, with participation also in research.

The 1995 awardee is Professor Gordon K. Weddle, professor of biology at Campbellsville College, Campbellsville, Kentucky.

Dr. Weddle is known for his reality-based learning approach to biology. To strengthen this, he has his students participate in scientific research as undergraduates. He has led the college in a plan to involve undergraduates in research as part of their curriculum. He instituted a research methods course that emphasizes professional development toward the capacity to deal with real-world problems as faced by practicing biologists. Many of his students have gone on to success in graduate work at research universities.

Dr. Weddle, active in national science organizations, has organized sessions and chaired them at national meetings.

His other strong commitment is to ecology and environmental issues. An expert on the ecology of stream fishes, he has established a stream ecology laboratory at the college. He has also worked with a large textile company in monitoring water quality and has been consulting ecologist for the Campbellsville waste treatment plant.

Dr. Weddle has demonstrated the qualities of scholarship, research, and particularly commitment to student achievement and growth that make him a well-qualified recipient for the award as Outstanding College/University Teacher for 1995.

Industrial Scientist of the Year Award

Mr. Robert H. Wombles—vice president of research and development and product applications, Ashland Corporation, Ashland, Kentucky. B.S., mathematics, Georgetown College; M.S., chemistry, Vanderbilt University. The Industrial Scientist Award is based on these criteria: "The recipient shall have made some significant contribution to industrial science in Kentucky. Contributions should be interpreted broadly, to mean contribution directly to commerce or industry, or to the improvement of the quality of life in the Commonwealth."

The 1995 awardee is Robert H. Wombles, vice president of research and development and product applications, Ashland Corporation, Ashland, Kentucky.

Mr. Wombles satisfies all elements of the criteria in abundance. A native of Kentucky, he received his bachelor's degree from Georgetown College. He then earned an M.S. degree at Vanderbilt in Organic Chemistry. His entire professional career of 20 years has been with the Ashland Corporation. After only 3 years with Ashland he was promoted from industrial scientist to research chemist.

Under his leadership Ashland became a major asphalt producer. Three years later he was promoted to group leader to direct studies of heavy hydrocarbons. His group helped develop a pitch known throughout the world as a premium product. Promoted to manager of the analytical section in 1986, he was responsible for research methods development and analytical services. By 1992 he was director of all research and development, a department of 109 employees. He was named vice president of research and development in 1994. In 1995 his role was expanded to include the automotive product and application laboratories.

Robert holds five patents and has been instrumental in the ability of Ashland to diversify into different areas making use of heavy hydrocarbons. He is a long-time member of national ASTM committees and is active in the petroleum division of the American Chemical Society.

He is a member of the statewide EPSCoR Committee, the committee responsible for coordinating research and education EPSCoR programs of five different federal agencies. He is also a member of the governing board of the Kentucky Academy of Science. His role in Kentucky science, particularly in this period when we are looking to science and technology to point the way to an improved economy, would be hard to overestimate.

Outstanding Secondary School Science Teacher Award

Mrs. Rose H. Caldwell—chair and teacher of mathematics, Paul Dunbar High School, Lexington, Kentucky. B.A., mathematics, M.S., mathematics, Rank I, mathematics, University of Kentucky. The Outstanding Secondary School Science Teacher Award is given for significant contributions to science teaching at the middle and high school levels in Kentucky.

The 1995 awardee is Rose H. Caldwell, mathematics co-chair and teacher at Paul Lawrence Dunbar High School, Lexington, Kentucky.

Nominators who have been in teaching for up to 25

years cite Mrs. Caldwell as the best mathematics teacher they have known throughout their careers. She uses innovative teaching techniques to make the math-learning environment fun and clearly rewarding to her students. She makes extensive use of positive-reinforcement techniques to encourage students to higher levels of achievement. She regularly takes many hours outside usual school hours, often in the evening, to help students wishing extra assistance in learning. She volunteered to teach a class at 6:45 A.M. although her administrative duties required her to be present at least until the end of the school day. When the math, science, and technology program at Dunbar needed a teacher for five students, Rose Caldwell volunteered.

Students respect and like Rose Caldwell because she has an outstanding background and excellent abilities in mathematics; she teaches the most advanced courses with such strength that her students enjoy them. Her ability to motivate is remarkable. Students work very hard without feeling that the tasks are onerous. She is able to relate mathematics to real-life, ordinary situations and occupations. Her students regard her as caring, selfless, fair, and concerned for all.

Her students' achievements are impressive. Last year 11 of the 30 students receiving the highest possible score on the AP placement examination, statewide, were Mrs. Caldwell's students. This year 20 of her 25 students who took the AP calculus examination earned the highest possible score.

Despite her extensive administrative and teaching responsibilities, she served on the committee that wrote the Curriculum Framework in mathematics for KERA and helped set the qualifying standards for mathematics portfolio scoring. She works closely with the University of Kentucky mathematics education program.

Mrs. Caldwell, eminently active and successful in all aspects of mathematics education at the senior high school level, has contributed substantially to mathematics education in the Commonwealth. She is superbly qualified for the present award.

Guidelines for Contributors to the *Transactions*

1. GENERAL

- A. Original papers based on research/review in science will be considered for publication in the *Transactions*; at least the first author must be a member of the Academy. Announcements, news, and notes will be included as received.
- B. Papers (in triplicate) may be submitted at any time to the editor.

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List in the cover letter your telephone/FAX numbers, your E-mail address, and the names, addresses, and phone numbers of two persons who are potential reviewers.

- C. Format/style of papers must conform to practices in recent issues of the *Transactions*, which are, in effect, a style manual. The running head at top right of each page should give name of author(s), a short version of paper title, and page number of total. Do not staple pages together.
- D. Papers should be submitted in hard copy and on a 3.5 inch disk, preferably in WordPerfect for Windows 5.2 or later version.
- E. Indent the first line of each paragraph (but not the first line of entries in the Literature Cited).

2. FORMAT

- A. Papers should be in 10 cpi type on white paper 8.5 × 11 inches, with margins at least 1 inch all around. Double-space throughout the paper (i.e., one full line of space between each two lines of text, literature cited, or tabular data). Do not justify right margins.
- B. Except for scientific names of genera and of infra-generic taxa, which should be typed in italics, the same type (roman) should be used throughout (i.e., one type size only, no bold).
- C. Sequence of sections in papers should, where appropriate, be as follows: title of paper, name/address of author(s), abstract, body of paper, footnotes, table captions, figure captions (all the preceding on consecutively numbered pages), tables, and figures.
- D. The first page should include the running head and, centered near the top of the sheet, the paper's title and the name and address of author(s). These should be followed immediately by the abstract.
- E. The abstract, not to exceed 200 words, should be concise, descriptive, and complete in itself without reference to the paper.
- F. The body of the paper should, where appropriate, include the following sections: Introduction, Materials

and Methods, Results, Discussion, Summary, Acknowledgements, and Literature Cited.

- G. No more than three levels of headings should be used: level 1, in capitals, centered; level 2, in capitals/low-ercase, flush left; level 3, in italics, a paragraph indent, with initial capital only (except proper nouns and adjectives), and followed by a period, the text then starting after one blank space.
- H. Personal communications (avoid if possible) should be indicated in the text as follows: (name, affiliation, pers. comm., date), e.g., (O.T. Mark, Wainwright College, pers. comm., 5 Jun 1995).
- I. Notes should follow the format established in *Transactions* 57(1).

3. STYLE

- A. In text, spell out one-digit numbers unless they are used with units of measure (four oranges, 4 cm) and use numerals for larger numbers; do not begin any sentence with a numeral.
- B. Use no footnotes except those for title page and tables. Footnotes, identified by consecutive superscript numbers, should be entered on a separate sheet.
- C. Measurements should be in metric and Celsius units. Define lesser-known symbols and give the meaning of acronyms at first use. Express time of day in the 24-hour system. Dates should be written day, month (abbreviated to three letters), year without internal punctuation. Units with multiple components should have individual components separated by a virgule (e.g., g/m² or g/m²/yr).
- D. Names of authors of binomials should be included but only at the first mention of the binomial. Cultivar names are not italicized but are enclosed in single quotes or preceded by cv.
- E. Useful guides for contributors to the *Transactions* are the following: *Scientific style and format: the CBE manual for authors, editors, and publishers*, 6th ed., Cambridge University Press, 1994; *The Chicago manual of style*, 14th ed., University of Chicago Press, 1993; *The ACS style guide*, American Chemical Society, Washington, DC, 1986; and *AIP style manual*, American Institute of Physics, New York, 1990.

4. IN-TEXT CITATION OF LITERATURE

- A. Cite publications in the text by author(s) and date—e.g., (Readley 1994); multiple citations should be in alphabetical order and separated by semi-colons—e.g., (Ashley 1987; Brown 1994; Foster 1975); multiple citations of works by one author(s) should be in chronological order—e.g., (Jones 1978, 1983); publications by one author(s) in the same year should be distinguished by a, b, c, etc.—e.g., (Smith 1994a, 1994b). For in-text references to works with one to three authors use names of all authors—e.g., (Jones, Smith, and Williams 1991); for works with four or more au-

thors use name of the first author followed by et al.—e.g., (Lee et al. 1985).

- B. Do not include any reference unless it has been published or accepted for publication (“in press”). In the latter case give the name of the accepting journal or the publisher/place of publication; use n.d. in place of a date for in-text citation of “in press” references, e.g., (Jones n.d.).

5. LITERATURE CITED

- A. List all authors of each entry. Do not abbreviate journal titles; abbreviations for these will be supplied by the editor.
- B. The first line of each reference should be typed flush left; the remaining lines should be indented.
- C. Examples of common types of references are given below.

JOURNAL ARTICLE

- Lacki, M.J. 1994. Metal concentrations in guano from a gray bat summer roost. *Transactions of the Kentucky Academy of Science* 55:124–126.

BOOK

- Ware, M., and R.W. Tare. 1991. *Plains life and love*. Pioneer Press, Crete, WY.

PART OF A BOOK

- Kohn, J.R. 1993. Pinaceae. Pages 32–50 in J.F. Nadel (ed). *Flora of the Black Mountains*. University of Northwestern South Dakota Press, Utopia, SD.

WORK IN PRESS

- Groves, S.J., I.V. Woodland, and G.H. Tobosa. n.d. *Deserts of Trans-Pecos Texas*. 2nd ed. Ocotillo Press, Yucca City, TX.

6. ILLUSTRATIONS

FIGURES (LINE DRAWINGS, MAPS, GRAPHS, PHOTOGRAPHS)

Figures must be camera-ready, glossy, black-and-white prints of high quality or laser prints of presentation qual-

ity. These should be designed to use available space effectively: a full page or part of one, or a full column or part of one. They should be mounted on heavy white board and covered with a protective sheet of paper; photographs to be grouped as a plate should have no space between them. Dimensions of plates must observe page proportions of the journal. Each illustration in a plate may be numbered as a separate figure or the entire plate may be treated as one figure. Include scale bars where appropriate. Lettering should be large enough to be legible after reduction; use lowercase letters for sections of a figure. Figure captions should be self-explanatory without reference to the text and should be entered on a page separate from the text. Number figures in Arabic numerals. Statistics presented in figures should be explained in the caption (e.g., means are presented \pm SE, $n = 7$).

TABLES

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NEWS

KAS Annual Meetings α

The 1996 annual meeting of the Kentucky Academy of Science will be held 14-16 November at Kentucky State University, Frankfort. The 1997 meeting will be held at Morehead State University, Morehead; the 1998 meeting, at Jefferson Community College, Southwest Campus, Louisville.

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TRANSACTIONS OF THE KENTUCKY ACADEMY OF SCIENCE



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Rare and Extirpated Plants and Animals of Kentucky

Kentucky State Nature Preserves Commission

Kentucky State Nature Preserves Commission,
801 Schenkel Lane, Frankfort, Kentucky 40601

ABSTRACT

The Kentucky State Nature Preserves Commission has updated and revised the rare plant and animal list last published in 1986. The new list includes 342 plant and 273 animal taxa that are rare in Kentucky or nationally, and 4 plant and 45 animal taxa considered to be extirpated from Kentucky or extinct.

INTRODUCTION

Kentucky's terrestrial, aquatic, and subterranean habitats support a broad array of plants and animals. Some groups achieve near maximum diversity within the commonwealth's borders. For example, freshwater unionids (mussels) are distributed worldwide but achieve their greatest diversity (297 taxa) in the United States (Williams et al. 1992), where Kentucky's fauna is the third richest (Cicerello, Warren, and Schuster 1991). Within the world's temperate regions, the North American freshwater fish fauna also is the most diverse with ca. 790 taxa (Page and Burr 1991). Only the faunas of Tennessee and Alabama exceed the more than 230 native taxa found in Kentucky (Burr and Warren 1986). Our freshwater crayfish fauna includes ca. 50 of the 322 taxa known from North America, which harbors the world's richest fauna (Bouchard 1978). Mammoth Cave, the world's largest cave system, has one of the richest cave faunas in the world (Barr 1985a). About 345 species of birds have been reported from Kentucky, including 169 confirmed breeding species (Monroe 1994). Although not exceptionally diverse for a temperate region, Kentucky's flora has over 2400 native plant taxa (Medley 1993), including three endemics.

Kentucky's environment has been pro-

foundly altered since European settlement over 200 years ago. Old-growth forests that covered ca. 90% of the state at settlement have been grazed, cut, and replaced with highly fragmented secondary forest tracts of greatly altered structure and composition that cover only half of the state (EQC 1992). Over 80% of Kentucky's 633,760 ha of wetlands have been drained or altered (KSWCC 1982), and the majority of those remaining are fragmented and degraded. Native grasslands—"barrens" to early settlers—may have once covered up to 1.5 million ha of western Kentucky (McInteer 1942, 1946) but have been reduced to a few remnant tracts totaling less than 500 ha. The bluegrass savanna-woodland natural community that historically occurred exclusively in the Bluegrass region has been essentially extirpated with only a few highly disturbed remnants still recognizable (Bryant et al. 1980). Essentially all of Kentucky's largest streams have been impounded or channelized for navigation or flood control (USACE 1993), and most smaller streams also have been physically or chemically altered. Kentucky's caves and springs, the most numerous in the United States (Hobbs 1992), have been directly and indirectly altered by human activities (Barr and Kuehne 1971; Hobbs 1992; Thrailkill 1985).

Concern over the impact of habitat alteration and loss on Kentucky's biodiversity resulted in the development of previous endangered species lists (Branson et al. 1981; KSNPC 1991, 1995; Warren et al. 1986). Recent interest in biodiversity has resulted in a special session on that topic at the 1991 Kentucky Academy of Science meeting, funding of the Heritage Land Conservation Fund (KRS 132.020, KRS Chapter 186, and KRS 224.10-250) by the 1994 General Assembly, and the Governor's Task Force on Biodiversity report (Taylor 1995). Previous lists were not supported by statute. Following the passage of the Rare Plant Recognition Act in 1994, however, Kentucky became one of 32 states (Griffin and French 1993) with the authority to create an official endangered plant list. Kentucky was one of only four states without an official state endangered animal list (Griffin and French 1993). The Kentucky Department of Fish and Wildlife Resources has the statutory authority (KRS 150.183) to create and adopt by administrative regulation lists of endangered animals (Glass 1992).

This list serves to identify native Kentucky plants and animals in need of protection, research, and/or management because they are rare, the number of populations known for a taxon is declining or low, or their distribution has been reduced. A list of plants and animals presumed extinct or extirpated from Kentucky also is presented to document losses, many of which are attributable to human activities. The overall goal of publishing these lists is to assist in the recovery and preservation of Kentucky's rich natural diversity.

METHODS

The Kentucky State Nature Preserves Commission (KSNPC), a state agency mandated to identify and protect natural areas, worked jointly with many scientific authorities to identify plant and animal taxa rare in the Commonwealth. KSNPC uses The Nature Conservancy's standardized Natural Heritage Program methodology (TNC 1988) to manage distributional and ecological information on selected taxa in map, manual, and computer files. These data are used by KSNPC to locate aggregations of rare taxa, natural communities, and other natural features for monitoring, protection, and acquisition. The methodology was

therefore ideally suited for the revision process outlined below.

Each taxon listed by KSNPC (1991) and many previously unlisted taxa were evaluated to determine their conservation status. The criteria used included, but were not limited to, the number, age, and accuracy of occurrences; historic and present geographic distribution; habitat requirements; habitat loss and relative threat of destruction; and ecological fragility. The information used to make the evaluation was that available as of 1 Mar 1995. Each taxon was assigned a conservation status, suggested for delisting, or recommended for addition to the list of taxa presumed to be extinct or extirpated from Kentucky. Plants and animals were presumed to be extinct or extirpated when habitat loss was pervasive and/or concerted efforts by knowledgeable biologists to collect or observe specimens within appropriate habitat failed. The resultant list and statuses were submitted to knowledgeable individuals for peer review and suggestions for additions and deletions. All comments received were considered and in many cases discussed with the reviewer before final statuses were assigned.

Exceptions to this process involve taxa officially listed or under status review for listing by the United States Fish and Wildlife Service (USFWS 1989, 1993a, 1993b, 1994). Taxa of federal concern that are not considered rare in Kentucky are included on the list with their federal status (i.e., *Speyeria diana*, *Cycleptus elongatus*, *Polyodon spathula*, *Cryptobranchus alleganiensis alleganiensis*, *Dendroica cerulea*, *Lanius ludovicianus migrans*, and *Neotoma magister*). Non-breeding birds (e.g., *Accipiter gentilis*, *Charadrius melodus*, *Contopus borealis*, and *Sterna hirundo*) occurring regularly as migrants or visitors in Kentucky are not included on the list. Finally, taxa that have been extirpated from Kentucky but persist elsewhere are presented in the list of extinct and extirpated taxa.

The Rare Plant Recognition Act of 1994 (KRS 146.600-619) authorizes KSNPC to create administrative regulations outlining criteria for listing endangered and threatened plants and adopting lists of these taxa. This law specifically states that these lists are not to impede the development or use of public or private land. KSNPC started the administrative

rule-making process in 1995; an official list should be promulgated in 1996. There are several differences between the rare plants listed herein and those designated under the Act. The Act list will include only endangered and threatened plants, will require public comment, and will be revised or updated at least every 4 years. The plant list herein uses additional status categories, relies upon peer review instead of public comment, and will be updated annually, with a complete revision published every 4 years.

STATUS CATEGORIES

The intent of assigning status designations is to (1) indicate the degree of rarity of the taxon, (2) indicate the degree of threat to the continued survival of the taxon, and (3) aid in establishing conservation priorities. The KSNPC status designations assigned herein have no legal or statutory implication but rather were established as tools to monitor the survival potential of the taxon. The four KSNPC status categories utilized are coded in capital letters and defined as follows:

Endangered (E). A taxon in danger of extirpation and/or extinction throughout all or a significant part of its range in Kentucky.

Threatened (T). A taxon likely to become endangered within the foreseeable future throughout all or a significant part of its range in Kentucky.

Special Concern (S). A taxon that should be monitored because (a) it exists in a limited geographic area in Kentucky, (b) it may become threatened or endangered due to modification or destruction of habitat, (c) certain characteristics or requirements make it especially vulnerable to specific pressures, (d) experienced researchers have identified other factors that may jeopardize it, or (e) it is thought to be rare or declining in Kentucky but insufficient information exists for assignment to the threatened or endangered status categories.

Historical (H). A taxon that has not been reliably reported in Kentucky since 1975.

Federal status categories and definitions used include the following:

Endangered (E). "... any species ... in danger of extinction throughout all or a signif-

icant portion of its range ..." (USFWS 1992).

Threatened (T). "... any species ... likely to become an endangered species within the foreseeable future throughout all or a significant portion of its range" (USFWS 1992).

Proposed Endangered (PE). A taxon proposed for listing as endangered.

Proposed Threatened (PT). A taxon proposed for listing as threatened.

Category 1 (C1). Status review taxa for which the United States Fish and Wildlife Service "has substantial information on hand to support the biological appropriateness of proposing to list as endangered or threatened" (USFWS 1993b).

Category 2 (C2). Status review taxa for which information now in possession of the United States Fish and Wildlife Service "indicates that proposing to list as endangered or threatened is possibly appropriate, but for which conclusive data on biological vulnerability and threat are not currently available to support proposed rules" (USFWS 1993b). The USFWS is currently redefining its use of status review categories (R.G. Biggins, USFWS, pers. comm., 14 Aug 1995).

Category 3A. Taxa for which the USFWS "has persuasive evidence of extinction" (USFWS 1993b).

INTRODUCTION TO THE PLANT AND ANIMAL LISTS

Sources consulted for the plant names are: Anderson (1990); Crum, Anderson, and Buck (1990); Kartesz (1994); Kartesz and Thieret (1991); and McKinney (1992). The sources consulted for the common and scientific names of animals are as follows: gastropods—Hubricht (1985) and Turgeon et al. (1988); unionids—Gordon (1995) and Turgeon et al. (1988); crustaceans—Barr (1968), Holsinger (1972), USFWS (1994), and Williams et al. (1989); insects—Barr (1959, 1985b, 1994), Garrison (1991), Krekeler (1973), McCafferty (1981, 1994), Opler and Malikul (1992), Schuster (1993), Schweitzer (1989), and USFWS (1993a, 1994); fishes—Page and Burr (1991), Page et al. (1992), Robins et al. (1991), and Warren (1992); amphibians and reptiles—Collins (1990), Frost (1985), and King and Burke (1989); birds—American Ornitholo-

Table 1. Endangered, threatened, special concern, and historical plants and animals of Kentucky.

		STATUS				STATUS	
		KSNPC US				KSNPC US	
NONVASCULAR PLANTS							
				<i>Aster concolor</i>			T
				Eastern silvery aster			
<i>Sphagnum quinquefarium</i>	E			<i>Aster drummondii</i> var. <i>texasus</i>			T
A sphagnum moss				Texas aster			
<i>Tortula norvegica</i>	E			<i>Aster hemisphericus</i>			E
Tortula				Tennessee aster			
				<i>Aster phyllolepis</i>			S
VASCULAR PLANTS							
				Western silky aster			
<i>Acer spicatum</i>	E			<i>Aster pilosus</i> var. <i>priceae</i>			T
Mountain maple				White heath aster			
<i>Aconitum uncinatum</i>	T			<i>Aster saxicastellii</i>			T C2
Blue monkshood				Rockcastle aster			
<i>Adiantum capillus-veneris</i>	T			<i>Aureolaria patula</i>			T C2
Southern maidenhair-fern				Spreading false foxglove			
<i>Adlumia fungosa</i>	E			<i>Baptisia australis</i> var. <i>minor</i>			S
Climbing fumitory				Blue wild indigo			
<i>Aesculus pavia</i>	T			<i>Baptisia bracteata</i> var. <i>leucophaea</i>			S
Red buckeye				Cream wild indigo			
<i>Agalinis obtusifolia</i>	E			<i>Baptisia tinctoria</i>			T
Ten-lobed false foxglove				Yellow wild indigo			
<i>Agalinis skinneriana</i>	E	C2		<i>Bartonia virginica</i>			T
Pale false foxglove				Yellow screwstem			
<i>Ageratina luciae-brauniae</i>	S	C2		<i>Berberis canadensis</i>			E
Lucy Braun's white snakeroot				American barberry			
<i>Agrimonia gryposepala</i>	T			<i>Berchemia scandens</i>			E
Tall hairy groovebur				Supplejack			
<i>Amianthium muscitoxicum</i>	T			<i>Botrychium matricariifolium</i>			E
Fly-poison				Matricary grapefern			
<i>Amsonia tabernaemontana</i> var. <i>gattingeri</i>	T	C2		<i>Botrychium oneidense</i>			E
Eastern blue-star				Blunt-lobed grapefern			
<i>Anemone canadensis</i>	H			<i>Boykinia aconitifolia</i>			T
Canada anemone				Brook saxifrage			
<i>Angelica triquinata</i>	E			<i>Cabomba caroliniana</i>			T
Filmy angelica				Carolina fanwort			
<i>Apios priceana</i>	E	T		<i>Calamagrostis canadensis</i> var. <i>macouniana</i>			E
Price's potato-bean				Blue-joint reed grass			
<i>Arabis missouriensis</i>	E			<i>Calamagrostis porteri</i> ssp. <i>insperata</i>	E		E C2
Missouri rock cress				Reed bent grass			
<i>Arabis perstellata</i>	T	E		<i>Calamagrostis porteri</i> ssp. <i>porteri</i>			T
Braun's rock cress				Porter's reed grass			
<i>Aristida ramosissima</i>	H			<i>Callirhoe alcaeoides</i>			H
Branched three-awn grass				Clustered poppy-mallow			
<i>Armoracia lacustris</i>	T			<i>Calopogon tuberosus</i>			E
Lake cress				Grass-pink			
				<i>Calycanthus floridus</i> var. <i>glaucus</i>			T
				Sweetshrub			

Table 1. (continued).

	STATUS			STATUS	
	KSNPC	US		KSNPC	US
<i>Calylophus serrulatus</i> Yellow evening primrose	H		<i>Chelone obliqua</i> var. <i>obliqua</i> Red turtlehead	E	
<i>Carex aestivalis</i> Summer sedge	E		<i>Chelone obliqua</i> var. <i>speciosa</i> Rose turtlehead	S	
<i>Carex alata</i> Broadwing sedge	T		<i>Chrysogonum virginianum</i> Green-and-gold	E	
<i>Carex atlantica</i> ssp. <i>capillacea</i> Howe sedge	E		<i>Chrysosplenium americanum</i> American golden-saxifrage	E	
<i>Carex austrocaroliniana</i> Tarheel sedge	S		<i>Cimicifuga rubifolia</i> Appalachian bugbane	T	C2
<i>Carex buxbaumii</i> Brown bog sedge	E		<i>Circaea alpina</i> Small enchanter's-nightshade	S	
<i>Carex comosa</i> Bristly sedge	H		<i>Clematis crispa</i> Blue jasmine leather-flower	T	
<i>Carex crawei</i> Crawe's sedge	S		<i>Coeloglossum viride</i> var. <i>virescens</i> Long-bract green orchis	H	
<i>Carex crebriflora</i> Coastal Plain sedge	T		<i>Collinsonia verticillata</i> Whorled horse-balm	E	
<i>Carex decomposita</i> Epiphytic sedge	T		<i>Comptonia peregrina</i> Sweet-fern	E	
<i>Carex gigantea</i> Large sedge	T		<i>Conradina verticillata</i> Cumberland-rosemary	E	T
<i>Carex hystericina</i> Porcupine sedge	H		<i>Convallaria montana</i> American lily-of-the-valley	E	
<i>Carex jorii</i> Cypress-swamp sedge	E		<i>Corallorrhiza maculata</i> Spotted coralroot	E	
<i>Carex juniperorum</i> Cedar sedge	E		<i>Coreopsis pubescens</i> Star tickseed	S	
<i>Carex lanuginosa</i> Woolly sedge	E		<i>Crataegus engelmannii</i> Engelmann's hawthorn	H	
<i>Carex leptonevia</i> Finely-nerved sedge	E		<i>Cymophyllus fraserianus</i> Fraser's sedge	E	
<i>Carya aquatica</i> Water hickory	T		<i>Cyperus plukenetii</i> Plukenet's cyperus	H	
<i>Castanea dentata</i> American chestnut	E		<i>Cypripedium candidum</i> Small white lady's-slipper	E	
<i>Castanea pumila</i> Allegheny chinkapin	T		<i>Cypripedium kentuckiense</i> Kentucky lady's-slipper	S	C2
<i>Castilleja coccinea</i> Scarlet indian paintbrush	E		<i>Cypripedium parviflorum</i> Small yellow lady's-slipper	T	
<i>Ceanothus herbaceus</i> Prairie redroot	T		<i>Cypripedium reginae</i> Showy lady's-slipper	H	
<i>Cheilanthes alabamensis</i> Alabama lip fern	E		<i>Delphinium carolinianum</i> Carolina larkspur	T	
<i>Cheilanthes feei</i> Fee's lip fern	E		<i>Deschampsia cespitosa</i> ssp. <i>glauca</i> Tufted hair grass	E	

Table 1. (continued).

	STATUS			STATUS	
	KSNPC	US		KSNPC	US
<i>Deschampsia flexuosa</i>		T	<i>Gentiana alba</i>		E
Crinkled hair grass			Yellow gentian		
<i>Dichanthelium boreale</i>		S	<i>Gentiana decora</i>		S
Northern witch grass			Showy gentian		
<i>Didiplis diandra</i>		S	<i>Gentiana puberulenta</i>		E
Water-purslane			Prairie gentian		
<i>Dodecatheon frenchii</i>		S	<i>Glandularia canadensis</i>		T
French's shooting-star			Rose verbena		
<i>Draba cuneifolia</i>		E	<i>Glyceria acutiflora</i>		T
Wedge-leaf whitlow-grass			Sharp-scaled manna grass		
<i>Drosera brevifolia</i>		E	<i>Gnaphalium helleri</i> var.		
Dwarf sundew			<i>micradenium</i>		H
<i>Drosera intermedia</i>		H	Small rabbit-tobacco		
Spoon-leaved sundew			<i>Gratiola pilosa</i>		T
<i>Dryopteris carthusiana</i>		S	Shaggy hedge-hyssop		
Spinulose wood fern			<i>Gratiola viscidula</i>		S
<i>Dryopteris ludoviciana</i>		H	Short's hedge-hyssop		
Southern shield wood fern			<i>Gymnopogon ambiguus</i>		S
<i>Echinodorus berteroi</i>		T	Bearded skeleton grass		
Burhead			<i>Gymnopogon brevifolius</i>		E
<i>Echinodorus parvulus</i>		E	Shortleaf skeleton grass		
Dwarf burhead		C2	<i>Halesia tetraptera</i> var. <i>tetraptera</i>		E
<i>Eleocharis olivacea</i>		S	Common silverbell		
Olivaceous sedge			<i>Hedeoma hispidum</i>		T
<i>Elodea nuttallii</i>		H	Rough pennyroyal		
Waterweed			<i>Helianthemum bicknellii</i>		T
<i>Elymus svensonii</i>		S	Plains frostweed		
Svenson's wild rye		C2	<i>Helianthemum canadense</i>		E
<i>Eriophorum virginicum</i>		E	Canada frostweed		
Tawny cotton-grass			<i>Helianthus eggertii</i>		T
<i>Eryngium integrifolium</i>		E	Eggert's sunflower		PT
Blue-flower coyote-thistle			<i>Helianthus silphoides</i>		E
<i>Erythronium rostratum</i>		S	Silphium sunflower		
Golden-star			<i>Heracleum maximum</i>		E
<i>Eupatorium maculatum</i>		H	Cow-parsnip		
Spotted joe-pye-weed			<i>Heteranthera dubia</i>		S
<i>Eupatorium semiserratum</i>		E	Grassleaf mud-plantain		
Small-flowered thoroughwort			<i>Heteranthera limosa</i>		S
<i>Eupatorium steelei</i>		E	Blue mud-plantain		
Steele's joe-pye-weed			<i>Heterotheca subaxillaris</i> var.		
<i>Euphorbia mercurialina</i>		T	<i>latifolia</i>		T
Mercury spurge			Broad-leaf golden-aster		
<i>Fimbristylis puberula</i>		T	<i>Hexastylis contracta</i>		E
Hairy fimbristylis			Southern heartleaf		C2
<i>Forestiera ligustrina</i>		S	<i>Hexastylis heterophylla</i>		S
Upland privet			Variable-leaved heartleaf		

Table 1. (continued).

	STATUS			STATUS	
	KSNPC	US		KSNPC	US
<i>Hieracium longipilum</i>	T		<i>Lesquerella globosa</i>	T	C2
Hairy hawkweed			Lesquereux's bladderpod		
<i>Houstonia serpyllifolia</i>	E		<i>Lesquerella lescurii</i>	S	
Michaux's bluets			Lescur's bladderpod		
<i>Hydrocotyle americana</i>	E		<i>Leucothoe recurva</i>	E	
American water-pennywort			Fetterbush		
<i>Hydrolea ovata</i>	E		<i>Liatris cylindracea</i>	T	
Ovate fiddleleaf			Slender blazingstar		
<i>Hydrophyllum virginianum</i>	S		<i>Lilium philadelphicum</i>	T	
Virginia waterleaf			Wood lily		
<i>Hypericum adpressum</i>	H	C2	<i>Lilium superbum</i>	T	
Creeping St. John's-wort			Turk's cap lily		
<i>Hypericum crux-andreae</i>	T		<i>Limnobium spongia</i>	T	
St. Peter's-wort			American frog's-bit		
<i>Hypericum nudiflorum</i>	H		<i>Liparis loeselii</i>	T	
Pretty St. John's-wort			Loesel's twayblade		
<i>Hypericum pseudomaculatum</i>	H		<i>Listera australis</i>	E	
Large spotted St. John's-wort			Southern twayblade		
<i>Iris fulva</i>	E		<i>Listera smallii</i>	T	
Copper iris			Kidney-leaf twayblade		
<i>Isoetes butleri</i>	E		<i>Lobelia appendiculata</i> var.		
Butler's quillwort			<i>gattingeri</i>	E	
<i>Isoetes melanopoda</i>	E		Gattinger's lobelia		
Blackfoot quillwort			<i>Lobelia nuttallii</i>	T	
<i>Juglans cinerea</i>	S	C2	Nuttall's lobelia		
White walnut			<i>Lonicera dioica</i> var. <i>orientalis</i>	H	
<i>Juncus articulatus</i>	S		Wild honeysuckle		
Jointed rush			<i>Lonicera reticulata</i>	E	
<i>Juncus elliotii</i>	E		Grape honeysuckle		
Bog rush			<i>Ludwigia hirtella</i>	E	
<i>Juncus filipendulus</i>	T		Hairy ludwigia		
Long-styled rush			<i>Lycopodiella appressa</i>	E	
<i>Juniperus communis</i> var. <i>depressa</i>	T		Southern bog club-moss		
Ground juniper			<i>Lycopodiella inundata</i>	E	
<i>Koeleria macrantha</i>	E		Northern bog club-moss		
June grass			<i>Lycopodium clavatum</i>	E	
<i>Lathyrus palustris</i>	T		Running-pine		
Vetchling peavine			<i>Lysimachia fraseri</i>	E	C2
<i>Lathyrus venosus</i>	S		Fraser's loosestrife		
Smooth veiny peavine			<i>Lysimachia radicans</i>	H	
<i>Leavenworthia exigua</i> var. <i>laciniata</i>	T	C2	Trailing loosestrife		
Glade cress			<i>Lysimachia terrestris</i>	E	
<i>Leavenworthia torulosa</i>	T		Swamp-candles		
Necklace glade cress			<i>Maianthemum canadense</i>	T	
<i>Leiophyllum buxifolium</i>	H		Wild lily-of-the-valley		
Sand-myrtle			<i>Maianthemum stellatum</i>	E	
			Starflower false Solomon's-seal		

Table 1. (continued).

	STATUS			STATUS	
	KSNPC	US		KSNPC	US
<i>Malus angustifolia</i>	S		<i>Oenothera oakesiana</i>	H	
Southern crabapple			Evening primrose		
<i>Malvastrum hispidum</i>	T		<i>Oenothera perennis</i>	E	
Hispid false mallow			Small sundrops		
<i>Marshallia grandiflora</i>	E	C2	<i>Oenothera triloba</i>	T	
Barbara's-buttons			Stemless evening-primrose		
<i>Matelea carolinensis</i>	E		<i>Oldenlandia uniflora</i>	E	
Carolina anglepod			Clustered bluets		
<i>Melampyrum lineare</i> var. <i>latifolium</i>	T		<i>Onosmodium molle</i> ssp.		
American cow-wheat			<i>hispidissimum</i>	E	
<i>Melampyrum lineare</i> var. <i>pectinatum</i>	E		Hairy false gromwell		
American cow-wheat			<i>Onosmodium molle</i> ssp. <i>molle</i>	E	
<i>Melanthium parviflorum</i>	E		Soft false gromwell		
Small-flowered false hellebore			<i>Onosmodium molle</i> ssp. <i>occidentale</i>	E	
<i>Melanthium virginicum</i>	E		Western false gromwell		
Virginia bunchflower			<i>Orobanche ludoviciana</i>	H	
<i>Melanthium woodii</i>	T		Louisiana broomrape		
False hellebore			<i>Orontium aquaticum</i>	T	
<i>Minuartia cumberlandensis</i>	E	E	Goldenclub		
Cumberland sandwort			<i>Oxalis priceae</i>	H	
<i>Minuartia glabra</i>	T		Price's yellow wood sorrel		
Appalachian sandwort			<i>Parnassia asarifolia</i>	E	
<i>Mirabilis albida</i>	E		Kidney-leaf grass-of-parnassus		
Pale umbrella-wort			<i>Parnassia grandifolia</i>	E	
<i>Monarda punctata</i>	E		Largeleaf grass-of-parnassus		
Spotted beebalm			<i>Paronychia argyrocoma</i>	E	
<i>Monotropsis odorata</i>	T	C2	Silverling		
Sweet pinesap			<i>Paspalum boscianum</i>	S	
<i>Muhlenbergia bushii</i>	E		Bull paspalum		
Bush's muhly			<i>Paxistima canbyi</i>	T	C2
<i>Muhlenbergia cuspidata</i>	T		Canby's mountain-lover		
Plains muhly			<i>Pedicularis lanceolata</i>	H	
<i>Muhlenbergia glabrifloris</i>	S		Swamp lousewort		
Hair grass			<i>Perideridia americana</i>	T	
<i>Myriophyllum heterophyllum</i>	S		Eastern eulophus		
Broadleaf water-milfoil			<i>Phacelia ranunculacea</i>	S	
<i>Myriophyllum pinnatum</i>	T		Blue scorpion-weed		
Cutleaf water-milfoil			<i>Philadelphus inodorus</i>	T	
<i>Najas gracillima</i>	S		Mock orange		
Thread-like naiad			<i>Philadelphus pubescens</i>	E	
<i>Nemophila aphylla</i>	T		Hoary mock orange		
Small-flower baby-blue-eyes			<i>Phlox bifida</i> ssp. <i>bifida</i>	T	
<i>Nestronia umbellula</i>	E		Cleft phlox		
Conjurer's-nut			<i>Phlox bifida</i> ssp. <i>stellaria</i>	T	C2
<i>Oenothera linifolia</i>	E		Starry cleft phlox		
Thread-leaf sundrops			<i>Plantago cordata</i>	H	
			Heartleaf plantain		

Table 1. (continued).

	STATUS			STATUS	
	KSNPC	US		KSNPC	US
<i>Platanthera cristata</i>	T		<i>Rhododendron canescens</i>	E	
Yellow-crested orchid			Hoary azalea		
<i>Platanthera integrilabia</i>	T	C2	<i>Rhynchosia tomentosa</i>	E	
White fringeless orchid			Hairy snout-bean		
<i>Platanthera psycodes</i>	E		<i>Rhynchospora globularis</i>	S	
Small purple-fringed orchid			Globe beaked-rush		
<i>Poa saltuensis</i>	E		<i>Rhynchospora macrostachya</i>	E	
Drooping blue grass			Tall beaked-rush		
<i>Podostemum ceratophyllum</i>	S		<i>Rubus canadensis</i>	E	
Threadfoot			Smooth blackberry		
<i>Pogonia ophioglossoides</i>	E		<i>Rubus whartoniae</i>	T	C2
Rose pogonia			Wharton's dewberry		
<i>Polygala cruciata</i>	E		<i>Rudbeckia subtomentosa</i>	E	
Cross-leaf milkwort			Sweet coneflower		
<i>Polygala nuttallii</i>	H		<i>Sabatia campanulata</i>	E	
Nuttall's milkwort			Slender marsh-pink		
<i>Polygala polygama</i>	T		<i>Sagittaria graminea</i>	T	
Racemed milkwort			Grass-leaf arrowhead		
<i>Polymnia laevigata</i>	E		<i>Sagittaria rigida</i>	E	
Tennessee leafcup			Sessile-fruit arrowhead		
<i>Pontederia cordata</i>	T		<i>Salix amygdaloides</i>	H	
Pickereel-weed			Peachleaf willow		
<i>Potamogeton illinoensis</i>	S		<i>Salix discolor</i>	H	
Illinois pondweed			Pussy willow		
<i>Potamogeton pulcher</i>	T		<i>Salvia urticifolia</i>	E	
Spotted pondweed			Nettle-leaf sage		
<i>Prenanthes alba</i>	E		<i>Sambucus racemosa</i> ssp. <i>pubens</i>	E	
White rattlesnake-root			Red elderberry		
<i>Prenanthes aspera</i>	E		<i>Sanguisorba canadensis</i>	E	
Rough rattlesnake-root			Canada burnet		
<i>Prenanthes barbata</i>	E	C2	<i>Saxifraga michauxii</i>	T	
Barbed rattlesnake-root			Michaux's saxifrage		
<i>Prenanthes crepidinea</i>	T		<i>Saxifraga micranthidifolia</i>	E	
Nodding rattlesnake-root			Lettuce-leaf saxifrage		
<i>Psoralidium tenuiflorum</i>	E		<i>Saxifraga pensylvanica</i>	H	
Few-flowered scurf-pea			Swamp saxifrage		
<i>Ptilimnium capillaceum</i>	T		<i>Schisandra glabra</i>	E	
Mock bishop's-weed			Bay starvine		
<i>Ptilimnium nuttallii</i>	E		<i>Schizachne purpurascens</i>	T	
Nuttall's mock bishop's-weed			Purple-oat		
<i>Pycnanthemum albescens</i>	E		<i>Schwalbea americana</i>	H	E
White-leaved mountain-mint			Chaffseed		
<i>Pyrola americana</i>	H		<i>Scirpus expansus</i>	E	
American wintergreen			Woodland beak-rush		
<i>Ranunculus ambigens</i>	S		<i>Scirpus fluviatilis</i>	E	
Water-plantain spearwort			River bulrush		

Table 1. (continued).

	STATUS			STATUS	
	KSNPC	US		KSNPC	US
<i>Scirpus hallii</i>	E	C2	<i>Spiraea alba</i> var. <i>alba</i>	E	
Hall's bulrush			Narrow-leaved meadowsweet		
<i>Scirpus heterochaetus</i>	E		<i>Spiraea virginiana</i>	T	T
Slender bulrush			Virginia spiraea		
<i>Scirpus microcarpus</i>	E		<i>Spiranthes lucida</i>	T	
Small-fruit bulrush			Shining ladies'-tresses		
<i>Scirpus verecundus</i>	E		<i>Spiranthes magnicamporum</i>	T	
Bashful bulrush			Great Plains ladies'-tresses		
<i>Scleria ciliata</i> var. <i>ciliata</i>	E		<i>Spiranthes odorata</i>	E	
Fringed nut-rush			Sweetscent ladies'-tresses		
<i>Scleria muehlenbergii</i>	H		<i>Sporobolus clandestinus</i>	T	
Pitted nut-rush			Rough dropseed		
<i>Scutellaria saxatilis</i>	T		<i>Sporobolus heterolepis</i>	E	
Rock skullcap			Northern dropseed		
<i>Sedum telephioides</i>	T		<i>Stachys eplingii</i>	E	
Allegheny stonecrop			Epling's hedge-nettle		
<i>Sida hermaphrodita</i>	S		<i>Stellaria fontinalis</i>	T	C2
Virginia-mallow			Water stichwort		
<i>Silene ovata</i>	E	C2	<i>Stellaria longifolia</i>	S	
Ovate catchfly			Longleaf stitchwort		
<i>Silene regia</i>	E		<i>Streptopus roseus</i> var. <i>perspectus</i>	E	
Royal catchfly			Rosy twistedstalk		
<i>Silphium laciniatum</i> var. <i>laciniatum</i>	E		<i>Symphoricarpos albus</i>	E	
Compassplant			Snowberry		
<i>Silphium laciniatum</i> var. <i>robinsonii</i>	T		<i>Talinum calcaricum</i>	E	
Compassplant			Limestone fameflower		
<i>Solidago albopilosa</i>	T	T	<i>Talinum teretifolium</i>	T	
White-haired goldenrod			Roundleaf fameflower		
<i>Solidago buckleyi</i>	S		<i>Taxus canadensis</i>	T	
Buckley's goldenrod			Canadian yew		
<i>Solidago caesia</i> var. <i>curtisii</i>	T		<i>Tephrosia spicata</i>	E	
Curtis' goldenrod			Spiked hoary-pea		
<i>Solidago puberula</i>	S		<i>Thaspium pinnatifidum</i>	T	
Downy goldenrod			Cutleaf meadow-parsnip		
<i>Solidago roanensis</i>	T		<i>Thermopsis mollis</i>	E	
Roan Mountain goldenrod			Soft-haired thermopsis		
<i>Solidago shortii</i>	E	E	<i>Thuja occidentalis</i>	T	
Short's goldenrod			Northern white-cedar		
<i>Solidago simplex</i> ssp. <i>randii</i>	S		<i>Torreyochloa pallida</i>	E	
Rand's goldenrod			Pale manna grass		
<i>Solidago squarrosa</i>	H		<i>Toxicodendron vernix</i>	E	
Squarrose goldenrod			Poison sumac		
<i>Sparganium eurycarpum</i>	E		<i>Tragia urticifolia</i>	E	
Large bur-reed			Nettle-leaf noseburn		
<i>Sphenopholis pensylvanica</i>	S		<i>Trepocarpus aethusae</i>	E	
Swamp wedgescale			Trepocarpus		

Table 1. (continued).

	STATUS			STATUS	
	KSNPC	US		KSNPC	US
<i>Trichostema setaceum</i> Narrow-leaved bluecurls	E		<i>Zizaniopsis miliacea</i> Southern wild rice	T	
<i>Trientalis borealis</i> Northern starflower	E			ANIMALS	
<i>Trifolium reflexum</i> Buffalo clover	E		Gastropods		
<i>Trifolium stoloniferum</i> Running buffalo clover	T	E	<i>Anguispira rugoderma</i>	S	
<i>Trillium nivale</i> Snow trillium	E		<i>Pine Mountain disc</i>		
<i>Trillium pusillum</i> var. <i>ozarkanum</i> Ozark least trillium	E	C2	<i>Antroselatus spiralis</i>	S	
<i>Trillium pusillum</i> var. <i>pusillum</i> Least trillium	E	C2	<i>Shaggy cavesnail</i>	S	C2
<i>Trillium undulatum</i> Painted trillium	T		<i>Glyphyalinia raderi</i>		
<i>Triplasis purpurea</i> Purple sand grass	E		<i>Maryland glyph</i>		
<i>Ulmus serotina</i> September elm	S		<i>Glyphyalinia rhoadsi</i>	T	
<i>Utricularia macrorhiza</i> Greater bladderwort	E		<i>Sculpted glyph</i>		
<i>Vallisneria americana</i> Eel-grass	S		<i>Leptoxis praerosa</i>	S	C2
<i>Vernonia noveboracensis</i> New York ironweed	S		<i>Onyx rocksnail</i>		
<i>Veronica americana</i> American speedwell	H		<i>Lithasia armigera</i>	S	C2
<i>Viburnum lentago</i> Nannyberry	H		<i>Armored rocksnail</i>		
<i>Viburnum nudum</i> Possum haw viburnum	E		<i>Lithasia geniculata</i>	S	C2
<i>Viola septemloba</i> var. <i>egglestonii</i> Eggleston's violet	S		<i>Ornate rocksnail</i>		
<i>Viola walteri</i> Walter's violet	T		<i>Lithasia salebrosa</i>	S	C2
<i>Vitis rupestris</i> Sand grape	T		<i>Muddy rocksnail</i>		
<i>Woodsia appalachiana</i> Mountain woodsia	E		<i>Lithasia verrucosa</i>	S	C2
<i>Xerophyllum asphodeloides</i> Eastern turkeybeard	H		<i>Varicose rocksnail</i>		
<i>Xyris difformis</i> Carolina yellow-eye-grass	E		<i>Mesodon chilhoweensis</i>	S	
<i>Zizania palustris</i> var. <i>interior</i> Indian wild rice	H		<i>Queen crater</i>		
			<i>Mesodon panselenus</i>	S	
			<i>Virginia bladetooth</i>		
			<i>Mesodon wetherbyi</i>	S	
			<i>Clifty covert</i>		
			<i>Mesomphix rugeli</i>	T	
			<i>Wrinkled button</i>		
			<i>Pilsbryna</i> sp.	E	
			<i>A snail (undescribed)</i>		
			<i>Pleurocera alveare</i>	S	C2
			<i>Rugged hornsnailed</i>		
			<i>Pleurocera curta</i>	S	C2
			<i>Shortspire hornsnailed</i>		
			<i>Rabdotus dealbatus</i>	T	
			<i>Whitewashed rabdotus</i>		
			<i>Rhodacme elatior</i>	S	C2
			<i>Domed ancylid</i>		
			<i>Triodopsis dentifera</i>	T	
			<i>Big-tooth whitelip</i>		
			<i>Triodopsis multilineata</i>	T	
			<i>Striped whitelip</i>		

Table 1. (continued).

	STATUS			STATUS	
	KSNPC	US		KSNPC	US
<i>Vertigo bollesiana</i>	E		<i>Plethobasus cyphus</i>	S	
Delicate vertigo			Sheepnose		
<i>Vertigo clappi</i>	E		<i>Pleurobema clava</i>	E	E
Cupped vertigo			Clubshell		
<i>Vitrinizonites latissimus</i>	T		<i>Pleurobema oviforme</i>	E	C2
Glassy grapeskin			Tennessee clubshell		
Unionid Bivalves (Mussels)			<i>Pleurobema plenum</i>	E	E
			Rough pigtoe		
<i>Alasmidonta atropurpurea</i>	E	PE	<i>Pleurobema pyramidatum</i>	E	C2
Cumberland elktoe			Pyramid pigtoe		
<i>Alasmidonta marginata</i>	T	C2	<i>Potamilus capax</i>	E	E
Elktoe			Fat pocketbook		
<i>Anodontoides denigratus</i>	E	C2	<i>Potamilus purpuratus</i>	E	
Cumberland papershell			Bleufer		
<i>Cumberlandia monodonta</i>	E	C2	<i>Ptychobranchus subtentum</i>	T	
Spectaclecase			Fluted kidneyshell		
<i>Cyrogenia stegaria</i>	E	E	<i>Quadrula cylindrica cylindrica</i>	T	C2
Fanshell			Rabbitsfoot		
<i>Epioblasma brevidens</i>	E	PE	<i>Simpsonaias ambigua</i>	T	C2
Cumberlandian combshell			Salamander mussel		
<i>Epioblasma capsaeformis</i>	E	PE	<i>Toxolasma lividus</i>	E	C2
Oyster mussel			Purple lilliput		
<i>Epioblasma obliquata obliquata</i>	E	E	<i>Toxolasma texasensis</i>	E	
Catspaw			Texas lilliput		
<i>Epioblasma torulosa rangiana</i>	E	E	<i>Villosa fabalis</i>	E	C2
Northern riffleshell			Rayed bean		
<i>Epioblasma triquetra</i>	S	C2	<i>Villosa lienosa</i>	S	
Snuffbox			Little spectaclecase		
<i>Fusconaia subrotunda subrotunda</i>	T		<i>Villosa ortmanni</i>	T	C2
Long-solid			Kentucky creekshell		
<i>Lampsilis abrupta</i>	E	E	<i>Villosa trabalis</i>	E	E
Pink mucket			Cumberland bean		
<i>Lampsilis ovata</i>	E		<i>Villosa vanuxemensis</i>	T	
Pocketbook			Mountain creekshell		
<i>Lasmigona compressa</i>	E		Crustaceans		
Creek heelsplitter					
<i>Lasmigona subviridis</i>	E	C2	<i>Barbicambarus cornutus</i>	S	
Green floater			Bottlebrush crayfish		
<i>Lexingtonia dolabelloides</i>	H	C2	<i>Bryocamptus morrisoni elegans</i>	T	
Slabside pearlymussel			A copepod		
<i>Obovaria retusa</i>	E	E	<i>Caecidotea barri</i>	E	C2
Ring pink			Clifton Cave isopod		
<i>Pegias fabula</i>	E	E	<i>Cambarellus puer</i>	E	
Little-wing pearlymussel			A dwarf crayfish		
<i>Plethobasus cooperianus</i>	E	E	<i>Cambarellus shufeldtii</i>	S	
Orange-foot pimpleback			Cajun dwarf crayfish		

Table 1. (continued).

	STATUS			STATUS	
	KSNPC	US		KSNPC	US
<i>Cambarus ornatus</i>	S		<i>Ophiogomphus howei</i>	S	C2
A crayfish			Pygmy snaketail		
<i>Cambarus parvoculus</i>	E		<i>Papaipema eryngii</i>	E	C2
A crayfish			Rattlesnake-master borer moth		
<i>Cambarus veteranus</i>	S	C2	<i>Phyciodes batesii</i>	T	C2
A crayfish			Tawny crescent		
<i>Gammarus bousfieldi</i>	E	C2	<i>Pseudanophthalmus abditus</i>	T	
Bousfield's amphipod			A cave beetle		
<i>Macrobrachium ohione</i>	E		<i>Pseudanophthalmus audax</i>	T	C2
Ohio shrimp			Bold cave beetle		
<i>Orconectes australis</i>	T		<i>Pseudanophthalmus caecus</i>	T	C2
A crayfish			Clifton Cave beetle		
<i>Orconectes bisectus</i>	T		<i>Pseudanophthalmus calcareus</i>	T	C2
Crittenden crayfish			Limestone Cave beetle		
<i>Orconectes inermis</i>	T		<i>Pseudanophthalmus catoryctos</i>	T	C2
A crayfish			Lesser Adams Cave beetle		
<i>Orconectes jeffersoni</i>	E	C2	<i>Pseudanophthalmus conditus</i>	T	C2
Louisville crayfish			Hidden cave beetle		
<i>Orconectes lancifer</i>	E		<i>Pseudanophthalmus exoticus</i>	H	C2
A crayfish			Exotic cave beetle		
<i>Orconectes palmeri</i>	E		<i>Pseudanophthalmus frigidus</i>	T	C2
A crayfish			Icebox Cave beetle		
<i>Orconectes pellucidus</i>	S		<i>Pseudanophthalmus globiceps</i>	T	C2
A crayfish			Round-headed cave beetle		
<i>Palaemonias ganteri</i>	E	E	<i>Pseudanophthalmus horni</i>	S	C2
Mammoth Cave shrimp			Garman's cave beetle		
<i>Procambarus viaeviridis</i>	T		<i>Pseudanophthalmus hypolithos</i>	T	C2
A crayfish			Stone-dwelling cave beetle		
<i>Stygobromus vitreus</i>	S		<i>Pseudanophthalmus inexpectatus</i>	T	
An amphipod			A cave beetle		
Insects			<i>Pseudanophthalmus major</i>	T	C2
			Beaver Cave beetle		
			<i>Pseudanophthalmus parvus</i>	T	C2
			Tatum Cave beetle		
<i>Cheumatopsyche helma</i>	H	C2	<i>Pseudanophthalmus pholeter</i>	T	C2
Helma's net-spinning caddisfly			Greater Adams Cave beetle		
<i>Dryobius sexnotatus</i>	T	C2	<i>Pseudanophthalmus pubescens</i>		
Sixbanded longhorn beetle			<i>intrepidus</i>	T	
<i>Litobrancha recurvata</i>	S		A cave beetle		
A burrowing mayfly			<i>Pseudanophthalmus puteanus</i>	T	C2
<i>Lordithon niger</i>	H	C2	Old Well Cave beetle		
Black lordithon rove beetle			<i>Pseudanophthalmus rogersae</i>	T	C2
<i>Lytrosia permagnaria</i>	E	C2	Rogers' cave beetle		
A geometrid moth			<i>Pseudanophthalmus scholasticus</i>	T	C2
<i>Madeophylax</i> sp.	S		Schoolhouse Cave beetle		
A caddisfly (undescribed)			<i>Pseudanophthalmus simulans</i>	T	C2
<i>Nicrophorus americanus</i>	T	E	Cub Run Cave beetle		
American burying beetle					

Table 1. (continued).

	STATUS			STATUS	
	KSNPC	US		KSNPC	US
<i>Pseudanophthalmus tenebrosus</i> Stevens Creek Cave beetle	T	C2	<i>Etheostoma maculatum</i> Spotted darter	T	C2
<i>Pseudanophthalmus troglodytes</i> Louisville cave beetle	T	C2	<i>Etheostoma microlepidum</i> Smallscale darter	E	
<i>Pyrgus wyandot</i> Appalachian grizzled skipper	T	C2	<i>Etheostoma nigrum susanae</i> Johnny darter	T	C2
<i>Speyeria diana</i> Diana fritillary	--	C2	<i>Etheostoma parvipinne</i> Goldstripe darter	S	
<i>Speyeria idalia</i> Regal fritillary	H	C2	<i>Etheostoma pellucidum</i> Eastern sand darter	S	C2
<i>Stenonema bednariki</i> A heptageniid mayfly	S		<i>Etheostoma proeliare</i> Cypress darter	T	
<i>Stylurus notatus</i> Elusive clubtail	H	C2	<i>Etheostoma pyrrhogaster</i> Firebelly darter	S	
Fishes			<i>Etheostoma sagitta spilotum</i> Arrow darter	S	
<i>Acipenser fulvescens</i> Lake sturgeon	E	C2	<i>Etheostoma swaini</i> Gulf darter	S	
<i>Alosa alabamae</i> Alabama shad	E		<i>Etheostoma tippecanoe</i> Tippecanoe darter	S	
<i>Amblyopsis spelaea</i> Northern cavefish	S	C2	<i>Fundulus chrysotus</i> Golden topminnow	E	
<i>Atractosteus spatula</i> Alligator gar	E		<i>Fundulus dispar</i> Starhead topminnow	E	
<i>Clinostomus funduloides</i> Rosyside dace	S		<i>Hybognathus hayi</i> Cypress minnow	E	
<i>Cycleptus elongatus</i> Blue sucker	--	C2	<i>Hybognathus placitus</i> Plains minnow	S	C2
<i>Cyprinella camura</i> Bluntnose shiner	S		<i>Hybopsis amnis</i> Pallid shiner	H	
<i>Cyprinella venusta</i> Blacktail shiner	S		<i>Ichthyomyzon castaneus</i> Chestnut lamprey	S	
<i>Erimystax insignis</i> Blotched chub	E		<i>Ichthyomyzon fossor</i> Northern brook lamprey	T	
<i>Erimyzon sucetta</i> Lake chubsucker	T		<i>Ichthyomyzon gagei</i> Southern brook lamprey	H	
<i>Esox niger</i> Chain pickerel	S		<i>Ichthyomyzon greeleyi</i> Mountain brook lamprey	T	
<i>Etheostoma chienense</i> Relict darter	E	E	<i>Ictiobus niger</i> Black buffalo	S	
<i>Etheostoma cinereum</i> Ashy darter	T	C2	<i>Lampetra appendix</i> American brook lamprey	T	
<i>Etheostoma fusiforme</i> Swamp darter	E		<i>Lepomis marginatus</i> Dollar sunfish	E	
<i>Etheostoma lynceum</i> Brighteye darter	S		<i>Lepomis miniatus</i> Redspotted sunfish	T	

Table 1. (continued).

	STATUS			STATUS	
	KSNPC	US		KSNPC	US
<i>Lota lota</i>	S		<i>Scaphirhynchus albus</i>	E	E
Burbot			Pallid sturgeon		
<i>Macrhybopsis gelida</i>	H	C1	<i>Thoburnia atripinnis</i>	S	
Sturgeon chub			Blackfin sucker		
<i>Macrhybopsis meeki</i>	H	C1	<i>Typhlichthys subterraneus</i>	S	
Sicklefin chub			Southern cavefish		
<i>Menidia beryllina</i>	T		<i>Umbra limi</i>	T	
Inland silverside			Central mudminnow		
<i>Moxostoma poecilurum</i>	S		Amphibians		
Blacktail redhorse					
<i>Nocomis biguttatus</i>	S		<i>Amphiuma tridactylum</i>	E	
Hornyhead chub			Three-toed amphiuma		
<i>Notropis hudsonius</i>	S		<i>Cryptobranchus alleganiensis</i>		
Spottail shiner			<i>alleganiensis</i>	--	C2
<i>Notropis maculatus</i>	T		Eastern hellbender		
Taillight shiner			<i>Eurycea longicauda guttolineata</i>	T	
<i>Notropis</i> sp.	E	E	Three-lined salamander		
Palezone shiner (undescribed)			<i>Hyla avivoca</i>	T	
<i>Notropis</i> sp.	E		Bird-voiced treefrog		
Sawfin shiner (undescribed)			<i>Hyla cinerea</i>	S	
<i>Noturus exilis</i>	E		Green treefrog		
Slender madtom			<i>Hyla gratiosa</i>	S	
<i>Noturus hildebrandi</i>	S		Barking treefrog		
Least madtom			<i>Hyla versicolor</i>	S	
<i>Noturus phaeus</i>	S		Gray treefrog		
Brown madtom			<i>Plethodon cinereus</i>	S	
<i>Noturus stigmosus</i>	S		Redback salamander		
Northern madtom			<i>Plethodon wehrlei</i>	E	
<i>Percina evides</i>	S		Wehrle's salamander		
Gilt darter			<i>Rana areolata circulosa</i>	S	
<i>Percina macrocephala</i>	T	C2	Northern crawfish frog		
Longhead darter			<i>Rana pipiens</i>	S	
<i>Percina squamata</i>	E	C2	Northern leopard frog		
Olive darter			Reptiles		
<i>Percopsis omiscomaycus</i>	S				
Trout-perch			<i>Apalone mutica mutica</i>	S	
<i>Phenacobius uranops</i>	S		Midland smooth softshell		
<i>Phoxinus cumberlandensis</i>	T	T	<i>Chrysemys picta dorsalis</i>	S	
Blackside dace			Southern painted turtle		
<i>Platygobio gracilis</i>	S	C2	<i>Clonophis kirtlandii</i>	E	C2
Flathead chub			Kirtland's snake		
<i>Polyodon spathula</i>	--	C2	<i>Elaphe guttata guttata</i>	S	
Paddlefish			Corn snake		
<i>Rhinichthys cataractae</i>	E		<i>Eumeces anthracinus anthracinus</i>	T	
Longnose dace			Northern coal skink		

Table 1. (continued).

	STATUS			STATUS	
	KSNPC	US		KSNPC	US
<i>Eumeces anthracinus pluvialis</i> Southern coal skink	E		<i>Botaurus lentiginosus</i> American bittern	H	
<i>Eumeces inexpectatus</i> Southeastern five-lined skink	S		<i>Bubulcus ibis</i> Cattle egret	S	
<i>Farancia abacura reinwardtii</i> Western mud snake	S		<i>Casmerodius albus</i> Great egret	E	
<i>Lampropeltis triangulum elapsoides</i> Scarlet kingsnake	S		<i>Certhia americana</i> Brown creeper	E	
<i>Macroclemys temminckii</i> Alligator snapping turtle	T	C2	<i>Chondestes grammacus</i> Lark sparrow	T	
<i>Nerodia cyclopion</i> Mississippi green water snake	E		<i>Circus cyaneus</i> Northern harrier	T	
<i>Nerodia erythrogaster neglecta</i> Copperbelly water snake	S	PT	<i>Cistothorus platensis</i> Sedge wren	S	
<i>Nerodia fasciata confluens</i> Broad-banded water snake	E		<i>Corvus corax</i> Common raven	E	
<i>Ophisaurus attenuatus longicaudus</i> Eastern slender glass lizard	T		<i>Corvus ossifragus</i> Fish crow	S	
<i>Pituophis melanoleucus melanoleucus</i> Northern pine snake	T	C2	<i>Dendroica cerulea</i> Cerulean warbler	--	C2
<i>Sistrurus miliarius streckeri</i> Western pigmy rattlesnake	T		<i>Dendroica fusca</i> Blackburnian warbler	T	
<i>Thamnophis proximus proximus</i> Western ribbon snake	T		<i>Dolichonyx oryzivorus</i> Bobolink	S	
<i>Thamnophis sauritus sauritus</i> Eastern ribbon snake	S		<i>Egretta caerulea</i> Little blue heron	E	
Birds			<i>Empidonax minimus</i> Least flycatcher	E	
<i>Accipiter striatus</i> Sharp-shinned hawk	S		<i>Fulica americana</i> American coot	H	
<i>Actitis macularia</i> Spotted sandpiper	E		<i>Gallinula chloropus</i> Common moorhen	T	
<i>Aimophila aestivalis</i> Bachman's sparrow	E	C2	<i>Haliaeetus leucocephalus</i> Bald eagle	E	E
<i>Ammodramus henslowii</i> Henslow's sparrow	S	C2	<i>Ictinia mississippiensis</i> Mississippi kite	S	
<i>Anas discors</i> Blue-winged teal	E		<i>Ixobrychus exilis</i> Least bittern	T	
<i>Ardea herodias</i> Great blue heron	S		<i>Junco hyemalis</i> Dark-eyed junco	S	
<i>Asio flammeus</i> Short-eared owl	E		<i>Lanius ludovicianus migrans</i> Migrant loggerhead shrike	--	C2
<i>Asio otus</i> Long-eared owl	E		<i>Lophodytes cucullatus</i> Hooded merganser	T	
<i>Bartramia longicauda</i> Upland sandpiper	H		<i>Nyctanassa violacea</i> Yellow-crowned night-heron	T	

Table 1. (continued).

	STATUS			STATUS	
	KSNPC	US		KSNPC	US
<i>Nycticorax nycticorax</i> Black-crowned night-heron	T		<i>Corynorhinus rafinesquii</i> Rafinesque's big-eared bat	T	C2
<i>Pandion haliaetus</i> Osprey	T		<i>Corynorhinus townsendii virginianus</i> Virginia big-eared bat	E	E
<i>Passerculus sandwichensis</i> Savannah sparrow	S		<i>Mustela nivalis</i> Least weasel	S	
<i>Phalacrocorax auritus</i> Double-crested cormorant	H		<i>Myotis austroriparius</i> Southeastern myotis	E	C2
<i>Pheucticus ludovicianus</i> Rose-breasted grosbeak	S		<i>Myotis grisescens</i> Gray myotis	E	E
<i>Picoides borealis</i> Red-cockaded woodpecker	E	E	<i>Myotis leibii</i> Eastern small-footed myotis	E	C2
<i>Podilymbus podiceps</i> Pied-billed grebe	E		<i>Myotis sodalis</i> Indiana myotis	E	E
<i>Poocetes gramineus</i> Vesper sparrow	E		<i>Neotoma magister</i> Allegheny woodrat	--	C2
<i>Rallus elegans</i> King rail	E		<i>Nycticeius humeralis</i> Evening bat	T	
<i>Riparia riparia</i> Bank swallow	S		<i>Peromyscus gossypinus</i> Cotton mouse	T	
<i>Sterna antillarum athalassos</i> Interior least tern	E	E	<i>Sorex cinereus</i> Masked shrew	S	
<i>Thryomanes bewickii</i> Bewick's wren	S		<i>Sorex dispar blitchi</i> Long-tailed shrew	E	
<i>Tyto alba</i> Barn owl	S		<i>Spilogale putorius</i> Eastern spotted skunk	S	
<i>Vermivora chrysoptera</i> Golden-winged warbler	T		<i>Ursus americanus</i> Black bear	S	
<i>Vireo bellii</i> Bell's vireo	S				
<i>Wilsonia canadensis</i> Canada warbler	S				
Mammals					
<i>Clethrionomys gapperi maurus</i> Kentucky red-backed vole	S	C2			

gists' Union (1957, 1983, 1989); mammals—Hall (1981), Jones et al. (1992), and Wilson and Reeder (1993).

DISCUSSION

This list includes 342 plant and 273 animal taxa considered rare in Kentucky (Tables 1 and 2). Based on generally accepted estimates

of the number of native taxa extant in Kentucky, the following approximate percent of the groups indicated can be considered Endangered, Threatened, of Special Concern, or Historic: plants—13.9%, gastropods—8.9%, unionid bivalves—42.9%, fishes—28.5%, amphibians and reptiles—27.5%, breeding birds—27.7%, and mammals—21.9%. Al-

Table 2. The diversity and conservation status of the major groups of organisms in Kentucky.

Number of Kentucky Species or Taxa ¹	Plants	Gastropods	Bivalves	Crustaceans	Insects	Fishes	Amphibians	Reptiles	Breeding Birds	Mammals
Native	2467	257	122	unknown	unknown	230	51	52	166	69
Exotic	787	11	3	unknown	unknown	17	0	1	3	5
KSNPC Monitored as Rare	342	23	36	20	36	63	10	18	46	14
KSNPC Endangered	152	3	25	9	2	17	2	4	16	6
KSNPC Threatened	103	6	7	5	24	14	2	6	10	3
KSNPC Special Concern	49	14	3	6	5	28	6	8	16	5
KSNPC Historic	38	0	1	0	5	4	0	0	4	0
Presumed Extinct or Extirpated	4	0	19	0	1	9	0	1	10	5
Extant / Extirpated Federally Endangered or Threatened	9/0	0/0	11/8	1/0	1/0	4/0	0	0	3 ² /3	3/3
Extant Federally Proposed Endangered or Threatened	1	0	3	0	0	0	0	1	0	0
Extant Federal Status Review Category 2 (C2) / Category 1 (C1)	29 ³ /0	9 ⁴ /0	13/0	4/0	30/0	12/0	1/0	3/0	4 ⁵ /0	6/0
Extirpated Federal Status Review Category 2 (C2)	0	0	1	0	0	2	0	0	1	0

Table 2. (continued).

- 1 Numbers refer to plant, bivalve, and fish taxa, or gastropod, amphibian, reptile, bird, and mammal species.
- 2 *Falco peregrinus* is extirpated as a native species and is not counted here.
- 3 *Delphinium exaltatum*, *Eriogonum longifolium* var. *harperi*, *Litsea aestivalis*, *Penstemon deamii*, and *Rudbeckia triloba* var. *pinnatifida* have been reported from Kentucky but are not considered part of the flora because voucher specimens are not available; they are not counted here.
- 4 *Catinella gelida* and *Vertigo hubrichti* are known from Kentucky only as fossils and are not counted here. *Vertigo ovata* is a Category 2 species in New Mexico and is secure in Kentucky.
- 5 *Accipiter gentilis*, *Contopus borealis*, and *Sterna hirundo* do not breed in the state and are not counted here. *Laterallus jamaicensis* has never been reported from Kentucky and is not counted here.

though extensive, the list does not adequately treat or include several groups of organisms found in Kentucky. The thallophytes, bryophytes, insects, amphipods, isopods, and other groups also are important elements of our natural heritage but are poorly known in Kentucky. This revision is the first to attempt to incorporate taxa from select invertebrate groups (e.g., terrestrial gastropods and beetles) and mosses for which sufficient information is available to indicate that listing is warranted. Researchers are encouraged to undertake studies that will provide information needed to determine the status of members of these and other poorly known groups in Kentucky.

All taxa listed are being monitored by KSNPC. Information about delisted taxa and many others not included also is maintained in manual files so that we can respond to unforeseen changes in distribution or status. For example, a list of plants that are candidates for listing can be obtained upon request from KSNPC.

Four plants and 45 animals are presumed extinct or extirpated from Kentucky (Tables 2 and 3). Most of the extinct or extirpated animals are unionids or fishes that have experienced range-wide declines caused by habitat destruction, stream modification, and pollution (Warren and Burr 1994; Williams et al. 1992). Because extirpation or extinction is difficult to prove definitively, the list should remind biologists that these plants and animals could be encountered during field activities.

We invite recommendations from knowledgeable individuals regarding native taxa they believe deserve a status change or should be added to or deleted from the list. Each recommendation should include the scientific name of the organism, its habitat requirements, collection information (e.g., localities, number of specimens, dates, disposition of specimens), historic and present distribution, whether the taxon has been specifically sought during field work, threats, and recommended KSNPC status. Recommendations should be forwarded to the Director, KSNPC, who will pass the information on to appropriate staff members for timely review and response.

KSNPC intends to publish a new list of rare and extirpated plants and animals in the *Transactions of the Kentucky Academy of Sci-*

Table 3. Plants and animals presumed extinct or extirpated from Kentucky.

US STATUS		US STATUS	
PLANTS		<i>Plethobasus cicatricosus</i>	E
		White wartyback	
<i>Caltha palustris</i> var. <i>palustris</i>		<i>Quadrula fragosa</i>	E
Marsh marigold		Winged mapleleaf	
<i>Orbexilum stipulatum</i>	3A	<i>Quadrula tuberosa</i>	
Stipuled scurf-pea		Rough rockshell	
<i>Physostegia intermedia</i>		Insects	
Slender dragon-head			
<i>Polytaenia nuttallii</i>		<i>Pentagenia robusta</i>	3A
Prairie parsley		Robust pentagenian burrowing mayfly	
ANIMALS			
Unionid Bivalves (Mussels)		Fishes	
<i>Dromus dromas</i>	E	<i>Crystallaria asprella</i>	
Dromedary pearlymussel		Crystal darter	
<i>Epioblasma arcaeformis</i>	3A	<i>Erimystax x-punctatus</i>	
Sugarspoon		Gravel chub	
<i>Epioblasma biemarginata</i>	3A	<i>Etheostoma clarum</i>	
Angled riffleshell		Western sand darter	
<i>Epioblasma flexuosa</i>	3A	<i>Etheostoma microperca</i>	
Leafshell		Least darter	
<i>Epioblasma florentina florentina</i>	E	<i>Etheostoma vivax</i>	
Yellow blossom		Scaly sand darter	
<i>Epioblasma florentina walkeri</i>	E	<i>Hemitremia flammea</i>	C2
Tan riffleshell		Flame chub	
<i>Epioblasma haysiana</i>	3A	<i>Moxostoma lacerum</i>	
Acornshell		Harelip sucker	
<i>Epioblasma lewisii</i>	3A	<i>Moxostoma valenciennesi</i>	C2
Forkshell		Greater redhorse	
<i>Epioblasma obliquata perobliqua</i>	E	<i>Percina burtoni</i>	
White catpaw		Blotchside logperch	
<i>Epioblasma personata</i>	3A	Reptiles	
Round combshell			
<i>Epioblasma propinqua</i>	3A	<i>Masticophis flagellum flagellum</i>	
Tennessee riffleshell		Eastern coachwhip	
<i>Epioblasma sampsonii</i>			
Wabash riffleshell		Birds (* extirpated as nesting species)	
<i>Epioblasma stewardsoni</i>	3A		
Cumberland leafshell		<i>Anhinga anhinga</i>	
<i>Epioblasma torulosa torulosa</i>	E	Anhinga	
Tuberclad blossom		<i>Aquila chrysaetos</i> *	
<i>Hemistena lata</i>	E	Golden eagle	
Cracking pearlymussel		<i>Campephilus principalis</i>	E
<i>Leptodea leptodon</i>	C2	Ivory-billed woodpecker	
Scaleshell			

Table 3. (continued).

US STATUS		US STATUS	
<i>Chlidonias niger</i> *	C2	Mammals	
Black tern			
<i>Conuropsis carolinensis</i>		<i>Bos bison</i>	
Carolina parakeet		American bison	
<i>Ectopistes migratorius</i>		<i>Canis lupus</i>	E
Passenger pigeon		Gray wolf	
<i>Elanoides forficatus</i>		<i>Canis rufus</i>	E
American swallow-tailed kite		Red wolf	
<i>Falco peregrinus</i> *	E	<i>Cervus elaphus</i>	
Peregrine falcon		Elk	
<i>Tympanuchus cupido</i>		<i>Felis concolor cougar</i>	E
Greater prairie-chicken		Eastern cougar	
<i>Vermivora bachmanii</i>	E		
Bachman's warbler			

ence every 4 years. The present list will be updated annually by submitting a note to the *Transactions* listing status and name changes. Interested persons can contact KSNPC at any time for the current status of Kentucky's rare plants and animals.

We reiterate the hope expressed by Warren et al. (1986) that this updated list of Kentucky's rare plants and animals will assist developers and decision makers in reaching informed decisions concerning the most effective use of Kentucky's natural resources. By focusing attention on the rarest elements of our natural heritage, we can avoid the unnecessary destruction of our diverse flora and fauna.

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Transmission of Helminths in Lambs on a Farm in Central Kentucky in 1993 and 1994

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ABSTRACT

On a farm in central Kentucky, naturally infected lambs ($n = 49$) were euthanized and examined at necropsy periodically (May to August) in 1993/1994 to determine transmission patterns of internal parasites for these years. The lambs were born over about a 1-month period from mid-March to early or mid-April. Lambs were from field 21 in 1993 and 1994 and nearby new lots I, II, III, and IV in 1994. Nematodes and cestodes were identified and enumerated for each lamb; nematode eggs per gram of feces were also counted. In addition, for field 21 ewes in 1993, weekly counts of nematode eggs per gram of feces were determined to delineate periparturient patterns. Species of helminths recovered from the lambs totalled 13 in 1993 and 11 in 1994 for field 21 and 8 to 10 for new lots I, II, III, and IV in 1994. Highest counts of nematodes were in July and August. The predominant species was the stomach worm, *Haemonchus contortus*. Eggs per gram counts for the ewes in field 21 in 1993 peaked in late April, which was about 6 weeks after parturition of the first ewes.

INTRODUCTION

The major species of gastrointestinal parasites (nematodes and cestodes) in lambs in central Kentucky are acquired from infective larval stages on pasture. For some species, these stages can overwinter on pasture. However, the major contamination of the environment with larvae is from parasite eggs deposited in feces of ewes and which increase in spring in the periparturient period (Herd et al. 1983; Michel 1974). Internal parasites can be the cause of poor health and even death, particularly in lambs. The main purpose of our investigation was to follow transmission of gastrointestinal parasites over 1993/1994 in spring lambs on a farm in central Kentucky. Also, the study was done for comparisons with similar research on the same farm in 1986/1987 (Lyons et al. 1987, 1992).

MATERIALS AND METHODS

The sheep in our study were mainly Cheviot/Polypay crossbreds. Flocks of these sheep were established over 30 years ago at the main research farm in the Department of Veterinary Science, University of Kentucky. The only anthelmintic usage over the last 20 years in the flock has been occasional treatment of rams and replacement females. In the course of the study, none of the sheep was treated with an antiparasitic compound.

In 1993, all sheep—ewes ($n = 3$) and lambs

($n = 19$)—were from one field (F) (F 21). Counts of nematode eggs per gram of feces (EPGs) were done weekly from 9 March to 15 June for the ewes and at necropsy for the lambs. Parturition occurred between 14 March and 12 April. Lambs, designated as testers, were euthanized and examined for parasites at intervals (1 to 8 per month) from 11 May to 16 August. Data for the lambs on their age at and the date of necropsy are recorded (Table 1).

For the 1994 study, EPG counts were done for the lambs but not for the ewes. Tester lambs were used from F 21 ($n = 8$) and from new lot (NL) I ($n = 4$), NL II ($n = 5$), NL III ($n = 4$), and NL IV ($n = 9$). Parturition of the ewes was from 13 March to 8 April. The tester lambs were euthanized (1 to 14 per month) from 17 May to 16 August. Data for the tester lambs on age and other information at necropsy are presented in Table 2.

Necropsy examination of the tester lambs included the abomasum, small intestine, cecum, and large intestine for immature and mature helminths. Techniques for recovering and identifying helminths and for EPG counts were as previously published (Drudge and Szanto 1963; Lyons et al. 1991). Briefly, the entire contents and three rinses of the abomasum and each portion of the intestines were emptied into separate containers and fixed with formaldehyde. Artificial digestive

Field 21- Ewes - 1993

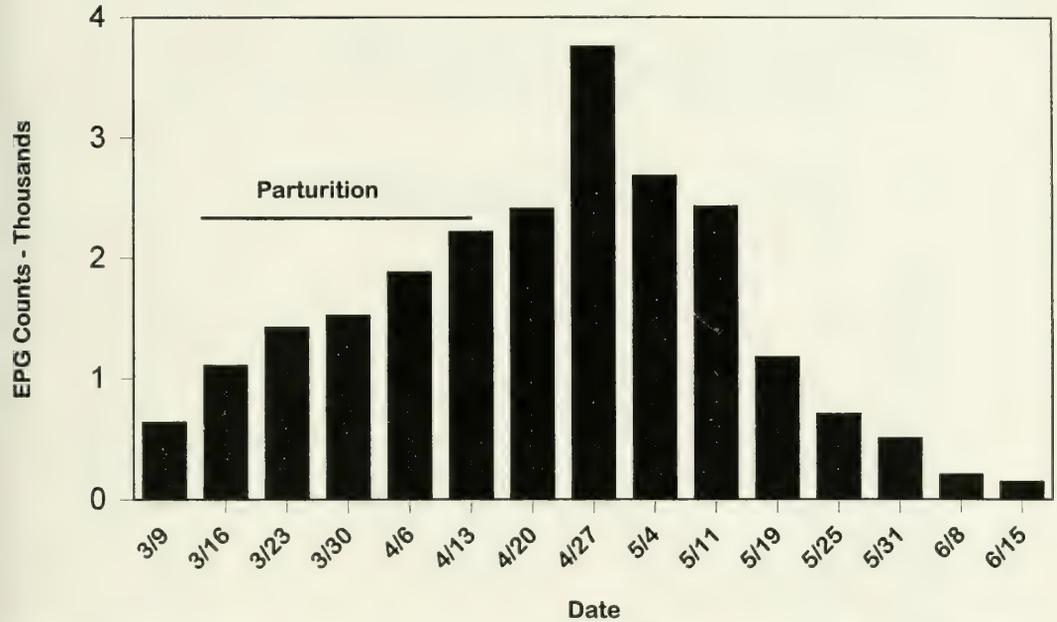


Figure 1. Weekly average counts of nematode eggs per gram of feces (EPG) for F 21 ewes (n = 13) during the periparturient period 9 Mar to 15 Jun 1993 in central Kentucky.

Field 21 Tester Lambs - 1993

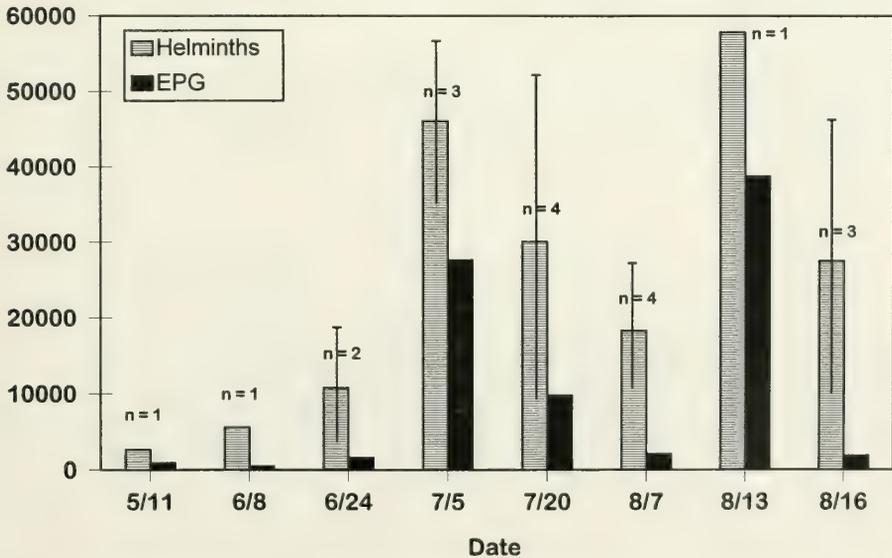


Figure 2. Average (\pm SD) (where n \geq 2) or total (where n = 1) number of helminths (nematodes and cestodes) and of nematode eggs per gram of feces (EPG) for F 21 tester lambs (n = 19) in 1993 in central Kentucky.

Table 1. Species of helminths recovered at necropsy from Field 21 lambs in 1993.

Helminth species	Lamb no. (date killed; age in days)								
	1 (5/11:128)	2 (6/8:84)	3 (6/24:100)	4 (6/24:98)	5 (7/5:111)	6 (7/5:111)	7 (7/5:111)	8 (7/20:124)	9 (7/20:124)
Nematodes									
<i>Haemonchus</i> spp. (imm)	67	1,213	7,241	1,900	12,080	15,867	8,820	2,800	2,400
<i>H. contortus</i>	14	506	2,207	1,040	31,347	17,620	19,480	1,493	200
<i>Ostertagia</i> spp. (imm)	200	1,147	2,040	47	866	1,273	2,774	234	1,227
<i>O. circumcincta</i> ♂	534	553	247	80	1,260	700	420	400	200
<i>O. trifurcata</i> ♂		80	40	0	200	0	0	0	0
<i>Ostertagia</i> spp. ♀	960	973	640	160	3,847	1,140	1,233	200	0
<i>Trichostrongylus</i> spp. (imm)	7	0	20	20	7	7	0	0	7
<i>T. axei</i>	127	0	167	13	867	327	414	27	260
<i>T. colubriformis</i>	0	20	60	0	247	14	80	80	280
<i>T. vitrinus</i>	20	0	0	0	0	167	0	0	0
<i>Nematodirus</i> spp. (imm)	0	200	820	433	640	1,000	1,193	160	367
<i>N. spathiger</i> ♂	320	160	327	440	400	40	220	707	1,340
<i>Nematodirus</i> spp. ♀	127	220	280	180	500	60	260	753	1,440
<i>Cooperia</i> spp. (imm)	0	33	200	273	760	527	527	113	413
<i>C. curticei</i>	0	300	1,640	500	5,447	1,560	3,307	3,373	5,833
<i>C. oncophora</i>	0	0	20	0	20	0	0	0	0
<i>Strongyloides papillosus</i>	60	0	20	7	67	20	40	40	40
<i>Oesophagostomum</i> spp. (imm)	0	0	0	0	0	0	7	0	0
<i>O. columbianum</i>	0	0	0	0	0	0	0	0	7
<i>Trichuris</i> spp. (imm)	0	4	0	0	0	0	0	0	0
<i>T. ovis</i>	3	81	185	157	145	239	169	110	189
Total nematodes	2,519	5,503	16,154	5,250	58,700	40,561	38,944	10,490	14,203
Cestodes									
<i>Moniezia</i> spp.	131	59	11	0	1	0	0	1	114
Total helminths	2,650	5,562	16,165	5,250	58,701	40,561	38,944	10,491	14,317

Table 1. Extended.

Helminth species	Lamb no. (date killed; age in days)									
	10 (7/26:122)	11 (7/26:121)	12 (8/7:140)	13 (8/7:139)	14 (8/7:138)	15 (8/7:137)	16 (8/13:123)	17 (8/16:142)	18 (8/16:137)	19 (8/16:137)
Nematodes										
<i>Haemonchus</i> spp. (imm)	2,400	2,840	220	80	1,093	440	2,607	833	554	1,020
<i>H. contortus</i>	21,527	43,053	380	0	993	4,746	40,680	3,687	7	247
<i>Ostertagia</i> spp. (imm)	1,833	434	1,160	974	420	607	600	647	147	133
<i>O. circumcincta</i> ♂	1,000	1,213	100	180	220	320	1,207	293	40	100
<i>O. trifurcata</i> ♂	0	0	40	180	120	100	7	40	20	67
<i>Ostertagia</i> spp. ♀	1,227	2,247	193	240	200	640	2,207	533	60	200
<i>Trichostrongylus</i> spp. (imm)	0	0	80	100	87	20	0	0	100	40
<i>T. axei</i>	1,227	660	667	947	1,027	1,027	1,200	1,660	846	1,054
<i>T. colubriformis</i>	220	127	120	460	220	207	400	407	687	1,613
<i>T. vitrinus</i>	0	0	0	0	0	0	0	0	0	0
<i>Nematodirus</i> spp. (imm)	267	153	93	927	607	400	200	833	2,093	4,027
<i>N. spatuliger</i> ♂	1,300	753	1,087	1,220	2,013	2,007	407	3,020	307	3,220
<i>Nematodirus</i> spp. ♀	1,147	793	874	707	1,407	2,800	600	3,066	387	3,207
<i>Cooperia</i> spp. (imm)	200	120	120	360	1,407	213	820	1,067	353	1,093
<i>C. curticei</i>	4,587	5,507	6,554	5,773	19,034	5,613	5,846	30,287	2,767	11,053
<i>C. oncophora</i>	0	0	20	0	0	0	0	0	0	0
<i>Strongyloides papillosus</i>	40	200	40	60	200	0	800	7	73	7
<i>Oesophagostomum</i> spp. (imm)	0	0	14	0	0	0	0	0	7	0
<i>O. columbianum</i>	0	2	12	15	13	3	28	58	22	11
<i>Trichouris</i> spp. (imm)	0	7	0	0	0	0	0	0	0	0
<i>T. ovis</i>	140	149	142	193	159	207	74	93	145	168
Total nematodes	37,115	58,258	11,916	12,416	29,220	19,350	57,683	46,531	8,615	27,260
Cestodes										
<i>Moniezia</i> spp.	44	5	19	0	39	253	108	27	0	37
Total helminths	37,159	58,263	11,935	12,416	29,259	19,603	57,791	46,558	8,615	27,297

Field 21 Tester Lambs - 1994

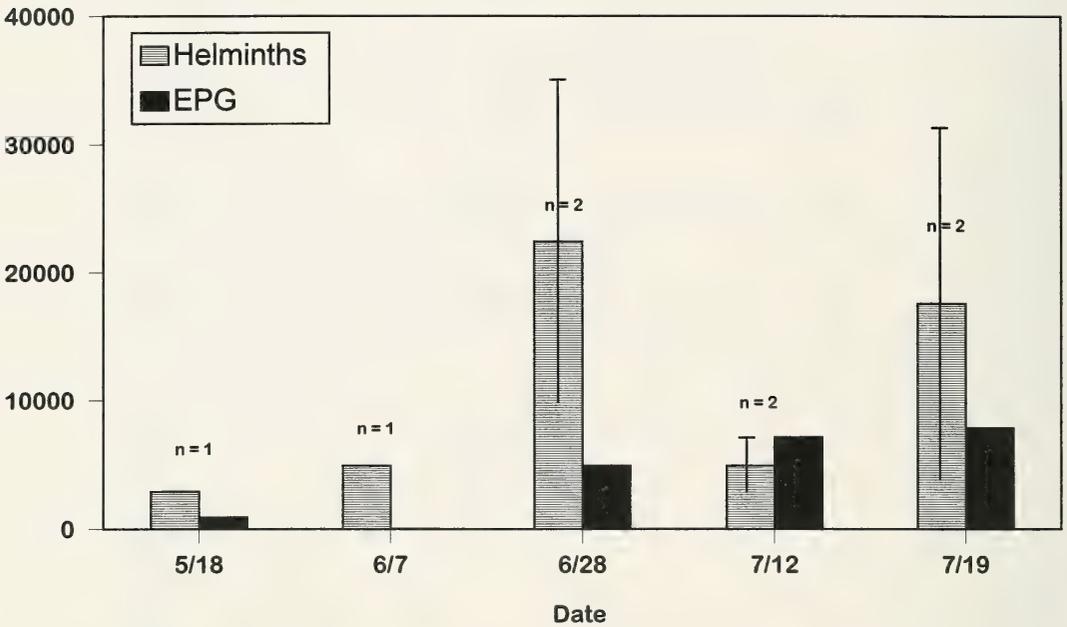


Figure 3. Average (\pm SD) (where $n \geq 2$) or total (where $n = 1$) number of helminths (nematodes and cestodes) and nematode eggs per gram of feces (EPG) for F 21 tester lambs ($n = 8$) in 1994 in central Kentucky.

New Lot I, II, III, & IV Tester Lambs - 1994

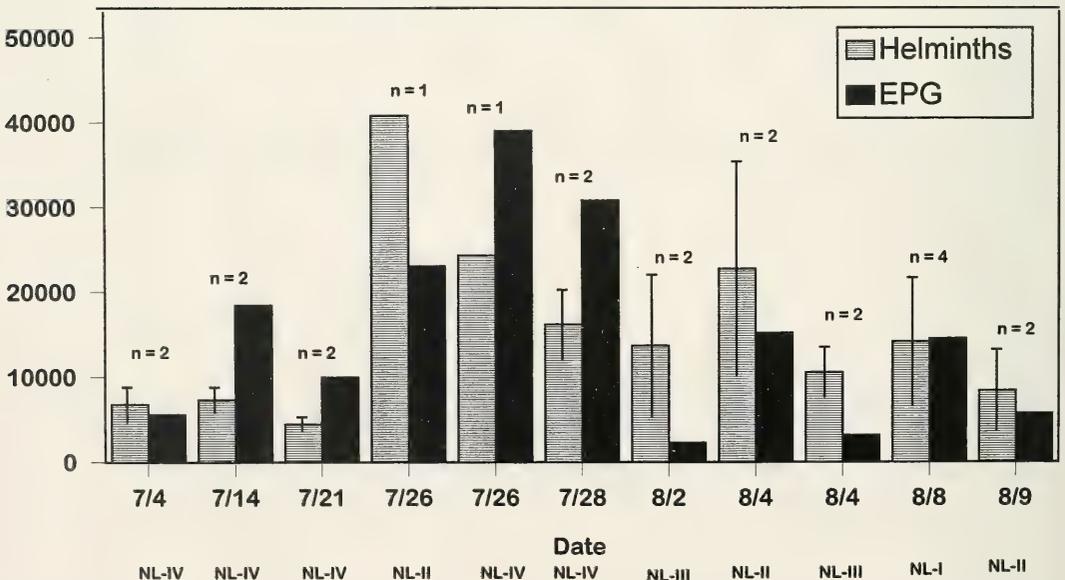


Figure 4. Average (\pm SD) (where $n \geq 2$) or total (where $n = 1$) number of helminths (nematodes and cestodes) and nematode eggs per gram of feces (EPG) for NL I, II, III, and IV tester lambs ($n = 22$) in 1994 in central Kentucky.

Table 2. Species of helminths recovered at necropsy from Field 21 Tester Lambs in 1994.

Helminth species	Lamb no. (date killed: age in days)							
	20 (5/18:140)	21 (6/7:161)	22 (6/28:105)	23 (6/28:106)	24 (7/12:119)	25 (7/12:113)	26 (7/19:118)	27 (7/19:117)
Nematodes								
<i>Haemonchus</i> spp. (imm)	140	20	383	4,830	580	100	1,023	2,247
<i>H. contortus</i>	220	0	1,646	21,486	1,096	4,040	4,586	7,467
<i>Ostertagia</i> spp. (imm)	250	1,380	136	2,816	0	23	43	1,457
<i>O. circumcincta</i> ♂	420	30	100	1,003	3	163	383	833
<i>O. trifurcata</i> ♂	60	0	20	0	0	0	6	3
<i>Ostertagia</i> spp. ♀	600	90	180	1,606	20	183	590	640
<i>Trichostrongylus</i> spp. (imm)	0	120	0	0	0	0	0	203
<i>T. axei</i>	40	430	103	1,000	106	43	93	223
<i>T. colubriformis</i>	0	370	280	603	160	40	100	800
<i>Nematodirus</i> spp. (imm)	480	1,590	1,067	2,410	450	67	380	850
<i>N. spathiger</i> ♂	250	160	823	600	20	743	1,230	3,027
<i>Nematodirus</i> spp. ♀	240	40	640	1,400	0	900	1,503	4,063
<i>Cooperia</i> spp. (imm)	20	80	87	3	0	0	43	7
<i>C. curticei</i>	0	360	820	0	183	80	160	2,610
<i>C. oncophora</i>	0	20	0	0	0	0	0	0
<i>Strongyloides papillosus</i>	40	0	100	0	40	0	0	3
<i>Trichuris</i> spp. (imm)	0	1	11	4	5	2	1	5
<i>T. ovis</i>	0	31	388	241	293	388	3	375
Total nematodes	2,760	4,902	6,784	38,002	2,959	6,772	10,205	24,813
Cestodes:								
<i>Moniezia</i> spp.	147	0	14	0	74	33	0	64
Total helminths	2,907	4,902	6,798	38,002	3,030	6,805	10,205	24,877

juice was used to recover parasites in the lining of the abomasum and small intestine; this material was added to the contents of the gastrointestinal tract. Later, aliquot samples from the contents of each container were taken and examined under a dissecting microscope for internal parasites, which were recovered, identified, and enumerated. EPG counts were by a technique of using saturated sodium chloride solution to float the helminth eggs.

RESULTS AND DISCUSSION

For the 1993 study of F 21, weekly EPG counts for the ewes (Fig. 1) gradually increased until 27 April when a sharp rise occurred. This was about 6 weeks after the first ewes gave birth and about 2 weeks after parturition of the remaining ewes ceased. Thereafter, EPG counts gradually declined and were less than pre-lambing low values by late May and early to mid-June.

Data on the 1993 F 21 tester lambs are shown for total counts of helminths and EPG (Fig. 2). Values were lowest in May and June with a sharp increase in early July. There was a decline in late July and early August, fol-

lowed by peak numbers on 13 August and a sharp decline on 16 August. The EPG counts, though somewhat lower, had the same general pattern as the total helminth counts. Specific numbers and species ($n = 13$) of helminths recovered in 1993 tester lambs are shown in Table 1. The predominant species of nematode, in numbers of specimens, was *Haemonchus contortus*, the main component of the overall infection of the total helminths found. The next species present in greatest numbers, in descending order, were *Cooperia* spp., *Nematodirus* spp., and *Ostertagia* spp.

For the 1994 tester lambs, counts of total helminths and EPG at necropsy are summarized for F 21 (Fig. 3) and NL I, II, III, and IV (Fig. 4). F 21 lambs had the highest numbers on 28 June, much less on 12 July, but second highest on 19 July. For these same lambs, EPG counts were highest in July. NL lambs had the highest total helminth counts on 26 July for NL I, on 4 August for NL II, on 2 August for NL III, and on 26 July for NL IV. For each containment area the total numbers of species of helminths (nematodes and cestodes) were: F 21 (11) (Table 2); and

Table 3. Species of helminths recovered at necropsy from New Lot I, II, III, and IV lambs in 1994.

Helminth species	Lamb no. (date killed; age in days)											
	28 (8/8:143) NL-I	29 (8/8:142) NL-I	30 (8/8:141) NL-I	31 (8/8:141) NL-I	32 (7/26:126) NL-II	33 (8/4:140) NL-II	34 (8/4:140) NL-II	35 (8/9:145) NL-II	36 (8/9:140) NL-II	37 (8/2:131) NL-III	38 (8/2:131) NL-III	
Nematodes												
<i>Haemonchus</i> spp. (imm)	0	0	0	600	0	400	0	0	0	0	260	160
<i>H. contortus</i>	2,673	5,860	13,123	7,610	38,073	12,510	30,584	11,391	4,296	13,250	2,416	2,416
<i>Ostertagia</i> spp. (imm)	0	0	0	0	0	0	0	0	0	60	40	40
<i>O. circumcincta</i> ♂	0	67	200	0	213	0	400	0	0	0	0	40
<i>O. trifurcata</i> ♂	0	0	0	0	203	0	0	0	0	0	0	20
<i>Ostertagia</i> spp. ♀	0	20	0	0	607	200	3	0	0	20	40	40
<i>Trichostrongylus</i> spp. (imm)	0	0	0	3	0	0	0	0	0	0	0	0
<i>T. axei</i>	0	7	0	0	220	600	10	200	123	870	1,070	1,070
<i>T. colubriformis</i>	80	140	346	400	320	63	120	200	110	43	23	23
<i>Nematodirus</i> spp. (imm)	153	450	153	1,020	0	0	0	0	0	240	86	86
<i>N. spathiger</i> ♂	760	2,517	2,916	3,617	1	0	0	0	0	2,120	1,760	1,760
<i>Nematodirus</i> spp. ♀	380	2,567	3,326	7,040	0	20	0	0	260	2,903	1,373	1,373
<i>Cooperia</i> spp. (imm)	0	0	0	0	0	0	0	0	0	0	0	0
<i>C. curticei</i>	0	120	26	0	600	203	120	0	47	323	180	180
<i>Capillaria</i> spp.	0	0	0	0	20	0	0	0	0	10	3	3
<i>Strongyloides papillosus</i>	0	0	0	0	0	0	0	0	3	23	0	0
<i>Trichouris</i> spp. (imm)	1	2	7	1	15	2	5	0	1	1	0	2
<i>T. ovis</i>	21	117	167	109	513	28	245	0	137	15	10	10
Total nematodes	4,068	11,867	20,264	20,400	40,784	14,026	31,487	11,791	4,977	20,137	7,223	7,223
Cestodes												
<i>Moniezia</i> spp.	0	3	0	0	20	0	0	0	0	0	0	0
Total helminths	4,068	11,870	20,264	20,400	40,804	14,026	31,487	11,791	4,977	20,137	7,223	7,223

Table 3. Extended.

Helminth species	Lamb no. (date killed—age in days)										
	39 (8/4,121) NL-III	40 (8/4,136) NL-III	41 (7/4,110) NL-IV	42 (7/4,110) NL-IV	43 (7/4,120) NL-IV	44 (7/4,120) NL-IV	45 (7/21,111) NL-IV	46 (7/21,123) NL-IV	47 (7/26,131) NL-IV	48 (7/28,118) NL-IV	49 (7/28,118) NL-IV
Nematodes											
<i>Haemonchus</i> spp. (imm)	260	0	0	0	20	0	690	40	0	400	203
<i>H. contortus</i>	2,164	7,863	7,009	4,560	5,870	5,530	2,233	3,264	23,637	15,620	10,926
<i>Ostertagia</i> spp. (imm)	0	0	0	0	0	3	170	0	0	13	3
<i>O. circumcincta</i> ♂	363	1,823	253	13	493	417	180	523	200	803	640
<i>O. trifurcata</i> ♂	0	200	0	0	23	20	23	0	0	3	10
<i>Ostertagia</i> spp. ♀	400	1,603	474	207	857	620	167	330	0	1,407	893
<i>Trichostrongylus</i> spp. (imm)	3	0	0	0	0	0	3	0	0	0	0
<i>T. axei</i>	240	203	23	206	6	7	130	20	24	237	0
<i>T. colubriformis</i>	20	0	43	200	0	40	226	20	80	163	0
<i>Nematodirus</i> spp. (imm)	183	3	20	0	0	0	27	0	0	0	0
<i>N. spathiger</i> ♂	1,987	400	40	0	80	60	220	60	43	100	163
<i>Nematodirus</i> spp. ♀	2,643	420	40	0	123	40	140	20	0	220	103
<i>Cooperia</i> spp. (imm)	20	0	0	0	0	0	0	0	0	20	0
<i>C. curticei</i>	220	40	60	0	0	20	83	20	100	60	0
<i>Capillaria</i> spp.	0	0	7	0	0	0	0	0	0	0	0
<i>Strongyloides papillosus</i>	3	0	0	0	0	0	0	0	0	0	0
<i>Trichouris</i> spp. (imm)	7	1	13	0	5	8	20	10	6	10	5
<i>T. ovis</i>	28	2	191	210	210	128	152	111	200	191	131
Total nematodes	8,541	12,558	8,173	5,396	7,687	6,893	4,464	4,418	24,290	19,247	13,077
Cestodes:											
<i>Moniezia</i> spp.	0	0	13	0	9	11	12	6	3	7	12
Total helminths	8,541	12,558	8,186	5,396	7,696	6,904	4,476	4,424	24,293	19,254	13,089

NL I (8), II (10), III (10), and IV (10) (Table 3). For F 21 lambs, the same four species were found in the same sequence of numbers present as those in 1993. Combined data on the NL lambs revealed the same four most prevalent species as in F 21 lambs. However, the order, from highest to lowest numbers, was *H. contortus*, *Nematodirus* spp., *Ostertagia* spp., and *Cooperia* spp.

Meteorological data at the research farm indicate similarities in air temperature and precipitation for 1993 and 1994, although total annual rainfall was about 13 cm higher in 1993 than in 1994. Air temperatures were highest in June, July, and August for both years. The months with the highest total precipitation were June, November, and August for 1993 and April, March, and August for 1994.

There was no obvious difference in seasonal transmission patterns of internal parasites of tester lambs for the 4 years of studies: 1986 (Lyons et al. 1987), 1987 (Lyons et al. 1992), and 1993/1994 (present paper). General increase in numbers of specimens with progression of age of the lambs and season were obvious. However, almost predictable individual variation in numbers of parasites acquired was evident in lambs examined on the same or similar dates. For 1986, 1987, and 1993, when EPG counts for ewes were done in the periparturient period, two constants were apparent. First, the counts were highest in about the second month after the first ewes lambed. Second, the highest counts of helminths in tester lambs were first found at about 2 months after the highest EPG counts in ewes. These findings substantiate the general knowledge that the main source of infective larvae on pasture for infection of lambs is related to

the tremendous increase of parasite eggs passed in feces of ewes after lambing (Herd et al. 1983; Michel 1974). This justifies anthelmintic treatment of ewes in spring before the grazing season.

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Longitudinal Studies of Family Size and the Human Sex Ratio

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ABSTRACT

In 1992, we obtained family size and sex ratio data from 1000 students in Ogden College of Science, Technology and Health, Western Kentucky University, by using the same study format that was followed at the university ca. 10 and 20 years ago. The objectives were to compare findings of the studies made at decadal intervals, to explore further the effects of composition of sexes of existing children on family size, and to explore relations among past, present, and projected generations. The results showed that the average number of children per family decreased for successive generations within studies and for successive studies. Preferences for both sexes and for males influenced family size in all studies. In the three successive studies, when both sexes were present in the first two children, families stopping with two children were 6.0%, 6.7%, and 9.4% greater, respectively, than when the first two children were of the same sex. Also, more families stopped having additional children when the first two were males than when they were females. For the projected generation, preferred families included both sexes and more males. Results from the three studies showed that the most desired family size consisted of two children, one of each sex with the male born first.

INTRODUCTION

The sex ratio of children has been found to influence family size. Two sex ratio patterns influence parents' decisions to have additional children. Presence of both sexes in the first two or three children was associated with fewer additional children in studies conducted in Britain (Thomas 1951), the United States (Gray 1972; Gray and Morrison 1974), and China (Gray, Hurt, and Wu 1995). Presence of more male children reduced the likelihood of additional children in Korean families (Park 1978). Male preference was evident in studies of families in the United States (Gray 1982) and in China (Gray, Hurt, and Wu 1995). In those studies, higher percentage of one-child families consisted of a male rather than a female child or more two-child families consisted of two males rather than two females.

College students in Brazil (Gray, Bortolozzi, and Hurt 1979), China (Gray, Hurt, and Wu 1995), and the United States (Gray and Morgan 1976) and high school students in Japan (Gray, Duckworth, and Nakajima 1980) expressed specific preferences for family sizes and composition of sexes of their children. The strongest preference was two-child families including both sexes with the male born first.

The population of students enrolled in Ogden College of Science, Technology and

Health, Western Kentucky University (WKU), was studied originally in 1969 (Loyd and Gray 1969) and in 1972 (Gray 1972); it was studied again in 1982 (Gray 1982). Transgenerational analyses, involving the parental, present, and projected generations, were made on the sex ratio and the influence of the composition of sexes of children on family size. Consistency of the results across generations gave credibility to study of the projected generation and indicated that preferences for composition of sexes of children extended beyond a single generation.

Objectives of the present study were to revisit the WKU student population, to verify findings of the two previous studies, to explore further the effects of composition of sexes of existing children on family size, and to study relations among the past, present, and projected generations.

POPULATION AND PROCEDURES

Since previous studies of the WKU students were conducted ca. 1970 (Gray 1972; Loyd and Gray 1969) and early 1980s (Gray 1982), the 1992 sampling permitted decadal observations. Sex data were obtained from 1000 (equal numbers of females and males) white American students in various science classes as was done in the previous studies. Each student completed an information form providing data on the family's parental and present gen-

Table 1. Longitudinal analysis of family sizes and secondary sex ratios (male per 100 females) over generations and time.

Generation	Previous studies		Present study
	1972 ¹	1982 ²	1992
Family size	----- Number of children -----		
Parental	4.56	4.73	4.24
Present	3.32	3.70	2.70
Projected	2.46	2.60	2.35
Secondary sex ratio	- Number of males per 100 females -		
Parental	103	103	105
Present	101	101	97
Projected	138	133	136

¹ Loyd and Gray (1969) and Gray (1972).

² Gray (1982).

erations as well as the student's projected generation.

For the parental generation, information was collected on the number of children by sex in both the maternal and paternal parents' families, permitting average family sizes and sex ratios to be calculated. For the present generation, information was obtained on sex of children by order of birth, enabling exploration of the effects of composition of sexes of children on family size. For the projected generation, each student respondent reported the desired number of children and sex by order of birth. These data were used to calculate average family size and sex ratio by order of birth.

RESULTS AND DISCUSSION

Results of the present study indicate that the average number of children in families represented by WKU students continues to decrease (Table 1). The decrease in family size was apparent from comparisons of the generations included in this study and from comparisons of these results with those reported for the previous studies. The decrease in number of children from 4.24 in the parental generation to 2.70 in the present generation was ca. 1.5 children per family. A further decrease of ca. 0.35 children per family was reported between the present and projected generations. Comparisons with the previously reported results indicated that family size was continuing to decrease over time. The increase in family size between 1972 and 1982 likely occurred because enhanced financial aid

Table 2. Longitudinal influence of composition of sexes of existing children on family size in the present generation over time.

Sex composition	Percentage of families who ceased having children		
	Previous studies		Present study
	1972 ¹	1982 ²	1992
First child			
f		3.2	13.6
m		6.7	11.6
Total		5.0	12.6
First two children			
ff		19.8	38.4
mm		26.8	42.8
Same sex	32.4	23.3	40.6
Different sex	38.4	30.0	50.0
Total	35.4	26.2	45.3
First three children			
fff		36.6	50.8
mmm		34.7	62.1
Same sex		35.6	56.4
Different sex		37.7	54.6
Total		36.6	55.5

¹ Loyd and Gray (1969) and Gray (1972).

² Gray (1982).

programs made college more accessible to children from larger families (Gray 1982).

Secondary sex ratios (males:100 females) observed for the parental and present generations were within ranges reported for other populations. The lower ratio for the present (97:100) than for the parental (105:100) generation may result from a bias in the sampling procedure. If the ratio is greater than 100:100, as indicated by the parental generation results and as reported for other populations (Stern 1960), the ratio for the present generation would have been biased downward by the inclusion of equal numbers of female and male respondents (Loyd and Gray 1969).

Influence of sexes of existing children on parents' decision to have additional children was studied in the present generation (Table 2). The percentage of families stopping with one child was slightly, but not significantly, higher (13.6% vs. 11.6%) when the child was female than male. In the 1982 study (Gray 1982), a significantly lower percentage of one-child families consisted of female rather than male children (3.2% vs. 6.7%). In the recently reported China study (Gray, Hurt, and Wu 1995), sex of the child had no significant influence on the frequency of one-child families.

Table 3. Longitudinal comparisons of desired family size, combination, and permutation of sexes of children in the projected generation over time. Figures are percentages.

Number of children	Combination of sexes	Previous studies		Present study	Permutation of sexes ³	Previous studies		Present study
		1976 ¹	1982 ²	1992		1976 ¹	1982 ²	1992
0		7.5	7.0	3.6				
1	1f	0.8	0.9	3.2				
	1m	3.0	2.2	3.0				
	Total	3.8	3.1	6.2				
2	2f	1.0	0.4	1.4				
	1f1m	39.9	39.2	46.6	fm mf	5.4 34.6	6.4 32.8	7.0 39.5
	2m	4.0	4.0	7.7				
	Total	44.9	43.6	55.7				
3	3f	0.4	0.2	0.3				
	2f1m	2.8	6.1	4.4	ffm fmf mff	0.5 1.0 1.4	0.6 2.2 3.4	0.7 2.0 1.7
	1f2m	18.5	16.8	17.6	fmm mfm mmf	1.5 9.1 8.0	0.7 8.9 7.3	0.8 10.2 6.6
	3m	1.2	2.4	1.2				
	Total	22.9	25.5	23.5				
4	4f	0.0	0.2	0.0				
	3f1m	0.3	0.2	0.3				
	2f2m	11.8	13.0	5.9	mfmf	6.2	7.2	2.9
	1f3m	3.0	2.0	2.4				
	4m	1.1	0.6	0.5				
	Total	16.2	16.0	9.1				
>4		4.7	5.0	1.6				

¹ Gray and Morgan (1976)² Gray (1982).³ For families of four children, data are presented for the mfmf permutation only.

Considering the first two children, a higher percentage of families ceased having children when both sexes were represented (50.0%) than when the first two children were of the same sex (40.6%). This impact of both sexes in the first two children was slightly greater than the same effect in the previous two studies. More specifically, the effects of both sexes on percentages of families stopping with two children were 6.0% (38.4 vs. 32.4), 6.7% (30.0 vs. 23.3), and 9.4% (50 vs. 40.6) in the three successive studies (Table 2). Other studies (Gray 1972; Gray and Morrison 1974; Thomas 1951) have reported fewer additional children when both sexes were represented in the first two children. Also, in the present study, more families stopped having children when the first two were males (42.8%) than when they were females (38.4%), providing further evidence of preference for males.

For families with three existing children, the decision to have additional children appeared to be independent of whether existing children were of the same or different sexes unless the same sex consisted of three females. For families with three daughters 50.8% ceased having children, whereas for families with three sons 62.1% had no additional children. Male preference has been reported for populations in China (Gray, Hurt, and Wu 1995), Korea (Park 1978), and the United States (Gray 1982).

In addition to providing information on family members in the parental and present generations, each respondent was asked to indicate her/his preference for family size and composition of sexes of the children. The results (Table 3) showed that 3.6% of the respondents wanted no children. This percentage was about one-half that reported in the

two previous studies. In the present study, the proportion of respondents wanting one child (6.2%) was about twice that of the previous studies. However, combining the percentages of respondents wanting either no children or one child gave essentially the same proportion (ca. 10%) for each of the three studies. The most desired family size consisted of two children. The percentage of respondents (55.7) wanting two children was ca. 11% higher than in the two previous studies. The percentage of respondents (23.5%) preferring three children was similar to that of the previous studies. The proportions of respondents wanting four or more than four children were much lower than in the earlier studies. Combining all the desired family sizes and their frequencies resulted in an average desired family size of 2.35 children, which is slightly lower than averages reported in the previous studies (Table 1). Average numbers of children desired by college students in other populations have ranged from 1.65 in China (Gray, Hurt, and Wu 1995) to 4.88 in Nigeria (Gray, Hurt, and Oyewole 1983).

The respondents expressed strong preference for both sexes of children; however, preferences for the same sex were stronger for all male than for all female children (Table 3). Almost one-half (46.6%) of desired families consisted of two children with one male and one female. Respondents expressed strong preference for the male child to be born first, e.g., the mf and fm orders in two-child families were preferred by 39.5% and 7.0% of respondents, respectively. The mf sequence preference continued in family sizes of three and four children resulting in mfm and mfmf as the most desired orders. Preferences for composition of sexes of children in desired families were similar to those expressed in the previous studies. Preference for the male being born first was consistent with results from other studies (Abdulla, Al-Rubeai, and Gray 1984; Gray, Bortolozzi, and Hurt 1979; Gray, Duckworth, and Nakajima 1980).

For desired families, the combinations and permutations of sexes of children were analyzed to determine the sex ratio by order of birth within families (Table 4). With the exception of one-child families where desired families included slightly fewer males than females, preferences were strong for first and

Table 4. Longitudinal comparisons of sex ratios (males per 100 females), by order of birth, resulting from desired family size, combination, and permutation of sexes of children in the projected generation over time.

Desired family size	Order of birth:	--- Number of males per 100 females ---			
		First	Second	Third	Fourth
Previous study 1976 ¹					
1		454	—	—	—
2		610	26	—	—
3		761	104	118	—
4		494	92	208	51
Previous study 1982 ²					
1		300	—	—	—
2		536	32	—	—
3		681	98	99	—
4		589	82	160	41
Present study 1992					
1		94	—	—	—
2		555	36	—	—
3		513	83	122	—
4		700	72	138	88

¹ Gray and Morgan (1976).

² Gray (1982).

third children to be males and second and fourth children to be females. Combining desired family sizes and sexes of children resulted in an overall sex ratio of 136 males to 100 females (Table 1). Comparable sex ratios for the two previous studies were 138 and 133. Sex ratios for desired families in other populations have ranged from 110 in China (Gray, Hurt, and Wu 1995) to 167 in Nigeria (Gray, Hurt, and Oyewole 1983). These ratios were based upon desired families as opposed to actual families. However, the preferred combinations of sexes of children, e.g., one male and one female (Table 4), were the same combinations that limited family size (Table 2). These and similar results from other studies indicate that preferences for one sex would further imbalance the human sex ratio. Goodman (1961) showed that birth control combined with preference for one sex could influence the sex ratio. The 106 males:100 females sex ratio commonly accepted for white Americans (Stern 1960) may have resulted from this preference for males.

These longitudinal studies were based upon white American students largely from south central Kentucky. Family size generally decreased for successive generations within each

study and for the successive studies. In the present study higher percentages of families ceased having children after one, two, or three children than in the previous studies. The likelihood of additional children decreased when existing children included either both sexes or males. Sex ratios were similar among the three studies.

Respondents in each study expressed preferences for two-child families with the male being born first. For larger families, this pattern was continued, resulting in an mfmf order. Realized preferences for smaller families would be a factor in human population control. However, realized preferences for combinations of sexes resulting in an imbalance of the sex ratio would have further implications.

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Comparison of *Cryptococcus neoformans* Isolates from Clinical and Environmental Collections in South Central Kentucky and Surrounding Areas

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ABSTRACT

Cryptococcus neoformans is an opportunistic encapsulated yeast responsible for the invasive disease cryptococcosis in immunocompromised hosts. The importance of epidemiological studies on cryptococcosis has increased since the beginning of the AIDS epidemic. *Cryptococcus neoformans* exists in two varieties with four serotypes: *C. neoformans* var. *neoformans* (serotypes A and D) and *C. neoformans* var. *gattii* (serotypes B and C). In this research, *C. neoformans* var. *neoformans* has been associated with pigeon feces in those months having an average temperature of 17.8°C and above. Clinical and environmental isolates of *C. neoformans* were grouped into their variety status utilizing canavanine-glycine-bromthymol blue agar. Polyclonal antisera against *C. neoformans* serotypes A, B, C, and D were pooled from challenged rabbits. Serotyping *C. neoformans* isolates by using the polyclonal antisera resulted in 57% (20 of 35) of the serotypes confirmed with a direct immunofluorescent assay utilizing a single monoclonal antibody (E1). Assay data suggest that all *C. neoformans* obtained from regional hospitals (26 of 26) and those isolated from the environment (9 of 9) belong to the A serotype group. These data provide information on the origin of cryptococcosis in our region and may be beneficial to immunocompromised individuals.

INTRODUCTION

Cryptococcosis is a potentially harmful fungal disease in humans and animals. Usually it is listed as an opportunistic infection; however, the causative yeast, *Cryptococcus neoformans*, does occur in healthy persons (Sugar 1991). Cryptococcosis has recently been recognized as the most life-threatening mycosis in patients with Acquired Immunodeficiency Syndrome (AIDS) (Clancy et al. 1990). This mycosis generally manifests itself as meningitis, with the respiratory tract as the portal of entry for the yeast (Ellis and Pfeiffer 1990). Meningitis and to a lesser extent pneumonia have proven to be life-threatening manifestations of cryptococcosis, although morbidity may be due to involvement of virtually any organ system. Among the many species of *Cryptococcus*, the etiologic agent in virtually all cases of human cryptococcosis is *C. neoformans*. Previously, rare cases of infection were thought to be caused by *C. albidus* and *C. laurentii* (Krumholz 1972; Lynch et al. 1981); however, recent studies utilizing fluorescent antibody techniques suggest the need for a reassessment of past literature with respect to infections caused by *Cryptococcus* species other than *C. neoformans* (Sigmund et al. 1991).

On the basis of antigenic determinants of

the polysaccharide capsule, four serotypes (A, B, C, and D) of *C. neoformans* have been recognized (Evans 1950; Wilson et al. 1968). Subsequent studies, using type specific antisera, have shown that serotypes A and D share similarities differentiating them from serotypes B and C, which are similar to one another.

Cryptococcus neoformans has a perfect or teleomorphic state, *Filobasidiella neoformans*, with two varieties. The teleomorph *F. neoformans* var. *neoformans* corresponds to the anamorph (asexual state) *C. neoformans* var. *neoformans* serotypes A and D. The teleomorph *F. neoformans* var. *bacillisporus* corresponds to the anamorph *C. neoformans* var. *gattii* serotypes B and C (Kwon-Chung 1975, 1976a, 1976b).

Extensive research conducted on epidemiological differences between these varieties (Bennett et al. 1977; Kwon-Chung and Bennett 1984) has shown that *C. neoformans* serotypes B and C are prevalent only in tropical and subtropical regions, whereas 85-100% of *C. neoformans* serotypes A and D are found in Europe and North America, excluding southern California, where serotypes B and C represented 41% of the isolates (Kwon-Chung et al. 1978).

Among dissimilarities between the varie-

ties—including epidemiologic, biochemical, and genetic aspects—the most striking is in their ecology. Cultures of *C. neoformans* var. *neoformans* are regularly isolated throughout the world from pigeon excreta and waste from other birds including chickens, parrots, sparrows, starlings, turtledoves, canaries, and skylarks (Leitz 1991). Until recently, isolates of *C. neoformans* var. *gattii* serotypes B and C have been cultured only from clinical specimens. Environmental isolations established that *C. neoformans* var. *gattii* may have a specific ecological association with the tree *Eucalyptus camaldulensis* (Ellis and Pfeiffer 1990), which could explain its tropical and subtropical distribution. Investigations have shown that creatinine assimilation in *C. neoformans* plays a role in these ecological differences (Kwon-Chung 1991). The biochemical basis for the differences was found in the deiminase enzyme system common to both varieties. Upon deimination of creatinine, two compounds are produced: methylhydantoin and ammonia, the latter of which may raise the pH of the surrounding environment. A regulatory system that has evolved in *C. neoformans* var. *neoformans* but not in *C. neoformans* var. *gattii* is repression of creatinine deiminase once small amounts of ammonia are detected. This mechanism of creatinine metabolism seems to suit the conditions of avian guanos in which *C. neoformans* var. *neoformans* occurs (Kwon-Chung 1991).

In our study, we prepared antiserum against each of the four *C. neoformans* serotypes A, B, C, and D. Environmental samplings of plant materials and pigeon roosts were analyzed to determine the serotypes most prevalent in the sampling area and to identify natural habitats for serotypes A and D. Serological comparison of clinical specimens, obtained from regional hospitals, with the environmental isolates was conducted utilizing polyclonal antisera and a highly specific monoclonal antibody against the cryptococcal capsular polysaccharide (Dromer et al. 1993; Dromer et al. 1987)).

MATERIALS AND METHODS

Environmental Samples

Random environmental samples were collected monthly from October 1991 through

December 1992; the average temperature of each month was recorded. The environmental test area was a 30-km sampling site along Highway 31-W including Bowling Green, Woodburn, and Franklin, Kentucky.

Plant collections, as well as pigeon roost samples, were collected throughout the 15 months. Plant collections from selected sites included tree bark, tree leaves, and tassels and stalks from corn and sorghum; they were kept in plastic bags.

Plants to be sampled were swabbed with the culturette collection system (American Scientific Products), which contained modified Stuarts Bacterial Transport Medium. Samples taken from pigeon roosts were nest material, feces, soil, and swabs of surrounding surfaces; they were stored as described for the plant collections.

Sample preparation. An 11 g portion of each plant collection was suspended in 99 ml of 0.1M phosphate buffer (pH 6.8), shaken on an Eberbach shaker at 270 cycles/min for 5 min, and allowed to stand for 10 min. A 0.1 ml aliquot of the dilution was spread-plate inoculated onto *Guizotia abyssinica* medium (niger seed agar) (Staib and Seeliger 1966) containing 0.5% diphenyl as a mold inhibitor. All plates were incubated at 26°C and observed daily for phenoloxidase-producing colonies, which synthesize melanin. To prepare the sample collections from pigeon roosts a 1.0 g portion of the sample was suspended into 99 ml of phosphate buffer and treated as described for plant materials. Sample collections acquired with the culturette collection system were either directly plated onto agar or suspended in 9 ml 0.1 M phosphate buffer (pH 6.8) prior to plating. After plating, the medium was incubated at 26°C and observed daily for phenoloxidase-producing colonies.

All isolates were stored on 2% (w/v) dextrose, 1% neopeptone agar slants and maintained at 26°C. For long-term storage, 3-day slant cultures were kept at -70°C.

Clinical Isolates

Clinical isolates were obtained from University of Louisville School of Medicine, Department of Pathology, Louisville, Kentucky; University of Kentucky School of Medicine, Department of Pathology, Lexington, Kentucky; T.J. Samson Community Hospital, Glas-

gow, Kentucky; Greenview Hospital, Bowling Green, Kentucky; and Vanderbilt University Medical Center, Department of Pathology, Nashville, Tennessee. These hospitals are within a 240-km radius of Bowling Green.

All isolates were initially tested for production of phenoloxidase and urease (Urease Test Media, Difco Laboratories). Those environmental isolates presumptively identified as *C. neoformans* were further characterized by the API 20C, as directed by the manufacturer. Excluding Greenview Hospital and T.J. Samson Community Hospital, which utilized the Vitek system and Microscan, respectively, all remaining participating hospitals confirmed *C. neoformans* with API 20C. All clinical and environmental isolates of *C. neoformans* were identified to variety with canavanine-glycine-bromthymol blue (CGB) agar prepared according to the methods of Kwon-Chung et al. (1982).

Preparation of Vaccines

Cultures of *C. neoformans* var. *neoformans* strains A68 and D52 and of *C. neoformans* var. *gattii* strains B112 and C18 were donated by K.J. Kwon-Chung (National Institute of Allergy and Health, Bethesda, Maryland). Strain B112 was a mutant of the original Evans Strain 1523 (Ellis and Pfeiffer 1990).

Each of the four *C. neoformans* serotypes was grown in 300 ml of 2% (w/v) dextrose/1% neopeptone broth on a rotary shaker at 140 RPM for 72 hours at 26°C. Cells were pelleted by centrifugation at 1200 g and washed twice in 0.85% saline. Cell suspensions were heat-killed by submersion in a water bath at 100°C for 1 hour, pelleted, and resuspended in 20 ml of sterile 0.85% saline. Vaccines were prepared from heat-killed cells by adjusting the number of cells/ml to 2×10^8 as determined by hemocytometer counts.

Immunization of Rabbits

Rabbits weighing 2 kg or more were given intravenous injections of 2×10^8 cells in sterile 0.85% saline. All rabbits received a course of six injections consisting of two 3-day series, 4 days apart, then no injections for 2 weeks. The course of injections was repeated four times, and the rabbits were given one injection a day for 14 consecutive days. Seven days after the last injection, the rabbits were bled and

the antisera pooled. Those weighing less than 2 kg (1800–1999 g) were given 10^8 cells per injection.

Absorption and Agglutination

Heterologous titers were determined by slide agglutination with suspensions of cells corresponding to McFarland standards 1 and 3. Slide agglutinations were carried out by mixing 0.05 ml of cells (2×10^8 cells/ml) and 0.05 ml of antisera, diluted with 0.85% NaCl, on a glass slide. Agglutination titers were observed after 5, 10, and 15 min on a Fisher Clinical Rotator, model 341, rotating 120 times/min. Antibody titers were expressed as final dilutions of antiserum with agglutinating cells.

Serotyping of isolates was performed with antiserum absorbed with a mixture of cells from the other serotypes. The volume of packed cells was equal to the volume of antiserum to be absorbed. Three absorptions were incorporated for each of the four antisera. A fourth absorption was required if the antisera cross reacted with more than one of the four serotypes. Following serotyping of isolates, utilizing polyclonal antiserum, all *C. neoformans* serotypes were confirmed with a monoclonal antibody against type A cryptococcal cells conjugated with fluorescein isothiocyanate. The monoclonal antibody technique was developed and generously carried out by Dr. Françoise Dromer (Institute Pasteur, Paris, France) (Dromer et al. 1993, 1987).

RESULTS

Environmental Isolates

Isolation of *C. neoformans* from environmental sources occurred only on months with an average temperature of 17.8°C and above. Nine environmental isolates were collected from May 1992 through September 1992 from the sampling area (Table 1). Attempts to isolate *C. neoformans* from barks and leaves of various trees and other plant materials were unsuccessful. *Cryptococcus laurentii* was isolated in April 1992 from a Kentucky coffeetree (*Gymnocladus dioica*) on the campus of Western Kentucky University in Bowling Green.

Clinical Isolates

Twenty-six clinical isolates were obtained during our study (Table 2). All were acquired

Table 1. *Cryptococcus neoformans* isolates from environmental sample collections conducted October 1991 through December 1992.

Date	Avg. mon temp. °C	Sample collection	Location	Isolate
12 Oct 91	Oct 15.2	Sorghum tassel, stalk and leaf	Woodburn	None
18 Oct 91		Pigeon feces	Bowling Green	None
3 Nov 91	Nov 11.9	Tree bark	Franklin	None
3 Nov 91		Tree bark	Bowling Green	None
10 Nov 91		Pigeon feces	Bowling Green	None
11 Dec 91	Dec 5.4	Pigeon feces	Bowling Green	None
2 Feb 92	Feb 6.6	Pigeon feces	Bowling Green	None
2 Feb 92		Pigeon feces	Bowling Green	None
9 Mar 92	Mar 8.6	Pigeon feces	Bowling Green	None
6 Apr 92	Apr 14.3	Tree swabs	Bowling Green	None
11 May 92	May 17.8	Thistle swabs	Bowling Green	None
20 May 92	May 17.8	Bark, tree swabs, pine cones	Bowling Green	None
29 May 92		Pigeon feces	Bowling Green	SSE3, SSE4
20 June 92	Jun 22.2	Pigeon roost swabs	Bowling Green	SSE5, SSE6
21 Jul 92	Jul 26.4	Pigeon feces	Bowling Green	PE1, PE2
12 Sep 92	Sep 20.4	Pigeon feces	Franklin	FE1
14 Sep 92		Pigeon roost swabs	Bowling Green	None
14 Sep 92		Pigeon feces	Bowling Green	None
24 Oct 92	Oct 14.5	Pigeon feces	Franklin	None
24 Oct 92		Thistle swabs	Woodburn	None
24 Oct 92		Pigeon feces	Bowling Green	None
28 Nov 92	Nov 8.9	Pigeon feces	Franklin	None
9 Dec 92	Dec 3.7	Pigeon feces	Bowling Green	None

during the 15-month course, except those obtained from Vanderbilt Medical School, which had been stored frozen at -70°C for periods not exceeding 2 years.

Identification of Isolates

All nine isolates producing phenoloxidase on niger seed agar, modified with 0.5% diphenyl and giving a positive urease test, were confirmed by the API 20C system (Kwon-Chung and Rhodes 1986; Polacheck et al. 1990). Seven gave an API 20C profile index number of 2557373, which confirmed the identification as *C. neoformans*. The two remaining isolates (SSE5 and SSE2) gave an index num-

ber of 2757373, which also indicated *C. neoformans*.

All clinical and environmental isolates were identified to their variety by utilizing CGB agar. A positive test, indicated by pH change from 5.8 (greenish yellow) to at least 7.0 (cobalt blue), was exhibited by all isolates tested. Thus, all clinical and environmental isolates were identified as *C. neoformans* var. *neoformans*.

Absorption and Agglutination

The heterologous titers of the non-absorbed antisera failed to reach levels described in past literature (Ikeda et al. 1982; Wilson et al.

Table 2. *Cryptococcus neoformans* obtained from regional hospitals.

Hospital	Isolates	Isolate identification
University of Louisville School of Medicine, Louisville, KY	4	UL-1, UL-2, UL-3, UL-4
University of Kentucky School of Medicine, Lexington, KY	5	UK-1, S120, X77, 136R, S325
T.J. Samson Community Hospital, Glasgow, KY	1	TJ-1
Greenview Hospital, Bowling Green, KY	1	GH-1
Vanderbilt Medical School, Nashville, TN	15	V1334, V1347, V6415, V1459, V1733, V2153, V3493, V3933, V2667, V3176, V2409, V5928, V6824, V6626, V6627

1968). Rabbits injected with the A68 cells and those injected with the D52 cells gave the highest heterologous titers, 256 and 128, respectively. A dramatic decrease in titers of the antisera was experienced after absorptions were performed. Antisera for A and D serotyping expressed homologous titers of 16, whereas B and C antisera gave homologous titers of 4 and 8, respectively, which correlates with their low heterologous titer. The polyclonal antisera developed in rabbits were utilized and separated all isolates into either A, D, or AD serotypes. From the 35 total isolates obtained, 57% (20 of 35) were typed as being serotype A, 17% (6 of 35) were D serotype, 20% (7 of 35) typed AD, and 5% (2 of 35) were untypeable (Table 3). After data were obtained from monoclonal antibody typing, variability in the polyclonal antisera was discovered. All 35 isolates were found to be serotype A by utilizing a direct immunofluorescence assay with a single monoclonal antibody (E1) specific for cryptococcal polysaccharide (E1) specific for cryptococcal polysaccharide (data obtained from Dr. Françoise Dromer, Institute Pasteur, Paris, France) (Table 3).

DISCUSSION

In the collection of *C. neoformans* from the environment, an average monthly temperature 17.8°C and above appeared to be required for isolation of the yeast. Further observation of samples collected that did possess *C. neoformans* seemed to suggest the yeast would best be found in areas where a high available water was present. In terms of preventing exposure for HIV infected persons or those who have severe immunosuppression, these individuals should avoid environments with abundant pigeon feces. Samples collected from arid locations, and expected to contain *Cryptococcus*, did not contain the yeast in numbers high enough to isolate. Those samples possessed high mold counts, which may have inhibited the recovery of any *C. neoformans*. Attempts to isolate *C. neoformans* from plant collections were unsuccessful; however, *C. laurentii* was found to be associated with the Kentucky coffeetree, isolated in April 1992, when the average monthly temperature was 14.3°C.

By use of the CGB medium described by Kwon-Chung et al. (1982), the presumptive serotype group was determined for each of the 35 isolates obtained our study. The results of

Table 3. Serotypic identification of *Cryptococcus neoformans* isolates.

Isolate	Variety CGB agar ¹	Polyclonal antisera	Serotype Monoclonal antibody
SSE6	—	A	A
PE1	—	A	A
FE1	—	A	A
UL-2	—	A	A
UL-3	—	A	A
GH1	—	A	A
V5928	—	A	A
V1733	—	A	A
V6627	—	A	A
V2409	—	A	A
V1459	—	A	A
V1347	—	A	A
V3176	—	A	A
V2153	—	A	A
V6824	—	A	A
V3933	—	A	A
V2667	—	A	A
V6626	—	A	A
V3493	—	A	A
V1334	—	A	A
SSE2	—	D	A
SSE5	—	D	A
UK-1	—	D	A
S325	—	D	A
TJ-1	—	D	A
UL-4	—	D	A
SSE1	—	AD	A
SSE3	—	AD	A
SSE4	—	AD	A
PE2	—	AD	A
S120	—	AD	A
UL-1	—	AD	A
136R	—	AD	A
X77	—	Untypeable	A
V6415	—	Untypeable	A

¹ Negative test denotes *C. Neoformans* var. *neoformans*.

this testing show that 100% of *C. neoformans* isolated in the environmental sampling area and all regional hospitals were *C. neoformans* var. *neoformans* serotype A or D. These data are more than needed in hospitals since treatment appears to be the same for both varieties; however, in epidemiological studies more precise data require serological testing in order to differentiate serotypic groups within the species.

In typing the 35 strains of *C. neoformans* with the polyclonal antisera, only 57% were typed accurately in comparison to data obtained from the immunofluorescent monoclonal antibody (Table 3). Similar inaccuracies were noted by Bennett et al. (1977) when typing 106 strains with their polyclonal antisera.

The above authors found five isolates (4.7%) untypeable and four isolates (3.7%) reacting with A and D typing sera.

To omit these discrepancies when when serotyping *C. neoformans*, a monoclonal antibody should be used; this has proven to be a reproducible and reliable way of screening multiple isolates (Spiropulu et al. 1989). The difference between the conventional serotyping methods and the direct immunofluorescence assay using a monoclonal antibody was mainly the disappearance of the ambiguous AD serotype along with any strains that cannot be typed. All the environmental isolates corresponded serologically to the clinical isolates obtained from regional hospitals. Some degree of serotypic variability was experienced in that 43% of the isolates did not type specifically by conventional polyclonal antisera methods.

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Scientists of Kentucky

Introduction

Kentucky's contributions in the sciences were established remarkably early. Under the brilliant leadership of Horace Holley, Transylvania University in Lexington established one of the finest medical schools in America despite the frontier conditions still prevalent in the Bluegrass during his administration (1818–1825). Even beyond medicine, however, one historian concluded that Transylvania left “an enduring legacy. Having introduced quality science instruction into the curriculum of the early 19th century college, Transylvania's Academic Department had made significant contributions—ones as important as those of the more prestigious Medical Department.”^a

Although Transylvania's eminence in the sciences is over, the state's long heritage of scientific endeavor has endured into the present century and has counted individuals from all regions of the Commonwealth among its ranks. A chronicle of these men and women would seem appropriate for the *Transactions of the Kentucky Academy of Science*.

The most immediate problem with such a plan is, what constitutes a scientist of Kentucky? Is it an issue of birthplace, schooling, or subject matter? The great Bluegrass bibliophile John Wilson Townsend grappled with this issue some years ago with reference to literature. “What is a Kentucky book?” he asked. “Surely,” he concluded, “a Kentucky book is one written by a Kentuckian about Kentucky or Kentuckians and printed in Kentucky; surely it is a book written by a Kentuckian upon any subject under the sun, and published in any clime; surely it is one written in Kentucky by a citizen of any other state or country, regardless of the subject or place of publication, for, in general, I have regarded the birthplace of a piece of literature more important than that of the author.”^b Town-

send's rather broad guidelines will be adapted and adopted for the scientists to be included in this series.

Ever since Thomas S. Kuhn's *Structure of Scientific Revolutions* (1962; 2nd rev. ed., 1970), it has become unfashionable to speak of the cumulative, linear march of scientific progress. Current historians of science are more apt to talk about the “paradigmatic changes” occurring within various disciplines and the gestalt perceptual transformations that have caused fundamental changes in those fields. But any given discovery in science is seldom as revolutionary as it often appears; while some paradigms may change, many others remain intact. Thus, there is still much to be said for the contributions of one's predecessors in any scientific discipline. Even Newton, a man not given to modesty, once said: “If I have seen further (than you and Descartes) it is by standing upon the shoulders of Giants.”^c

Kentucky cannot claim Newton, but in geology, biology, medicine, and many other disciplines, the Commonwealth has produced its fair share of shoulders upon which the present generation of researchers may stand. It is to the appreciation of this basic fact that this series is dedicated.

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ENDNOTES

- a. Eric Christianson, “The Conditions for Science in the Academic Department of Transylvania University, 1799–1857,” *The Register of the Kentucky Historical Society* 79 (Autumn 1981):325.
- b. *Kentucky in American Letters, 1784–1912*, vol. 1 (Cedar Rapids, Iowa: The Torch Press, 1913), p. xi.
- c. Letter to Robert Hooke, 5 February 1675.

Scientists of Kentucky

Thomas Vaughan Morrow, 1804–1850: The Apostle of Eclecticism¹

It is ironic that, on 14 April 1804 near Fairview, Christian (now Todd) County, Kentucky, Thomas Vaughan Morrow (Figure 1) was born in the same log cabin that would bring Jefferson Davis into the world just 4 years later. The fact that the same domicile would provide shelter to a committed abolitionist like Morrow and the president of the Confederacy speaks to a region torn in two by issues of slavery and secession. Such an environment spawned characters of conviction and flamboyance. Morrow was such a man. Although America was beset by serious social and political divisions during the first half of the 19th century, it was also engaged in sectarian medical controversies that at times proved as violent as the struggle over slavery. The life of Thomas Vaughan Morrow provides a picture of this contentious period of American medicine and perhaps even a glimpse into the therapeutic future.

Morrow, like so many of the early Kentucky pioneers, was of Scotch Presbyterian descent. Heading westward into the region shortly after the Revolutionary War, the Morrow clan rose to positions of distinction in the early Republic. Jeremiah Morrow, Thomas Vaughan Morrow's cousin, was Ohio's 10th governor, serving from 1823–1826.

It was in Kentucky, however, that young Thomas would receive his early education and launch his career. He studied for a time at the famous Transylvania University in Lexington. He then removed to New York City where he graduated from a regular or allopathic medical school, after which he attended and received a degree from the Reformed Medical College. Here Morrow met the school's founder Wooster Beach (1794–1868), an event that would change his professional life.

Beach was a Connecticut-born physician who became acquainted with the use of me-

dicinal plants from an herb doctor named Jacob Tidd. It was an influence that would remain with Beach even after his graduation from University of the State of New York. Beach's fervent devotion to botanicals over the harsh heroic bleeding, blistering, and purging practiced by his allopathic counterparts caused him to start a number of short-lived schools based upon a creed of "reformed or vegetable system of medical practice."^a Beach was not the first to call for a gentler form of therapeutics based upon medicinal plants; Samuel Thomson (1769–1843) had done much the same with his *New Guide to Health, or Botanic Family Physician* (1825). Unlike Thomson's approach to medicine, which promoted the notion that every man could be his own physician by insisting that "the practice of physic requires a knowledge that cannot be got by reading books,"^b Beach held that creating such a system of unschooled medicine "would be an attempt as ridiculous as it is impossible."^c Thus Beach and his colleagues launched into aggressive efforts at establishing institutions with the mission of training physicians devoted to the cause of what they called Reformed Medicine. Denouncing the standard practices of their allopathic counterparts who called for debilitating doses of more "active" remedial agents like calomel (a purgative composed of mercurous chloride) and antimony (a chemical variously administered as *antimonii chloridum*, the caustic blistering agent commonly called "butter of antimony," or as *antimonii et potassii tartras*, more descriptively named "tartar emetic" and used to induce vomiting), these reformers could present themselves to the public as the champions of therapeutic common sense and moderation.^d

One of the most important of these reform-minded physicians was Thomas Vaughan Morrow. At first it appeared as though Morrow was destined for obscurity. Upon graduation from Beach's Reformed Medical College, he returned to his home state to open a practice in Hopkinsville, Kentucky. His residence in

¹ This paper was originally delivered at the annual meeting of the Ohio Academy of Medical History held at Wright State University, 30 Mar 1996.



Figure 1. Thomas Vaughan Morrow. Photograph courtesy of the Lloyd Library, Cincinnati, Ohio.

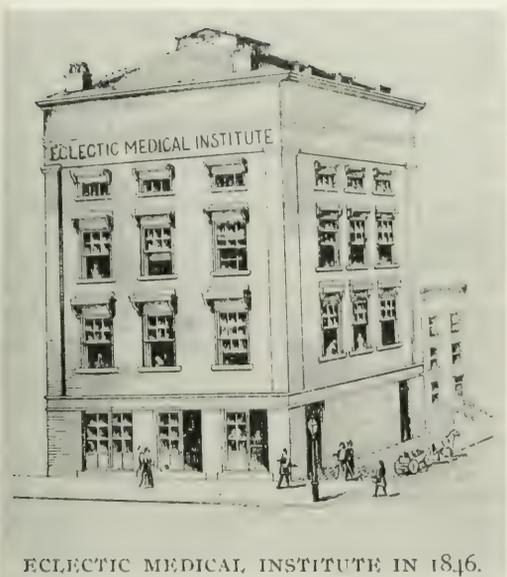
the Commonwealth was short-lived, however. Morrow's well known opposition to slavery and an altercation with a man named Pennington, whom he had wounded in a brawl, sent the strapping 250-pound, 6-foot doctor back to New York where Beach had subsequently founded the Reformed Medical Society of the United States. One of the first acts of this body was to call for the establishment of a medical school at Worthington, Ohio. The person chosen to head that effort was Thomas Vaughan Morrow.^e

Cautious in their granting of new charters to an ever growing number of self-proclaimed healers, the Ohio legislature agreed to amend the original 1819 charter of Worthington College to permit a medical department provided that Dr. Morrow would submit to an examination by an established professor of medicine

from Philadelphia.^f Morrow agreed, he passed this scrutiny, and the school opened its doors to seven medical students in December 1830.^g The early years of the Medical Department at Worthington College were met with modest success. By 1836, under Morrow's leadership as president, the school had four faculty members, 40 students, and a monthly journal appropriately titled the *Western Medical Reformer*, which Morrow edited.

The school suffered from serious long-term problems, however. Derided by Samuel Thomson as trying to profit from a system of medicine he had founded and opposed by regular practitioners, these medical reformers had few allies. Their woes were exacerbated by a financial panic in 1837 that forced the temporary suspension of the *Western Medical Reformer*. Finally, in fall 1839, accusations of "grave robbing" were sparked by members of the Cramm family of Marietta, Ohio. Arriving too late to claim the body due to poor roads and difficult travel, the Cramms found their relative had been taken to a potter's field. There they discovered the grave site disturbed and the body missing. With tempers flared, a mob assembled and descended first upon Morrow's home. A search behind the house revealed the corpse of "a negro ensheathed in a shock of freshly-cut corn," heightening suspicions that the body of Mrs. Cramm was to be found at Worthington College. Morrow and other faculty members quickly took arms in an effort to defend the building. When the crowd obtained a key to the school, however, Morrow saw the futility in further resistance and asked that his faculty be allowed to leave safely with any "movable college property" that could be carried away. The discovery of what appeared to be the body of Mrs. Cramm on a dissecting table within the College spelled the end of the medical department.^h

Despite the dramatic confrontation with the townspeople and the loss of its charter in 1840, the Worthington school limped along until 1842 when Alexander H. Baldrige (1795–1874) suggested that Morrow reap the advantages of Cincinnati's more cosmopolitan environment.ⁱ Morrow, well aware that there was little future for a medical school in Worthington, took the advice. During the winter of 1842–1843 he delivered his first series of lectures in Cincinnati. He was joined shortly



ECLECTIC MEDICAL INSTITUTE IN 1846.

Figure 2. The Eclectic Medical Institute, Cincinnati, Ohio, 1846. Photograph courtesy of the Lloyd Library, Cincinnati, Ohio.

thereafter by Lorenzo E. Jones and a Dr. Jordan. Morrow was committed to establishing a school of Reformed Medicine, and by 1844 the upper rooms of the “Hay Scales House” on Sixth and Vine streets were offering medical courses under the instruction of Morrow, Baldrige, Jones, and Jordan.

Morrow realized that if the school was to survive it would need the privileges and status of a chartered college. Borrowing from an earlier usage of the term “eclectic” by the famous naturalist Constantine Rafinesque (1783–1840) from Transylvania University,^j Morrow asked the Ohio legislature to establish an Eclectic Medical Institute.^k The petition was initially greeted with opposition by those who viewed the school as a threat to the allopathic Ohio Medical College also in Cincinnati. Nevertheless, with the persistent efforts of Senator Ephraim Eckley, the legislature finally adopted a bill incorporating the Eclectic Medical Institute on 10 March 1845 (Figure 2).

Thus eclecticism was born with Thomas Vaughan Morrow as its leading champion. In his *Western Medical Reformer* Morrow proclaimed the credo of the school: “Our college will be strictly what its name indicates—*Eclectic*—excluding all such medicines and such

remedies as, ‘under the ordinary circumstances of their judicious use, are liable to produce evil consequences or endanger the future health of the patient.’”^l Historian Ronald L. Numbers observed that “their claim to be eclectic was not merely rhetorical. At one time or another they borrowed from just about every system available, including homeopathy, hydropathy, phrenology—even allopathy.”^m Such brazen appropriations of theory and practice opened the school up to considerable criticism. Typical were the sarcastic comments of the allopathic *Medical and Surgical Reporter*: “All the ‘ics,’ ‘tics,’ ‘lics,’ ‘isms,’ ‘cisms,’ ‘ists,’ and ‘pathies,’ are said to be compounded into what is called Eclectic, which is therefore the most comprehensive of them all, and at the same time the least original. Most other fallacies spring up at once,” continued the *Reporter*, “create a great sensation and often stagger and stun the intelligent, by the startling novelty of their propositions, bewilder the unwary by the immensity of their premises, and then die out. But the Eclectics keep themselves alive by swallowing everything that happens to turn up, until they have become like Macbeth’s caldron, an extraordinary conglomeration of such incompatibles as Injun doctoring, Dutch homeopathy, water cure, electropathy, physiomedicalism, etc.”ⁿ

Such vituperation was both unkind and inaccurate. The eclectics accepted a number of principles from a variety of medical systems, but their adherence to a *materia medica* made up primarily of vegetable products did distinguish them from most other practitioners. Modern historians have appreciated the difference: “Instead of learning how to administer the common mercurial remedies,” wrote professor Numbers, “the eclectic student spent his time discovering the medicinal properties of indigenous plants, which from our vantage point seems to have been a wise choice.”^o

Morrow himself spoke passionately and tellingly on the need for medical reform in antebellum America. Addressing the 1843 class of Cincinnati’s Reformed Medical School, Morrow asked, “How often do we see the unfortunate patient subjected to a long course of treatment, with scarcely any adequate management whatever for the skin, while the stomach and bowels are dosed with strong and irritating drugs, which are calculated to deter-

mine the circulating fluids to the internal organs and concentrate the excitability there, and consequently withdraw them from the surface, and tend to produce an inequilibrium [sic] in the harmonious balances of the system, which is any thing but desirable." For Morrow it was the vegetable kingdom, not harsh mineral purgatives, blistering agents, and mercurials, which provided "that splendid control over disease, which God and Nature designed that it should."⁸

It was over such a system of therapeutics that Morrow presided. Recognition of Morrow's role in the founding of eclecticism comes from an unlikely source, a Virginia-born physician name George Washington Lafayette Bickley (1823–1867). This southern fire-eater, who came to the Institute in October 1852 to assume the chair of *Materia Medica*, Therapeutics, and Medical Botany, dubbed the Kentucky abolitionist Morrow as "the father of Eclecticism in the West." Citing Morrow's dedication to the Worthington enterprise, his personal assumption of financial responsibilities in its behalf, his editorial leadership of "the only scientific reform journal west of the Ohio," and his perseverance in "defending the Thermopolæ of rational medicine," Bickley secured Morrow's place in the history of eclecticism and American sectarian medicine.⁹

Yet Morrow was as much a product of his time as he was a medical pathfinder. Morrow and his sectarian counterparts represented a host of herbal, Indian, physiomedical, and homeopathic schools of medical thought that were the outgrowths of a populism which sought to maximize the freedom of medical practice in order to offer the widest array of therapeutic choice to the public. Among the strongest and best organized were the eclectics, whose emphasis of an indigenous *materia medica* resonated with a society that praised the natural superiority of all things American. Morrow exalted in the relaxation of statutory regulation for physicians. "State after state has marched forward to the noble work," he hailed, "and blotted out, *it is hoped forever*, from the statute-books all laws granting exclusive privileges to one class of medical practitioners to oppress another; thus placing each before the community on its own proper basis."¹⁰

From the founding of the Eclectic Medical Institute to his untimely death from dysentery

on 16 July 1850,¹¹ Thomas Vaughan Morrow was the luminary of sectarian medicine in the Midwest. During this time he served as the Dean of the Faculty and taught courses in anatomy, physiology, the theory and practice of medicine (which at that time included pathology), and clinical medicine.¹² The Institute prospered and matured during these years, graduating 212 eclectic physicians from 1846 to 1850. By guiding the school through its frail infancy, Morrow provided the basis for what has been acknowledged as one of the largest medical colleges in 19th-century America, a school that during the 1850s provided more than a quarter of all physicians practicing in Ohio.¹³

More than just the head of the Institute, Morrow also provided national leadership. Although his earlier efforts at consolidating the various factions of botanic practitioners had failed, he worked ceaselessly to establish an eclectic organization of national proportions. Opening correspondence with "the leading Medical Reformers of different shades of sentiment," Morrow, with tactful diplomacy, brought together a convention of Reformed practitioners in Cincinnati on 25 May 1848.¹⁴ This led to a second meeting on 15 May 1849, when a National Eclectic Medical Association was established with Morrow as its president.

Morrow did not live long enough to see many of his ambitious projects through. The eclectic cause would be taken up by others such as John King (1813–1893), John Milton Scudder (1829–1894), and John Uri Lloyd (1849–1936). Under their leadership eclecticism would carry on.

Although advances in the etiology of disease and microbiology would leave the eclectics behind the therapeutic revolution of the late 19th and early 20th centuries, Morrow's contribution to American medicine is very real. Ronald Numbers makes a strong case for the importance of the Institute in the following: "The educated irregulars, particularly among the homeopaths and the eclectics, simply were not the incompetent charlatans their enemies made them out to be. Judged by almost any criterion—quality of faculty, physical facilities, length of terms, or time devoted to clinical instruction and anatomical dissection—the Eclectic Medical Institute [founded by Thomas Vaughan Morrow] was at least the peer of the average regular school. Admittedly it

was not the best in the country, but it was far from the worst. And it may well be that the eclectic physician sent out from Cincinnati in the 1840s and 1850s did their patients less harm than many doctors who proudly displayed the most orthodox credentials.”^w

By the time of his death, Morrow was the undisputed dean of eclecticism. This philosophy was spread to the American heartland by the guidance and dedication of Morrow, a 19th century Paul of Tarsus, rather than through the efforts of his teacher Wooster Beach. Moreover, as historian John Haller pointed out, Morrow was the first among a series of “men of strong personality who insisted that eclecticism was not so much a scientific system as it was a comprehensive mass of science embracing everything connected with the healing art.”^x His role in establishing the Eclectic Medical Institute as the “mother” of reformed medicine ensured that the sect would become more than a brief footnote in the annals of American medical history; indeed the Institute served as the “mecca of eclectic thinking” for nearly a century.^y His editorial direction first with the *Western Medical Reformer* from 1836 to 1838 and then with its successor the *Eclectic Medical Journal* in 1849 disseminated eclectic ideas across the country. Although as a scientist and author he was neither profound nor prolific,^z his contribution of administrative skills at both the institutional and national levels provided the sect with the direction it needed during its vulnerable early years. These are the chief contributions of a man accurately described as the “most inspiring apostle of Eclecticism.”^{aa}

Perhaps another way to view Morrow is not as the pioneer of a bygone medical school but as a precursor to what appears to be a rising botanical movement now referred to as phytomedicine. As the public increasingly turns to over-the-counter herbal remedies and Europe leads the way in serious phytomedical research through Germany's Commission E and the European Scientific Cooperative for Phytotherapy, the medical/pharmaceutical professions in America are slowly waking up to the fact that there should be more to their armamentarium than synthetics and chemotherapies. Noting that most of today's pharmacy school graduates are woefully unprepared to answer consumer questions regarding the efficacy of

an increasing assortment of herbal products, Lilly Distinguished Professor of Pharmacognosy at Purdue University Varro E. Tyler has called upon the profession to “do some homework to bring themselves up to speed as competent advisors.”^{bb}

This new emphasis upon the systematic investigation into the therapeutic prospects of vegetable products has shown itself in a number of ways: in a growing number of journals such as *Alternative Complementary Therapies*, *HerbalGram*, *Phytomedicine*, and *Protocol Journal of Botanical Medicine*; in passage of the “Dietary Supplement Health and Education Act of 1994” (often simply called DSHEA); and in an increasing number of scholarly symposiums and workshops on the subject.

The American Pharmaceutical Association's 143rd annual meeting at Nashville in March 1996 offered a considerable number of presentations dealing with phytomedicinal topics. There were three addresses on this theme in the AIHP (American Institute of the History of Pharmacy) section of contributed papers. In addition, the AIHP sponsored a symposium on “Herbal Remedies and Their Regulation.” The capstone, however, was an afternoon session held as the finale of the conference and attended by several hundred pharmacists. Dr. Ara Der Marderosian of the Philadelphia College of Pharmacy led a panel of scholars in an instructive and enthusiastic discussion of phytotherapeutics. As the audience was treated to a series of slides showcasing research in medicinal plant products, botany and medicine seemed to be renewing their marriage vows. The session clearly demonstrated that phytotherapeutics offer much more than an outmoded “weeds and seeds” approach to pharmacy and that modern phytomedicine is far more sophisticated than any New Age counterculture mystic could possibly appreciate. By the time the session was over the room seemed imbued with the approving spirit of Thomas Vaughan Morrow. That Tuesday afternoon on 12 March 1996 the botanico-medical movement was resurrected.

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ENDNOTES

- a. See Beach's own discussion in numerous editions of his *American Practice of Medicine*, first published in 1833. The actual phrase quoted here appeared as a heading in the introduction to his later editions such as *The American Practice Condensed, or The Family Physician* (New York: Clark, Austin & Smith, 1857), p. xi. For details on Wooster Beach and his Reformed System of Medicine see Alex Berman, "Wooster Beach and the Early Eclectics," *Univ. Mich. Med. Bull.* 24 (July 1958):277-286.
- b. Samuel Thomson, *New Guide to Health, or Botanic Family Physician*, 9th ed. (Columbus, OH: J. Pike, 1833), p. 10.
- c. Wooster Beach, *The American Practice of Medicine* (New York: Betts & Anstice, 1833), p. 11.
- d. An excellent account detailing the excesses of the regular physicians during this period is available in Alex Berman, "The Heroic Approach In 19th Century Therapeutics," *Bull. Am. Soc. Hosp. Pharm.* (Sep-Oct 1954):321-327.
- e. Alexander Wilder, *History of Medicine* (Augusta, ME: Maine Farmer Publishing, 1904), pp. 483-484. G.W.L. Bickley suggests that the initial duties of establishing the medical department at Worthington College fell upon a Dr. John J. Steele but that Morrow took over after Steele became "dissatisfied." See G.W.L. Bickley's "History of the Eclectic Medical Institute and Its Ethical Peculiarities," *Eclectic Med. J.* (Feb 1857):60.
- f. Alexander Wilder, *History of Medicine* (Augusta, ME: Maine Farmer Publishing, 1904), p. 515.
- g. Harvey Wickes Felter, *History of the Eclectic Medical Institute* (Cincinnati: Alumna Association, 1902), pp. 10-11.
- h. For details of this colorful episode see Harvey Wickes Felter, *History of the Eclectic Medical Institute* (Cincinnati: Alumna Association, 1902), pp. 16-17, and Alexander Wilder, *History of Medicine* (Augusta, ME: Maine Farmer Publishing, 1904), pp. 516-517. There is some discrepancy as to the exact date of this uprising. Wilder gives it as spring 1840; Felter, autumn 1839. Most historians have accepted Felter's date as the more reliable. See Alex Berman, "Wooster Beach and the Early Eclectics," *Univ. Mich. Med. Bull.* 24 (July 1958):280; and John S. Haller Jr., *Medical Protestants: The Eclectics in American Medicine, 1825-1939* (Carbondale: Southern Illinois University Press, 1994), pp. 81-82.
- i. G.W.L. Bickley, "History of the Eclectic Medical Institute and Its Ethical Peculiarities," *Eclectic Med. J.* (Feb 1857):61.
- j. Alex Berman offers some interesting discussion regarding Rafinesque's eclectic connections in his "A Striving for Scientific Respectability: Some American Botanics and the Nineteenth-Century Plant Materia Medica," *Bull. Hist. Med.* 30 (Jan-Feb 1956):23-31.
- k. The first use of the term eclectic in reference to a specific school of medical practice came from Dr. Thomas Cooke who had established the Eclectic Botanic Medical Association of Pennsylvania in 1840. See Alexander Wilder, *History of Medicine* (Augusta, ME: Maine Farmer Publishing, 1904), pp. 535-536.
- l. Although Rafinesque used the term in a different sense, in this essay "eclecticism" refers exclusively the 19th-century medical philosophy founded by Wooster Beach that called primarily for the use of indigenous American botanicals in its therapeutics.
- m. Quoted in Harvey Wickes Felter, *History of the Eclectic Medical Institute* (Cincinnati: Alumna Association, 1902), p. 21.
- n. Ronald L. Numbers, "The Making of an Eclectic Physician: Joseph M. McElhinney and the Eclectic Medical Institute of Cincinnati," *Bull. Hist. Med.* 47 (Mar-Apr 1973):157.
- o. Quoted in Alex Berman, "Wooster Beach and the Early Eclectics," *Univ. Mich. Med. Bull.* 24 (July 1958):281.
- p. Ronald L. Numbers, "The Making of an Eclectic Physician: Joseph M. McElhinney and the Eclectic Medical Institute of Cincinnati," *Bull. Hist. Med.* 47 (Mar-Apr 1973):162.
- q. T.V. Morrow, *A Few Thoughts on the Necessity of Medical Reformation* (Cincinnati: Derrough, 1844), pp. 12, 16.
- r. G.W.L. Bickley, "History of the Eclectic Medical Institute," *Eclectic Med. J.* (Mar 1857):105-106. The full series ran in four parts; from the January through the April issues of the *Eclectic Medical Journal*.
- s. Quoted in Alexander Wilder, *History of Medicine* (Augusta, ME: Maine Farmer Publishing, 1904), p. 511.
- t. Vaughan is buried in the Wesleyan Cemetery, Northside, Cincinnati, Ohio.
- u. Harvey Wickes Felter, *History of the Eclectic Medical Institute* (Cincinnati: Alumna Association, 1902), pp. 79-80.
- v. Ronald L. Numbers, "The Making of an Eclectic Physician: Joseph M. McElhinney and the Eclectic Medical Institute of Cincinnati," *Bull. Hist. Med.* 47 (Mar-Apr 1973):160.
- w. Alexander Wilder, *History of Medicine* (Augusta, ME: Maine Farmer Publishing, 1904), pp. 573-576.
- x. Ronald L. Numbers, "The Making of an Eclectic Physician: Joseph M. McElhinney and the Eclectic Medical Institute of Cincinnati," *Bull. Hist. Med.* 47 (Mar-Apr 1973):166.
- y. John S. Haller Jr., *Medical Protestants: The Eclectics in American Medicine, 1825-1939* (Carbondale: Southern Illinois University Press, 1994), p. 242.
- z. John S. Haller Jr., *Medical Protestants: The Eclectics in American Medicine, 1825-1939* (Carbondale: Southern Illinois University Press, 1994), p. 216.
- aa. The only extant sources for Morrow's writings are contained in the two eclectic journals he edited and in Ichabod G. Jones's *The American Eclectic Practice*

of Medicine, 2 vols. (Cincinnati: Moore, Anderson, 1853–1854). Jones (1807–1857), who had been a colleague of Morrow's at Worthington, appended "The Posthumous Writings of the Late Prof. T. V. Morrow on the Theory and Practice of Physic" to volume one of that work.

- aa. Ralph Taylor, "The Formation of the Eclectic School in Cincinnati," *Ohio State Archaeol. Hist. Quart.* 51 (Oct–Dec 1942):282.
- bb. Varro E. Tyler, "What Pharmacists Should Know About Herbal Remedies," *J. Am. Pharm. Assoc.* 36 (January 1996):29–37.

Performance of a Constructed Wetland for On-Site Wastewater Treatment

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ABSTRACT

A subsurface flow (SSF) constructed wetland system was operated in Lexington, Kentucky, to treat wastewaters from a single household. The system was monitored monthly for a period of 1 year for temperature, pH, dissolved oxygen (DO), biochemical oxygen demand (BOD₅), total suspended solids (TSS), nitrate nitrogen (NO₃-N), ammonia nitrogen (NH₄-N), orthophosphate (PO₄ ion), and fecal coliform (FC) bacteria. It provided satisfactory reductions in concentrations of TSS, NH₄-N, and BOD₅. NH₄-N level in the septic tank was 86.67 mg/liter then dropped to 34.63 over the first 7 m of the bed and reached 10.76 mg/liter at the discharge end of the system (21.3 m). Over the same bed distance (21.3 m), counts of fecal bacteria were reduced about 99.93% but still averaged 811 colonies/100 ml, which exceeds the concentration of 200 colonies/100 ml of wastewater (reference level established by EPA) at the discharge end of the wetland system. Dissolved oxygen increased from 0.22 mg/liter in the septic tank to 1.91 mg/liter at the beginning of the wetland system, then fluctuated through the system to reach 1.32 mg/liter in the effluent discharge. There was no significant difference between the various regions of the bed for NO₃-N removal. The wetland system shows promise for a novel treatment process for removal of BOD, TSS, and pathogenic bacteria (FC) that can provide a sustainable solution for treatment of septic tank effluent. Further research work may lead to improvements in the system design to increase efficiency for removal of NO₃, NH₄ and PO₄.

INTRODUCTION

Steiner and Combs (1993) indicated the need for alternative wastewater treatment systems in Kentucky due to the vulnerability of Kentucky's groundwater to pollution. At least half of Kentucky's aquifer systems occur in karst regions, which make these aquifers highly susceptible to contamination from the surface (Anonymous 1994). The treatment of domestic sewage is a problem confronting small communities throughout the U.S. (Wolverton 1987a). A promising solution to this problem is an emerging technology using subsurface flow (SSF) wetland systems with plants for wastewater treatment.

SSF wetland systems have the capacity to remove a large percentage of the total nitrogen and other pollutants in wastewater (Gersberg, Elkins, and Goldman 1983) and to satisfy regulatory effluent criteria (EPA 1993). They can be installed in any suitable location

proximal to the home, taking advantage of the land elevations. In SSF wetland systems, a major part of the treatment process for degradation of pollutants is attributed to the microorganisms living in a symbiotic relationship on and around root systems of the plants (Wolverton 1987b). During microbial degradation of pollutants, metabolites are produced which the plants absorb and utilize along with nitrogen, phosphorus, and other minerals as a food source. Microorganisms also use some or all metabolites released through plant roots as a food source. Recent advances in SSF wetland systems take advantage of high surface support media (such as rock-based filter beds) and their support of certain wetland plants with associated microbiota. The synergistic effect of this type of technology removes many of the substances contributing to BOD (e.g., NO₃, NO₂, NH₄, and PO₄ ions) from domestic sewage wastewaters. When plants such as cattail (*Typha latifolia*) are planted in the rock filter, their roots penetrate into the wastewa-

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ter, adding oxygen and producing an aerobic rhizosphere around the roots, thereby increasing biological activity.

An understanding of the effectiveness of the constructed SSF wetland treatment systems is the long-range goal of the present study, which provides an overview of preliminary characteristics important in considering treatment efficiency. Characteristics of the wastewater to be treated include BOD₅, TSS, nitrogen and phosphorus compounds, heavy metals, and microbial load (Wieder, Tchobanoglous, and Tuttle 1989). Currently, however, quality performance standards are based on BOD₅, TSS, and FC bacteria. Well-designed SSF wetland treatment systems typically can produce effluent water with BOD₅ values of 10 mg/liter and suspended solids concentrations below 10 mg/liter (Tchobanoglous 1987). A BOD₅ level of <20 mg/liter and TSS level of <20 mg/liter meet regulatory effluent criteria, according to current EPA regulations (EPA 1993).

The main objectives of the present study were to provide information on the treatment efficiency of an SSF constructed wetland used for single-home wastewater treatment. These monitoring data can be used to define the fate of wastewater constituents including their removal, retardation, transformation, and movement within the wetland system so that management decisions may be made regarding suitability of these systems for area installations and approval by the Health Department in Kentucky.

MATERIALS AND METHODS

A single-family dwelling (three-bedroom house of three people) in Lexington, Fayette County, Kentucky was studied as a model system. The wetland was a plastic-lined trench 21.34 m long, 1.22 m wide, and 0.46 m in depth. This type of SSF, commonly called a rock-plant filter, was developed by National Aeronautic and Space Administration (NASA) at the National Space Technologies Laboratory in Mississippi (Wolverton 1987b). The trench was partially filled with size 2 rock (crushed limestone) to a depth of ca. 0.36 m; the water level was maintained at 0.36 m; the trench was then covered with size 5 and 6 rock to a depth of 0.46 m. The inlet of the system was fed wastewater from the septic tank (Fig-

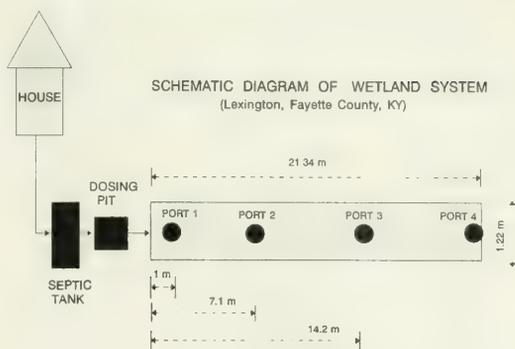


Figure 1. Schematic diagram of a subsurface flow (SSF) constructed wetland system for a three-bedroom house (Lexington, Fayette County, KY 1991). Note that sampling ports are 1 meter (port 1), 7.1 meter (port 2), 14.2 meter (port 3), and 21.3 meter (port 4) from the edge of the system.

ure 1). Cattail (*Typha latifolia*) was planted. The estimated wastewater flow throughout the system was 1.36 m³/day (360 gallon/day). Retention time in the SSF system was 4.39 days calculated on the basis of 360 gallons of wastewater flow throughout the system per day. Cattail rhizomes were planted in 1990, allowed to mature for several seasons, and were set out with one plant per each 0.37 m² of bed surface (70 plants were used for the system) for optimum efficiency (Gersberg, Elkins, and Goldman 1983).

The system was sampled three times over the course of a single day once a month from February 1991 to January 1992 (for technical reasons samples of April and June 1991 were not available for analysis). The samplings occurred in the morning, mid-day, and evening from fixed sampling ports throughout the system: the septic tank (ST), inlet port (port 1), two middle ports (ports 2 and 3, which represent 1/3 [7.1 m] and 2/3 [14.2 m] the system length, respectively), and the discharge end port (port 4).

The system was monitored monthly for temperature, pH, and dissolved oxygen (DO) in the field and analysed for biochemical oxygen demand (BOD₅) in the 5-day test, total suspended solids (TSS), nitrate nitrogen (NO₃-N), ammonia nitrogen (NH₄-N), orthophosphate (PO₄ ion), and FC bacteria. The water quality parameters of the collected samples were analysed at the Water Quality and En-

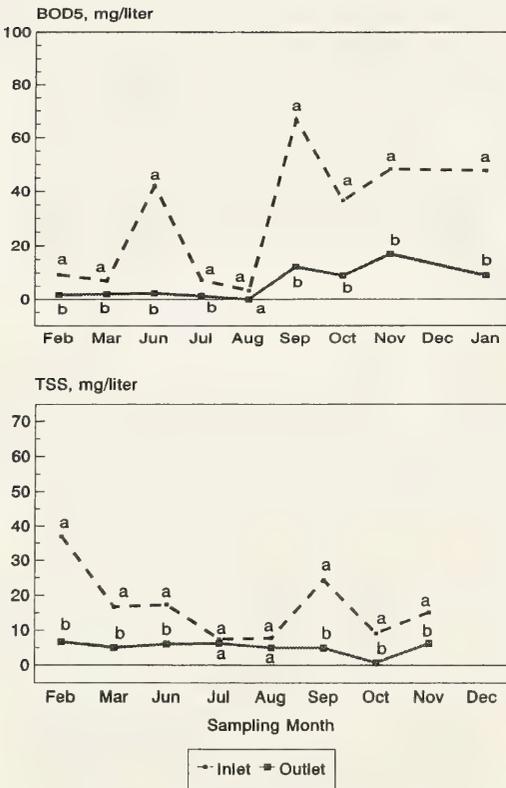


Figure 2. Mean concentration (n=30) of BOD₅ and TSS in influent and effluent wastewater from constructed wetland system for a three-bedroom house (Lexington, Fayette County, KY 1991) versus sampling month. Inlet and outlet concentrations having different letters are significantly different ($P < 0.05$).

Environmental Toxicology laboratory at Kentucky State University using standard methods (APHA 1992). Ammonia (NH₄-N) was determined by the selective ion electrode method 4500-F; BOD₅, by method 5210-B; nitrate (NO₃-N), by method 4500-NO₃-E; orthophosphate, by method 4500-P-E; pH was determined by the electrometric method (method 4500-H); and total suspended solids, by method 2540-D.

FC bacterial analysis was carried out in the Lexington-Fayette Health Department Laboratory using the membrane filter standard method no. 9222 (APHA 1992). All samples were collected 13 cm above the bottom of system from each sampling port to avoid disturbing any sediment and were analysed within 6 hours.

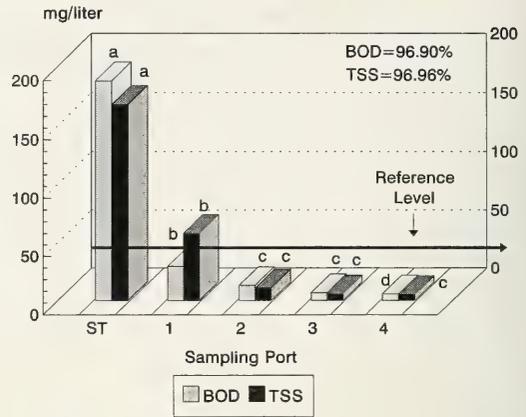


Figure 3. Concentration of BOD₅ and TSS (n=30) in wastewater from constructed wetland system for a three-bedroom house (Lexington, Fayette County, KY 1991) versus sampling port and septic tank (ST). Bars having different letters are significantly different ($P < 0.05$).

Data were analysed for each parameter by port distance with respect to the septic tank and by sampling months using analysis of variance (ANOVA) procedure (SAS Institute 1991). Means (n=30) were compared using Fisher's protected LSD test (Snedecor and Cochran 1967).

RESULTS AND DISCUSSION

BOD₅ and TSS effluent values for the studied system during all sampling months were below the 20 mg/liter reference level, which is a common permit requirement (EPA 1993) (Figure 2). Overall BOD₅ average value dropped significantly ($P < 0.05$) from 187.8 mg/liter in the septic tank (ST) to 5.7 mg/liter at the discharge of the system (port 4), which indicated an overall removal of 96.9% (Figure 3). TSS also dropped from 168.0 mg/liter (ST) to 5.10 mg/liter (discharge of the system), which indicated 96.9% removal (Figure 3).

Oxygen plays an important role for many changes in wastewater composition. One oxygen source in these wetland beds is roots of the cattails. Therefore, it is essential to bring the wastewater into direct contact with the root zone. Brix and Schierup (1990) indicated that oxygen may also be supplied as a result of air movement into the bed as the feedwater level falls during the flow-off period of an intermittent flow regime, and possibly as a result

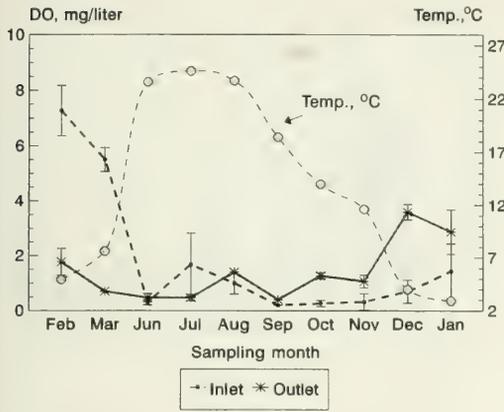


Figure 4. Dissolved oxygen (DO) concentrations in influent and effluent and temperature levels in wastewater from constructed wetland system for a three-bedroom house (Lexington, Fayette County, KY 1991). Vertical bars indicate \pm standard error.

of flow around the gravel particles and through air-filled pores.

Average DO, pH, and temperature in the septic tank were 0.22 mg/liter, 7.96, and 10° C, respectively. These values showed significant variation throughout the system. The DO concentration in this system averaged 0.22 mg/liter in samples collected from septic tank and 1.32 mg/liter in port 4. The relationship between DO and temperature by sampling month is illustrated in Figure 4. DO level increased significantly ($P < 0.05$) when temperature decreased (winter months).

Removal of FC bacteria is a concern of health officials (Wolverton 1987a). The system under study cannot satisfy the discharge requirements of FC removal, which often specifies < 200 colonies/100 ml of wastewater (EPA 1993). Table 1 shows that the overall average counts of FC dropped from $1.2 \times$

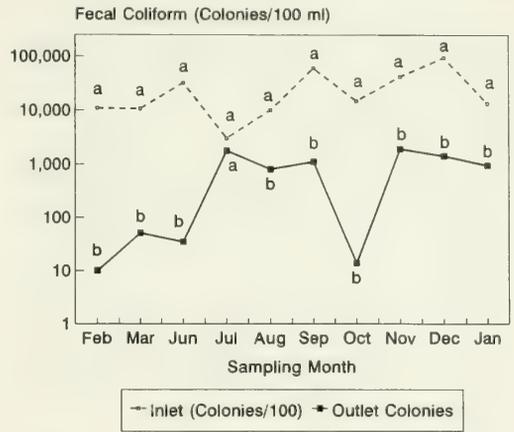


Figure 5. Mean counts of fecal coliform colonies ($n=30$) in influent and effluent wastewater from constructed wetland system for a three-bedroom house (Lexington, Fayette County, KY 1991) versus sampling month. Inlet and outlet counts having different letters are significantly different ($P < 0.05$).

$10^6/100$ ml wastewater in the septic tank to $0.8 \times 10^3/100$ ml at the end of the system (99.9% removal); but these remaining levels are still over the discharge requirements. FC counts in wastewater sampled from inlet and outlet versus sampling month (Figure 5) indicated significant differences.

Wetlands have the capacity to remove large percentages of total nitrogen in wastewater. Chemoautotrophic nitrifying bacteria, mainly *Nitrobacter* and *Nitrosomonas*, oxidize ammonia (NH_4) to nitrite (NO_2) and nitrate (NO_3), respectively. NO_3 and NO_2 are reduced by facultative bacteria to nitrous oxide (N_2O) and nitrogen gas (N_2) in the anaerobic denitrification process (Davido and Conway 1991). Oxygen consumption in this process is due to the direct microbial oxidation of organic mat-

Table 1. Impact of a constructed subsurface flow wetland system for three-bedroom house on some wastewater parameters¹ (Fayette County, Lexington, KY 1991).

Sampling port	Colonies/100 ml of water	Dissolved O ₂ (mg/liter)	PO ₄ (mg/liter)	pH	Temperature °C
Septic tank	1,200,000 \pm 505,000a	0.22 \pm 0.02c	—	7.96 \pm 0.3a	10.0 \pm 0.0d
Port 1	29,000 \pm 21,200b	1.91 \pm 1.46a	1.76 \pm 0.70a	7.51 \pm 0.2b	14.5 \pm 8.5a
Port 2	12,000 \pm 11,070b	1.40 \pm 1.07b	1.77 \pm 0.72a	7.46 \pm 0.2b	13.9 \pm 9.1b
Port 3	900 \pm 811b	1.85 \pm 1.18a	1.46 \pm 0.91b	7.30 \pm 0.1c	13.4 \pm 9.3bc
Port 4	800 \pm 717b	1.32 \pm 0.94b	1.20 \pm 1.10c	7.22 \pm 0.2c	13.2 \pm 8.7c

¹ Each value in the table is an average \pm SE of 10 months analysis. Values within a column having different letters are significantly different from each other, using Fisher's protected LSD ($P < 0.05$). Note that sampling ports are 1 meter (port 1), 7.1 meter (port 2), 14.2 meter (port 3), and 21.3 meter (port 4) from the edge of the system.

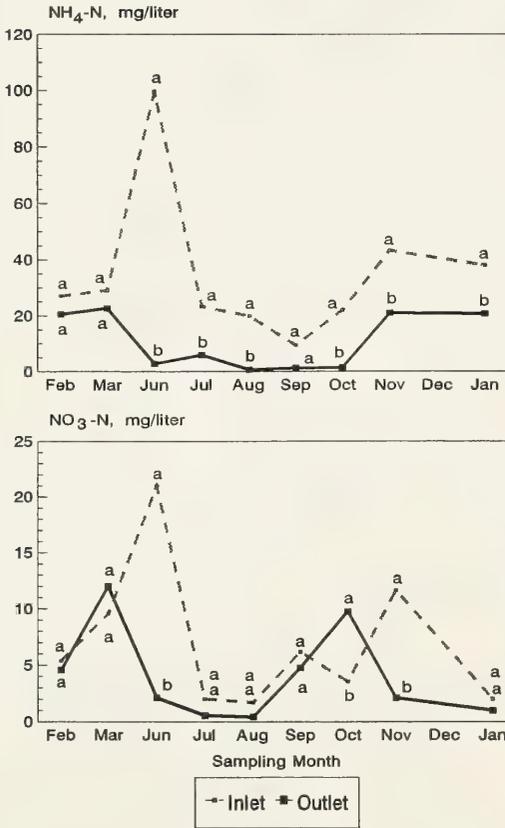


Figure 6. Mean concentration (n=30) of NH₄-N and NO₃-N in influent and effluent wastewater from constructed wetland system for a three-bedroom house (Lexington, Fayette County, KY 1991) versus sampling month. Inlet and outlet concentrations having different letters are significantly different ($P < 0.05$).

ter and oxidation of reduced substances. Systems with good aeration will likely have most of the nitrogen in the nitrate form. Generally, levels of NH₄-N were significantly lower in wastewater effluent than in influent, as indicated in Figure 6. This decrease in NH₄-N level should be accompanied by an increase in NO₃-N level during the same sampling period if nitrification process is taking place properly in the system. But what is clear in Figure 6 is that NO₃-N level in effluent wastewater was equal to the level in the influent from January to March (2 to 7.5° C) and also from July to September (18 to 24.5° C). These findings indicated that the system was not effective in reducing nitrate.

Figure 7 shows that NH₄-N concentrations

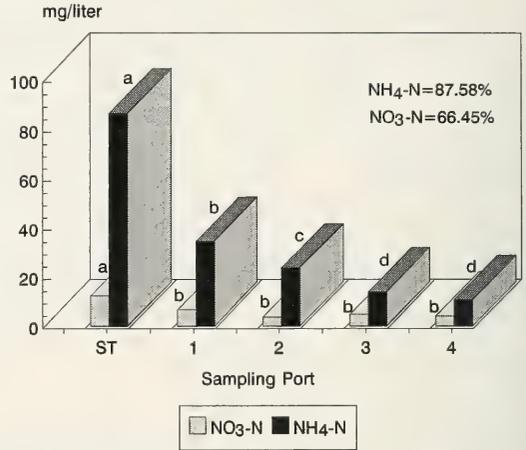


Figure 7. Levels of NH₄-N and NO₃-N (n=30) versus sampling port and septic tank (ST). Bars having the same letters are not significantly different ($P > 0.05$).

decreased from 86.7 mg/liter (septic tank) to all depths across the wetland system and reached 10.8 mg/liter at the discharge end of the system. This corresponds to 87.6% ammonia removal. On the other hand, the decrease in NH₄-N concentration should correspond with an overall NO₂-N and NO₃-N increase (Davido and Conway 1991) at all depths in the marsh. One may consider that the decrease in NH₄-N is due mainly to nitrification during aeration and that the decrease in NO₃-N is probably due to denitrification and absorption by wetland plants. Figure 7 indicates that NO₃-N was 12.4 mg/liter in the septic tank and then showed little variation throughout the system ranging from 6.7 mg/liter at the beginning of the system to 4.1 mg/liter at the system end with no significant differences.

Data obtained from the present study are start-up data; the system may not respond the same after several years of use. Generally, operation and maintenance of onsite systems are left to the homeowner. Maintenance of septic systems consists primarily of periodic pumping of the tank to remove the build-up of sludge in the tank. Many homeowners are not aware that septic tanks are designed to accommodate a particular daily water usage and that systems can malfunction from overload. Therefore, with increasing use of onsite systems, homeowner education and personal responsibility

have to be considered for the success of the SSF constructed wetland systems.

ACKNOWLEDGMENTS

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NOTES

Rediscovery of *Ammocrypta clara* (Percidae) in Kentucky; Range Extensions for Six Other Kentucky Fishes.—Recent fish surveys in the upper Kentucky and Green River basins and an examination of museum specimens resulted in rediscovery of *Ammocrypta clara*, which was presumed extirpated from Kentucky, and range extensions for six other species. Voucher specimens for each species, except *Cottus bairdi* (at the Branson Museum of Zoology, Eastern Kentucky University, Richmond), are housed at the Kentucky State Nature Preserves Commission (KNP), Frankfort, or at Southern Illinois University at Carbondale (SIUC).

Ammocrypta clara, western sand darter.—KNP uncat. (1), North Fork Kentucky River downstream from Frozen Creek, Breathitt Co., 1 Jun 1995; KNP uncat. (2), Green River at River Styx, Mammoth Cave National Park (MCNP), Edmonson Co., 7 Sep 1995. In Kentucky, the western sand darter has been collected only from the Green River at Greensburg, Green Co., in 1890, the Cumberland River at Mill Springs, Wayne Co., in 1925, and Wolf Creek (Big Sandy River) near Lovely, Martin Co., in 1938 (1, 2). The specimens reported herein were collected downstream from islands over clean sand in shallow (< 0.5 m) water with slow or no current. Additional sampling in the North Fork failed to reveal additional specimens, but ca. 40 seine hauls at the Green River site yielded four specimens. Both populations are extremely localized and rare as judged from our failure to secure specimens from 24 sites sampled in the Green River in MCNP in 1990 (3) or from 19 additional mainstem North Fork Kentucky River sites sampled in 1995. The western sand darter formerly was considered extirpated from Kentucky (4) but will be added to the list as endangered. Persistent collecting with small mesh seines in suitable habitat and at night should further clarify its range and conservation status in Kentucky.

Ammocrypta pellucida, eastern sand darter.—KNP uncat. (1), Quicksand Creek immediately upstream from the North Fork Kentucky River, Breathitt Co., 14 Jul 1995; KNP uncat. (1), North Fork Kentucky River at KY 28, Perry Co., 15 Aug 1995; KNP uncat. (1), North Fork Kentucky River ca. 2.4 stream km downstream from Cedar Creek, Breathitt Co., 18 Aug 1995. The only previous record of the eastern sand darter in the North Fork Kentucky River drainage is based on 16 specimens collected at the mouth of Quicksand Creek in 1925 (University of Alabama Ichthyological Collection 3960) (1). Our collections confirm the existence of the eastern sand darter in Quicksand Creek and extend its range upstream into Perry County. We released seven additional specimens at Quicksand Creek and 10 individuals (representing three age classes) at the Cedar Creek site that we assumed were *A. pellucida*; however, some of these specimens could have been *A. clara*. All collections were made during low-flow from shallow (< 0.5 m), slow-

flowing pools/runs with extensive sand deposits. The eastern sand darter is of special concern in Kentucky (4).

Cottus bairdi, mottled sculpin.—KNP uncat. (3), Upper Devil Creek (North Fork Kentucky River) ca. 200 stream m upstream from Bear Pen Creek, Wolfe Co., 18 Aug 1995. The mottled sculpin is sporadic to occasional from the Kentucky River to the Big Sandy River, with most populations located along or near the Pottsville Escarpment (1). Previously known from the Kentucky River drainage upstream as far as Station Camp Creek, Estill and Jackson cos. (1), the mottled sculpin now is known to inhabit the North Fork Kentucky River drainage. The record for *Cottus caroliniae* from Laurel Fork, Harlan County, in the upper Middle Fork Kentucky River basin (5) (EKU 1034) was re-examined and is referable to *C. bairdi*. Although habitat seemingly suitable for *C. bairdi* is common on the Cumberland Plateau, the latter record is only the third clearly from this region of Kentucky.

Fundulus catenatus, northern studfish.—KNP uncat. (2), Line Fork (North Fork Kentucky River) 1.3 stream km upstream from Big Branch, Letcher Co., 12 Jun 1995; KNP uncat. (1), North Fork Kentucky River at Big Branch, Perry Co., 6 Jul 1995. In the Kentucky River drainage the northern studfish is known only from the Dix River drainage, possibly as a result of stream capture or bait-bucket introduction (1, 6). The collections reported herein and three additional specimens released at the Perry County site extend the range of the northern studfish in the Kentucky River basin upstream more than 400 river km. Both occurrences probably resulted from bait-bucket introductions.

Percina shumardi, river darter.—KNP uncat. (1), South Fork Kentucky River at Matton Cr., Lee/Owsley cos., 26 Sep 1995. Except in the Big Sandy, Salt, and Tradewater rivers and Tygarts Creek, river darters are sporadic and uncommon in all of Kentucky's major rivers (1, 7). Formerly known only from the lower Red River in the Kentucky River drainage (8, 9), the river darter now is known to inhabit the lower South Fork Kentucky River. The specimen was collected from a slow-flowing pool (ca. 0.5-1.25 m deep) underlain with mixed sand, pebble, and gravel and scattered cobble and boulders in association with the fishes *Ammocrypta pellucida*, *Extrarius aestivalis*, *Hybopsis amblops*, *Percina caprodes*, *P. copelandi*, *P. evides*, *P. maculata*, and *P. oxyrhynchus*.

Phoxinus erythrogaster, southern redbelly dace.—KNP uncat. (5), Cope Fork (North Fork Kentucky River) at Little Falling Rock Branch, Breathitt Co., 15 Aug 1995; KNP uncat. (5), Hell Creek (North Fork Kentucky River) ca. 7.3 air km NE of Beattyville, Lee Co., 17 Aug 1995; KNP uncat. (5), Upper Devil Creek (North Fork Kentucky River) ca. 200 stream m upstream from Bear Pen Creek, Wolfe Co., 18 Aug 1995. The southern redbelly dace, previously unreported from the North Fork Kentucky River drainage (1), is an occasional and common

inhabitant of small, spring-fed, upland streams in the eastern two-thirds of the state.

Pimephales vigilax, bullhead minnow.—SIUC 23175 (5), North Fork Kentucky River at Rock Lick Creek, Breathitt Co., 7 Jul 1993; KNP uncat. (3), North Fork Kentucky River at Wolf Creek, Breathitt Co., 6 Apr 1995; KNP uncat. (4), North Fork Kentucky River at Lick Branch, Perry Co., 30 Jun 1995; SIUC 20929 (3), South Fork Kentucky River at Hacker Branch, Owsley Co., 18 Aug 1992. The bullhead minnow is occasional to generally distributed in the Kentucky River drainage, where previous headwater records are limited to the Middle Fork (1). These are the first records for the South and North forks.

The rediscovery of the western sand darter and the eastern sand darter in Quicksand Creek reinforces the need to thoroughly and repeatedly sample historic collection sites and the specific habitat of species presumed extirpated from Kentucky (1, 4). Despite being reasonably well collected (1, 5, 10, 11, 12, 13, 14, 15), the upper Kentucky River drainage, particularly the North Fork where we made 117 collections in 1995, yielded several range extensions. Most are from headwater streams or the mainstems of large rivers, which are noted for their potential for ichthyofaunal discoveries (7). Although often ignored or difficult to sample for small species that comprise the bulk of piscine biodiversity, these habitats are likely to yield additional rediscoveries and range extensions.

Our appreciation is extended to former KSNPC staff members A.L. Covert and M.A. Patterson for field assistance; to D.A. Etnier, University of Tennessee, for confirming the *A. clara* identification; to B.M. Burr, Southern Illinois University at Carbondale, for reviewing the manuscript; and to Robert McCance, Jr., Director, KSNPC, for supporting this effort.

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Unusual Dimensions of Amur Honeysuckle (*Lonicera maackii*, Caprifoliaceae).—Amur honeysuckle (*Lonicera maackii* (Rupr.) Herder; Caprifoliaceae), is a non-indigenous shrub now naturalized throughout much of eastern United States. Its average height in northern Kentucky in open habitats is 2–3 m; its width tends to equal its height. Average height in forested habitats of the region is 4–5 m; width is difficult to measure due to intertwining of shrub branches. Shrubs in forested habitats tend to branch less and have longer vertical opportunistic branches than those in open habitats. Published maximum dimensions of Amur honeysuckle are as follows: height 6 m, crown width 9 m, and stem diameter to 15 cm (4). We observed a population of Amur honeysuckle at the Terrace Park Nature Preserve, Hamilton Co., Ohio, where many individuals exceeded these dimensions. The Terrace Park Nature Preserve is on a floodplain of the Little Miami River. Much of the preserve supports a bottomland hardwood forest with *Acer negundo*, *Carya cordiformis*, and *Celtis occidentalis* as the dominant tree species. Amur honeysuckle is dominant in the understory.

In a single subjectively placed 150 m² plot, the following measurements were obtained: maximum shrub height 8.0 m; maximum crown width 9.8 m; maximum stem diame-

ter 17.4 cm; maximum shrub base diameter 49.0 cm; basal area of shrub bases 54.5 m²/ha; basal area of stems 22.1 m²/ha; shrub density 0.1 shrubs/m²; and stem density 0.3 stems/m². Because of the difficulty in interpreting core samples from Amur honeysuckle, a single shrub that represented average size was cut and a complete cross-section was removed for aging. The cross-section indicated that the shrubs are about 40 years old.

One of us (JOL) traveled to northeastern China in September 1994 to study Amur honeysuckle in its native habitat. He found the species primarily in river bottom communities. On a floodplain 30 km N of The Changbai Research Station, the largest shrub he measured had the following dimensions: shrub height 5.0 m; crown width 5.7 m; base diameter 15.0 cm; and maximum stem diameter 6.7 cm.

Basal area of Amur honeysuckle stems in Terrace Park approximates that of an entire forest community. For example, Bryant (1, 2) reported total basal areas of 35.5 m²/ha and 31.0 m²/ha for old growth forests in nearby Kentucky. Luken et al. (3) calculated an average basal area of 25.8 ± 1.9 m²/ha from various second-growth woodlands in northern Kentucky.

Amur honeysuckle has clearly become an important component of the landscape in southern Ohio and northern Kentucky. The shrub may comprise a major amount of biomass in the forest understory. It may also be affecting population dynamics of canopy trees.

We thank Randy Haller, Arborist for the Village of Terrace Park, for his assistance and permission to study in the preserve; and Linda Kuddes for aid in data collection.

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New County Records for the West Virginia White (Lepidoptera: Pieridae: *Pieris virginiensis*) in Kentucky.—The West Virginia white, *Pieris virginiensis* W. H. Edwards, has a single flight period lasting from late March to early May in Kentucky (1). Opler and Malikul (2) showed *P. virginiensis* as being distributed throughout the eastern third of Kentucky with a disjunct population oc-

curing in the general area around Louisville, Jefferson County, Kentucky. More recently, numerous new records for this species have been found throughout the state, although none has been reported for the Inner Bluegrass subsection (C. Covell Jr., pers. comm.). During spring 1995 we searched five counties in the Inner Bluegrass. Subsequently, new records for *P. virginiensis* were confirmed for four of these counties.

ANDERSON COUNTY—On 12 Apr 1995, two individuals (one male, one female) were collected along Wildcat Creek, a first-order stream at elevations of 180 to 198 m. The collection site is 4 km from State Highway 62 along County Highway 1510 on the eastern boundary of the county.

CLARK COUNTY—On 8 Apr 1995 12 individuals (nine males, three females) were collected along an unnamed, intermittent first-order stream at an elevation of 242 m. This site is on Grime's Mill Road, 1.6 km south of county highway 418 on the southwestern boundary with Fayette County. We saw ca. 50 individuals of *P. virginiensis*. Six species of butterflies (mourning cloak, *Nymphalis antiopa*; cabbage butterfly, *Pieris rapae*; Juvenal's dusky wing, *Erynnis juvenalis*; tiger swallowtail, *Papilio glaucus*; eastern tailed blue, *Everes comyntas*; and spring azure, *Celastrina argiolus*) and two species of diurnal moths (grapevine epimenis, *Psychomorpha epimenis*; and orange wing, *Mellilla xanthometata*) were seen along the stream with *P. virginiensis*. We visited this site on 18 Mar 1995 but found no *P. virginiensis* flying at that time.

FAYETTE COUNTY—On 8 Apr 1995, individuals were sighted at the confluence of the above intermittent stream and Boone Creek, a second-order stream that forms the boundary of Clark and Fayette counties. On 29 Apr 1995 a population was observed at the Raven Run Nature Sanctuary along a first-order stream at elevations of 197 to 273 m. This location is 2.4 km east of county highway 1975 (Jack's Creek Road) near the extreme southeastern boundary of the county. No specimens were collected from Fayette County.

WOODFORD COUNTY—On 25 Apr 1995 two individuals (one male, one female) were collected along Buck Run, a first-order stream at elevations of 167 to 182 m. This site is 1.2 km southwest of county highway 1964 along the western boundary of the county.

These four county records document the presence of *P. virginiensis* in the north-central portion of Kentucky. It is very probable that additional spring collecting will reveal that *P. virginiensis* is much more widely distributed within the Inner Bluegrass and north-central Kentucky than is currently known.

Voucher specimens from Anderson, Clark, and Woodford counties were deposited in the collection of Lepidoptera at the University of Louisville.

We thank Converse Griffith, James Wagner, and Dave Wooster for their comments on this manuscript and Charles Covell Jr. for confirming our species identification.

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terflies of North America: a natural history and field guide. Stanford Univ. Press, Stanford, CA. (2) Opler, P.A. 1992. A field guide to eastern butterflies. Houghton Mifflin Co., Boston, MA.—**Michael J. Lauer, James J. Krupa,** and

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KAS Annual Meetings

The 1996 annual meeting of the Kentucky Academy of Science will be held 14-16 November at Kentucky State University, Frankfort. The 1997 meeting will be held at Morehead State University, Morehead; the 1998 meeting, at Jefferson Community College, Southwest Campus, Louisville.

PUBLICATIONS

Two 1995 checklists by P.J. Harmon et al. concerning the vascular flora of West Virginia have been reprinted: *Checklist of the Vascular Flora of West Virginia* (\$5.00, including shipping) and *Checklist of the Wetland Vascular Plants of West Virginia* (\$4.00, including shipping). To order, send request and a check for the purchase price to Sue Kennedy, West Virginia Natural Heritage Program, DNR, P.O. Box 67, Elkins, WV 26241; phone (304) 637-0245.

Aquatic and Wetland Plants of Kentucky, by Ernest O. Beal and John W. Thieret, has been reprinted. To order, send request and a check for \$24.68 (including shipping; Kentucky residents add \$1.32 for tax) to Kentucky State Nature Preserves Commission, 801 Schenkel Lane, Frankfort, KY 40601; phone (502) 573-2886.

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